

ECOLOGICAL EVALUATION OF PROPOSED
DISCHARGE OF DREDGED MATERIAL FROM
OAKLAND HARBOR INTO OCEAN WATERS
(PHASE III B OF -42-FOOT PROJECT)

Volume 1 - Analyses and Discussion

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PREFACE

This is Volume 1 of a two-volume report that presents information gathered to determine the suitability of ocean disposal of sediments dredged from Oakland Harbor. This volume contains project background, materials and methods, results, discussion, and conclusions. Volume 2 contains Appendixes A through N, which provide details of the data analyses and full presentation of the data and results.

SUMMARY

The Water Resources Development Act of 1986 (Public Law 99-662) authorized the U.S. Army Corps of Engineers (USACE) San Francisco District, to deepen and widen the navigational channels of the Oakland Inner and Outer Harbors to accomodate deeper-draft vessels. The USACE is considering several disposal options for the dredged material removed during these channel improvements including open-water disposal. Dredged material proposed for open-water disposal must be evaluated to determine the potential impacts of the disposal activity on the water column and disposal site environments. The USACE requested that Battelle/Marine Sciences Laboratory (MSL) conduct studies to evaluate open-water disposal options for Oakland Harbor sediments. This request developed into the Oakland Harbor Phase III Program.

Because of the number of sites and associated evaluations, Phase III was divided into three projects: Oakland Harbor Phase III A (Inner Harbor deepening from -38 ft to -42 ft MLLW), Phase III B (Outer Harbor deepening to -42 ft MLLW), and Phase III 38 ft (Inner Harbor deepening from -35 ft to -38 ft MLLW). This report summarizes the collection, chemical analysis, toxicity testing, and bioaccumulation analysis of sediments conducted during the Oakland Harbor Phase III B Project, initiated in November 1990.

During Phase III B, sediments representing dredged material from 18 Oakland Harbor stations were tested with and compared to sediments from six reference areas representing potential disposal sites (in the absence of a single designated disposal site). Core samples of sediment targeted for potential dredging operations were collected from the Oakland Harbor stations, while surface sediments were collected from reference and control sites. Test treatments (potential dredged material), reference treatments, and control treatments were tested for physical and chemical parameters, water column effects, dredged sediment toxicity, and bioaccumulation potential.

Physical and chemical analyses of sediment consisted of conventional parameters [grain size, total volatile solids (TVS), total organic carbon (TOC), oil and grease, and total petroleum hydrocarbons (TPH)], metals, polynuclear aromatic hydrocarbons (PAH), chlorinated pesticides and polychlorinated biphenyls (PCB), and butyltin compounds. The results of the physical analyses for sediment conventionals showed that the highest concentrations of these parameters were present in test treatments O-C7, O-C6, O-C8, reference treatment R-BF, and O-C13 (in descending order). Generally, the finer-grained sediments were associated with higher TOC, TVS, oil and grease, and TPH levels. Results of chemical analyses showed some interesting and

similar trends for some parameters. Test treatments O-C8, O-C7, R-BF, and O-C13 had the highest total metal concentrations of all samples. Levels of organic compounds, determined by the sum of butyltin compounds, Aroclor 1254, and PAH concentrations were found to be the highest in sediment treatments O-C13, O-C6, O-C8, and O-C7. Chlorinated pesticides and PCBs other than Aroclor 1254 were not present in significant amounts in any of the sediments tested. In contrast to the consistently high contaminant concentrations in sediment treatments O-C6, O-C7, O-C8, and O-C13, four treatments consistently showed low concentrations of the parameters measured: O-C5, O-C9, O-C12, and I-C3.

To evaluate water column effects, suspended-particulate-phase (SPP) tests were conducted using the mysid shrimp *Holmesimysis sculpta*, the speckled sanddab *Citharichthys stigmaeus*, and larvae of the mussel *Mytilus edulis*. SPP tests of four sediment composites representing the bulk of Outer Harbor sediments showed that none were acutely toxic to the species tested. To evaluate dredged sediment toxicity, solid-phase toxicity tests were conducted using the bent-nose clam *Macoma nasuta*, the polychaete worm *Nephtys caecoides*, the speckled sanddab *C. stigmaeus*, and the amphipod *Rhepoxynius abronius*. Acute toxicity was noted in two of the solid-phase tests: the 10-day *R. abronius* test and the 10-day *N. caecoides* test. Significant decreases in *R. abronius* and *N. caecoides* survival were observed in certain test treatments, depending on the choice of reference treatment used for comparison. Treatment O-C1 showed significant *R. abronius* mortality relative to all six reference treatments; O-C1 was also the only treatment that was acutely toxic to both *R. abronius* and *N. caecoides* relative to a reference treatment (R-AM).

Bioaccumulation was evaluated by exposing *M. nasuta* and *N. caecoides* to solid-phase treatments for 28 days and then measuring the contaminants of concern present in their tissues. The concentrations of contaminants in the tissues were compared to existing Food and Drug Administration (FDA) limits, and were also compared using Dunn's Test to determine whether statistically significant ($\alpha = 0.10$) levels of contaminants were present in organisms exposed to test sediments relative to those exposed to reference sediments. Bioaccumulation results showed that FDA limits, where available for compounds of interest, were not exceeded, but that statistically significant levels of contaminants were present in the tissues of *M. nasuta* and *N. caecoides* exposed to test treatments relative to the six references. Three PAHs (naphthalene, fluoranthene, and pyrene) accumulated in *M. nasuta* and *N. caecoides* regardless of which reference the test treatments were compared to. Metals also bioaccumulated in both *M. nasuta* and *N. caecoides* from treatments relative to references, but the significant treatments were different for *M. nasuta* than for *N. caecoides*. The number and significance of the comparisons depends on the reference to which the test treatments are compared.

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CONTENTS

SUMMARY	v
1.0 INTRODUCTION	1.1
2.0 MATERIALS AND METHODS	2.1
2.1 SEDIMENT AND TEST ORGANISM COLLECTION	2.1
2.1.1 Oakland Harbor Core Samples	2.1
2.1.2 Reference and Control Samples	2.4
2.1.3 Test Organism Collection	2.4
2.2 SEDIMENT SAMPLE PREPARATION	2.5
2.2.1 Laboratory Glassware and Equipment Preparation	2.6
2.2.2 Geologic Description of Cores	2.6
2.2.3 Preparation of Solid-Phase Samples	2.6
2.2.4 Preparation of Suspended-Particulate-Phase	2.8
2.3 SEDIMENT AND TISSUE CHEMISTRY PROCEDURES	2.8
2.3.1 Convention Sediment Measurements	2.11
2.3.2 Semivolatile Organic Compounds	2.13
2.3.3 Chlorinated Pesticides and PCBs	2.14
2.3.4 Metals	2.14
2.3.5 Butyltins	2.15
2.4 TOXICOLOGICAL TESTING PROCEDURES	2.16
2.4.1 10-Day Solid-Phase Flow-Through Test with <i>N. caecoides</i> and <i>M. nasuta</i>	2.17
2.4.2 28-Day Solid-Phase Flow-Through Test with <i>N. caecoides</i> and <i>M. nasuta</i>	2.19
2.4.3 10-Day Solid-Phase Static Test with <i>R. abronius</i>	2.19
2.4.4 10-Day Solid-Phase Flow-Through Test with <i>C. stigmaeus</i>	2.21

2.4.5 96-h Suspended-Particulate-Phase Static Test with <i>C. stigmarus</i> .	2.22
2.4.6 96-h Suspended-Particulate-Phase Static Test with <i>H. sculpta</i>	2.23
2.4.7 48-h Suspended-Particulate-Phase Static Test with Larval <i>M. edulis</i>	2.24
2.5 DATA ANALYSIS AND INTERPRETATION	2.26
2.5.1 Randomization	2.26
2.5.2 Statistical Analysis of Solid-Phase Tests	2.27
2.5.3 Statistical Analysis of SPP Tests	2.27
2.5.4 Statistical Analysis of Bioaccumulation	2.28
2.6 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES	2.28
2.6.1 Sample Tracking and Storage	2.29
2.6.2 Sediment and Tissue Chemistry Quality Control Procedures	2.29
2.6.3 Toxicological Testing Quality Control Procedures	2.31
3.0 RESULTS	3.1
3.1 SEDIMENT SAMPLING RESULTS	3.1
3.2 GEOLOGIC DESCRIPTIONS	3.7
3.2.1 Older Bay Mud Unit	3.15
3.2.2 Sand Deposits	3.16
3.2.3 Younger Bay Mud	3.16
3.3 SEDIMENT CHEMISTRY	3.17
3.3.1 Sediment Conventional Measurements	3.17
3.3.2 Polynuclear Aromatic Hydrocarbons	3.27
3.3.3 Chlorinated Pesticides and Polychlorinated Biphenyls	3.27
3.3.4 Metals	3.27
3.3.5 Butlytin	3.35
3.4 TOXICOLOGICAL TESTING RESULTS	3.37

3.4.1 10-Day Flow-Through Solid-Phase Test with <i>N. caecoides</i>	3.39
3.4.2 28-Day Flow-Through Solid-Phase Test with <i>N. caecoides</i> and <i>M. nasuta</i>	3.41
3.4.3 10-Day Static Solid-Phase Test with <i>R. abronius</i>	3.41
3.4.4 10-Day Flow-Through Solid-Phase Test with <i>C. stigmaeus</i>	3.42
3.4.5 96-h Static Suspended-Particulate-Phase Test with <i>C. stigmaeus</i>	3.45
3.4.6 96-h Static Suspended-Particulate-Phase Test with <i>H. sculpta</i>	3.45
3.4.7 48-h Static Suspended-Particulate-Phase Test with Larval <i>M. edulis</i>	3.45
3.5 TISSUE BIOACCUMULATION	3.47
3.5.1 Polynuclear Aromatic Hydrocarbon Bioaccumulation in <i>M. nasuta</i>	3.49
3.5.2 Pesticide and PCB Bioaccumulation in <i>M. nasuta</i>	3.54
3.5.3 Metals Bioaccumulation in <i>M. nasuta</i>	3.54
3.5.4 Butyltin Bioaccumulation in <i>M. nasuta</i>	3.55
3.5.5 Polynuclear Aromatic Hydrocarbon Bioaccumulation in <i>N. caecoides</i>	3.58
3.5.6 Pesticide and PCB Bioaccumulation in <i>N. caecoides</i>	3.59
3.5.7 Metals Bioaccumulation in <i>N. caecoides</i>	3.62
3.5.8 Butyltin Bioaccumulation in <i>N. caecoides</i>	3.66
4.0 DISCUSSION AND CONCLUSIONS	4.1
4.1 GEOLOGIC EVALUATIONS	4.1
4.2 SEDIMENT CHEMISTRY	4.1
4.3 TOXICOLOGY AND BIOACCUMULATION	4.2
4.3.1 Comparison of Test Treatments to the Reference Sediment R-AC	4.4
4.3.2 Comparison of Test Treatments to the Reference Sediment R-AM	4.4

4.3.3 Comparison of Test Treatments to the Reference Sediment R-BF	4.7
4.3.4 Comparison of Test Treatments to the Reference Sediment R-OS	4.7
4.3.5 Comparison of Test Treatments to the Reference Sediment R-PC	4.10
4.3.6 Comparison of Test Treatments to the Reference Sediment R-PF	4.10
4.4 CONCLUSIONS	4.13
4.4.1 Water Column	4.13
4.4.2 Deposited Sediment Toxicity	4.14
4.4.3 Bioaccumulation	4.14
5.0 REFERENCES	5.1

FIGURES

1.1	Oakland Harbor Phase III B Study Area	1.2
2.1	Components of the 4-in. and 12-in. Vibratory-Hammer Coring Devices	2.2
2.2	Flow-Through Aquarium for <i>M. nasuta</i> , <i>N. caecoides</i> , and <i>C. stigmaeus</i>	2.18
2.3	Static Amphipod Testing Jars	2.20
3.1	Oakland Harbor Phase III B Sampling Stations	3.2
3.2	Reference Sediment Sampling Sites in the Bay Farm Borrow Area Oakland Harbor Phase III B	3.5
3.3	Reference Sediment Sampling Site Near Alcatraz Island, Oakland Harbor Phase III B	3.6
3.4	Offshore Reference Sediment Sampling Sites, Oakland Harbor Phase III B	3.8
3.5	Location of Sequim Bay, Washington, Control Station (C-SB)	3.10
3.6	Location of West Beach, Whidbey Island, Washington, Control Station	3.11
3.7	Location of Tomales Bay, California, Control Station	3.12
3.8	Location of Cross-Section A-A', Oakland Harbor Phase III B	3.13
3.9	Oakland Outer Harbor Cross-Section A-A', Oakland Harbor Phase III B	3.14
3.10	Grain Size Distribution in Sediment Samples, Oakland Harbor Phase III B	3.20
3.11	Concentrations of TOC, Oakland Harbor Phase III B	3.20
3.12	Relationship of Sediment TOC to Grain Size	3.21
3.13	Total Volatile Solids in Sediment	3.22
3.14	Relationship Between TVS and Grain Size	3.22
3.15	Relationship Between TOC and TVS	3.23
3.16	Total Oil and Grease in Sediment, Oakland Harbor Phase III B	3.24
3.17	Total TPH in Sediment, Oakland Harbor Phase III B	3.24
3.18	Linear Regressions Showing Relationship of Oil and Grease to (a) TOC, (b) TVS, and (c) Grain Size	3.25

3.19	Linear Regression Showing the Relationship of TPH to (a) Oil and Grease, (b) TOC, and (c) TVS	3.26
3.20	Concentration of (a) LPAH and (b) HPAH in Sediment, Oakland Harbor Phase III B	3.28
3.21	Linear Regression Between Total LPAH and HPAH, Oakland Harbor Phase III B	3.29
3.22	PCB Aroclor 1254 in Sediments, Oakland Phase III B	3.29
3.23	Concentration of Silver in Sediments, Oakland Harbor Phase III B	3.31
3.24	Concentration of Arsenic in Sediments, Oakland Harbor Phase III B	3.31
3.25	Concentration of Cadmium in Sediments, Oakland Harbor Phase III B	3.32
3.26	Concentration of Chromium in Sediments, Oakland Harbor Phase III B	3.32
3.27	Concentration of Copper in Sediments, Oakland Harbor Phase III B	3.33
3.28	Concentration of Mercury in Sediments, Oakland Harbor Phase III B	3.34
3.29	Concentration of Nickel in Sediments, Oakland Harbor Phase III B	3.34
3.30	Concentration of Lead in Sediments, Oakland Harbor Phase III B	3.36
3.31	Concentration of Selenium in Sediments, Oakland Harbor Phase III B	3.36
3.32	Concentration of Zinc in Sediments, Oakland Harbor Phase III B	3.37
3.33	Concentration for Monobutyltin in Sediments, Oakland Harbor Phase III B ..	3.38
3.34	Concentration of Dibutyltin in Sediments, Oakland Harbor Phase III B	3.38
3.35	Concentration of Tributyltin in Sediments, Oakland Harbor Phase III B	3.39

TABLES

2.1	Analytical Chemistry Requirement for Oakland Harbor Phase III B Sediment Samples	2.9
2.2	Analytical Chemistry Requirements for Oakland Harbor Phase III B Tissue Samples	2.10
2.3	Grain Size Fractions Measured	2.12
2.4	Analytical Method and Corresponding Metal for Each Sample Matrix	2.15
3.1	Core Sampling Information for the Oakland Harbor Phase III B Project	3.3
3.2	Reference Sediment Sampling Information, Oakland Harbor Phase III B	3.9
3.3	Summary of Sediment Samples for Testing, Oakland Harbor Phase III B	3.18
3.4	Conventional Sediment Measurement Results (Oil and Grease and TPH in mg/kg dry weight; grain size, TOC, and TVS in percent dry weight)	3.19
3.5	Results of the 10-Day Flow-Through Solid-Phase Test with <i>N. caecoides</i>	3.40
3.6	ANOVA Results for the 10-Day Flow-Through Solid-Phase Test with <i>N. caecoides</i>	3.40
3.7	Results of the 28-Day Flow-Through Solid-Phase Test with <i>M. nasuta</i> and <i>N. caecoides</i>	3.42
3.8	Results of the 10-Day Static Solid-Phase Test with <i>R. abronius</i>	3.43
3.9	ANOVA Results for the 10-Day Static Solid-Phase Test with <i>R. abronius</i>	3.43
3.10	Results of the 10-Day Flow-Through Solid-Phase Test with <i>C. stigmaeus</i>	3.44
3.11	ANOVA Results for the 10-Day Flow-Through Solid-Phase Test with <i>C. stigmaeus</i>	3.44
3.12	Results of the 96-h Static Suspended-Particulate-Phase Test with <i>C. stigmaeus</i>	3.46
3.13	T-Test and LC50 Determinations for the 96-h Static Suspended-Particulate-Phase Test with <i>C. stigmaeus</i>	3.47
3.14	Results of the 96-h Static Suspended-Particulate-Phase Test with <i>H. sculpta</i>	3.48
3.15	T-Test and LC50 Determinations for the 96-h Static Suspended-Particulate-Phase Test with <i>H. sculpta</i>	3.49

3.16	Results of the 48-h Static Suspended-Particulate-Phase Test with <i>M. edulis</i>	3.50
3.17	T-Test and LC50 Determinations for the 48-h Static Suspended-Particulate-Phase Test Based on Percent Survival with <i>M. edulis</i>	3.51
3.18	T-Test and EC50 Determinations for the 48-h Static Suspended-Particulate-Phase Test Based on Proportion Normal with <i>M. edulis</i>	3.51
3.19	Mean PAH Concentrations in the Tissues of <i>M. nasuta</i> (µg/kg dry weight)	3.52
3.20	PAHs in Tissues of <i>M. nasuta</i> That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means	3.53
3.21	Mean Metals Concentrations in Tissues of <i>M. nasuta</i>	3.55
3.22	Metals in Tissues of <i>M. nasuta</i> That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means	3.56
3.23	Mean Butyltin Concentrations In Tissues of <i>M. nasuta</i> (µg/kg dry weight)	3.58
3.24	Butyltins in Tissues of <i>M. nasuta</i> That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means	3.59
3.25	Mean PAH Concentrations in Tissues of <i>N. caecoides</i> (µg/kg dry weight)	3.60
3.26	PAHs in Tissues of <i>N. caecoides</i> That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means	3.61
3.27	Mean Metals Concentrations in the Tissues of <i>N. caecoides</i> (mg/kg dry weight)	3.63
3.28	Metals in Tissues of <i>N. caecoides</i> That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means	3.64
3.29	Mean Butyltin Concentration in Tissues of <i>N. caecoides</i> (µg/kg dry weight)	3.67
3.30	Butyltins in Tissues of <i>N. caecoides</i> That Are Significantly Different From Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means	3.68
4.1	Summary of Geological Descriptions	4.2
4.2	Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Alcatraz Disposal Site Reference R-AC	4.5
4.3	Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Alcatraz Island Environs Reference R-AM	4.6

4.4	Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Bay Farm Reference R-BF	4.8
4.5	Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Deep Off-shelf Reference R-OS	4.9
4.6	Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Point Reyes Coars Reference R-PC	4.11
4.7	Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Point Reyes Fine Reference R-PF	4.12

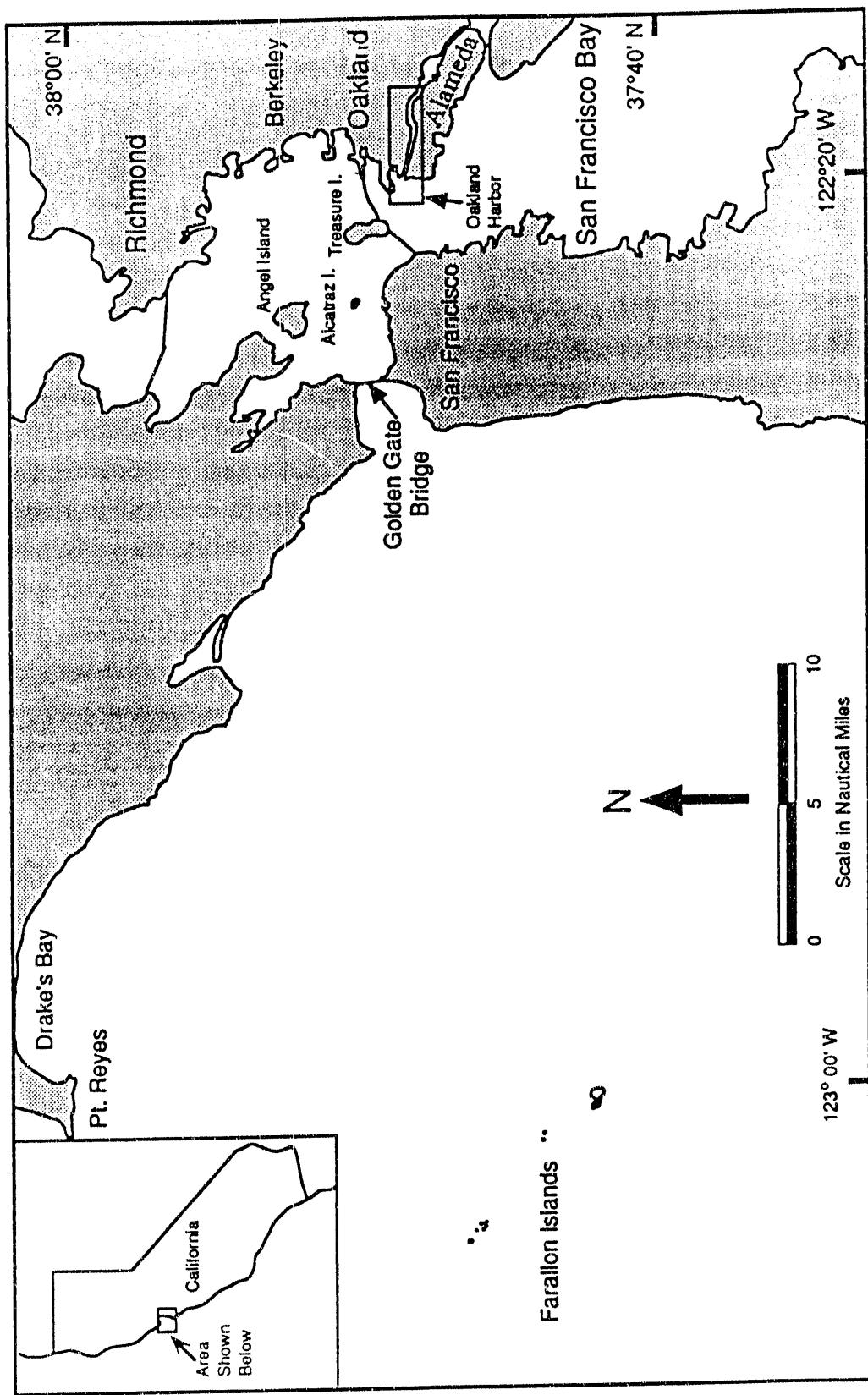
1.0 INTRODUCTION

Oakland Harbor is located on the eastern shoreline of central San Francisco Bay in Alameda County, between the cities of Oakland and Alameda, California (Figure 1.1). Oakland Harbor and its access channels are no longer wide or deep enough to accommodate modern deeper-draft vessels. The Water Resources Development Act of 1986 (Public Law 99-662) authorized the U.S. Army Corps of Engineers (USACE), San Francisco District, to deepen and widen the navigation channels in Oakland Harbor. Several options for disposal of the material from this dredging project are under consideration by USACE. Those options include disposal within San Francisco Bay, at open-ocean sites, or at uplands disposal sites.

Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (MPRSA), Public Law 92-532, specifies that all proposed disposal of dredged material into open water be evaluated to determine the potential environmental impacts of those activities. To comply with those requirements, the potential harmful effects of the dredged material must be evaluated by chemical characterization, toxicity testing, and bioaccumulation testing prior to dredging and disposal.

Between March 1988 and February 1990, Battelle/Marine Sciences Laboratory (MSL),^(a) operating under contract to USACE, completed three studies to evaluate the acceptability of Oakland Harbor sediments for the open ocean disposal option: the Oakland Harbor 38-Foot, 42-Foot Phase I, and 42-Foot Phase II Projects (Word et al. 1988, 1990a,b). Those studies included sediment chemistry analysis, solid- and suspended-phase sediment toxicity tests, and 10-day bioaccumulation measurements. The 1988-1990 Oakland Harbor 38-Foot, 42-Foot Phase I, and 42-Foot Phase II evaluations were conducted under the guidance of the 1977 *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters; Implementation Manual for Section 103 of Public Law 92-532 (Marine Protection, Research, and Sanctuaries Act of 1972)* (1977 Implementation Manual) published jointly by USACE and the U.S. Environmental Protection Agency (EPA). Since the above tests were completed, the Implementation Manual was revised by EPA and USACE, and released in January 1990 as *Draft Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters* (EPA/USACE 1990). The revised version is hereinafter referred to as the Draft Implementation Manual.

(a) The Marine Sciences Laboratory is part of the Pacific Northwest Laboratory, which is operated for the U.S. Department of Energy by Battelle Memorial Institute.



PHASE III B

1.2

FIGURE 1.1. Oakland Harbor Phase III B Study Area

In 1990, USACE requested that MSL sample additional sites and resample other sites included in the Oakland Harbor 38-Foot, Phase I, and Phase II studies, and subject the sediment samples to chemical and toxicological evaluations following the revised protocols in the Draft Implementation Manual. The intent of this program was to evaluate the dredged material that would be removed during widening and deepening Oakland Harbor to -38 ft Mean Lower Low Water (MLLW) or -42 ft MLLW. This work was done during the Oakland Harbor Phase III Program, which was divided into two parts, A and B. The objective of Phase III, Part A (the Oakland Harbor Phase III A Project) was to evaluate sediment samples from Oakland Inner Harbor through toxicity and bioaccumulation testing with marine organisms. Those evaluations were conducted in June 1990, and are presented in a separate document. The present report covers Oakland Harbor Phase III, Part B, hereinafter referred to as the Oakland Harbor Phase III B Project (or Phase III B). The Oakland Harbor Phase III B Project primarily evaluates sediment from deepening Oakland Outer Harbor and entrance channel to -42 ft MLLW. A companion sampling and analytical program, conducted in September 1990 provided data for the Oakland Inner Harbor 38-Foot Project, which is also contained in a separate document.

The study area for the Oakland Harbor Phase III B Project included 13 sites in Oakland Outer Harbor, 2 sites near the entrance to Oakland Inner Harbor, and 3 sites in Oakland Inner Harbor. The latter three Inner Harbor sites were added to Phase III B to repeat sampling and testing during Phase III A. The objectives of the Oakland Harbor Phase III B Project were to collect sediment samples from the proposed dredging sites and to subject the samples to chemical, biological toxicity, and bioaccumulation analysis following the guidelines in the Draft Implementation Manual. The testing guidelines require that reference and control sediment samples be collected and tested. The experimental control sediments allow evaluation of the health and normal behavior of the test organisms. The testing of reference sample(s) allows the biological responses and contaminant levels of a proposed dredged sediment to be compared to those of a potential disposal area. Because a potential disposal area has not been selected for Phase III B sediments, six different reference samples were evaluated during the program.

Chemical analyses included measurements of EPA priority pollutant metals and organics [polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides] as well as butyltins and conventional parameters. Biological toxicity tests included controlled laboratory exposures of sensitive marine organisms to the solid phase and suspended-particulate phase of the dredged material. Four species were exposed to the solid phase (the polychaete *Nephtys caecoides*, benthose clam *Macoma nasuta*, amphipod *Rhepoxynius abronius*, and juvenile sanddab *Citharichthys stigmaeus*) and three species were exposed to the suspended-

particulate phase (the mysid *Holmesimysis sculpta*, juvenile sanddab *Citharichthys stigmaeus*, and larvae of the bay mussel *Mytilus edulis*). Bioaccumulation potential was determined through a 28-day exposure of *M. nasuta* and *N. caecoides* to the solid phase of the proposed dredged material followed by chemical analysis of the tissues for the above EPA priority pollutants and butyltins. Contaminant levels in organisms exposed to test sediments were statistically compared to contaminant levels in organisms exposed to reference sediments. The purpose of these analyses was to provide information required to address potential ecological effects resulting from ocean disposal of the dredged material.

Volume 1 of this report is divided into five sections. Section 2.0 presents the materials and methods for sample collection, sample handling and processing, geologic observations, biological tests, physical and chemical analyses, and data analysis, as well as the quality assurance/quality control (QA/QC) considerations associated with each of these measurements. Results of geologic observations, sediment chemistry, toxicity tests, and bioaccumulation are given in Section 3.0. Section 4.0 provides a discussion of the results and conclusions. References are given in Section 5.0.

Volume 2 of this report contains a series of appendixes presenting the following information:

Appendix A	Geologic description method
Appendix B	Geologic core data logs
Appendix C	Sediment chemistry and QC data
Appendices D-J	Bioassay results
Appendices K-L	Tissue chemistry and QC data
Appendices M-N	Statistical Analysis of Tissue Bioaccumulation.

2.0 MATERIALS AND METHODS

2.1 SEDIMENT AND TEST ORGANISM COLLECTION

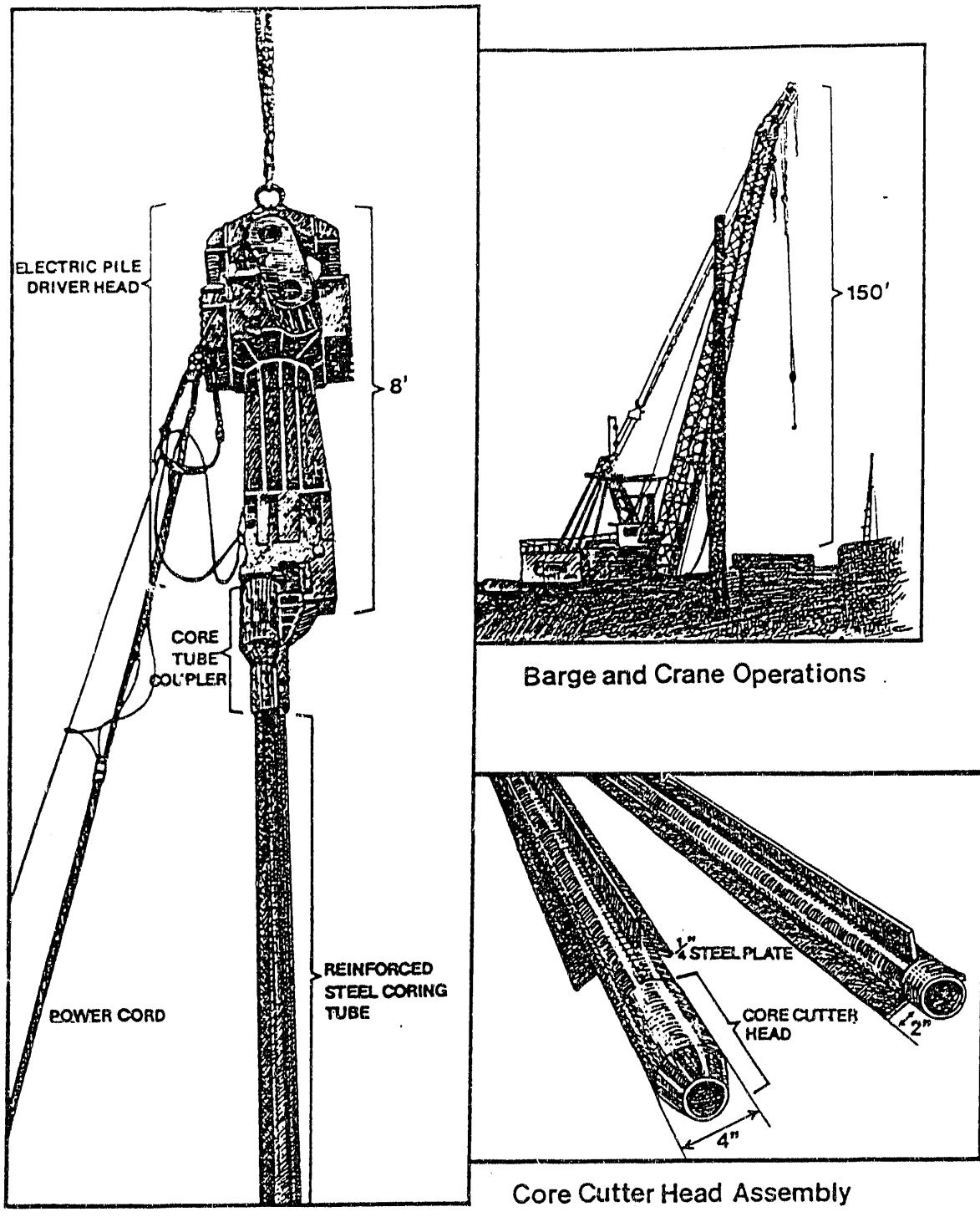
Sediment core samples were collected for the Oakland Phase III B Project to -44 ft MLLW (42 ft plus 2 ft overdepth) from 18 stations in Oakland Harbor. Sediment from six reference areas and grab or pipe dredge samples from four control sediment stations was also collected. Specific locations of sediment sampling sites are presented with the sampling results in Section 3.1. Core samples were taken for geological description, biological testing, and sediment chemistry. Reference and control samples were taken for biological testing and sediment chemistry. Sampling also included the collection of six animal species for use in solid-phase and suspended-particulate-phase (SPP) toxicity tests.

2.1.1 Oakland Harbor Core Samples

Navigation support for locating stations in Oakland Harbor, including a survey vessel and operator, was provided by Towill, Inc., of Concord, California. The surveyors used a laser/range azimuth positioning system (EDM-Geodimeter AGA-120, Wild T-2 one second theodolite). Towill provided corrected water depths at each station by measuring the actual depth with a recording fathometer (DE719-E), measuring the water surface elevation, and calculating the difference between the water surface elevation and 0 ft MLLW.

All stations were sampled to -44 ft MLLW using a 12-in. vibratory-hammer split corer, and all but two stations were sampled using a 4-in. vibratory-hammer corer. Both samplers were designed by MSL and Manson Construction (Figure 2.1). The 12-in. corer was used to collect the large volume of sediment needed for biological testing while minimizing contamination caused by excessive sample handling. The 4-in. cores were collected and stored in noncontaminating Lexan polycarbonate tubes to maintain the stratigraphic integrity of the sediment and to provide sediment for the chemical characterization from known depths at individual sites. Both coring systems had been used successfully in previous sampling programs in Oakland Harbor.

Detailed sampling records contained the station name, date, type of core (12-in. or 4-in.), replicate number, uncorrected water depth, tide height, corrected depth, required core length, sampling time, total core collected, and comments. Sediment samples were stored in a refrigerated truck at the staging area until all samples were collected. An inventory of samples was maintained as samples were loaded onto the truck. When sampling was completed, the inventory was confirmed on chain-of-custody forms. The custody forms were signed by the field leader, who



Vibratory-Hammer Core

FIGURE 2.1. Components of the 4-in. and 12-in. Vibratory-Hammer Coring Devices

kept one copy and sealed the others with the samples. The refrigerated truck then transported the samples to MSL in Sequim, Washington, where they were stored at 4°C.

Both the 12-in. and 4-in. core samplers were deployed from the Manson Construction derrick barge *Vasa*. The two sizes of cores were collected in a similar manner but the sediment samples were handled differently. The coring apparatus was attached to an electric vibratory hammer. The corer and the vibratory hammer were suspended by the crane on the derrick barge. When the coring apparatus was directly above the sampling site, the sampling gear was lowered through the water. When the end of the sampler reached the sediment surface, the vibratory hammer was switched on, unless the sediment was so soft that the corer penetrated because of its weight. Vibrating continued until the sampler penetrated beyond project depth, indicated by the water surface level relative to marks on the outside of the core barrel (when the water level was at least the uncorrected depth plus the core required). The coring apparatus was then pulled from the sediment, detached from the vibratory hammer, and lowered onto the barge deck.

Sediment was collected to -44 ft MLLW using the 12-in. vibratory-hammer split corer. One replicate was collected per site. As each core was brought on board the barge platform, the hinged door of the core barrel was opened and the sediment measured from the mudline down to ensure that appropriate depth was reached. If the required core length was not collected, the barrel was emptied and another core was taken. If the required core length was collected, the sediment was marked at -44 ft MLLW and at -38 ft MLLW if the mudline was shallower. The fraction or volume to be contributed to the sample and/or composite was determined based on the volume of sediment necessary for laboratory testing.

Once the core segments were measured, the appropriate amounts of sediment were transferred, using a stainless steel spade, from the core barrel to an epoxy-coated container. Each sample container was labeled with the project name, station or composite designation, contributing station(s), vertical segment contributed (i.e., -38 to -44 ft), and sampling date(s). The containers were sealed and kept cool (~4°C) in a freezer on board the sampling vessel until loaded into a refrigerated truck at the end of the sampling day.

Sediment was also collected to -44 ft MLLW using the 4-in.-diameter vibratory-hammer core sampler. One core was collected per site. The core barrel was lined with a 3.125-in. (inner diameter) clear Lexan core liner that had been steam cleaned. When each core was brought on board the liner was pulled from the barrel and the sediment measured from the mudline down to determine if appropriate depth (-44 ft MLLW) was reached. If not, the liner was replaced, and

another sample taken. If the core was long enough, it was carefully carried to the cutting stand where it was capped, sealed, labeled, and cut into shorter sections, if necessary, to fit in the freezer. Each core label included an arrow pointing to the top of the core, the station designation, core section indicator (i.e., Section 1 of 2 and Section 2 of 2), length interval from the mudline (i.e., 0-3 ft), and sampling date. When each 4-in. core was labeled and sealed, it was kept cool (~4°C) in a freezer on board the sampling vessel until it was transferred to a refrigerated truck. An inventory of core samples was maintained on chain-of-custody forms as samples were transferred to the truck.

2.1.2 Reference and Control Samples

Sediment samples from reference sites were collected with a pipe dredge deployed from the *FV Cobra*, a charter boat owned and operated by Bob Smith Sportfishing. Sampling locations were determined by LORAN C and variable fix and range radar systems aboard the vessel. The vessel navigation systems were verified at known fixed locations such as the Golden Gate channel pilot buoy whenever possible. Reference sampling records were maintained in a log book, and consisted of station position, date, time, replicate, water depth, sediment type, and comments. All reference samples were kept cool in labeled coolers on board the sampling vessel until they were stored at 4°±2°C in a refrigerated truck.

The control sediment sampling sites were Sequim Bay, Washington; West Beach, Whidbey Island, Washington; and Dillon Beach/Tomales Bay, California. Experimental control sediment from Sequim Bay, Washington, was collected with a modified van Veen grab sampler (0.1 m²) deployed from an MSL research vessel. Control sediment from West Beach (*R. abronius* native control) and Dillon Beach (*N. caecoides* and *C. stigmaeus* native controls) was collected at the same time test organisms were collected. West Beach sediment was collected with a small, MSL-designed, anchor-dredge sampler that also obtains sufficient quantities of the organisms for test purposes. The dredge was deployed from MSL's 17-ft Boston Whaler in approximately 15 ft of water. The West Beach sampling location was determined by reference to shoreline features. Dillon Beach control sediment was collected by Brezina and Associates, using a shovel, at the same time *N. caecoides* and *C. stigmaeus* were collected. The Dillon Beach sediment was shipped overnight to MSL, where it was stored at 4°±2°C.

2.1.3 Test Organism Collection

Six species of marine organisms were used in Oakland Harbor Phase III B Project toxicity tests:

- Bentnose clam *Macoma nasuta*
- Polychaete *Nephtys caecoides*
- Phoxocephalid amphipod *Rhepoxynius abronius*
- Juvenile flatfish (sanddab) *Citharichthys stigmaeus*
- Juvenile mysid shrimp *Holmesimysis sculpta*
- Larvae of bay mussel *Mytilus edulis*

Most of the organisms were wild-captured individuals collected either by a commercial supplier or by MSL. Amphipods (*R. abronius*) were collected by MSL off West Beach, Whidbey Island, using the specially designed anchor dredge deployed from a 17-ft Boston Whaler. Sediment brought up with the dredge was sieved through a 2-mm mesh screen to remove large debris and predatory species. Amphipods were kept in coolers partially filled with their native sediment and seawater until they were delivered to a holding tank at MSL later on the sampling day. *M. nasuta* were collected from intertidal zones in Discovery Bay near Gardiner, Washington, by a commercial supplier using a shovel, sieve, and bucket. In the field, clams were kept cool in large tubs containing sediment and seawater from the collection site.

Brezina and Associates (Dillon Beach, California) supplied *N. caecoides*, *C. stigmaeus*, and *H. sculpta* individuals for toxicity testing. *N. caecoides* were collected from mud flats in Tomales Bay, California, using a shovel, bucket, and sieve. Worms were placed into clean coolers containing sediment and seawater from the collection site. Before overnight shipment to MSL, the seawater in each cooler was supersaturated with oxygen (22 ppm). The *C. stigmaeus* were collected from Tomales Bay, California, in 12 to 15 ft of water. *C. stigmaeus* were captured with a small trawl with a 0.25-in. mesh net with no cod end. The trawl was held close to the work boat so a dip net could be used to transfer the fish into double plastic bags containing oxygen-saturated seawater. *H. sculpta* were collected with a plankton dip net in Monterey Bay, California, and transferred to a holding container aboard the work boat. Brezina and Associates were responsible for sorting *H. sculpta* of the appropriate age and size class and shipping them to MSL in bags containing oxygen-saturated water.

2.2 SEDIMENT SAMPLE PREPARATION

Sediment sample preparation involves all steps in the laboratory between delivery of the samples to MSL and the preparation of samples for chemical and/or biological testing. Sample preparation was completed within the 14-day holding limit between the sampling date and toxicity test initiation. The following sections describe equipment preparation, geologic descriptions of core samples, homogenizing solid-phase samples, and SPP sample preparation.

2.2.1 Laboratory Glassware and Equipment Preparation

All glassware, stainless steel utensils, plastic, and other laboratory containers and equipment undergo stringent cleaning procedures to avoid contamination of samples. Glassware, including test containers, aquaria, and sediment transfer dishes were washed with warm, soapy water, rinsed five times with deionized water, then soaked in a 5% reagent grade nitric acid bath for a minimum of 4 h. After soaking, glassware was rinsed with deionized water five times and allowed to dry. Titanium tools, PVC, Nalgene, and other plastic items such as funnels were also washed and soaked in acid baths in the same manner as glassware.

Stainless steel bowls, spoons, spatulas, and other utensils were washed with warm, soapy water, rinsed five times with deionized water, and allowed to air dry. They were then rinsed with methylene chloride under a fume hood and the methylene chloride was allowed to evaporate under the hood.

Rubber stoppers and other porous materials were washed with warm, soapy water and rinsed five times with deionized water. These items were then "seasoned" by continuous soaking in or exposure to 0.45- μm -filtered seawater for at least 2 days prior to use.

Large pieces of laboratory equipment such as the epoxy-coated mixer used to mix sediment and epoxy-coated boards used to hold cores for geologic descriptions were washed with mild soap solution and thoroughly rinsed with tap water followed by deionized water.

2.2.2 Geologic Description of Cores

A geologic characterization of each Oakland Harbor Phase III B Project sampling site was conducted by a geologist. The description was performed on the 4-in. core that was collected and stored in the Lexan core tube. All core sections from one station were removed from storage and cut longitudinally with a circular saw. A linoleum knife was used to split the core open to expose the sediment stratigraphy. The geologist measured and described the core from top to bottom, recording data on a core data log. The geologic characterization protocol (Appendix A) was consistent with ASTM Method D2488-84 (ASTM 1984).

2.2.3 Preparation of Solid-Phase Samples

Solid-phase, also called bulk sediment or whole sediment, refers to the sediment itself, as opposed to suspended or dissolved phases. In biological tests, the solid-phase of sediments represents either dredged material once it has settled at an aquatic disposal site (test sediment), the existing environment of a disposal site without dredged material (reference sediment), or the

environment of a benthic test organism (control sediment). Solid-phase preparation also applies to samples for sediment chemistry. All solid-phase samples were thoroughly homogenized before use in biological tests or chemical analysis.

The procedure for homogenizing Oakland Harbor test sediment samples varied according to sediment type. Compacted clay sediments were separated into smaller pieces with a metal grater and then mixed either with stainless steel spoons or a mixer coated with a special epoxy paint. Silt, soft clay, and sandy sediments were mixed with spoons in stainless steel bowls. Sediment samples were mixed until uniform consistency and color were visible throughout the sediment in the bowl or mixer. Minimal amounts of 0.45- μm -filtered seawater were added as needed to achieve a homogeneous consistency. After mixing, sample aliquots for chemical analyses were placed in cleaned and labeled containers appropriate for the parameters to be measured. If solid-phase samples were not used immediately for testing or SPP preparation they were returned to the labeled, epoxy-coated metal pails for storage at 4°C. All sediments were homogenized, subsampled for chemistry, and used for testing within the recommended 14-day holding period.

The reference and control sediments were contained in an ice chest at 4°C until sieving. The sediments were placed onto stacked screens having mesh diameters of 0.5 to 1.0 mm set on top of a sieving stand. The sieving stand was designed to empty directly into a 55 gal, acid-washed aquarium containing approximately 15 gal of filtered seawater. A Simms Geyser submersible pump was placed in the aquarium to recirculate the water. Sediment that passed through these sieves was collected in the 55-gal aquarium. Organisms collected on the sieves were discarded, and the small amount of debris was returned to the sieved sediment. The sieved sediment was allowed to settle in the aquarium overnight at 4°C. After settling, the overlying water was siphoned off and the sediment was transferred to an epoxy-coated mixer for compositing. The sediment was mixed for 5 to 10 min or until a homogenous mixture was formed. At the end of the mixing period, the sediment was transferred from the mixer to the 55-gal aquarium and stored at 4°C until needed for testing. Between sieving of reference sediments, all equipment was thoroughly rinsed with 0.45- μm -filtered seawater to avoid potential cross-contamination between samples.

Sediment composites were combined in the field using material from the 12-in. core from each contributing station. These sediments were placed in 5-gal, epoxy-coated, metal pails and stored in freezers maintained at 4°C in the field. The composites were homogenized in an epoxy-coated mixer in the same manner as the single station solid-phase samples, and subsamples were removed for chemical analysis. However, the composites were also used for SPP testing.

2.2.4 Preparation of Suspended-Particulate-Phase

The SPP of sediment samples was used to evaluate water column effects of open water dredged material disposal. The SPP is the liquid supernatant that remains after mixing sediment with seawater and allowing heavier particles to settle out. Because the sample preparation does not involve filtration, this phase contains suspended particles as well as dissolved constituents. The SPP tests evaluate effects caused by both the physical presence of the suspended particles and the chemical toxicity of contaminants associated with the particles or dissolved fractions. The process is intended to approximate exposure conditions created as a result of materials being discharged through the water column during dredge-disposal operations.

The first step of SPP preparation was creating a 4:1 (volume:volume) water to sediment slurry in 1-L glass jars with Teflon-lined lids. The jars were marked at 200 mL and 400 mL. 0.45- μm -filtered seawater was added to the 200-mL mark, then homogenized sediment was added until the water was displaced to the 400-mL mark. The jar was filled to 1 L with filtered seawater. A set of 12 jars of sediment and water were placed on a shaker table and agitated for 30 min at a shaking rate of 120 to 150 cycles/min. After shaking, the slurry was poured into 500-mL Teflon containers with tightly fitted lids. These containers were placed in a centrifuge and spun for 10 to 15 min at approximately 1750 rpm. The 10-min centrifugation was necessary to ensure that test organisms would be visible at the first observation after exposure to SPP test treatments. After centrifugation, the supernatant was composited by pouring it into clean 10-gal aquaria and used then in the SPP tests as soon as possible. If SPP was not used immediately, the aquaria were stored at 4°C. The Teflon jars were rinsed out after each use and the above process was continued until an adequate amount of SPP was produced. Between SPP preparations, all glass and Teflon containers were appropriately cleaned according to procedures described in Section 2.2.1.

2.3 SEDIMENT AND TISSUE CHEMISTRY PROCEDURES

Sediment samples were analyzed for conventional sediment measurements (e.g., grain size, oil and grease). Chemical analyses were conducted on sediment samples for polynuclear aromatic hydrocarbons (PAHs), chlorinated pesticides, polychlorinated biphenyls (PCBs), metals, and butyltins. Table 2.1 lists the parameters for which the Oakland Phase III B sediment samples were analyzed, as well as analytical goals for detection limits, range of recovery, and relative precision. *N. caecoides* and *M. nasuta* tissue samples were analyzed for the same set of PAHs, chlorinated pesticides, PCBs, metals, and butyltins. Table 2.2 lists the parameters for which the Oakland

TABLE 2.1. Analytical Chemistry Requirements for Oakland Harbor Phase III B Sediment Samples

<u>Parameters</u>	<u>Detection Limits (a)</u> <u>(mg/kg dry wt)</u>	<u>Number of Samples</u>	<u>Range of Recovery (%)</u>	<u>Relative Precision (%)</u>
<u>Sediment Conventional</u>				
TOC	0.1%	35	--- (b)	10
Oil and Grease	20	36	---	10
TPH	20	36	---	15
Grain Size	NA (c)	35	NA	---
Total Solids	NA	35	---	---
<u>Metals</u>				
Ag	1.0	35	---	15
As	1.0	35	75 - 120	15
Cd	0.1	35	---	15
Cr	1.0	35	85 - 115	15
Cu	1.0	35	---	15
Hg	0.02	35	75 - 125	15
Ni	1.0	35	---	15
Pb	1.0	35	---	15
Se	0.1	35	75 - 115	15
Zn	1.0	35	---	15
<u>Organic Compounds</u>				
Butyltins	0.01	35	40 - 140	20
PCBs (d)	0.02	35	50 - 150	50
PAHs (e)	0.02	35	50 - 150	50
Pesticides (f)	0.002	35	50 - 150	50

(a) Target detection limits; all efforts were made to reach lowest practical detection limits.

(b) Not available.

(c) Not applicable.

(d) Reported as Aroclor equivalents 1242, 1248, 1254, and 1260 and total PCB. Analyzed using EPA Method 8080.

(e) All compounds on EPA Method 610 list. Analyzed using Method 8270 in SIM mode.

(f) All compounds on EPA Method 608 list. Analyzed using Method 8080.

TABLE 2.2. Analytical Chemistry Requirements for Oakland Harbor Phase III B Tissue Samples

<u>Parameters</u>	<u>Detection Limits (a) (mg/kg dry wt)</u>	<u>Number of Samples</u>	<u>Range of Recovery (%)</u>	<u>Relative Precision (%)</u>
Metals				
Ag	1.0	205	---(b)	15
As	1.0	208	75 - 120	15
Cd	0.1	205	---	15
Cr	1.0	205	85 - 115	15
Cu	1.0	208	---	15
Hg	0.02	205	75 - 125	15
Ni	1.0	208	---	15
Pb	1.0	205	---	15
Se	0.1	205	75 - 115	15
Zn	1.0	208	---	15
Organic Compounds				
Butyltins	0.01	260	40 - 140	20
PCBs (c)	0.02	260	50 - 150	50
PAHs (d)	0.02	260	50 - 150	50
Pesticides(e)	0.002	260	50 - 150	50

(a) Target detection limits; all efforts were made to reach lowest practical detection limits.

(b) Not available.

(c) Reported as Aroclor equivalents 1242, 1248, 1254, and 1260 and total PCB, EPA Method 8080.

(d) All compounds on EPA Method 610 list. Analyzed using Method 8270 in SIM mode.

(e) All compounds on EPA Method 608 list. Analyzed using Method 8080.

Phase III B tissue samples were analyzed, as well as analytical goals for detection limits, range of recovery, and relative precision.

The following sections briefly describe the methods used for analysis of sediments and tissues for the required physical and chemical parameters. Analyses followed established EPA procedures where applicable. Quality control samples included method blanks, matrix spike (MS) and matrix spike duplicate (MSD) analyses, standard reference materials (SRMs), analytical replicates, and compositing duplicates. The MS, MSD, and SRM samples were used to evaluate analytical accuracy. Analytical replicates were compared to evaluate analytical precision, as were the MS and MSD when applicable. The compositing duplicates were used to assess the efficiency of homogenizing sediment samples.

2.3.1 Conventional Sediment Measurements

Conventional sediment measurements consist of grain size, total organic carbon (TOC), total volatile solids (TVSs), oil and grease and total petroleum hydrocarbons (TPHs), and percent solids. The procedures for each of these analyses are discussed in the following paragraphs.

Grain size analysis was conducted by Soil Technology, Inc., of Bainbridge Island, Washington. Sixteen grain size fractions were determined by a combination of sieve and pipet techniques from the Puget Sound Estuary Program (PSEP) Protocols for Measuring Selected Environmental Variables in Puget Sound (PSEP 1986). These methods are consistent with ASTM D421 (ASTM 1978) and D422 (ASTM 1972) (with the substitution of the No. 140 Sieve for the No. 120 sieve). Table 2.3 presents the fractions measured.

Approximately 25 g of each sediment sample was analyzed for total solids while another 10- to 100-g aliquot was weighed for grain size analysis. To separate the coarser sand and gravel fraction from the silt/clay fraction, sediment was washed with distilled water through a 63.5- μm (4.0 phi) sieve into a 1-L graduated cylinder. The coarse fraction was dried, weighed, and shaken through a nest of sieves to yield the required seven coarse subfractions. Any material still passing the final 63.5- μm sieve was added to the previous fines in the 1-L graduated cylinder. The silt/clay fraction was then subdivided by a pipet technique based on Stoke's Law of differential settling velocities for different sized particles. The silt/clay fraction was disassociated by a dispersant (sodium hexametaphosphate) in distilled water in a 1-L graduated cylinder. At specified time intervals and specified depths below the surface, 20-mL aliquots were withdrawn from the graduated cylinder, delivered to a preweighed container, and dried at $90^\circ \pm 2^\circ\text{C}$ to a constant weight. Duplicate analysis of five samples was performed as a quality control measure. Other

TABLE 2.3. Grain Size Fractions Measured

<u>Grain Size (μm)</u>	<u>Phi</u>	<u>Screen Number</u>
3350	-2	6
2000	-1	10
1000	0	18
500	1.5	35
250	2	60
125	3	120
62.5	4	230
48	4.5	NA ^(a)
31.2	5	NA
23	5.5	NA
15.6	6	NA
7.8	7	NA
3.9	8	NA
1.9	9	NA
0.976	10	NA
0.4883	11	NA

(a) Not applicable.

quality control measures, such as spikes, SRMs, or minimum detection limits, do not apply to grain size analysis.

Total organic carbon is the amount of non-volatile, partially volatile, volatile, and particulate organic carbon compounds in a sample. Analysis of TOC was performed by Global Geochem in Canoga Park, California. Each sediment sample was dried and ball milled to a fine powder. Before combustion, inorganic carbon in the sample was removed by acidification. The TOC in sediment was then determined by measuring the carbon dioxide released during combustion of the sample (PSEP 1986; SW846 Method 9060, EPA 1986), reported as percent of dry weight. Quality control measures included two method blanks, triplicate analysis of two samples, and analysis of compositing duplicates.

Total oil and grease includes vegetable oils, animal fats, soaps, waxes, and any other carbon-hydrogen material extractable by the solvent Freon. Total petroleum hydrocarbons comprise the nonpolar mineral fraction of total oil and grease that is not removed by silica gel absorption. These analyses were performed by Twin City Testing at St. Paul, Minnesota. Infrared spectrophotometry (IR) was used to determine concentrations of oil and grease (Method 413.2, EPA 1979) and petroleum hydrocarbons (Method 418.1, EPA 1979). A 20-g aliquot of

sample was dried with anhydrous sodium sulfate, then extracted with Freon. For total oil and grease, sample extracts were scanned from 4000 to 600 cm^{-1} on an infrared spectrophotometer and the peak height measured at 2930 cm^{-1} . This wavelength represents the $-\text{CH}_2$ configurations of hydrocarbons and was the standard used to determine oil and grease. For total petroleum hydrocarbons, silica gel was added to the extract to remove the more polar animal- and vegetable-based oils. The extract was then shaken and allowed to settle. An aliquot was then removed and scanned the same way as the oil and grease sample. The relationship of peak height to oil concentration was determined by regressing the peak height versus a known concentration of fuel oil.

Total volatile solids are a measure of the fraction of total solids that are lost on ignition at a higher temperature than that used to determine total solids. The TVSs are used as an estimate for the amount of organic matter in the total solids. Operationally, TVSs are defined by the combustion temperature, and do not always represent the organic content of a sample because some of the more volatile organic material may be lost during drying and some inorganic material may be lost during combustion. Analysis of TVS was performed by the MSL using the method defined in PSEP (1986). Following that method, the sample was freeze-dried to constant weight and ball milled to a fine powder. A portion was then removed, weighed, and combusted at 550°C. The sample was cooled in a desiccator and then reweighed. The amount of sample lost during ignition was then defined as the volatile solids fraction.

Sediment samples used for determination of percent solids were prepared in one of two ways. (Each laboratory performs a percent solids analysis to determine a sample dry weight; different laboratories may use one of the two methods.) Pre-weighed wet samples are either freeze-dried over a period of 4 days or dried in an oven at 110°C for at least 8 h, and cooled in a desiccator. The ratio of dry weight to wet weight is multiplied by 100 to determine the percent solids.

2.3.2. Semivolatile Organic Compounds

The semivolatile organic compounds analyzed in sediments were the 16 PAHs listed in EPA Method 610. These compounds were extracted from sediments following EPA SW-846 Method 3540 (1986) using methylene chloride as the extraction solvent. A portion of the extract was used for PAH analysis by gas chromatography/mass spectroscopy in the Selective Ion Mode (GC/MS SIM) following EPA SW-846 Method 8270 (1986). Tissue extracts were run through gel permeation chromatography (GPC) prior to analysis to remove any additional interferences.

Analyses for PAHs in the Oakland Phase III B sediments and tissues were performed by Alden Analytical in Seattle, Washington.

Four PAH surrogate compounds were added to all samples prior to extraction. All PAH compounds were added to two samples in duplicate to assess accuracy and precision of the analyses. National Research Council of Canada (NRCC) SRM HS-4, a sediment sample with known PAH concentrations, was also analyzed for PAH compounds.

2.3.3 Chlorinated Pesticides and PCBs

Chlorinated pesticides and PCBs in sediments and tissues were quantified by gas chromatography/electron capture detection (GC/ECD) following EPA SW-846 Method 8080 (1986). The PCB and pesticide analyses for Oakland Phase III B sediment and tissue samples were performed by Alden Analytical in Seattle, Washington.

Chlorinated pesticides and PCBs were extracted simultaneously with the PAH compounds using EPA SW-846 Method 3540 (1986). The procedure involved a methylene chloride extraction using sonication extraction techniques. A portion of the methylene chloride extract was solvent exchanged to hexane, and interferences were removed by passing the extract through a column packed with 10 g of 7% deactivated alumina. Most samples required an additional cleanup treatment using GPC to remove other interferences. Analytical quantification was performed using GC/ECD analysis. Dibutylchlorendate (DBC) was the surrogate compound added to each sample before extraction to assess extraction efficiency.

A matrix spiking solution, consisting of either a subset of pesticides or one aroclor, was also added to the appropriate samples before extraction. Matrix spike/matrix spike duplicate analyses were conducted to assess accuracy and precision of the measurement. A method blank was analyzed along with this set of samples as well.

2.3.4 Metals

Ten metals were measured in sediments and tissues: silver (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn). All metals analyses were performed by the Pacific Northwest Laboratory (PNL) in Richland, Washington, and the MSL. Samples of sediment, *M. nasuta* tissue, and *N. caecoides* tissue were analyzed using a combination of three different methods: 1) energy-diffusive x-ray fluorescence (XRF), following internal PNL procedures; 2) Zeeman graphite-furnace atomic absorption spectroscopy (GFAA), following EPA SW-846 Method 7000 (1986) and the method of Bloom and Crecelius (1984); and 3) cold-vapor atomic absorption spectroscopy (CVAA),

according to EPA SW-846 Method 7471 (1986) and the method of Bloom and Crecelius (1983). The analytical methods for each sample matrix and corresponding metals for which each method was used are presented in Table 2.4.

To prepare sediment and tissues for analysis, samples were freeze-dried, then blended in a Spex mixer-mill. Approximately 5 g of mixed sample was ground in a ceramic ball mill. The XRF analysis was performed on a 0.5-g aliquot of dried, ground material pressed into a pellet with a diameter of 2 cm. For GFAA, and CVAA analyses, 0.2- to 0.5-g aliquots of dried homogenous sample went through an acid digestion process to separate and isolate the metals from the matrix.

Quality control measures for metals analyses included analysis of blanks (not applicable to XRF technique), triplicate analyses for each batch of up to 20 samples, and analysis of at least one SRM sample per 20 samples. The SRMs for sediment were SRM 1646, obtained from the National Institute of Science and Technology (NIST), and MESS-1 and PACS-1, obtained from the NRCC. The tissue SRMs included an oyster tissue, 1566a, obtained from NRCC.

2.3.5. Butyltins

Butyltin compounds in sediment and tissues were analyzed using gas chromatography/flame photometric detection (GC/FPD) following the methods of Unger et al. (1986). Butyltins in sediment and *M. nasuta* tissue were analyzed at MSL in Sequim, Washington, and butyltins in *N. caecoides* samples were analyzed at Battelle Ocean Sciences in Duxbury, Massachusetts.

Wet samples were extracted with methylene chloride and tropolone. Propyltin was added before extraction as a surrogate compound to assess extraction efficiency. The mono-, di-, tri- and tetra-butyltin compounds extracted from the sediment and tissues were derivatized to a less volatile, more thermally stable form (nonionic n-hexyl or n-pentyl derivatives).

TABLE 2.4. Analytical Method and Corresponding Metal for Each Sample Matrix

Sediment			<i>N. caecoides</i> Tissue			<i>M. nasuta</i> Tissue		
XRF	GFAA	CVAA	XRF	GFAA	CVAA	XRF	GFAA	CVAA
As	Ag	Hg	As	Ag	Hg	As	Ag	Hg
Cr	Cd		Cu	Cd		Cu	Cd	
Cu	Se		Ni	Cr		Ni	Cr	
Ni			Zn	Pb		Pb		
Pb				Se		Se		
Zn						Zn		

The extracts were passed through a florisil liquid chromatography column for cleanup, and the butyltins were quantified by GC/FPD. Concentrations were reported in $\mu\text{g}/\text{kg}$ dry weight of mono-, di-, tri- and tetra-butylin species as tin. The recently certified reference material for butyltins, NRCC SRM PACS-1, was analyzed with Oakland Phase III B sediment. Selected sediment and tissue samples were spiked with di- and tri-butylin prior to extraction to assess the accuracy of the procedure. Method blanks were analyzed with each batch of samples extracted.

2.4 TOXICOLOGICAL TESTING PROCEDURES

Biological tests to assess the ecological effects of aquatic disposal of dredged material from the Oakland Harbor Phase III B Project area were conducted on both the solid-phase and SPP tests at the MSL in Sequim, Washington. The MSL facilities provided the required conditions for flow-through solid-phase tests, static solid-phase tests, and static SPP tests. Laboratory equipment providing these testing conditions included a controlled-temperature environment, flow-through seawater supply, lighting control, and air supply.

The solid-phase tests, also called benthic bioassays, were used to assess the acute toxicity and bioaccumulation potential of dredged material after it settles at an aquatic disposal site. Four species of marine organisms were exposed to the sediment from the Oakland Harbor sampling sites, reference area sediment, and control sediment. Three acute toxicity tests were conducted: 1) a 10-day solid-phase flow-through acute toxicity test using the polychaete *N. caecoides*, 2) a 10-day solid-phase flow-through test using the juvenile sanddab *C. stigmaeus*, and 3) a 10-day solid-phase static test using the amphipod *R. abronius*. In previous Oakland Harbor testing programs, the bentnose clam *M. nasuta* was included in the 10-day acute solid-phase test. For Phase III B, *M. nasuta* were not tested in the 10-day exposure because there were not enough organisms available.

The bioaccumulation test was a 28-day exposure of *N. caecoides* and *M. nasuta*. The purpose of the 28-day bioaccumulation test was to assess the potential for bioaccumulation of contaminants from the sediment into the tissues of the organisms. The test treatments and procedures were similar to the 10-day test except they involved a longer exposure period, larger test population, and depuration processes for surviving *M. nasuta* and *N. caecoides*.

The SPP tests were used to assess the potential effects of discharging dredged material through the water column during disposal operations. The SPP tests evaluate effects caused by the physical presence of suspended particles and the toxicity of chemical contaminants associated with

the particles or dissolved into the water after release. Three marine species were used in these tests: mysids (*H. sculpta*), juvenile sanddabs (*C. stigmaeus*), and larvae of the bay mussel (*M. edulis*). The SPP treatments were prepared as described in Section 2.2.4. For each SPP treatment, there were three replicates of each of the four SPP concentrations: 0% (seawater), 10%, 50%, and 100% (SPP).

2.4.1 10-Day Solid-Phase Flow-Through Test with *N. caecoides* and *M. nasuta*

Prior to testing, *N. caecoides* were held in their native sediment in shallow pans covered with well-aerated 15°C seawater from a gravity-fed flow-through system. *M. nasuta* were held in large tanks of clean sediment with flow-through 15°C seawater. Temperature, pH, dissolved oxygen (DO), and salinity of water in each holding tank were monitored daily. The organisms were not fed during the holding period.

The flow-through test with *M. nasuta* and *N. caecoides* was conducted in 10-gal aquaria placed in random positions on water tables. Figure 2.2 shows the system used for flow-through tests. Each aquarium was filled with approximately 8 L of sand-filtered seawater via the flow-through system. The test sediment was added to a depth of 3 cm by measuring out the required amount (3870 mL) in a clean glass container, and using seawater to wash and distribute the sediment evenly over the tank's bottom. The flow-through system was initiated, and aquaria were allowed to fill to a total volume of approximately 36 L. For approximately 4 h, suspended materials in the aquaria were allowed to settle and the flow-through system was adjusted and calibrated to deliver 125 ± 10 mL/min of seawater flow to each aquarium. The system was allowed to run overnight before the organisms were added.

For the 10-day test, 20 *M. nasuta* and 20 *N. caecoides* were collected from the holding tanks and placed in each aquarium. The initiation time/date and the initials of the analyst who added the organisms to each tank were noted on each aquarium. Water quality parameters of salinity, temperature, DO, and pH were measured daily in at least one replicate of each treatment and recorded on water quality data sheets. (Water quality data are provided in Volume 2, Appendixes E-H). The water quality parameters and ranges established for the tests were

Dissolved Oxygen	≥ 4.0 mg/L
pH	ambient ± 0.5 units
Salinity	ambient $\pm 2.0\%$
Temperature	$15.0^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$
Flow Rates	125 ± 10 mL/min.

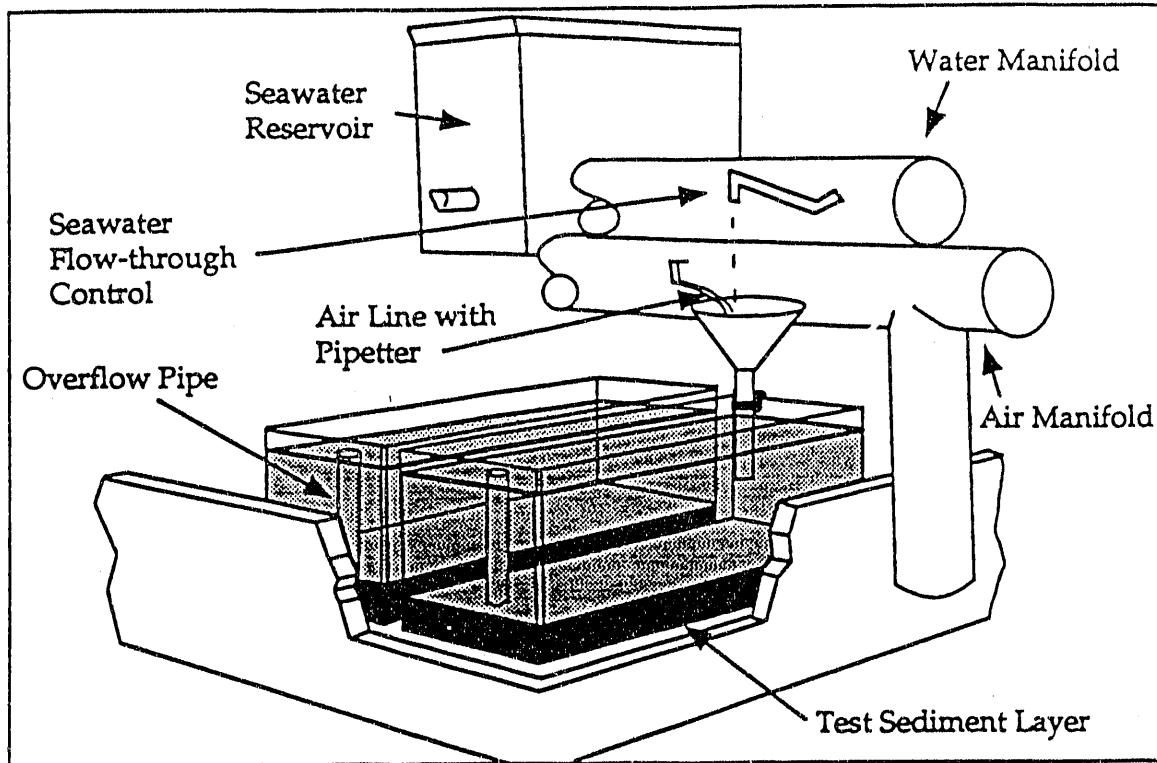


FIGURE 2.2. Flow-Through Aquarium for *M. nasuta*, *N. caecoides*, and *C. stigmaeus*

The number of dead organisms present was monitored daily. Dead organisms were removed but not replaced. If any dead *N. caecoides* were removed, the specimen was identified as to whether it was a whole animal or a head or tail portion. Daily observations of test animal behavior were made and recorded on data forms for each test. The number of *M. nasuta* on the sediment surface and the number of those with their siphons exposed were noted, as well as the number of *N. caecoides* on the sediment surface and the number of those with only their heads exposed.

At the end of the 10-day test, water quality measurements were taken in all tanks and the contents of each aquarium were gently passed through a 1.0-mm Nytex screen to recover the *N. caecoides* and *M. nasuta*. The organisms were placed in glass baking dishes labeled with the treatment number, and the number of dead and live of each species were counted. Acute toxicity was determined by observing whether the *N. caecoides* reacted to gentle probing. If there was no movement, the organism was considered dead. Acute toxicity in the *M. nasuta* was determined by observing and counting dead individuals. Those with gaping shells were considered dead. The mortality data were recorded on the Termination Forms. At least 10% of the mortality counts were confirmed by a second analyst.

2.4.2 28-Day Solid-Phase Flow-Through Test with *N. caecoides* and *M. nasuta*

The procedure for conducting the 28-day solid-phase flow-through test with *N. caecoides* and *M. nasuta* was identical to that of the 10-day test with three exceptions: 1) the number of organisms was increased to 25 *M. nasuta* and 30 *N. caecoides* because more individuals were needed to yield enough tissue for chemical analysis; 2) the exposure period was increased from 10 to 28 days; and 3) the surviving test organisms were depurated and sampled for chemical analysis. The ranges for water quality parameters as well as the test conditions, such as temperature and flow rate, were the same in the 28-day test as for the 10-day test. Water quality parameters were measured and mortality of the test organisms monitored at the same frequency for both tests.

When the 28-day test was terminated, the living *M. nasuta* and *N. caecoides* were collected for chemical evaluation of bioaccumulation. To ensure that tissue chemistry results would not be biased by contaminants associated with sediment grains in the digestive tract, the test organisms were allowed to depurate, or void the digestive tract, for 48 h following the 28-day exposure. The surviving *N. caecoides* from one test aquarium were placed in another flow-through 10-gal aquarium with approximately 2 in. of clean sediment in the bottom. Clean sediment was necessary for *N. caecoides* because these organisms require sediment to surround their tissues to survive. During the depuration period, the animals were not fed and the fecal material and debris were removed daily during water quality monitoring. The surviving *M. nasuta* were placed in a glass baking dish (without sediment), which was then placed in the depuration aquarium containing the *N. caecoides* from the same replicate. *M. nasuta* fecal material was siphoned from the baking dish daily during the depuration period. After 48 h of depuration, the *M. nasuta* shells were cleaned with a scrub brush, and the tissues were removed using titanium instruments and collected for chemical analysis. The *N. caecoides* were gently washed in clean seawater to remove external sediment grains and put in containers for chemical analysis.

2.4.3 10-Day Solid-Phase Static Test with *R. abronius*

R. abronius amphipods were captured by MSL off West Beach, Whidbey Island, Washington, using a specially designed anchor dredge deployed from a 17-ft Boston Whaler. Sediment brought up with the dredge was sieved through a 2-mm mesh screen to remove large debris and predatory organisms. Amphipods were kept in coolers partially filled with native sediment and seawater until they were delivered to a holding tank at MSL later on the sampling day. The amphipods were held in a large tank with their native sediment with flowing 15°C seawater. Organisms were not fed during the holding period, which was less than 2 weeks before test initiation.

The *R. abronius* test was conducted in 1-qt mason jars (Figure 2.3) placed in random positions on a water table maintained at 15°C. After the test sediment was mixed (Section 2.2.3) it was added to the jars to a depth of 2 cm, and then slowly filled with a 0.45- μm -filtered seawater to a volume of 750 mL. The jars were aerated and placed on the water table to stabilize temperature to test conditions. After sitting overnight, initial water quality parameters were measured in each container and recorded on water quality forms.

The test was initiated by adding 20 *R. abronius* to each test container. The amphipods were gently sieved from the holding tank into clean seawater, and counted into small transfer containers. The number of organisms was confirmed before they were transferred into the test container. The date and time of initiation were recorded on data forms. *R. abronius* were observed daily during the test, and the number of organisms floating on the surface, swimming in the jar, or on the sediment surface was recorded on observation forms. Amphipods that were

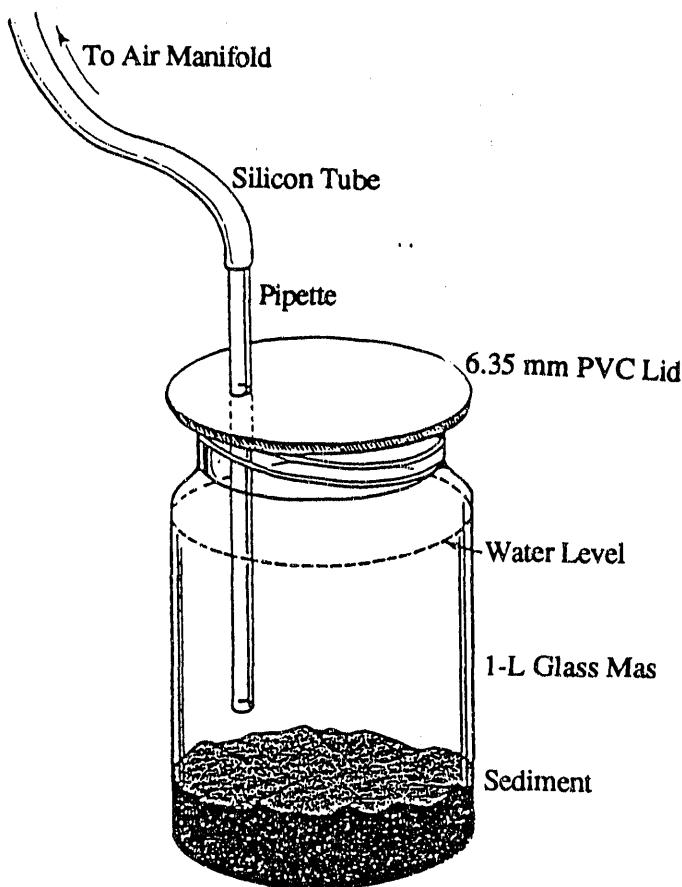


FIGURE 2.3. Static Amphipod Testing Jars

floating on the surface were gently pushed below the water surface with a pipet tip and observed as they either buried or did not rebury into the sediment.

Water temperature, salinity, pH, and DO were measured daily in one replicate of each treatment, and in all containers at initiation and termination of the bioassay. Acceptable ranges for water quality parameters during the experiment were

Dissolved Oxygen	≥ 4.0 mg/L
pH	ambient ± 0.5 units
Salinity	ambient $\pm 2.0\%$
Temperature	$15^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$.

At the end of the test (day 10), the contents of each jar were sieved through a 0.5-mm Nytex screen to collect the *R. abronius*. The organisms were placed in clean seawater in a glass dish labeled with the treatment and replicate number. The number of live and dead organisms in each dish was counted, and the presence or absence of body parts recovered at the end of the test was noted. If a *R. abronius* did not respond to gentle probing after several minutes of observation, it was considered dead. At least 10% of the mortality counts were confirmed by a second analyst.

2.4.4 10-Day Solid-Phase Flow-Through Test with *C. stigmaeus*

Prior to testing, *C. stigmaeus* were held in large tanks with a 3-in. layer of sediment on the bottom for at least 5 but no longer than 11 days prior to test initiation. The tanks were filled and supplied by flow-through seawater at test temperature (15°C). The sanddabs were fed freeze-dried krill twice a day. Temperature, pH, DO, and salinity of the holding tank water were monitored daily.

The solid-phase flow-through test for *C. stigmaeus* was conducted in 10-gal aquaria that were randomly positioned on the water tables. Approximately 8 L of sand-filtered seawater was added to each aquarium via the flow-through seawater system. Test sediment was added to a depth of 3 cm by measuring out 3870 mL in a clean glass container and using the seawater in the aquarium to distribute the sediment evenly. Each aquarium was filled over a period of approximately 4 h, allowing suspended particles to settle. Seawater was circulated overnight via the flow-through system at a flow rate of 125 mL/min (± 10 mL/min). Initial water quality parameters and flow-through rates were measured on every test container. Ten *C. stigmaeus* were collected from the holding tanks and placed in each aquarium. Initiation date, time, and analyst's initials were noted on the aquarium and on the data forms. The animals were checked after 2 h; dead or impaired organisms were removed and replaced. Organisms were considered impaired if they swam abnormally or were unable to orient themselves dorso-ventrally. Biological observations and the number of live and dead in each test container were recorded daily. Water

quality parameters and flow rates were measured daily in at least one replicate of each treatment and recorded on the water quality data sheets. Acceptable water quality parameters and ranges during the experiment were

Dissolved Oxygen	≥ 4.0 mg/L
pH	ambient ± 0.5 units
Salinity	ambient $\pm 2.0\%$
Temperature	$15^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$
Flow Rate	125 ± 10 mL/min.

During the test, all dead fish were removed and placed in individually labeled 50-mL centrifuge tubes and stored in the freezer. At the termination of the test, water quality parameters were measured on all replicates and the number of living and dead *C. stigmaeus* was counted and recorded on the Termination Form. Live fish from each treatment were placed in a clean, labeled glass jar and preserved in Davidson's solution for histopathological analysis, if required.

2.4.5 96-h Solid-Phase-Particulate Static Test with *C. stigmaeus*

The test chambers for the SPP test with *C. stigmaeus* were 10-gal aquaria that were randomly positioned on the water tables, with 20 to 24 aquaria per table. Test temperature was maintained by a circulating water bath on the water table. Aeration was provided through a glass pipet connected by silastic tubing to an overhead air manifold. Aquaria were labeled with a treatment code, concentration, and replicate number. The volume of test material in each aquarium was 16 L. To obtain the 100% SPP treatment, 16 L of 100% SPP was added directly to the aquarium; the 0% SPP treatment was 16 L of 0.45- μm -filtered Sequim Bay seawater. To prepare 16 L of each of the 10% and 50% SPP concentrations, appropriate volumes of 100% SPP and 0.45- μm -filtered Sequim Bay dilution water were mixed directly in the test aquaria.

Once all concentrations of an SPP treatment were prepared and all test containers were filled, aeration was started and initial water quality parameters were measured in all replicates. *C. stigmaeus* were then removed from the holding tanks using a net and added to each test container. Ten *C. stigmaeus* were placed in each container so that the test population for each concentration was 30 individuals (120 individuals per SPP treatment). Initiation time and date were documented on test containers and data record forms.

C. stigmaeus were not fed during the 96-h exposure. After initiation, DO, pH, salinity, and temperature were measured daily in at least one replicate. Acceptable ranges for the water quality parameters during the experiment were

Dissolved oxygen	$\geq 4.0 \text{ mg/L}$
pH	ambient ± 0.5 units
Salinity	ambient $\pm 2.0\text{‰}$
Temperature	$15.0^\circ\text{C} \pm 2.0^\circ\text{C}$.

Observations of *C. stigmaeus* activity and behavior in each test container were made at test initiation and at 4, 24, 48, and 72 h. A clean probe was used to determine the condition of any resting *C. stigmaeus*. An organism was considered dead if it did not respond to gentle probing. Dead organisms were removed and preserved in Davidson's fixative for potential histopathological analysis.

Before termination of the test at 96 h, water quality parameters were measured in all replicates. At 96 h, the number of live and dead organisms was counted in each test container. As a quality control check, a second analyst confirmed at least 10% of the mortality counts. Additionally, fish from SPP treatments where there appeared to be a toxicological effect on test organisms (either through mortality or behavioral abnormalities) were also preserved for histopathological analysis.

2.4.6 96-h Solid-Phase-Particulate Static Test with *H. sculpsa*

Prior to testing, *H. sculpsa* were held for at least 48 h in flow-through aquaria maintained at test temperature (15°C). *H. sculpsa* were fed finely ground, flaked fish food twice a day, and water quality parameters in the holding tanks were monitored daily.

The test containers for the *H. sculpsa* test were 2-L glass baking dishes placed in random positions on water tables. Test temperature was maintained by immersing these containers in a circulating water bath. Aeration was provided through a pipet connected by silastic tubing to an overhead air manifold. Appropriate volumes of 100% SPP and 0.45- μm -filtered Sequim Bay dilution water were added to clean glass gallon jars to make 0%, 10%, 50%, and 100% SPP concentrations for the *H. sculpsa* test. A total of 3000 mL was prepared for each dilution to allow 1000 mL in each of three replicate test chambers. The test containers were labeled with a treatment code, concentration, and replicate number.

As soon as containers were in place, gentle aeration was started to each one, and water quality measured in all replicates. *H. sculpsa* were then removed from the holding tanks using a wide-bore pipette. Ten individuals were added to each container so that the test population for each concentration was 30 individuals (120 individuals per treatment). The test initiation time and date were documented on data forms.

After test initiation, water quality parameters were measured daily in at least one replicate.

Acceptable ranges for the water quality parameters during the experiment were

Dissolved oxygen	$\geq 4.0 \text{ mg/L}$
pH	ambient ± 0.5 units
Salinity	ambient $\pm 2.0\%$
Temperature	$15.0^\circ\text{C} \pm 2.0^\circ\text{C}$.

Observations of test organisms were made at test initiation and at 4, 24, 48, and 72 h, using a light table to enhance visibility of the *H. sculpta*. During the 96-h exposure, *H. sculpta* were fed small amounts of ground flaked fish food at 4, 24, 48, and 72 h. Excess food was removed with a small pipet before daily observations, using extra caution not to disturb test animals. Molted exoskeletons and any particulates from the SPP solution that had precipitated out were also removed.

Before termination of the test at 96 h, water quality parameters were measured in all replicates. At 96 h, the number of live and dead animals was counted in each test container. An organism was considered dead if it did not respond to gentle probing. As a quality control check, a second analyst confirmed surviving test organisms in at least 10% of the mortality counts.

A 96-h reference toxicant test was also conducted to establish the health and expected response of the test organisms. *H. sculpta* were exposed to a seawater control plus four concentrations of zinc chloride (0.25, 0.50, 1.0, and 2.0 mg/L as Zn). There were three replicates of each treatment. The reference toxicant test was conducted in the same manner as the SPP tests.

2.4.7 48-h Solid-Phase-Particulate Static Test with Larval *M. edulis*

Prior to testing, adult *M. edulis* were held in flow-through tanks at ambient temperature until several days before the test, when they were transferred to 12°C filtered seawater and fed twice daily with algal paste. The test chambers for the bivalve larvae test were 1-qt Mason jars. The dilutions of SPP for the bivalve test (0%, 10%, 50%, and 100%) were prepared directly in labeled test containers. The dilution water consisted of Strait of Juan de Fuca seawater screened at $20 \mu\text{m}$. The final volume of test material in each container was 750 mL. Test chambers containing test material were placed in random positions on a water table and gentle aeration was started. Initial water quality parameters were measured in all replicates once the containers had reached test temperature ($16^\circ\text{C} \pm 2^\circ\text{C}$).

Adult *M. edulis* were induced to spawn by placing individuals in 16°C seawater for 2 h, then removing them from water and allowing them to dry for approximately 20 min. They were then returned to 16°C water that was quickly warmed to 20°C . Most adults had spawned within an

hour. Sperm from up to three males was pooled and screened through 35- μm mesh to remove debris. The sperm was then introduced to containers of egg suspension for fertilization. The egg suspensions were mixed frequently using a perforated plunger over a period of 90 to 140 min, after which development of the embryos was checked. Three egg suspensions with a high percentage of embryo development were pooled into a common stock for use in the test. The pooled egg suspension was screened through 75- μm mesh to remove debris, and then retained on a 20- μm screen to rinse away excess sperm. Finally, the eggs were rinsed from the 20- μm screen into a clean container and diluted with seawater.

To estimate fertilization success and embryo density, a 1-mL sample was removed from the container (after thorough mixing) and diluted to 100 mL with seawater. Three, 1-mL samples were removed from this 100:1 suspension, and the number of developing embryos and non-fertilized eggs was scored using a Sedgewick-Rafter counting chamber on a compound microscope at low magnification. The mean number of embryos from the replicate counts was multiplied by 100 (to correct for the dilution factor) to estimate the density of embryos in the egg stock. The resulting density of 11,133 embryos/mL was used to calculate the amount of stock to add to each test container as well as to calculate percent fertilization.

To initiate the test, 1.7 mL of bivalve embryo stock solution was pipetted into each test container to yield a density of 25 embryos per mL in the containers of test material. A perforated plunger was used to thoroughly mix the contents of the stock container before removing each aliquot with the pipette. The test initiation date and time were recorded on data record forms. To obtain the actual embryo stocking density, 10-mL subsamples were removed from 14 control containers (two replicates per treatment control) 1 h after test initiation, and after mixing the contents of the container with the perforated plunger. Each subsample was placed in a labeled vial, fixed with 1 mL of 5% formalin, and scored for the number of fertilized eggs.

Water quality parameters were measured in one replicate of each dilution 24 h after test initiation. Acceptable ranges for water quality parameters during the experiment were

Dissolved oxygen	$\geq 4.0\text{ mg/L}$
pH	ambient ± 0.5 units
Salinity	ambient $\pm 2.0\text{‰}$
Temperature	$16.0^\circ\text{C} \pm 2.0^\circ\text{C}$.

The bivalve test was terminated after 48 to 72 h, when development of D-shaped larvae predominated in control containers. Final water quality measurements were recorded for all replicates. Then, the contents of each chamber were homogenized with the perforated plunger, and a 10-mL sample was removed with a calibrated pipette and placed in a labeled vial containing 1 mL

of 5% formalin. Samples were scored for the appearance of normal D-shaped larvae, abnormally developed larvae, blastula-stage larvae, and total number of larvae. At least 10% of the counts were confirmed by a second analyst.

A 48-h reference toxicant test was also conducted to establish the health and expected response of the test organisms. *M. edulis* larvae were exposed to a seawater control plus four concentrations of copper sulfate (1 $\mu\text{g/L}$, 4 $\mu\text{g/L}$, 16 $\mu\text{g/L}$, and 64 $\mu\text{g/L}$ as Cu). There were two replicates of each treatment. The reference toxicant test was set up and conducted in the same manner as the SPP tests.

2.5 DATA ANALYSIS AND INTERPRETATION

Several statistical analyses were conducted to determine the magnitude and significance of toxicity and bioaccumulation in test treatments relative to reference treatments. Each statistical test is based upon a completely random design that allows unbiased comparison between treatments. The 1990 Draft Implementation Manual recommends Dunnett's Test for comparing test treatments to a single reference treatment. However, the Oakland Phase III B experiments were conducted with multiple reference treatments because a single reference most representative of a potential disposal site had not been selected. Because it is only appropriate for comparison to a single reference, Dunnett's Test could not be used for comparing Oakland Harbor Phase III B test sediments to all the reference sediments that were tested. In this case, Dunn's Test, a modification of Tukey's Honestly Significant Difference (HSD) Test, is appropriate and was used to compare the test treatments to all the reference treatments while maintaining the experiment-wise error rate. The alpha level was expanded to 0.1 for both bioaccumulation and toxicity comparisons in order to increase the comparison-wise error rate, which becomes smaller as the number of comparisons increases. This implies that the test is statistically less conservative, meaning that it is less likely to fail to detect a significant difference than comparisons at $\alpha = 0.05$. Comparisons conducted at $\alpha = 0.05$ are less sensitive to differences in means, resulting in fewer significant differences. Test design and specific statistical analysis procedures are discussed in the following sections.

2.5.1 Randomization

All solid phase and SPP toxicity tests were designed as completely random tests. Organisms were randomly allocated to treatments, and treatments were randomly positioned on water tables. A random number table for this purpose was generated for each toxicity test, using the discrete uniform random number generator in Lotus 123 spreadsheet. For the SPP tests, *C. stigmaeus* and *H. sculpta* individuals, and *M. edulis* larvae were randomly allocated to SPP

replicates for all concentrations. Special care was taken with *C. stigmatus* individuals in order to eliminate bias caused by variable mobility of the fish (otherwise, easily caught fish would be used earlier than more mobile fish).

2.5.2 Statistical Analysis of Solid Phase Tests

Solid phase toxicity of all sediment treatments was compared by analysis of variance (ANOVA) tests on the arcsine square-root of the proportion of organisms surviving the test. The arcsine square-root transformation stabilizes the within-class variances to meet the assumptions of the ANOVA. All treatments were compared using the conservative Dunn's Test for comparison of all means (Dunn 1961), using an experiment-wise error rate of $\alpha = 0.10$. Dunn's Test is similar to Tukey's HSD Test that had been used for previous Oakland Harbor projects, but Dunn's Test can accommodate unbalanced replicates. Both Dunn's and Tukey's HSD Tests are multiple-range comparisons that provide information about how each sediment treatment compares to all other treatments. These statistical methods allow comparison of a test treatment to more than one reference treatment when all are tested concurrently. Toxicity of a treatment was considered significant if it was statistically different from one or more reference treatments and if the survival in that treatment was $\geq 10\%$ lower than the control treatment for the test organism ($\geq 20\%$ lower than control for the amphipod *R. abronius*).

2.5.3 Statistical Analysis of SPP Tests

Two statistical tests are presented in the Draft Implementation Manual (EPA/USACE 1990) for the interpretation of SPP tests. The first test is a one-sided t-test between survival in control replicates and survival in the 100% SPP replicates. This test is performed only when survival in the 100% SPP is less than control (0% SPP) survival and when control survival is greater than 90% (indicating test validity). Prior to conducting the t-test, angular transformation (arcsine of the square root) of the proportion surviving in test replicates is performed to reduce possible heterogeneity of variance between control and 100% SPP mean survivals. The second test required by the Draft Implementation Manual is an LC50 calculation, the concentration of SPP that is lethal to 50% of the individuals tested. The LC50 values for these tests were calculated using the Trimmed Spearman Karber Method (Finney 1971). The Spearman Karber estimator is appropriate only if there is increasing mortality with increasing concentration and if 50% or greater mortality is observed in test solutions when normalized to control survival. If 50% mortality does not occur in the 100% SPP dilutions for any treatments, then LC50 values are reported as >100% SPP. The same method was used to calculate EC50 values (the concentration where 50% of the test

individuals show a certain effect) for the bivalve SPP test and LC50 values for all reference toxicant tests.

2.5.4 Statistical Analysis of Bioaccumulation

Before statistical analysis of *M. nasuta* and *N. caecoides* tissue concentrations, a visual inspection of the tissue chemistry data was performed. If visual inspection showed that a compound was undetected in the majority of replicate samples in test treatments or that the mean tissue concentration in the reference treatments was greater than that in the test treatments, no further analysis was performed. If the detected concentration of a compound exceeded that of any reference treatment, statistical analysis was performed. In all cases, detection limits were used in numerical calculations when a compound was undetected.

Treatments where contaminants of concern were detected were compared to all reference treatments using ANOVA and Dunn's Test for comparison of all means. Analytical detection limit values were used for replicates where the compound was not detected. Significant bioaccumulation of a contaminant was determined by the statistical grouping yielded by Dunn's Test at $\alpha = 0.10$.

2.6 QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

The QA/QC procedures followed for these studies were consistent with the Implementation Manuals (EPA/USACE 1977 and 1990) and the EPA protocols (PSEP 1986). The procedures followed were documented by Pacific Northwest Laboratory's (PNL) Quality Engineering Division as a QA Plan (Number EES-20, Revision 1). A member of PNL's quality engineering staff was present throughout each phase of these studies to ensure that accepted procedures were followed. The PNL Laboratory Record Books (LRB) were assigned to each portion of the study and served as records of day-to-day activities during the research. All entries in the LRBs were signed, dated, and reviewed by both the project manager and the quality assurance engineer. The following discussion summarizes QA/QC procedures followed for the three main portions of this study: sediment sampling, biological testing, and chemical testing. (All QA/QC evaluations are contained in Volume 2, Appendix C.)

2.6.1 Sample Tracking and Storage

All sediment samples were accompanied by chain-of-custody forms from the time of collection to receipt at MSL. After sample selection and compositing, a new set of custody forms was initiated for the sediment subsamples requiring chemical analyses. These accompanied the samples to the appropriate laboratory where the forms were signed and returned to the MSL project manager. Custody forms were also initiated for all tissue samples upon completion of the biological testing. These forms accompanied the samples to the appropriate laboratory for chemical analyses.

All sediment collected for these studies was stored in glass, cellulose acetate butyrate containers, or steel drums lined with 9-C-4-A-phenolic epoxy, a noncontaminating coating. Sediment cores and grab samples were stored at 4°C prior to biological testing. Subsamples for chemical analyses were obtained prior to biological testing. These subsamples were stored frozen until chemical analyses were performed. Samples for organic analyses were stored frozen up to 2 months (PSEP 1986). Samples for metals were freeze-dried upon receipt at the laboratory and held up to 6 months (PSEP 1986; USACE 1990).

Tissue samples were frozen immediately upon completion of the bioaccumulation tests. Samples for organic analyses were stored in precleaned glass jars with Teflon-lined lids and samples for metals analyses were stored in precleaned plastic jars. Samples for organic analyses were held up to 2 months prior to analysis and samples for metals were held up to 6 months prior to analysis.

2.6.2 Sediment and Tissue Chemistry Quality Control Procedures

Chemical testing procedures require that specific QA/QC protocols be followed. QA/QC guidelines specific to this project are provided in the Pacific Northwest Laboratory's (PNL) Quality Assurance Division as a QA Plan (Number EES-20, Revision 1). These guidelines include the following:

- analysis of a method blank with each batch of samples
- replicate analysis on at least 5% of the samples (triplicate analyses where possible) to assess analytical precision
- analysis of matrix spikes on 10% of the samples (where applicable) with appropriate compounds to assess accuracy
- analysis of standard reference materials (SRMs) at a frequency of 5%, if available for the analytes of interest and sample matrix

- archival of all instrument printouts (e.g., raw data and chromatograms from AA and GC analyses) for future review.

In actual practice, some of the specific guidelines listed in the QAP for analytical precision and accuracy were modified to apply to the most current methods employed by laboratories. The guidelines for detection limits, range of recovery, and relative precision are listed in Table 2.1 for sediments and in Table 2.2 for tissues.

Measurements of accuracy can be determined by analyzing matrix spikes of known concentrations, as well as SRMs that have been certified for the presence of specific parameters. Matrix spikes were analyzed for metals and for organic parameters, including oil and grease, petroleum hydrocarbons, PCBs, pesticides, and PAHs. Spikes generally are made up of a subset of the analytes of interest. Spike recoveries were calculated based on the difference between the amount spiked and the amount recovered in the sample, taking into account the amounts already present in the spiked sample. Spikes for metals were analyzed at a frequency of 5%. Spikes for organic compounds were generally analyzed in duplicate at a frequency of 5%. Surrogate compounds were added in known amounts to samples analyzed for PCBs, pesticides, PAHs and butyltins. Surrogate compounds are added to samples prior to extraction, and their recoveries are a measurement of the efficiency or procedural accuracy of the analysis. Analytical accuracy is also measured through the analysis of SRMs. Sediment SRMs were analyzed for metals and for organic compounds. Tissue SRMs were analyzed for metals. SRMs for organic compounds in tissue were not available for this study. SRMs are not associated with analysis of TOC, oil and grease and petroleum hydrocarbons, TVS, and grain size.

Measurements of precision were obtained through replicate analysis of selected sediment treatments. Analysis of replicates shows how precise or repeatable a result is. The measurement of precision is the industrial statistic "I" and relative percent difference (RPD) for duplicate analyses, and the relative standard deviation (RSD) for triplicate analyses. The "I" statistic is defined as the absolute value of the difference between duplicate measurements, divided by the sum of the duplicates. The RPD is defined as the absolute value of the difference between two duplicate measurements, divided by the mean of the duplicates, multiplied by 100. The RSD is defined as the sample standard deviation divided by the mean, multiplied by 100. All instrument printouts and other raw data generated using MSL analytical instruments are filed at MSL for future reference. Procedures and related data were written into the appropriate LRB. Raw data generated by offsite analytical facilities are retained at those facilities, but can be made available for inspection.

2.6.3 Toxicological Testing Quality Control Procedures

Test organisms were handled carefully during collection and transfer to test containers. Organisms shipped to MSL were gradually equilibrated to ambient surroundings, and kept in their native sediment whenever possible. Animals were fed, if necessary, before biological testing. Information on the collecting and handling of each test species is included in Section 2.1.3.

Selection of species was consistent with the Draft Implementation Manual and involved the use of juvenile forms, burrowing invertebrates, deposit feeding organisms, and a larval (planktonic) form. Representatives of all test organisms were taxonomically identified by qualified experts at MSL before use in bioassays.

During all bioassay tests, water quality parameters were measured to ensure that acceptable experimental conditions were maintained. These conditions included a stable temperature ($\pm 2.0^{\circ}\text{C}$), minimum DO of 4.0 mg/L, and 14 h of light per day. Salinity was allowed to vary $\pm 2.0\%$, and pH was allowed to vary ± 0.5 units within each test container during the bioassay period. These limits and values are consistent with those outlined in the Draft Implementation Manual. Water quality instruments were calibrated according to the manufacturer's specification or PNL protocols.

3.0 RESULTS

This section includes a discussion of sediment sampling results and geologic descriptions as well as detailed results of sediment chemistry, toxicological testing, and tissue chemistry.

3.1 SEDIMENT SAMPLING RESULTS

Sediment sampling for Oakland Harbor Phase III B occurred in November 1990. One day of mobilization of vessels and equipment was followed by 4 days of sampling in Oakland Harbor and the in-bay and offshore reference areas. Control sediment was collected at the same time. All sediment samples were collected following the procedures described in Section 2.1.

Sediment core samples were collected at 18 stations in Oakland Outer and Inner Harbors November 8 through 11, 1990. Oakland Outer Harbor Stations O-C1 through O-C13 and Stations I-C2 and I-C3 at the entrance to Oakland Inner Harbor comprise the Phase III B Project area (Figure 3.1). It should be noted that Phase III B Station I-C2 has a different location than sites designated I-C2 in other Oakland Phase III and previous Oakland programs. Inner Harbor Station I-C8 is a Phase III A station for which the entire suite of testing was repeated at the request of USACE, San Francisco District. Oakland Inner Harbor Stations I-C4 and I-T5 are Phase III A Project stations where sediment was collected and tested only for *N. caecoides* bioaccumulation during the Phase III B Project. One 12-in. and one 4-in. core were collected to at least -44 ft MLLW at all stations except I-C4 and I-T5, where only 12-in. cores were collected (Table 3.1). The 4-in. cores were not necessary at Stations I-T5 and I-C4 because geologic descriptions for those sites were done during Phase III A. Approximately 20 gal of sediment from between the mudline and -44 ft MLLW was collected from each 12-in. core from each Oakland Outer Harbor (O-C) station. Where sediment from the 12-in. core contributed to a sediment composite for SPP toxicity testing, the contributed volume is listed in Table 3.1. At each Oakland Inner Harbor (I-C) station, 16 to 20 gal of sediment was taken from the -38 to -44 ft MLLW segment of the 12-in. core. Sediment between the mudline and -39 ft MLLW from the Inner Harbor stations was tested during a previous sampling episode.

Sediment samples were collected from 19 sites in three offshore and three in-bay reference areas on November 6 and 7, 1990. The three in-bay reference areas all had multiple sampling sites. The Bay Farm Borrow Area (R-BF) consisted of four sites (Figure 3.2), Alcatraz Island environs (R-AM) consisted of eight sites within 0.5-nautical mile of Alcatraz Island (Figure 3.3), and the Alcatraz Island disposal site (R-AC) had four sites within its boundary (Figure 3.3).

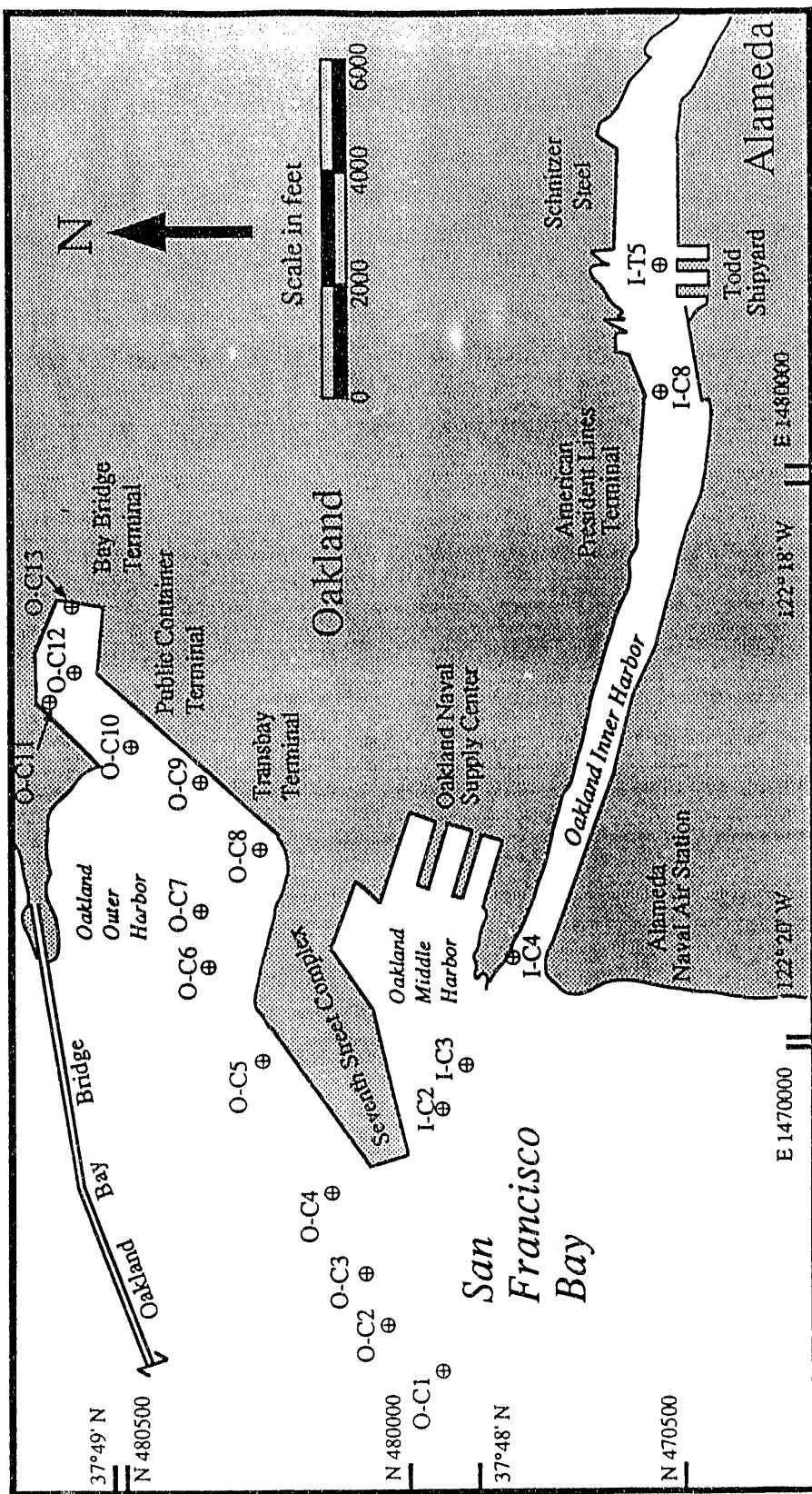


FIGURE 3.1. Oakland Harbor Phase III B Sampling Stations

TABLE 3.1. Core Sampling Information for the Oakland Harbor Phase III B Project

Station	Core Size (in.)	Replicate	Date	California State Plane			Core Required to -44 ft MLLW	Core Collected(ft)	Core Contribution to Composite	Mudline to -44 ft MLLW
				Coordinates (Zone III)	North (Y)	East (X)				
O-C1	12	1	09-Nov-90	479,282	1,464,194	38.1	5.9	11.9	4.0	to BC-6
O-C2	12	1	09-Nov-90	480,327	1,465,036	26.6	17.4	18.3	NC(a)	BC-6
O-C3	12	1	09-Nov-90	480,672	1,465,962	33.4	10.6	11.4	4.0	to BC-8
O-C4	12	1	09-Nov-90	481,290	1,467,348	36.3	7.7	9.4	6.0	to BC-8
O-C5	12	1	09-Nov-90	482,470	1,469,698	38.9	5.1	5.9	6.0	to BC-8
O-C6	12	1	08-Nov-90	483,388	1,471,343	29.4	14.6	18.0	3.0	to BC-5
O-C7	12	1	08-Nov-90	483,549	1,472,331	27.6	16.4	18.0	3.0	to BC-5
O-C8	12	1	08-Nov-90	482,539	1,473,386	37.7	6.3	8.5	3.0	to BC-5
O-C9	12	1	08-Nov-90	483,544	1,474,560	41.1	2.9	5.0	3.0	to BC-5
O-C10	12	1	08-Nov-90	484,723	1,475,192	38.3	5.7	5.7	3.0	to BC-7
O-C11	12	1	08-Nov-90	486,129	1,475,970	35.6	8.4	9.2	3.0	to BC-7
O-C12	12	1	08-Nov-90	485,726	1,476,500	39.0	5.0	7.2	3.0	to BC-7
O-C13	12	1	08-Nov-90	485,747	1,477,679	34.4	9.6	13.5	3.0	to BC-7
I-C2	12	1	11-Nov-90	479,295	1,468,801	37.2	6.8	8.3	4.0	to BC-6(b)
I-C3	12	1	09-Nov-90	478,886	1,469,591	35.7	8.3	9.7	4.0	to BC-6(b)
I-C8	12	1	11-Nov-90	475,477	1,481,313	37.7	6.3	7.9	NC	NC
I-C4	12	1	11-Nov-90	478,089	1,471,460	36.9	7.1	7.1	NC	NC
I-T5	12	1	11-Nov-90	475,475	1,483,525	30.4	13.6	7.3	NC	NC
O-C1	4	1	09-Nov-90	479,282	1,464,194	38.1	5.9	7.1	NC	NC
O-C2	4	1	09-Nov-90	480,327	1,465,036	26.6	17.4	19.3	NC	NC
O-C3	4	1	09-Nov-90	480,672	1,465,962	33.4	10.6	13.5	NC	NC
O-C4	4	1	09-Nov-90	481,290	1,467,348	36.3	7.7	8.5	NC	NC
O-C5	4	1	09-Nov-90	482,470	1,469,698	38.2	5.8	6.9	NC	NC
O-C6	4	1	08-Nov-90	483,388	1,471,343	29.4	14.6	14.1	NC	NC
O-C7	4	1	08-Nov-90	483,549	1,472,331	27.6	16.4	20.0	NC	NC
O-C8	4	1	08-Nov-90	482,539	1,473,386	37.7	6.3	9.9	NC	NC
O-C9	4	1	08-Nov-90	483,544	1,474,560	41.1	2.9	5.0	NC	NC
O-C10	4	1	08-Nov-90	484,723	1,475,192	38.3	5.7	10.0	11.1	NC
O-C11	4	1	08-Nov-90	486,129	1,475,970	35.6	8.4	8.4	10.0	NC
O-C12	4	1	08-Nov-90	485,726	1,476,500	39.0	5.0	5.0	9.9	NC
O-C13	4	1	08-Nov-90	485,747	1,477,679	34.4	9.6	9.6	9.9	NC

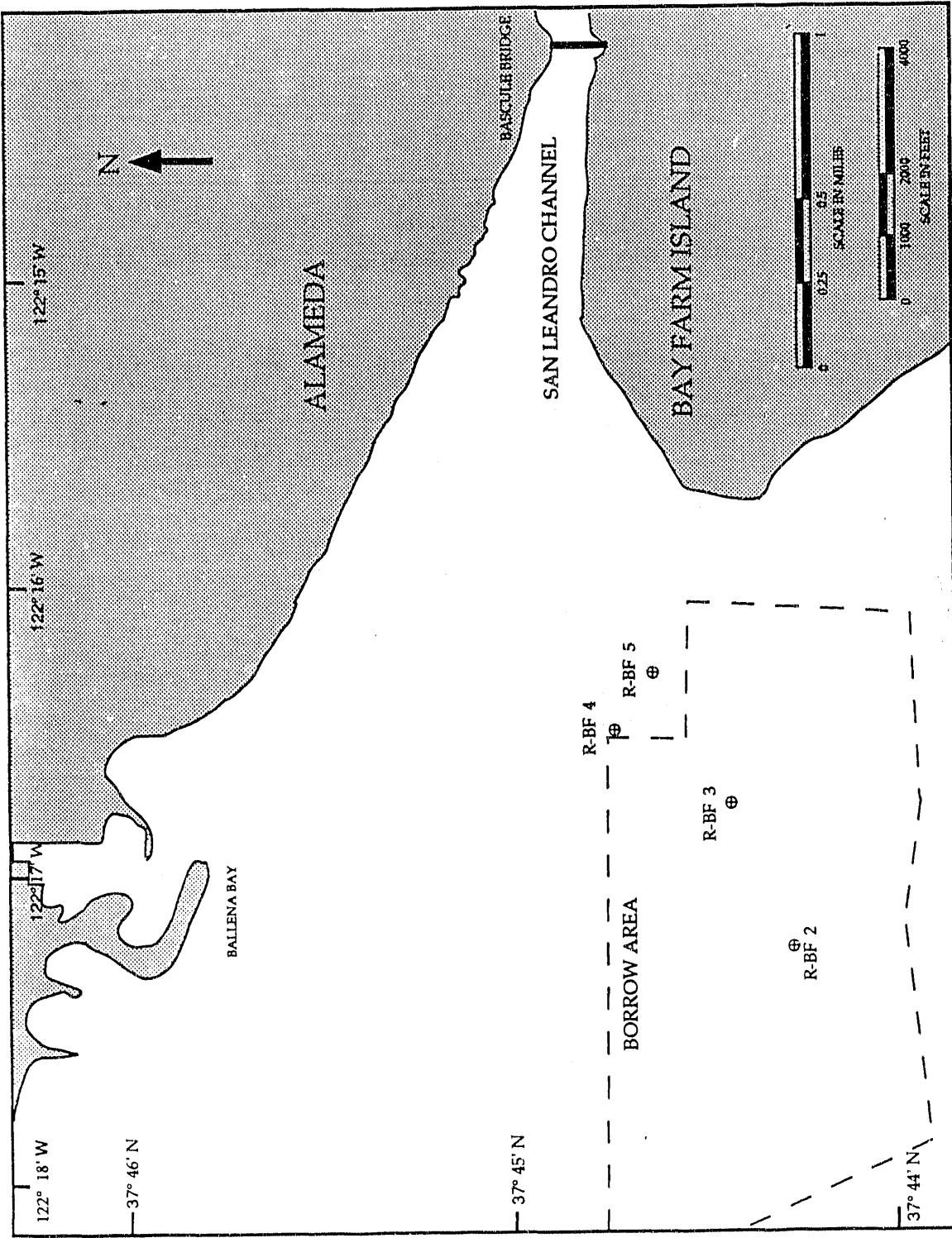
PHASE III B

TABLE 3.1. (contd)

Station	Core Size (in)	Replicate	Date	California State Plane		Depth (-ft MLLW)	Core Required to -44 ft MLLW (ft)	Core Collected (ft)	Mudline to -44 ft MLLW Contribution to Composite
				Coordinates (Zone III)	North (Y)				
I-C2	4	1	11-Nov-90	479,295	1,468,801	372	6.8	14.6	NC
I-C3	4	1	09-Nov-90	478,886	1,469,591	35.7	8.3	12.7	NC
I-C8	4	1	11-Nov-90	475,477	1,481,313	37.7	6.3	10.0	NC

(a) NC No contribution to a composite

(b) Contribution was from -38 to -44 ft MLLW



PHASE III B

FIGURE 3.2. Reference Sediment Sampling sites in the Bay Farm Borrow Area, Oakland Harbor Phase III B

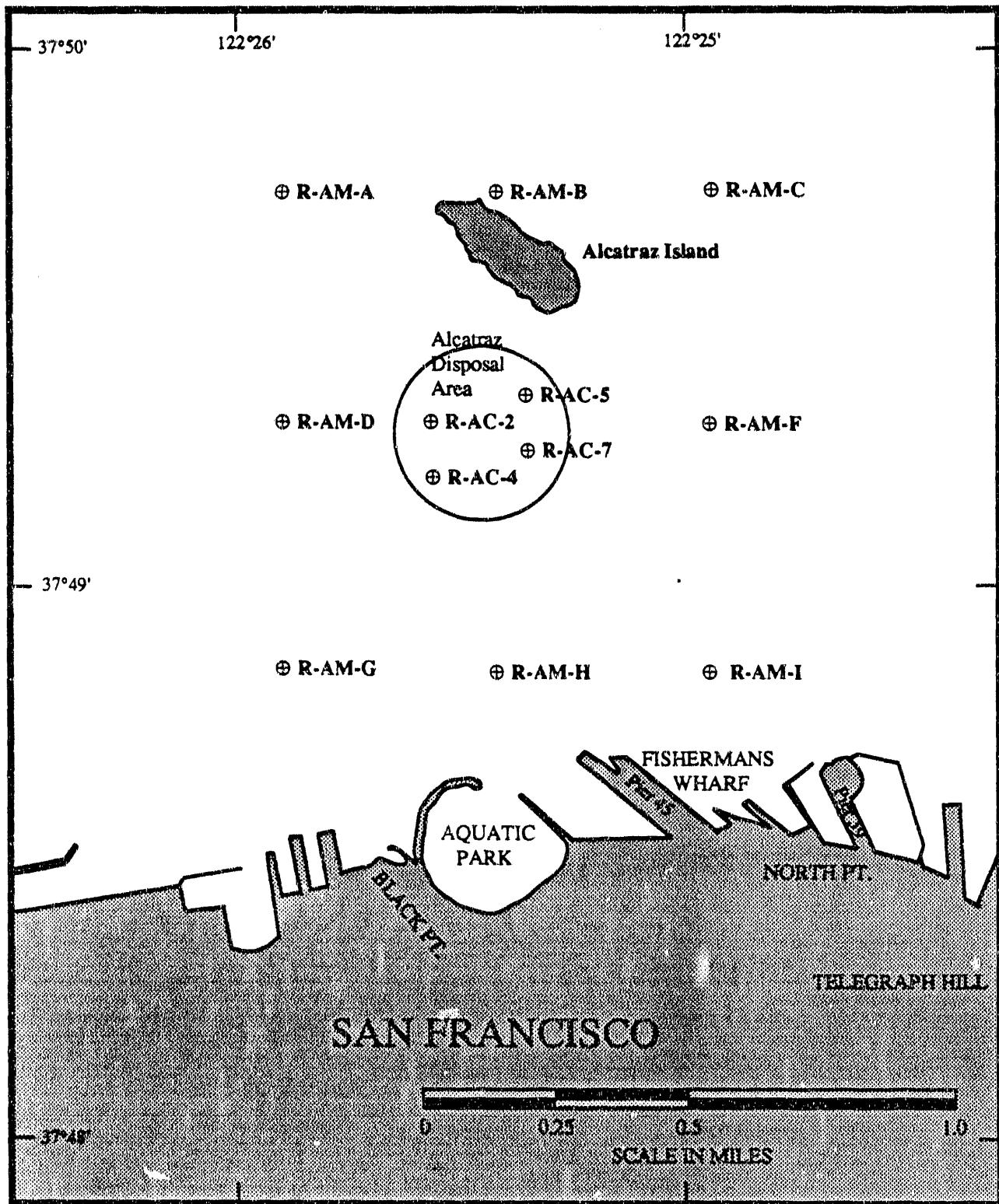


FIGURE 3.3. Approximate Locations for Reference Sediment Sampling Sites Near Alcatraz Island, Oakland Harbor Phase III B

Sediment was collected at each station in an area and composited with sediment from other stations in the area to obtain a representative sample. The offshore areas were Point Reyes Fine reference area (R-PF), Point Reyes Coarse reference area (R-PC), and the deep off-shelf reference area (R-OS) (Figure 3.4). The offshore reference areas consisted of one site each, with replicate samples collected in that area to obtain the required volume of sediment. Complete reference sediment sampling information is provided in Table 3.2.

Control sediment for use in solid-phase toxicity tests was collected from Sequim Bay, Washington (Figure 3.5); West Beach, Washington (Figure 3.6); and Tomales Bay, California (Figure 3.7), as described in Section 2.1.3. Sequim Bay control sediment (C-SB) was used both as an experimental control in all toxicity tests as well as the native control for *M. nasuta*. West Beach control sediment (C-WB) is the native sediment for the amphipod *R. abronius*. Native sediment for *N. caecoides* (C-NE) and *C. stigmaeus* (C-SD) were both collected from Tomales Bay, California. Control sediment collection occurred November 7 through 9, 1990.

3.2 GEOLOGIC DESCRIPTIONS

The following is a description of the geology of the Oakland Harbor Phase III B Project area, based on sediment characterization of 16 core samples collected in November 1990. Approximately 150 ft of core were described according to ASTM Procedure D2488-84: "Standard Practice for Description and Identification of Soils (Visual-Manual Procedure)" (ASTM 1984). Sediment characteristics that were logged include dilatancy of silt/clay, toughness of silt/clay, plasticity of silt/clay, sediment type (i.e., engineering classification), color, consistency (i.e., firmness), cementation, sedimentary structure, reaction with hydrochloric acid, maximum particle size, and odor. In addition, any other diagnostic features such as the presence of root traces, mollusk shells and/or related detritus, were noted. A detailed description of the materials and methods used for describing the cores is given in Volume 2, Appendix A. Copies of the core data logs and a key to the abbreviations used are presented in Volume 2, Appendix B.

Three geologic units are present in the Phase III B Project area: Older Bay Mud (OBM), Sand Deposits, and Younger Bay Mud (YBM) (USACE 1975). Selected Oakland Outer Harbor cores along cross section A-A' (Figure 3.8) were connected to construct a geologic cross section showing the relationship of the geologic units occurring in the outer harbor (Figure 3.9). The sediments in the Outer Harbor contained two of the three geologic units: the firm to hard marine deposits of the OBM, and the dark-colored, soft, marine deposits of the YBM. All three geologic units are described in detail below.

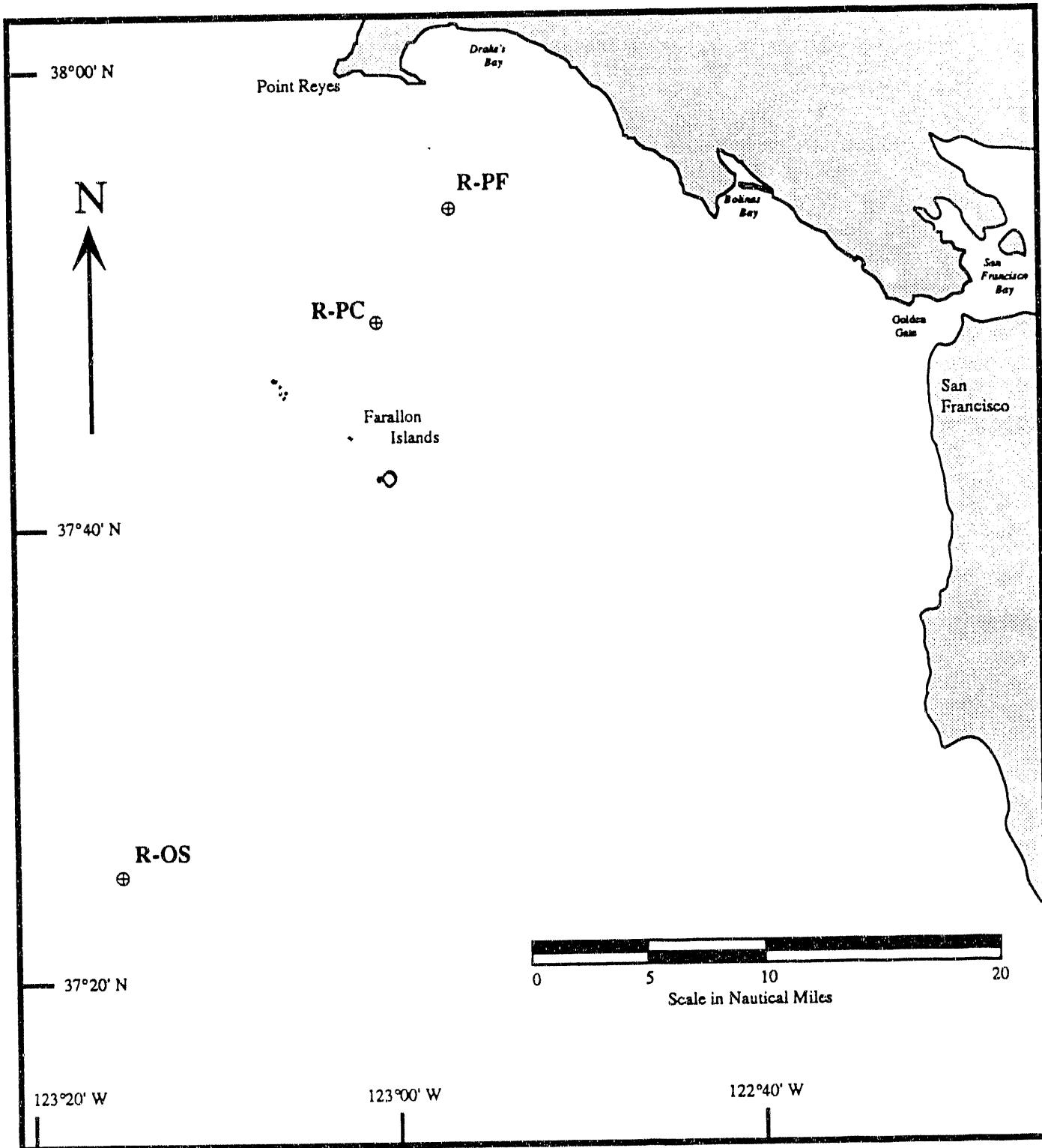


FIGURE 3.4. Offshore Reference Sediment Sampling Sites, Oakland Harbor Phase III B Project

TABLE 3.2. Reference Sediment Sampling Information, Oakland Harbor Phase III B Project (Sampling location coordinated obtained from vessel LORAN system)

PHASE III B

Station	Replicate	Sampling Date	Time	Sampling Location	Latitude (N)	Longitude (W)	Depth (ft)	Comments
R-BF-2	1	06-Nov-90	1204	37°44.26'	122°17.26'		30	no sample - wash out
R-BF-2	2	06-Nov-90	1218	37°44.26'	122°17.26'		31	silt, clay
R-BF-3	1	06-Nov-90	1230	37°44.43'	122°16.81'		37	silt, clay
R-BF-3	2	06-Nov-90	1238	37°44.44'	122°16.77'		38	silt, clay
R-BF-4	1	06-Nov-90	1247	37°44.72'	122°16.57'		38	silt, clay
R-BF-4	2	06-Nov-90	1255	37°44.71'	122°16.51'		38	silt, clay; Maldanid tubes and worms
R-BF-5	1	06-Nov-90	1307	37°44.62'	122°16.35'		38	silt, clay
R-AM-1	1	06-Nov-90	1412	37°49.04'	122°24.96'		56	med. coarse sand, some shell fragments
R-AM-F	1	06-Nov-90	1430	37°49.46'	122°24.84'		79	coarse sand, gravel, shell fragments
R-AM-C	1	06-Nov-90	1447	37°50.18'	122°25.01'		69	coarse sand, gravel, large shell fragments
R-AM-B	1	06-Nov-90	1513	37°49.98'	122°25.31'		64	coarse sand, gravel, small shell fragments
R-AM-A	1	06-Nov-90	1538	37°49.85'	122°25.98'		64	med. to coarse sand, gravel, shell fragments
R-AM-D	1	06-Nov-90	1555	37°49.06'	122°26.40'		66	med. to coarse sand, few shell fragments
R-AM-G	1	06-Nov-90	1623	37°48.90'	122°25.91'		39	med. to fine sand, silt
R-AM-H	1	06-Nov-90	1636	37°49.08'	122°25.59'		48	med. to fine sand, silt
R-AC-4	1	06-Nov-90	1655	37°49.10'	122°25.34'		60	fine to med. sand, shells and fragments
R-AC-2	1	06-Nov-90	1720	37°49.15'	122°25.35'		64	med. to coarse sand, shells
R-AC-5	1	06-Nov-90	1806	37°49.23'	122°25.26'		69	coarse sand, gravel, shells
R-AC-7	1	06-Nov-90	1828	37°49.20'	122°25.26'		62	coarse sand, silt, few small shell fragments
R-PF	1	06-Nov-90	2054	37°54.08'	122°57.02'		220	fine sand, silt
R-PF	2	06-Nov-90	2118	37°54.05'	122°57.06'		221	fine sand, silt
R-PF	3	06-Nov-90	2140	37°54.06'	122°57.02'		221	fine sand, silt
R-PF	4	06-Nov-90	2201	37°54.09'	122°57.06'		221	med. to fine sand, silt
R-PF	5	06-Nov-90	2218	37°54.05'	122°57.04'		222	fine sand, silt
R-PF	6	06-Nov-90	2250	37°54.08'	122°56.97'		222	fine sand, silt
R-PF	7	06-Nov-90	2301	37°54.00'	122°57.03'		223	fine sand, silt
R-PC	1	07-Nov-90	0000	37°48.96'	123°00.90'		249	fine sand, no silt
R-PC	2	07-Nov-90	0030	37°48.96'	123°00.92'		250	coarse sand, gravel; Saved as Point Reyes very coarse
R-PC	3	07-Nov-90	0045	37°49.00'	123°00.96'		250	fine to med. sand
R-PC	4	07-Nov-90	0110	37°48.98'	123°00.98'		250	fine sand
R-OS	1	07-Nov-90	0424	37°24.65'	123°15.10'		4200	fine sand, silt
R-OS	2	07-Nov-90	0650	37°24.33'	123°15.24'		NA	fine to med. sand, silt, some coarse sand

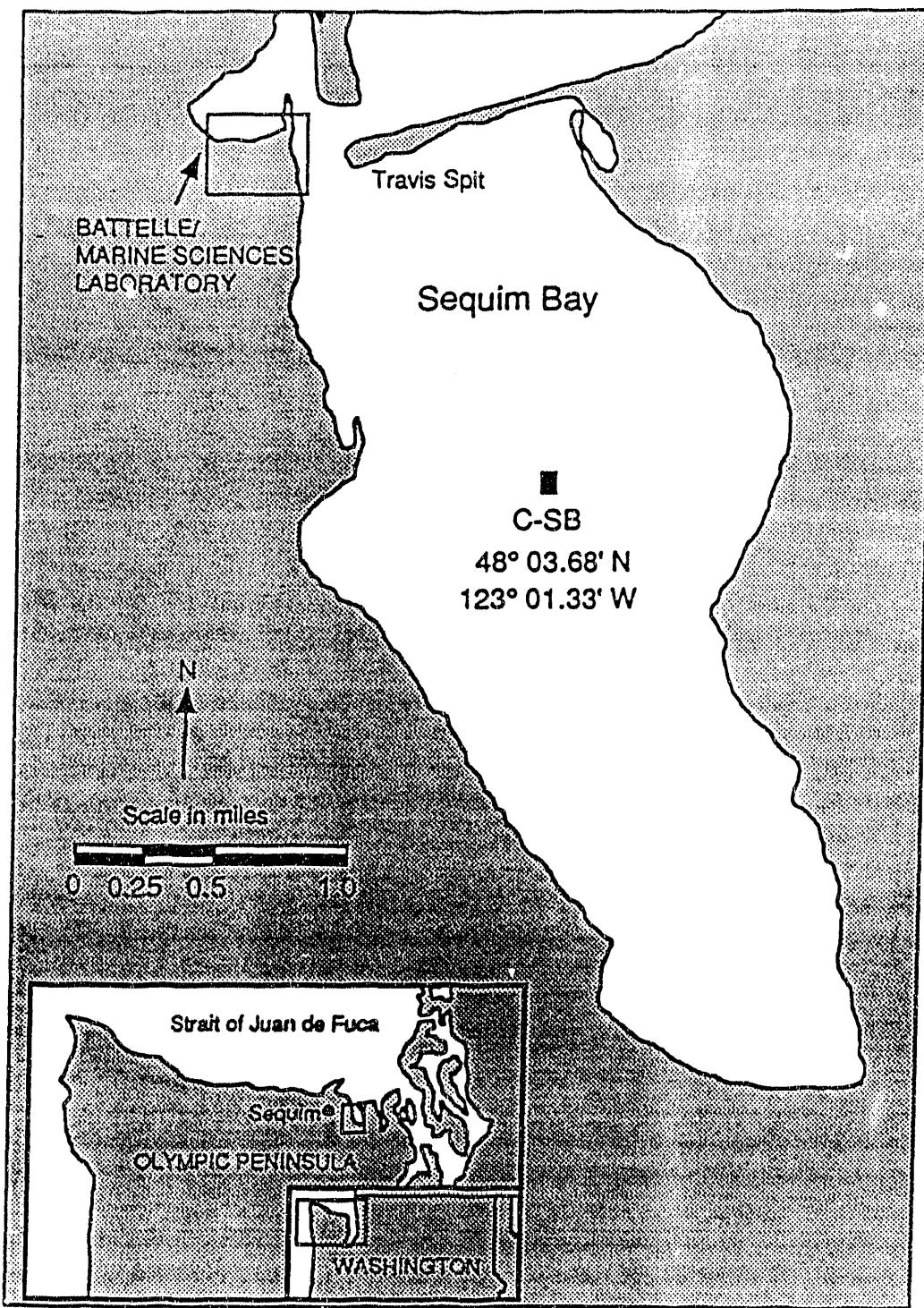


FIGURE 3.5. Location of Sequim Bay, Washington, Control Station (C-SB)

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3.10

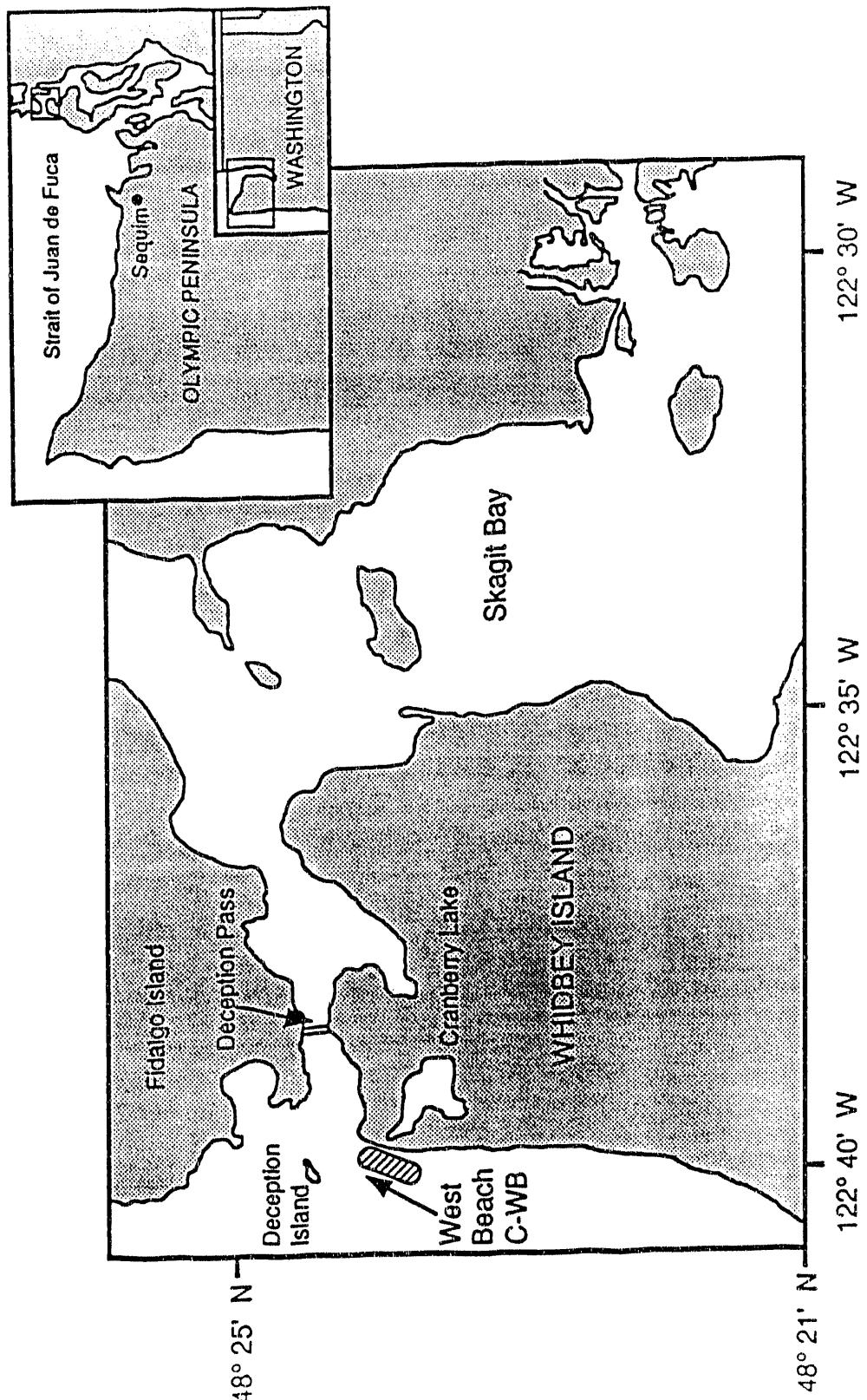


FIGURE 3.6. Location of West Beach, Whidbey Island, Washington, Control Station

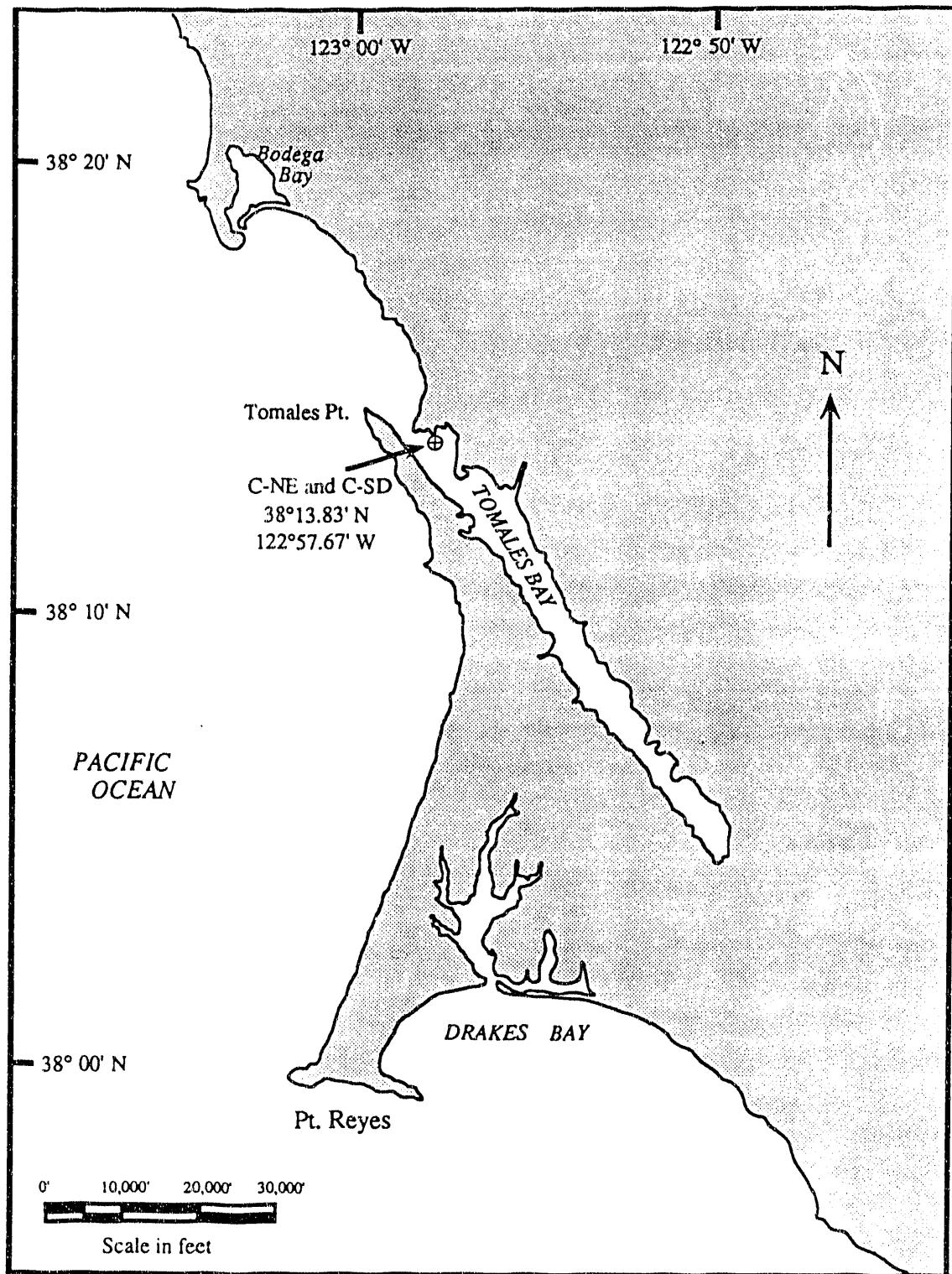
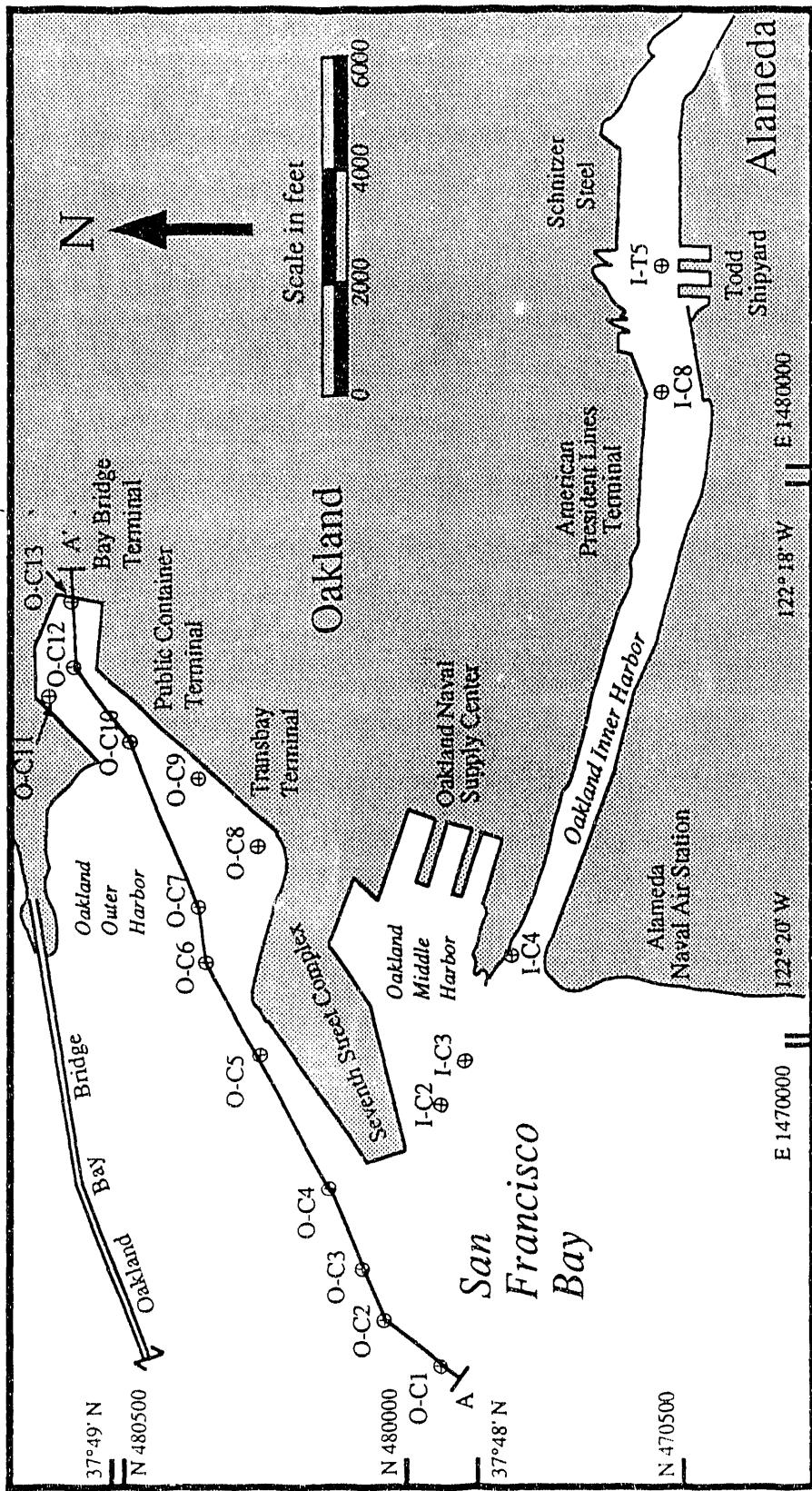


FIGURE 3.7. Location of Tomales Bay, California, Control Station



PHASE III B

FIGURE 3.8. Location of Cross-Section A-A', Oakland Harbor Phase III B

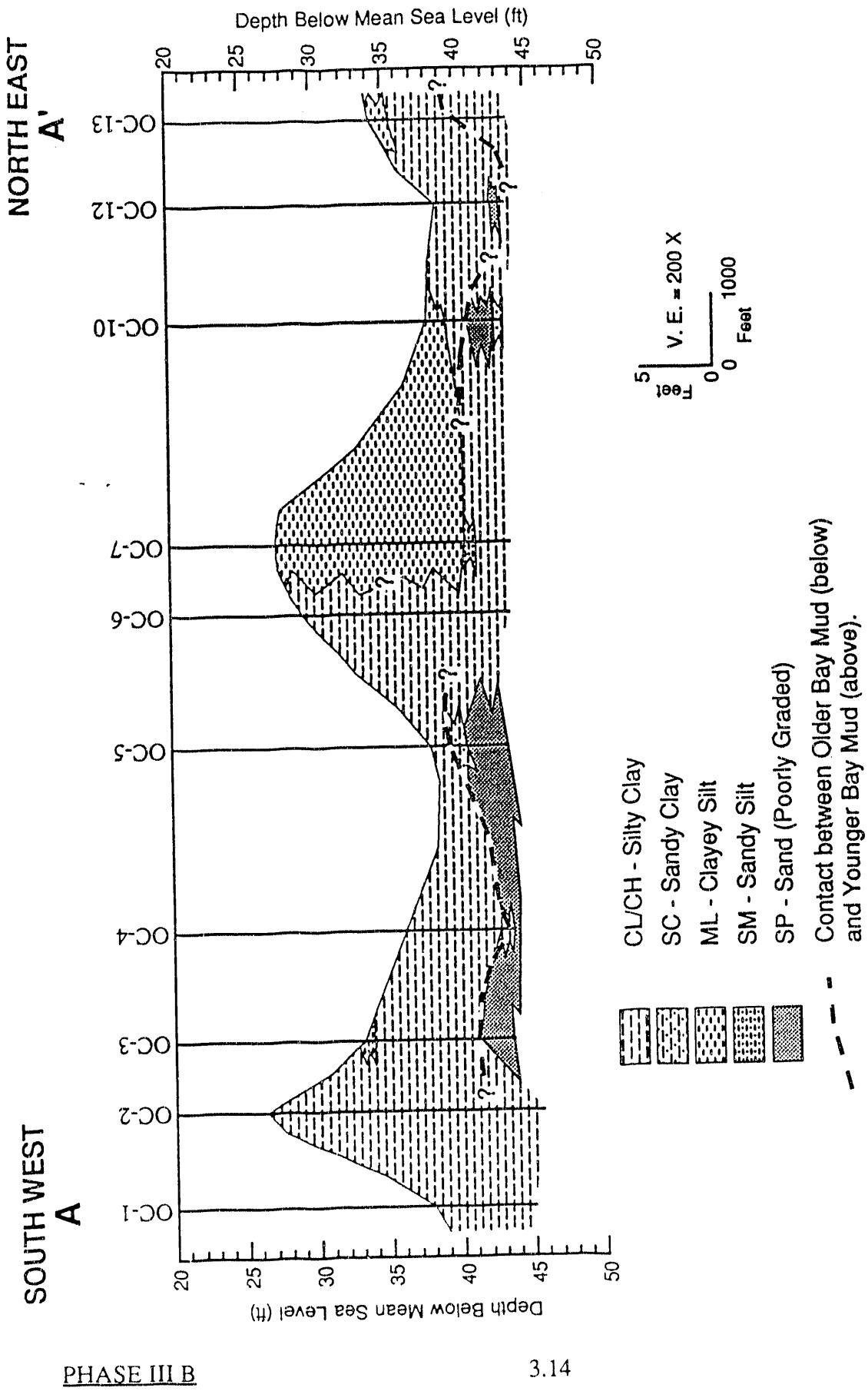


FIGURE 3.9. Oakland Outer Harbor Cross-Section A-A', Oakland Harbor Phase III B

3.2.1 Older Bay Mud Unit

The oldest unit is the OBM, consisting of deposits ranging from loose sands to hard, stiff silty clays. The OBM was probably deposited during the last glacial period when sea level resided as much as 335 ft below its present level; however, it is possible that some of the unit was formed during previous glacial and interglacial periods. The OBM unit is distinguished primarily by its firm to hard consistency and its color, particularly in the terrestrial sediments, which consist of various shades of red, yellow, and brown. These colors are consistent with an oxidizing environment associated with deposition by ancient rivers and streams that flowed westward toward the Pacific Ocean. The presence of deeply penetrating root traces is another indication of terrestrial conditions; deposits known as the Merritt Sands appear to belong to the terrestrial facies of the OBM. The marine portions of the OBM consist of drab-colored shades of green and gray. These colors, along with the presence of whole mollusk shells, are indicative of a low-energy, reducing, estuarine environment.

The highly oxidized and weathered appearance of the OBM, in combination with the presence of root traces and pedogenic, calcium-carbonate nodules, indicate that in places, the OBM unit underwent alteration during a period of subaerial soil development. A high degree of consolidation, in combination with the weathered and often bleached appearance of the OBM unit, suggests this unit is much older than the relatively recent estuarine sediments belonging to the YBM. No unusual odors were detected in the OBM unit. The compacted nature of the OBM may act as a barrier, preventing downward movement of the odors associated with contaminant migration. The top of the OBM appears to represent an erosional surface, but past dredging activities may also have modified its surface. The uneven surface of the OBM is apparent in the Oakland Outer Harbor cross section (Figure 3.9).

The OBM unit was identified at -40 to -43 ft MLLW in six Phase III B cores: O-C4, O-C5, O-C10, O-C11, O-C12, and O-C13. OBM was tentatively identified at -41.5 ft MLLW at O-C3. The contact between the OBM and the YBM units, where present, was based primarily on consistency and color. The OBM in Oakland Outer Harbor consisted of estuarine deposits of silty clay, sandy clay, and poorly graded sands having firm consistency and colored drab, pale shades of green and gray. Organic fragments and iron oxide mottles were observed in the OBM around -41 ft MLLW at Station O-C13, in the northeastern portion of the sample area. Coarse angular pebbles were present in another core, O-C11, also from the northeastern portion of the sample area. This may suggest a proximity to the terrestrial facies of the OBM, as sudden, periodic influxes of water could have introduced this material from upland sources. Other interfingering

facies and isolated lenses within the OBM might also be attributed to these influxes, or possibly to migrating delta fronts or periodic fluctuations in sea level.

3.2.2 Sand Deposits

The sand deposits are dark-colored, fine grained sands mixed with considerable silt and clay that lie between the OBM and the overlying YBM. The thickness of the sand deposits is highly variable, and is generally thicker along bay margins. The unit is believed to represent alluvial fans formed by fluvial current action during shoreline fluctuation at the end of the Pleistocene (USACE 1975). The discontinuous nature of the sand deposits and their similarity to the YBM make it difficult to differentiate between the two units. The sand deposit unit does not appear to be present in the Oakland Harbor Phase III B Project area.

3.2.3 Younger Bay Mud Unit

The YBM unit consists of mostly soft, dark-colored sediments deposited in an estuarine environment. These deposits were laid down as sea level rose following the last ice age, which ended approximately 12,000 years ago (Barry 1983). The YBM unit forms a continuous blanket across the harbor bottom, except where it has been removed by recent dredging or where recent sediments are kept in suspension by submarine currents. Inland exposures of the YBM are also found, suggesting that sea levels may have been higher in the past (USACE 1975). The YBM unit consists mostly of very soft to soft silty clays and clayey silts with minor amounts of organic material, fine sand, and shell fragments (USACE 1975). YBM sediment colors range from dark gray to dark olive gray to black. Dark colors, in combination with the odor of rotten eggs (i.e., hydrogen sulfide), are indications of chemically reducing conditions. The USACE (1975) has subdivided the YBM unit into a Semi-Consolidated Bay Mud member overlain by Soft Bay Mud member, distinguished by a sudden, characteristic change in consistency. While the firmness of the Phase III B sediments generally increased with depth, this appeared to be a result of compaction from overlying sediments rather than the boundary between the Semi-Consolidated Bay Mud and Soft Bay Mud members. The shallow nature of the sediment cores suggests that the primary unit represented in the sampling area is the Soft Bay Mud member of the YBM.

The YBM unit in the outer harbor consisted almost entirely of silty clay to clayey silt with an occasional interfingering sandy silt lens. These lenses could be attributed to a sudden influx of sediment-laden water, perhaps associated with runoff following severe storms. Mollusk shells were found within the YBM in three cores, O-C1, O-C3, and O-C4, all from the southwestern portion of the sample area. The thickness of the YBM varied in the outer harbor, with the thickest deposits occurring along the southwest and central portions of the cross section (Figure 3.9).

3.3 SEDIMENT CHEMISTRY

Thirty-six solid-phase sediment samples were prepared during the Oakland Harbor Phase III Project. Table 3.3 lists the samples and types of testing for which each sample was used. Sample preparation took place November 12 through 17, 1991, following the procedures described in Section 2.2. Two test sediments, O-C1 and O-C3, were wet-sieved because field notes indicated that live organisms (polychaetes) were present in the sediments when they were collected. However, the fact that no organisms were found during the sieving process suggests that organisms may have died and decayed in the week between sampling and processing, when samples were kept cold and sealed from exposure to air or water. The top 6 in. of the 4-in. core from I-C8 was analyzed separately at the request of USACE. This sample represents the contribution of soft fine-grained YBM (potentially higher in contaminants) to test treatment I-C8. This section presents the results of physical and chemical analyses conducted on the sediment samples. Following sections present the results of toxicity and bioaccumulation testing.

3.3.1 Conventional Sediment Measurements

Conventional sediment measurements are grain size, total organic carbon (TOC), total volatile solids (TVS), oil and grease, and total petroleum hydrocarbons (TPH). Grain size, TOC, and TVS are expressed as percent of the dry weight of the sample. Oil and grease and TPH concentrations are expressed as mg/kg dry weight. Complete results, quality control data, and quality control summaries are contained in Volume 2, Appendix C, Tables C.1 through C.6. Quality control criteria were met for all conventional analyses. A summary of conventional sediment measurements is presented in Table 3.4.

Grain size results (Table 3.4, Figure 3.10) show that Oakland Outer Harbor sediments were predominantly fine-grained ($\geq 50\%$ silt and clay). Exceptions were Station O-C5, O-C9, O-C12, and O-C10, with 13% to 48% silt and clay. In contrast, Oakland Inner Harbor samples were predominantly coarse-grained ($\geq 52\%$ sand and gravel), except for I-C2 (27% sand) at the entrance to the inner harbor. The reference sediment treatments were primarily sand ($\geq 60\%$) except for R-BF which had only 2% sand and 98% silt and clay. Control sediments C-NE, C-SD, and C-WB were $\geq 97\%$ sand, while C-SB was 11% sand and 89% fine material. The grain-size distribution of sediment composites BC-5, BC-6, BC-7, and BC-8 represent a mixture of their respective stations.

The concentrations of TOC (Table 3.4 and Figure 3.11) ranged from 0.01% in the core from Station I-T5 to 2.17% in C-SB. In general, higher TOC values were found in the fine-grained sediment. In fact, all of the stations with more than 50% fine-grained sediment also had more than 0.5% TOC. Figure 3.12 shows the significant linear regression representing this relationship

TABLE 3.3. Summary of Sediment Samples for Testing, Oakland Harbor Phase III B

<u>Sediment Treatment</u>	<u>Description</u>	<u>Sediment Chemistry</u>	<u>SPP Toxicity</u>	<u>Solid-Phase Toxicity</u>	<u><i>N. caecoides</i>/ <i>M. nasuta</i> Bioaccumulation</u>
BC-5	O-C6, O-C7, O-C8, O-C9	YES	YES	NO	NO
BC-6	O-C1, O-C3, I-C2	YES	YES	NO	NO
BC-7	O-C13, O-C12, OC-11, O-C10	YES	YES	NO	NO
BC-8	O-C5, O-C4	YES	YES	NO	NO
BC-8	O-C5, O-C4	YES ^(a)	NO	NO	NO
O-C13	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C13	-41.5 ft MLLW of 4-in core	YES ^(b)	NO	NO	NO
O-C12	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C11	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C10	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C9	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C8	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C8	Mudline to -44 ft MLLW	YES ^(a)	NO	NO	NO
O-C7	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C6	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C5	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C4	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C3	Mudline to -44 ft MLLW ^(b)	YES	NO	YES	YES
O-C2	Mudline to -44 ft MLLW	YES	NO	YES	YES
O-C1	Mudline to -44 ft MLLW ^(b)	YES	NO	YES	YES
I-C2	-38 to -44 ft MLLW	YES	NO	YES	YES
I-C3	-38 to -44 ft MLLW	YES	NO	YES	YES
I-C8	-38 to -44 ft MLLW, Phase III A Station	YES	NO	YES	YES
I-C8	Top 6" (-38 to -38.5 ft MLLW) of 4-in core	YES	NO	NO	NO
I-C8	-39 to -39.5 ft MLLW of 4-in core	YES ^(d)	NO	NO	NO
I-C4	-38 to -44 ft MLLW, Phase III A Station	YES	NO	NO	YES ^(e)
I-T5	Mudline to -37.7 ft MLLW, Phase III A	YES	NO	NO	YES ^(e)
R-AC	Alcatraz Disposal Site Reference	YES	NO	YES	YES
R-AM	Alcatraz Island Environs Reference	YES	YES	YES	YES
R-BF	Bay Farm Reference	YES	YES	YES	YES
R-OS	Deep Off-Shelf Reference	YES	NO	YES	YES
R-PC	Point Reyes Coarse Reference	YES	NO	YES	YES
R-PF	Point Reyes Fine Reference	YES	YES	YES	YES
C-SB	Sequim Bay Control	YES	NO	YES	YES
C-NE	<i>N. caecoides</i> Native Control	YES	NO	YES	YES
C-SD	<i>C. stigmaeus</i> Native Control	YES	NO	YES	NO
C-WB	West Beach (<i>R. abronius</i>) Control	YES	NO	YES	NO

(a) Compositing Duplicate.

(b) Sediment sample for oil and grease and total petroleum hydrocarbons only.

(c) Sediment was wet-sieved to remove organisms before it was homogenized for testing.

(d) Jar containing sample for oil and grease only was broken, sample was not analyzed.

(e) *N. caecoides* bioaccumulation testing only.

TABLE 3.4. Conventional Sediment Measurement Results (Oil and Grease and TPH in mg/kg dry weight; grain size, TOC, and TVS in percent dry weight)

PHASE III B

Sediment Treatment	Gravel >2000 μm	Sand 62.5-2000 μm	Silt 3.9-62.5 μm	Clay <3.9 μm	TOC	TVS	Oil and Grease	TPH
BC-7	0	40	23	37	0.55	4.83	150	87
O-C13	0	12	33	55	1.01	7.41	95	84
O-C13 41.5 ft MLLW	N/A	N/A	N/A	N/A	N/A	N/A	8	3
O-C12	0	64	14	22	0.15	2.51	24	31
O-C11	5	25	27	43	0.65	5.48	65	51
O-C10 (a)	0	52	19.5	28.5	0.51	4.23	91	66
BC-5	0	40	24	36	0.66	5.35	116	78
O-C9	0 ^(b)	87 ^(b)	7 ^(b)	6.5 ^(b)	0.03	0.99	12	3
O-C8	0 ^(b)	11 ^(b)	30.5 ^(b)	58 ^(b)	1.03	6.68	304	252
O-C7	0	6	35	59	1.11	7.15	251	159
O-C6	0	5	38	57	1.15	7.14	183	135
BC-8 (a)	0	63	17	21	0.33	2.99	63	39
O-C5	0	87	7	6	0.04	1.05	12 ^(b)	4 ^(b)
O-C4	0 ^(b)	19 ^(b)	38.5 ^(b)	42.5 ^(b)	0.93	5.55	116	70
BC-6	0	23	39	38	0.75	5.25	109	61
O-C3	0	28	32	40	0.64	6.46	84	39
O-C1	0	9	49	42	0.81	6.93	80	29
I-C2	0	27	35	40	0.87	6.39	138	99
O-C2	0	7	45	48	0.75	4.62	58	32
I-C3	0	92	5	5	0.06	1.23	1	5
I-C8	0	86	5	9	0.10	2.07	42	26
I-C8 Top 6"	0 ^(b)	52 ^(b)	18.5 ^(b)	30 ^(b)	0.48	3.93	137	97
I-C4	0	66	13	21	0.36	3.04	2	12
I-T5	0	85	4	11	0.01	1.38	130	106
C-SB	0	11	53	36	2.17 ^(b)	8.98 ^(b)	147	14U ^(c)
C-NE	0	97	1	2	0.08	1.30	3	0.6U
C-WB	0	98	0	2	0.09	1.08	72	0.6U
C-SD	0	99	0	1	0.05	1.83	1	0.7U
R-AC	0	98	0	2	0.05	1.20	7	5
R-AM	0	91	3	6	0.19	2.10	7	8
R-BF	0	2	35	63	1.10	9.61	92	40
R-OS	0	60	27	13	0.63 ^(b)	4.28 ^(b)	14 ^(b)	7 ^(b)
R-PC	0 ^(b)	97	0	3	0.15	1.67	0.7U	0.7U
R-PF	0 ^(b)	62.5 ^(b)	27 ^(b)	10.5 ^(b)	0.41	2.57	27	9

(a) All values are mean of compositing duplicates.

(b) Mean of analytical replicates.

(c) Undetected above given concentration.

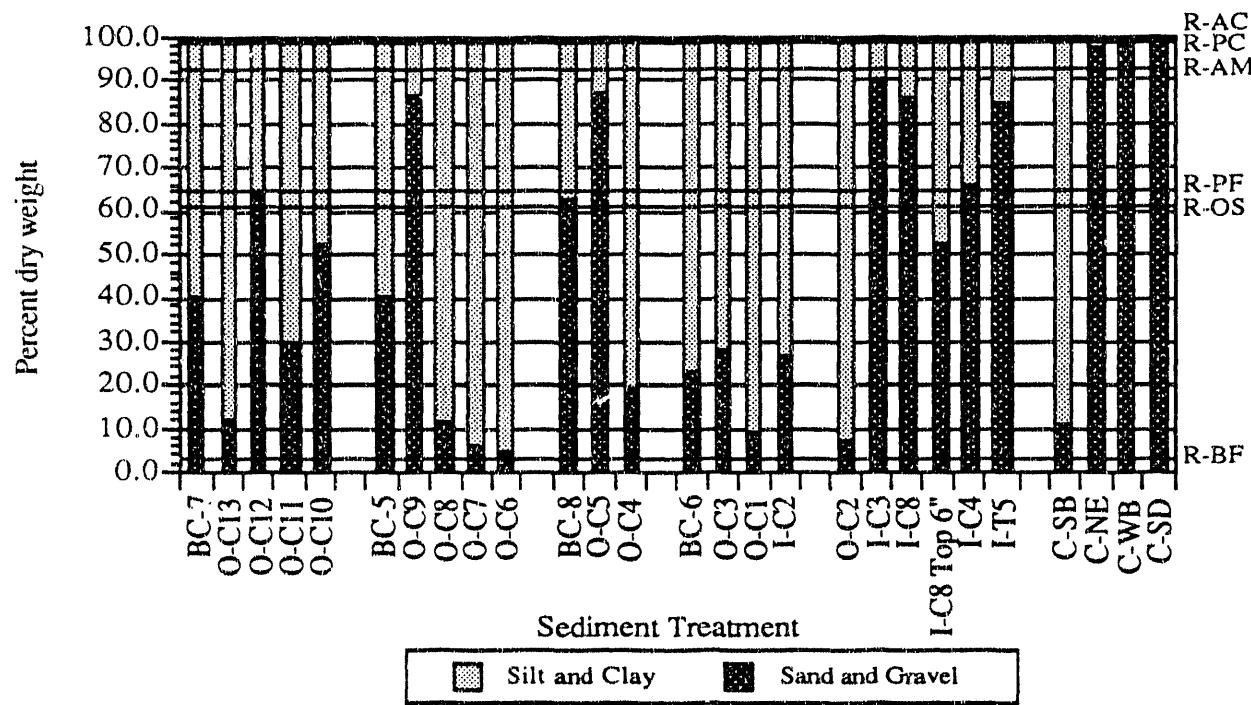


FIGURE 3.10. Grain Size Distribution in Sediment Samples, Oakland Harbor Phase III B
(Lines indicate the sand/silt boundary in reference sediments)

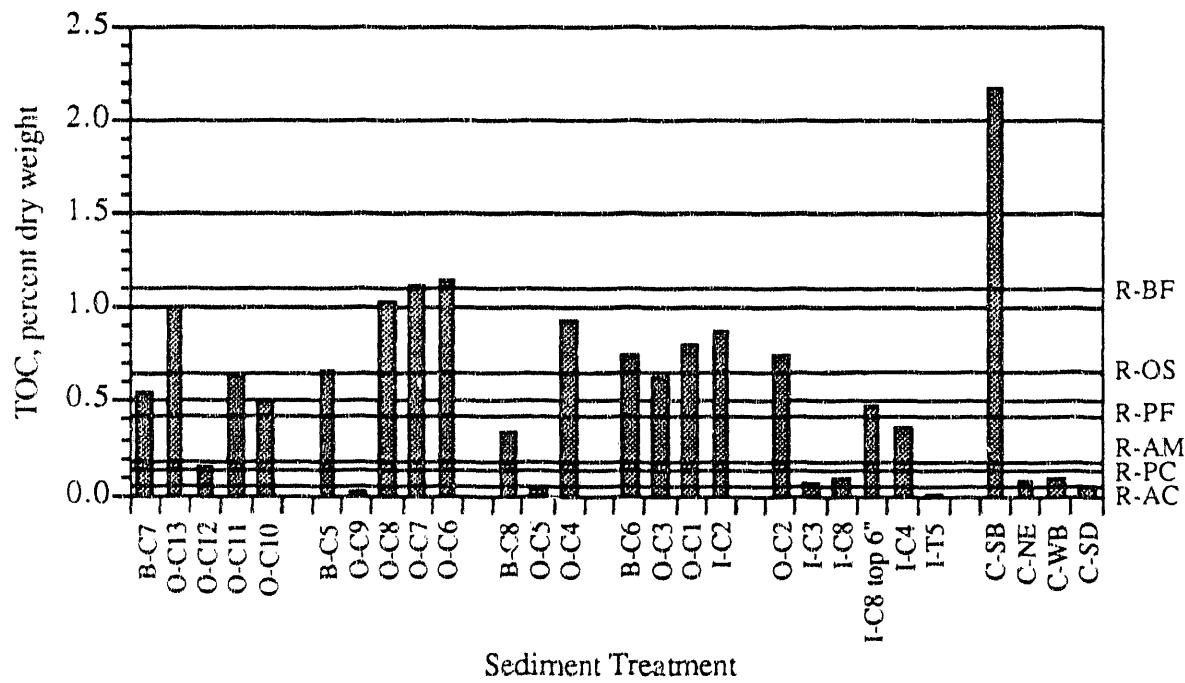


FIGURE 3.11. Concentrations of TOC in Sediment, Oakland Harbor Phase III B

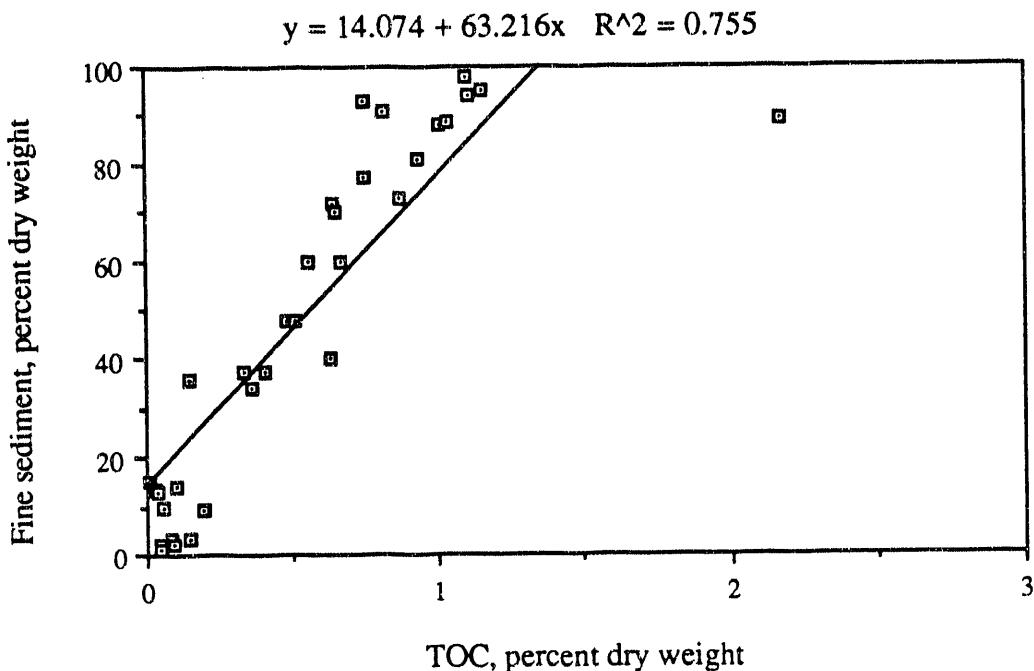


FIGURE 3.12. Relationship of Sediment TOC to Grain Size

between sediment grain size and TOC. Note that all the reference, control, and sediment treatments follow this correlation, with the exception of C-SB, where the 89% fine-grained sediment contained significantly more TOC.

The concentrations of TVS ranged from 1.05% at Station O-C5 to 9.61% in reference sediment R-BF, as shown in Figure 3.13. Control sediment C-SB and reference sediment R-BF had the highest TVS concentrations of 8.98% and 9.61%, respectively. The remaining three control and five reference treatments were similar to other sediment treatments, with TVS concentrations that ranged from 1.08% in C-WB to 9.61% in R-BF. All but one of the stations with more than 50% fine-grained sediment also had greater than 5% volatile solids. As with TOC, there is a significant linear relationship between TVS and percent of fine-grained sediment (Figure 3.14).

Figure 3.15 shows a linear regression representing the relationship between TOC and TVS. This figure shows that these two parameters are linearly related. Generally, as the percentage of TOC increased so did the percentage of TVS except for reference sediment R-BF, which had higher amounts of TVS than predicted by TOC, and control sediment C-SB that had more TOC than predicted by TVS.

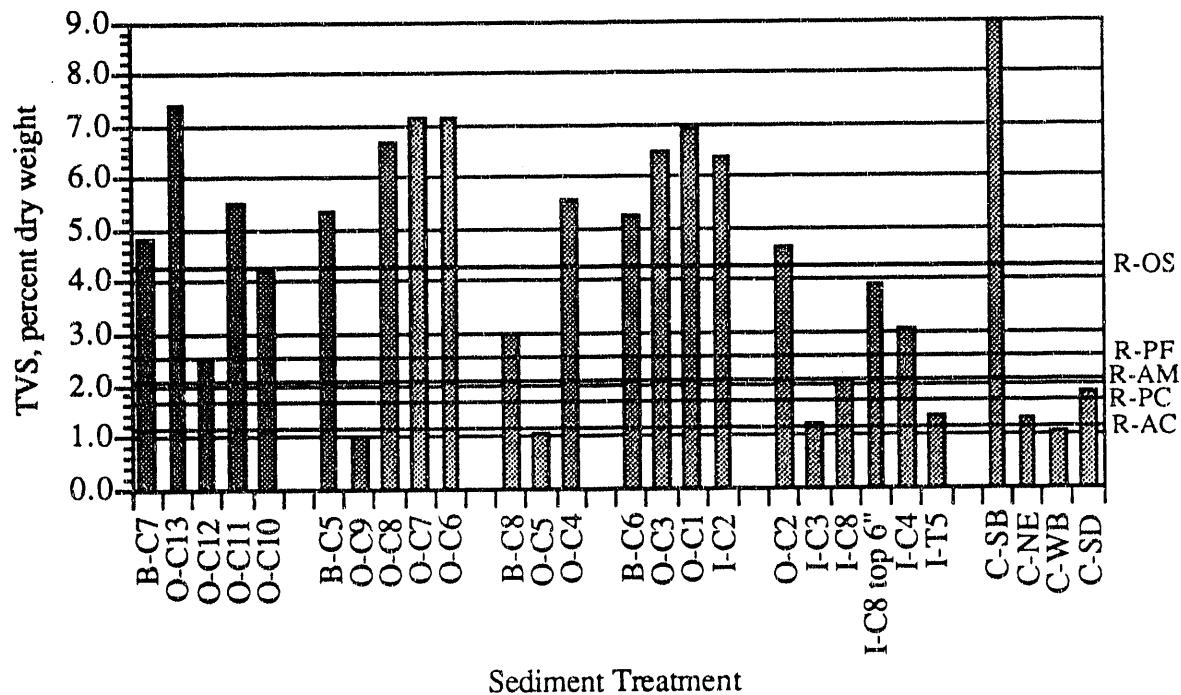


FIGURE 3.13. Total Volatile Solids In Sediment (Reference Station R-BF had a TVS value of 9.61% dry weight)

$$y = -5.3515 + 12.912x \quad R^2 = 0.885$$

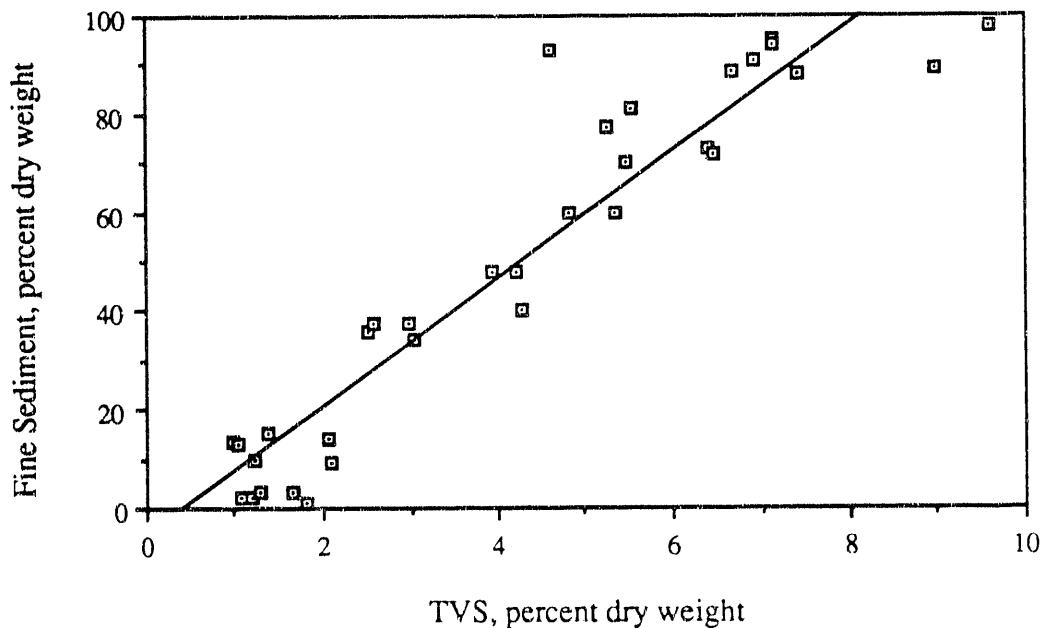


FIGURE 3.14. Relationship Between TVS and Grain Size

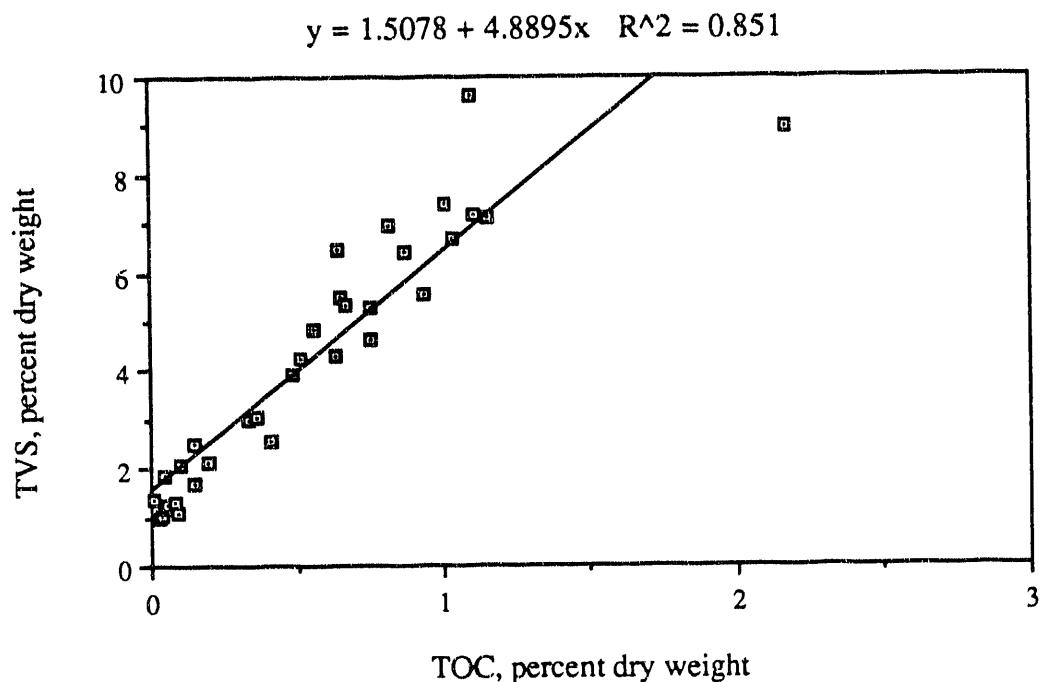


FIGURE 3.15. Relationship Between TOC and TVS

Oil and grease and TPH concentrations are presented in Table 3.4 and in Figures 3.16 and 3.17. Oil and grease concentrations in test sediments ranged from 1 mg/kg (dry weight) in I-C3 to 304 mg/kg in O-C8. In control and reference sediments, oil and grease was undetected above 0.7 mg/kg in R-AM and R-PC, ranging to 147 mg/kg in C-SB. Concentrations of TPH in test sediments ranged from 3 mg/kg in O-C13 (-41.5 ft MLLW) and O-C9, to 252 mg/kg in O-C8. In control and reference sediments, TPH was undetected above 1.4 mg/kg, and ranged to 40 mg/kg in R-BF. The relationship of oil and grease to TOC or TVS, while significant, was not as close as the relationship of oil and grease to grain size (Figure 3.18a, b, and c). Total oil and grease and TPH are closely related to each other but not to TOC or TVS (Figure 3.19a, b, and c). Treatments with the highest concentrations of oil and grease also had the highest concentrations of TPH. Reference and control treatments showed low TPH relative to oil and grease, suggesting the oil and grease is not of petroleum origin. The high TOC value and very low TPH value at C-SB suggests that the organic carbon found there does not have a high petroleum content. If the C-SB data point is removed from the plot in Figure 3.19b, R^2 increases to 0.425. The low TOC and 100 mg/kg TPH at I-T5 suggests that the organic carbon in this sediment is of petroleum-based origin.

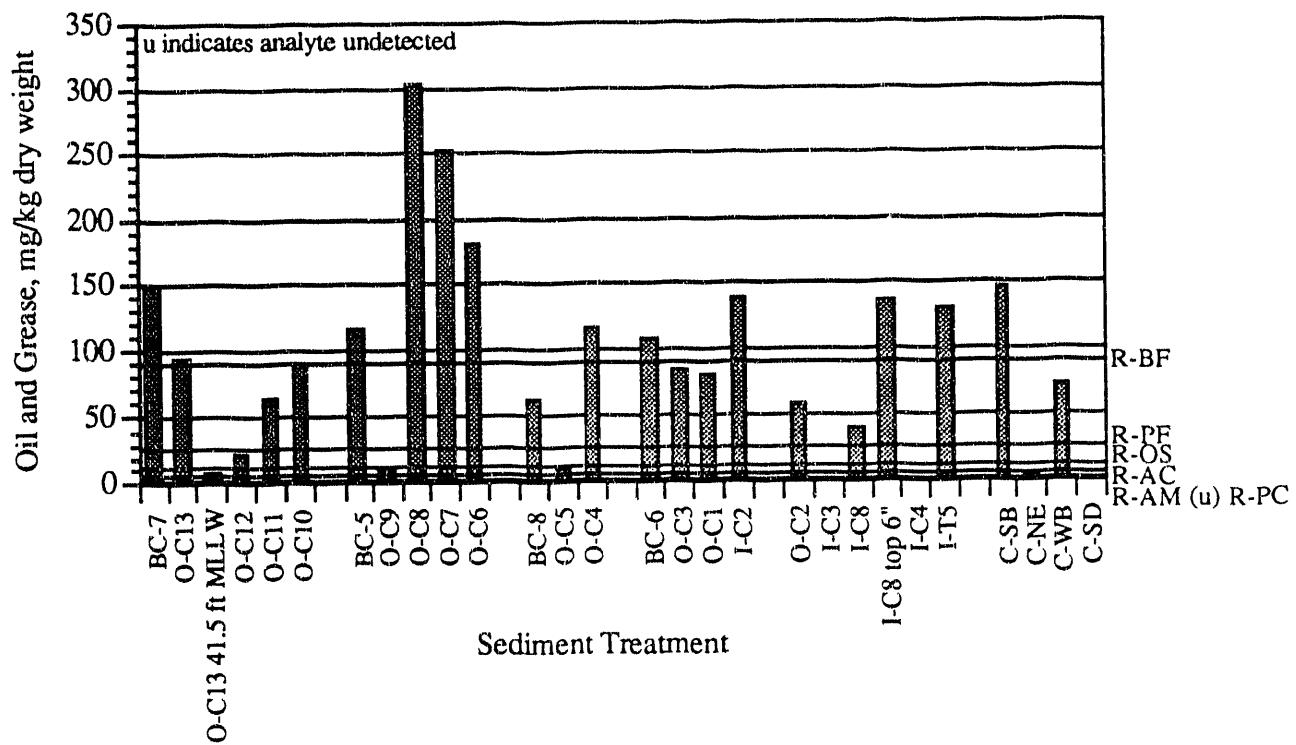


FIGURE 3.16. Total Oil and Grease in Sediment, Oakland Harbor Phase III B

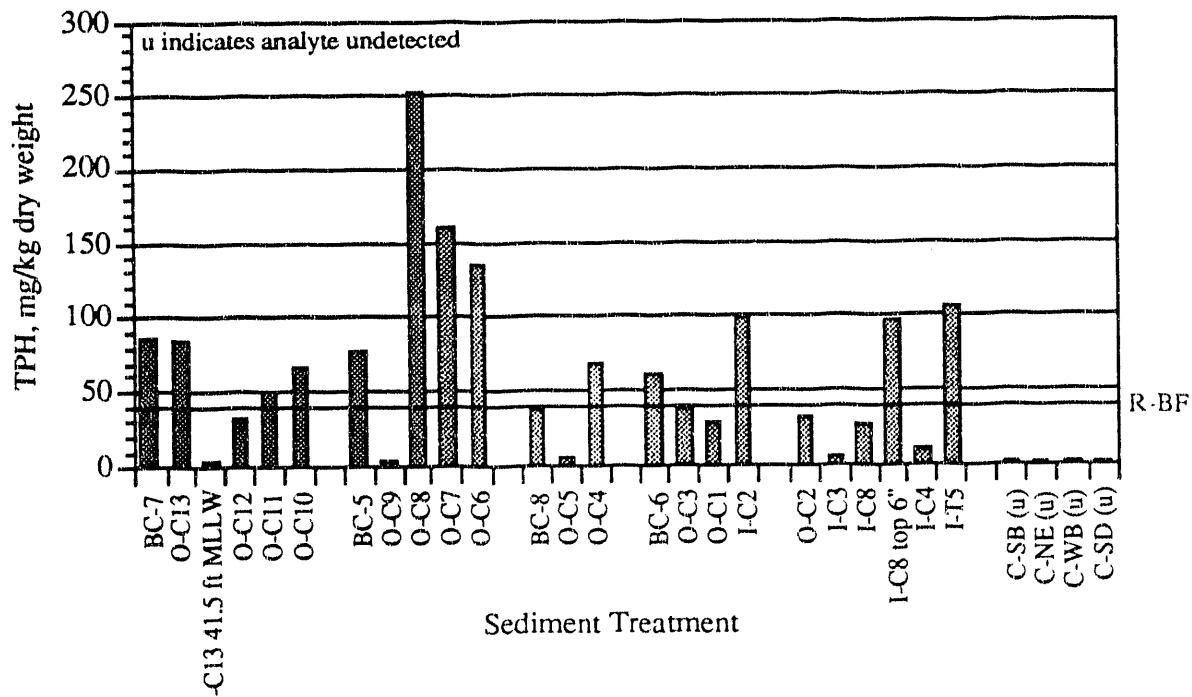


FIGURE 3.17. Total TPH in Sediment, Oakland Harbor Phase III B (TPH in reference sediments except R-BF were undetected above 10 mg/kg.)

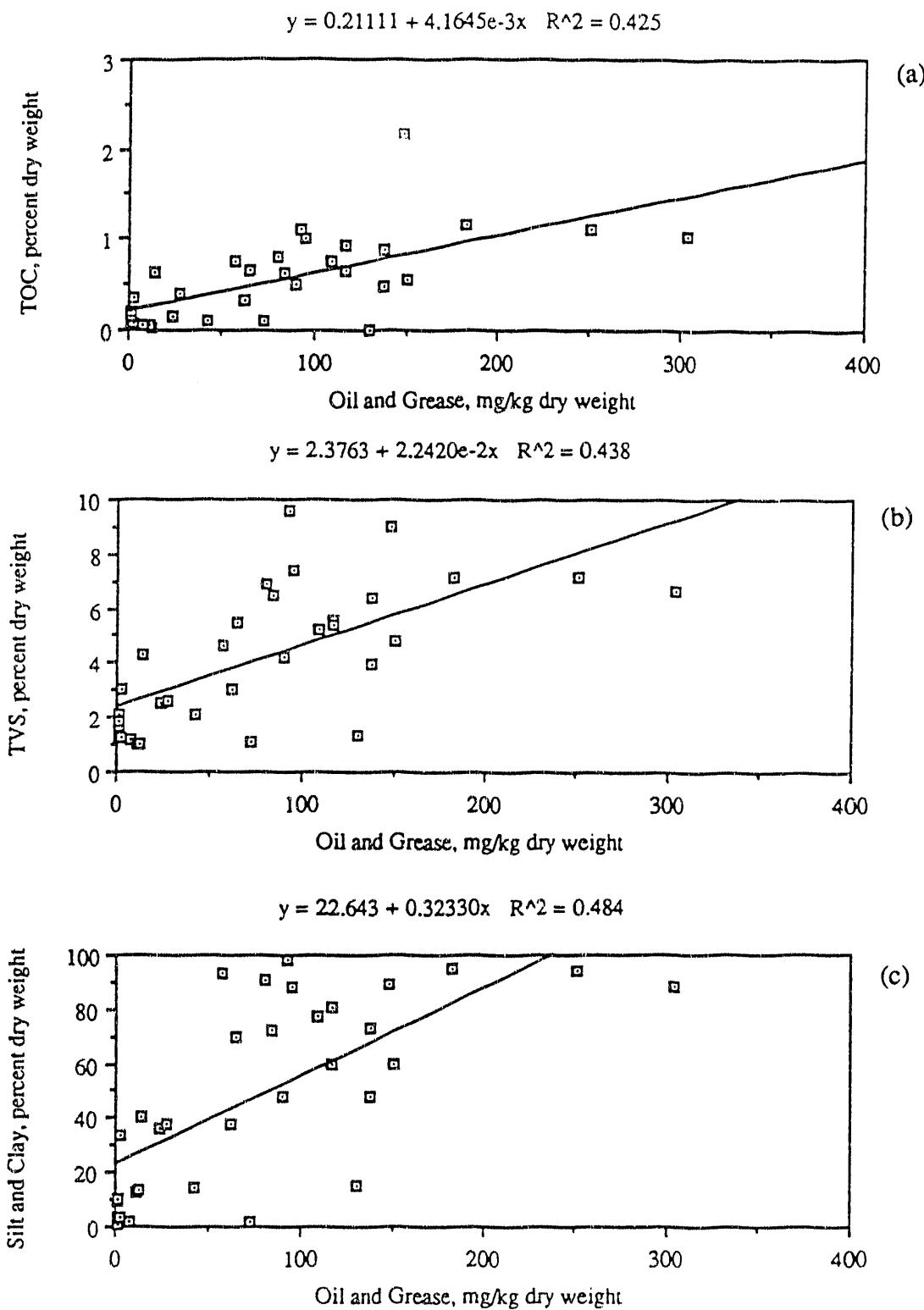


FIGURE 3.18. Linear Regressions Showing Relationship of Oil and Grease to a) TOC, b) TVS, and c) Grain Size

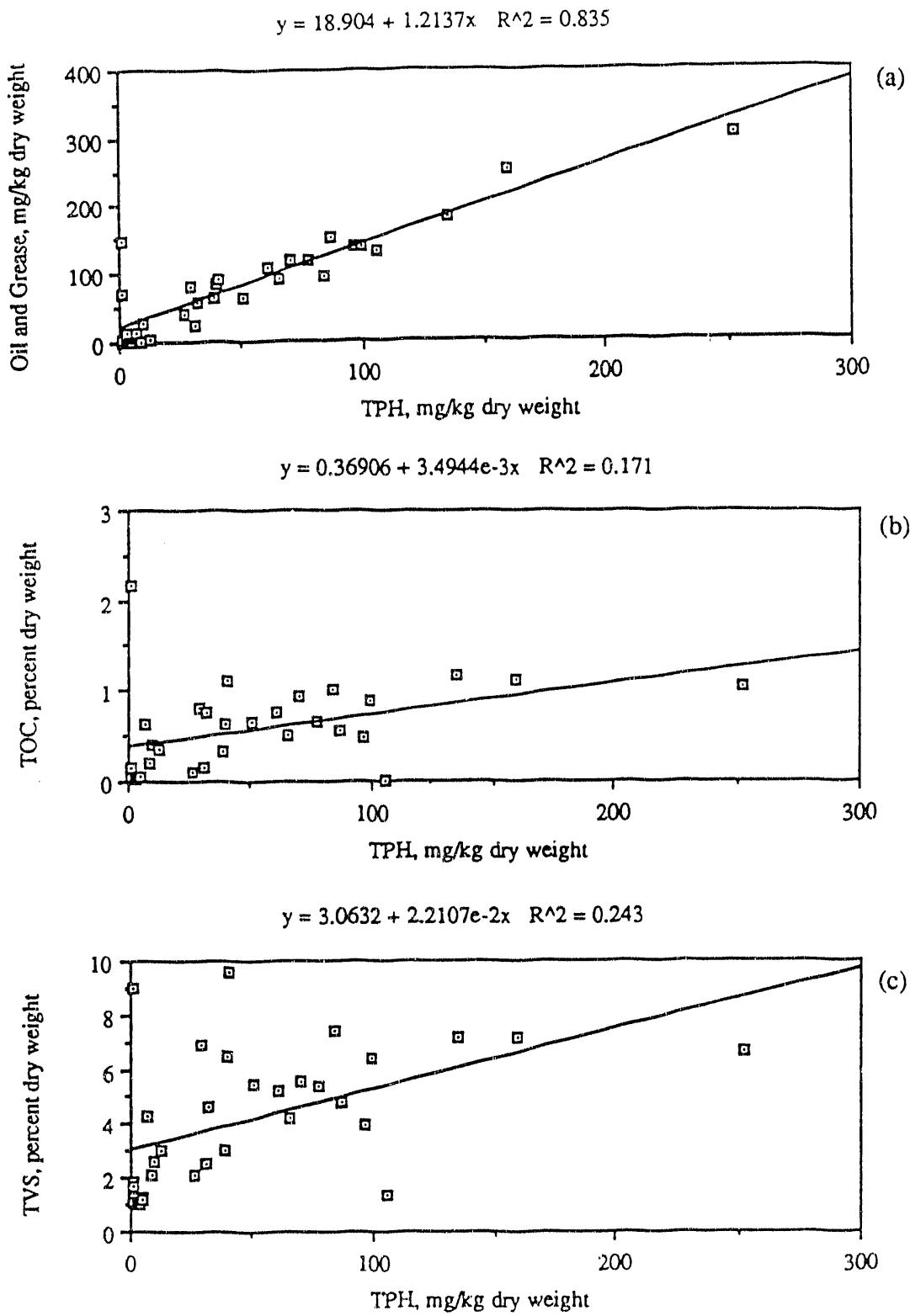


FIGURE 3.19. Linear Regression Showing the Relationship of TPH to a) Oil and Grease, b) TOC, and c) TVS

3.3.2 Polynuclear Aromatic Hydrocarbons

Both low molecular weight PAHs (LPAH) and high molecular weight PAHs (HPAH) contributed to the total PAH concentrations in Oakland Harbor sediments. Complete results of PAH analysis, quality control data, and a quality control summary are contained in Volume 2, Appendix C, Tables C.7 through C.12. In the Phase III B project area, concentrations of LPAH ranged from undetected to 495 $\mu\text{g}/\text{kg}$ dry weight, while concentrations of HPAH ranged from undetected to 2552 $\mu\text{g}/\text{kg}$ dry weight. The top 6 in. of Inner Harbor (Phase III A) Station I-C8, had little LPAH but had the highest HPAH concentration of 2824 $\mu\text{g}/\text{kg}$. It is likely that much of the PAH in test treatment I-C8 was contributed by the uppermost sediments at the site. Oakland Outer Harbor sediment treatments O-C4 and O-C13 had the highest LPAH concentrations (495 and 431 $\mu\text{g}/\text{kg}$), as well as high HPAH concentrations (2552 and 2407 $\mu\text{g}/\text{kg}$), respectively (Figure 3.20a and b). The stations located within Oakland Outer Harbor that had sediment with greater than 50% fine-grained material, greater than 5% TVS, and greater than 0.6% TOC, also had greater than 200 $\mu\text{g}/\text{kg}$ of LPAH and more than 1000 $\mu\text{g}/\text{kg}$ of HPAH. Figure 3.21 compares the total LPAH to the total HPAH in a linear regression.

3.3.3 Chlorinated Pesticides and Polychlorinated Biphenyls

Quality control criteria were met for chlorinated pesticide and polychlorinated biphenyl (PCBs) analyses. Only two pesticide compounds were detected in three Phase III B sediments. Results, quality control data, and quality control summaries for pesticide and PCB analyses are contained in Volume 2, Appendix C, Tables C.13 through C.19. Sediment treatment O-C13 had a value of 6 $\mu\text{g}/\text{kg}$ for 4,4'-DDD, O-C8 had a value of 8.1 $\mu\text{g}/\text{kg}$ for endrin, and O-C6 had a value of 6.8 $\mu\text{g}/\text{kg}$ for endrin. Aroclor 1254 was the only PCB that had concentrations above the detection limit. Concentrations of aroclor 1254 ranged from undetected values of 20 $\mu\text{g}/\text{kg}$ in 11 sediment treatments to 89 $\mu\text{g}/\text{kg}$ in O-C8 (Figure 3.22). All the control and reference sediments had undetected concentrations of 20 $\mu\text{g}/\text{kg}$. The stations with detectable values of chlorinated pesticides also had detectable values of the aroclor 1254. Sediment treatments where pesticides or PCBs were detected were predominantly fine-grained with relatively high TOC, oil and grease, and TPH.

3.3.4 Metals

Ten metals were measured in sediments from Oakland Inner and Outer Harbors and control and reference sediments. These are reported in mg/kg (ppm) dry weight. Complete results, quality control data, and a quality control summary are contained in Volume 2, Appendix C, Tables C.20 and C.21.

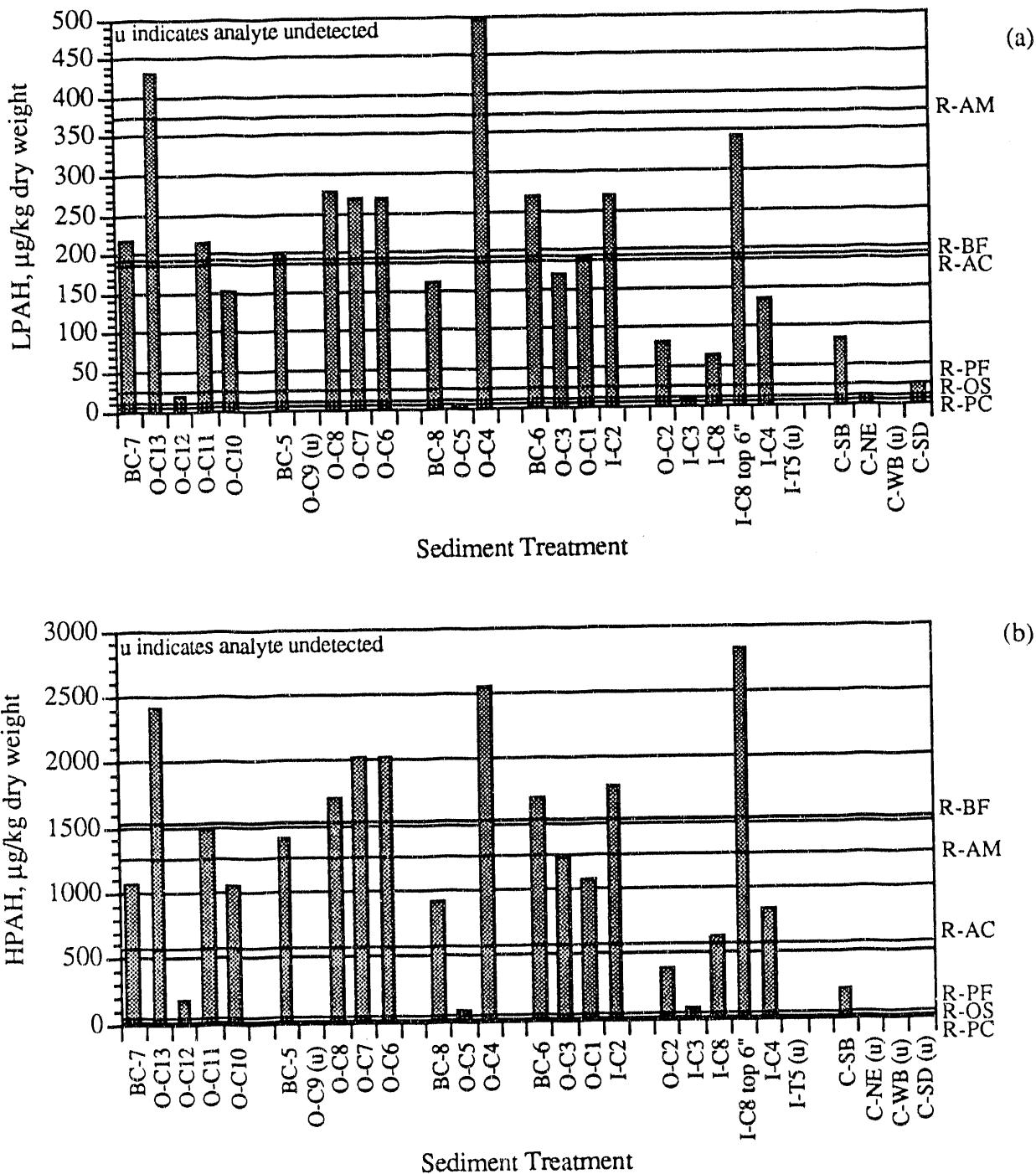


FIGURE 3.20. Concentration of a) LPAH and b) HPAH in Sediment, Oakland Harbor Phase III B

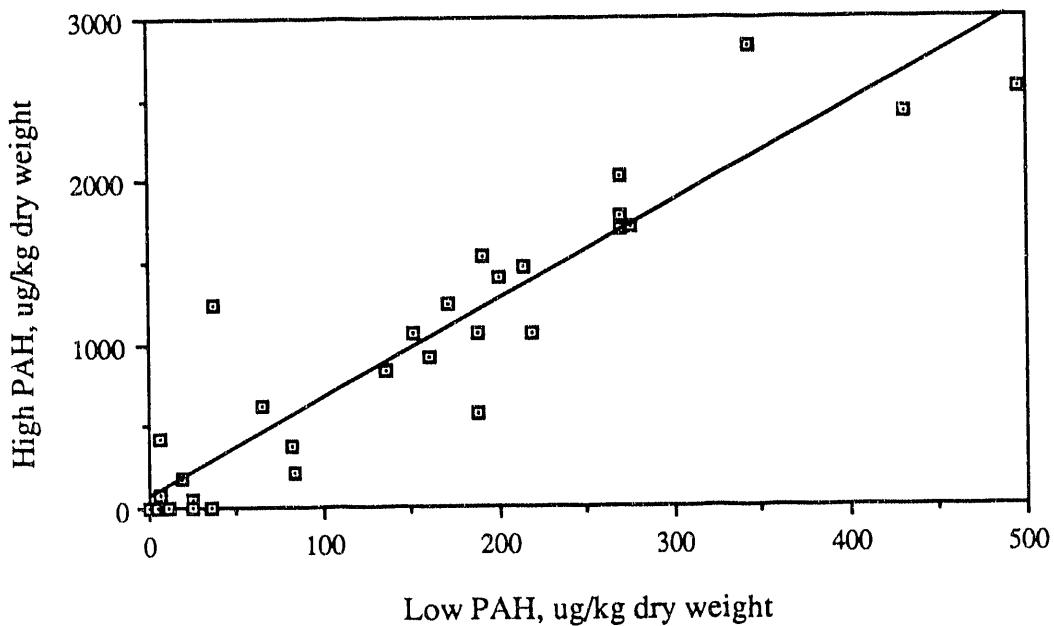


FIGURE 3.21. Linear Regression Between Total LPAH and HPAH, Oakland Harbor Phase III B

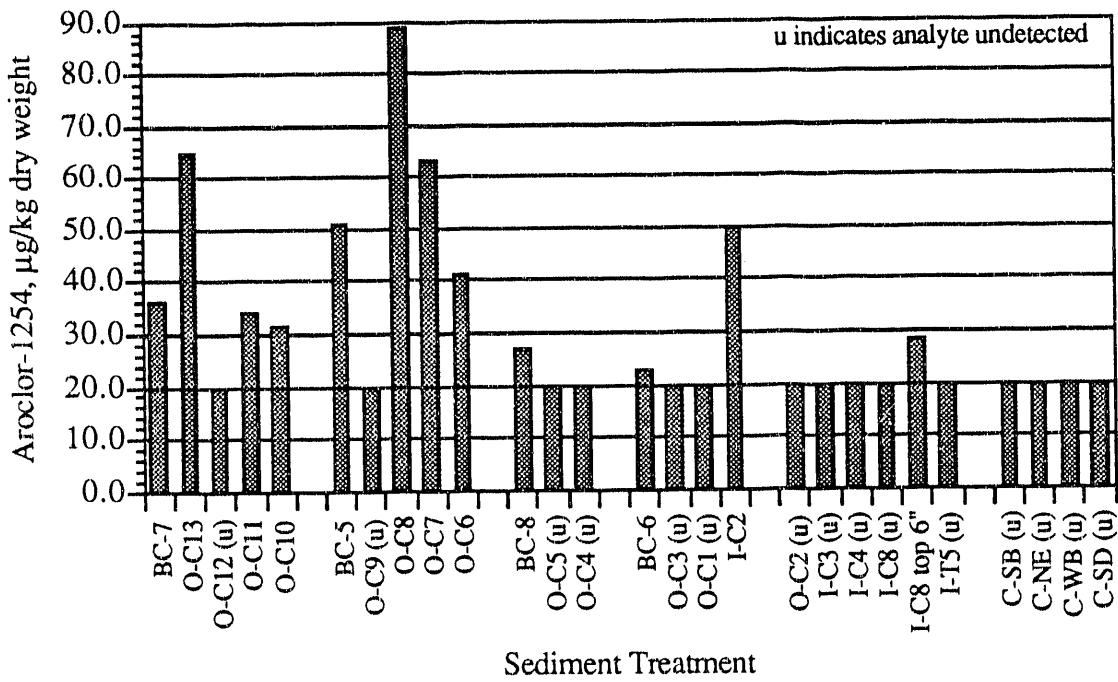


FIGURE 3.22. PCB Aroclor 1254 in Sediments, Oakland Phase III B (Aroclor 1254 was undetected above 20 ug/kg dry weight in all reference sediments)

Quality control criteria were met for all metals analyses. Because these metals are ubiquitous in the natural environment, the metal concentration for each treatment, including the six references and four control sediments, will be compared to a typical shale sediment (Krauskopf 1967).

Silver (Ag) concentrations (Figure 3.23) ranged from 0.026 mg/kg in sediment from Station I-T5 to 0.518 mg/kg at O-C8. Concentrations of silver in the six reference and four control sediments ranged from 0.005 mg/kg in C-SD and R-PC to 0.395 mg/kg in R-BF, a 79-fold difference. Five sediment treatments had Ag concentrations that exceeded R-BF (0.395 mg/kg). These sediment treatments included O-C8 (0.518 mg/kg), O-C13 (0.503 mg/kg), O-C7 (0.480 mg/kg) and O-C6 (0.433 mg/kg). Figure 3.23 shows that 19 sediment treatments also had Ag concentrations greater than that of typical shale soil (0.1 mg/kg) (Krauskopf 1967).

Concentrations of arsenic (As) ranged from 1.65 mg/kg at Station O-C9 to 12.4 mg/kg in O-C8 and are represented graphically in Figure 3.24. The six reference and four control sediments had As concentrations in the same range with values ranging from 2.40 mg/kg in C-WB to 11.7 mg/kg in C-SB. For comparison, the average As concentration in shale soil is 6.6 mg/kg. Sixteen of the sediment treatments analyzed had As concentrations exceeding the shale soil As concentration.

Cadmium (Cd) concentrations in sediment treatments (Figure 3.25) ranged from 0.082 mg/kg in O-C9 to 0.60 mg/kg in O-C13. Reference sediment R-PF had the highest cadmium concentration (2.48 mg/kg) followed by control sediment C-SB with a Cd concentration of 0.98 mg/kg. Fourteen test treatments, one control sediment (C-SB), and two reference sediments (R-OS and R-PF) had Cd concentrations exceeding the shale soil Cd concentration of 0.3 mg/kg.

Concentrations of chromium (Cr) in the sediment treatments are presented graphically in Figure 3.26. Chromium concentrations ranged from 175 mg/kg at O-C1 to 531 mg/kg at O-C5. The four control and six reference sediments had Cr concentrations that spanned most of this range with values ranging from 51 mg/kg at C-NE to 390 mg/kg at R-PF. Chromium concentrations in typical shale soil is 100 mg/kg dry weight (Krauskopf 1967). All of the sediments, including references and controls (except C-NE), contained Cr concentrations above 100 mg/kg. Seven sediment treatments had Cr concentrations four to five times greater than shale soil. In contrast to other metals, sediment treatment O-C5 had the highest concentration of Cr followed by O-C9 and I-C3.

Copper (Cu) concentrations (Figure 3.27) ranged from 12.1 mg/kg in I-C3 to 70.1 mg/kg in O-C8, a fivefold difference. Four sediment treatments, O-C8 (70.1 mg/kg), O-C13 (69.1 mg/kg), O-C7 (65.4 mg/kg), and O-C6 (64.9 mg/kg), had Cu concentrations higher than shale soil

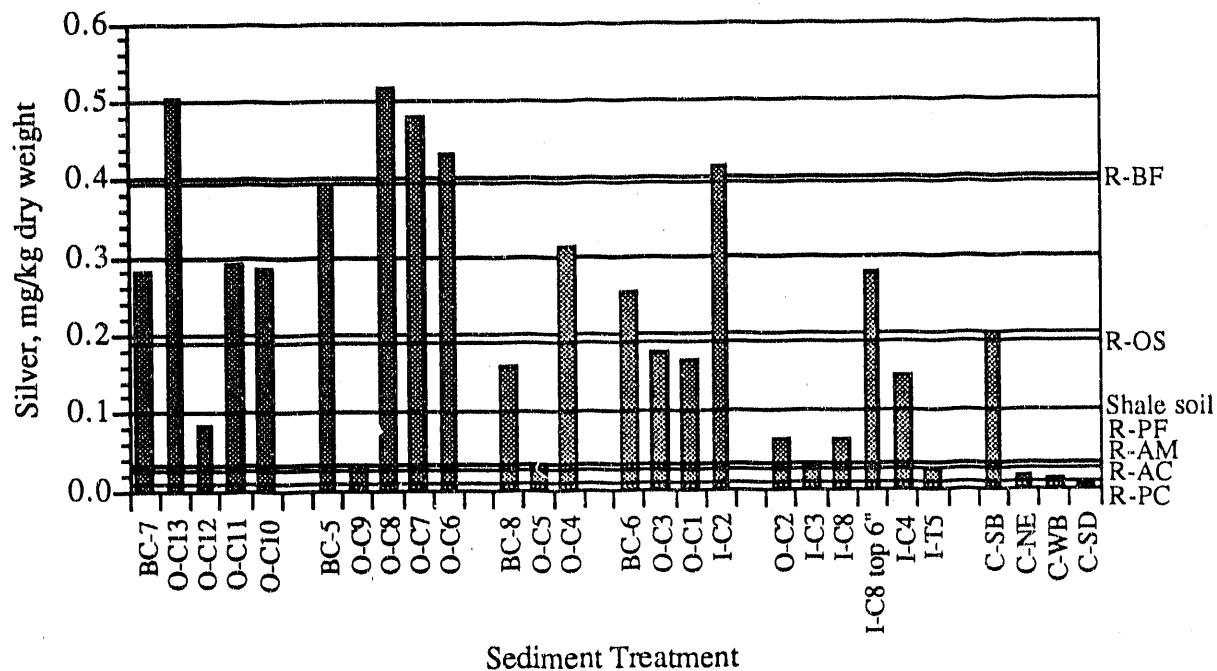


FIGURE 3.23. Concentration of Silver in Sediments, Oakland Harbor Phase III B

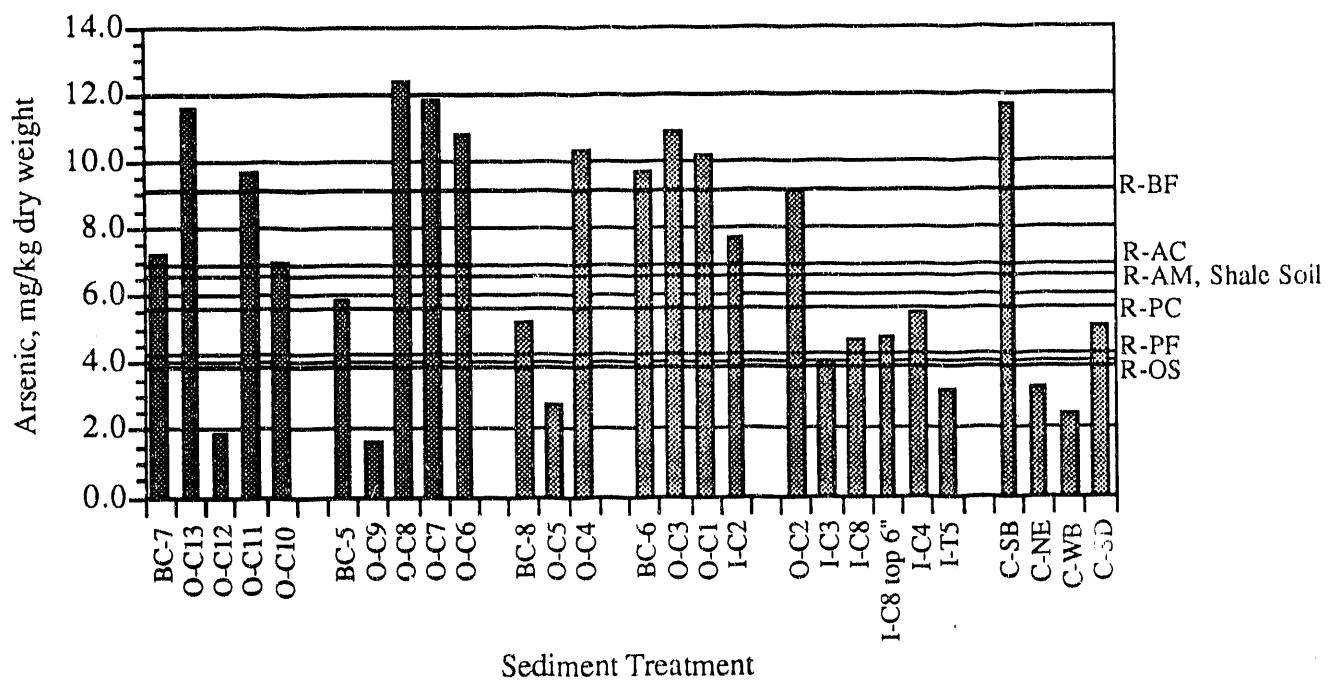


FIGURE 3.24. Concentration of Arsenic in Sediments, Oakland Harbor Phase III B

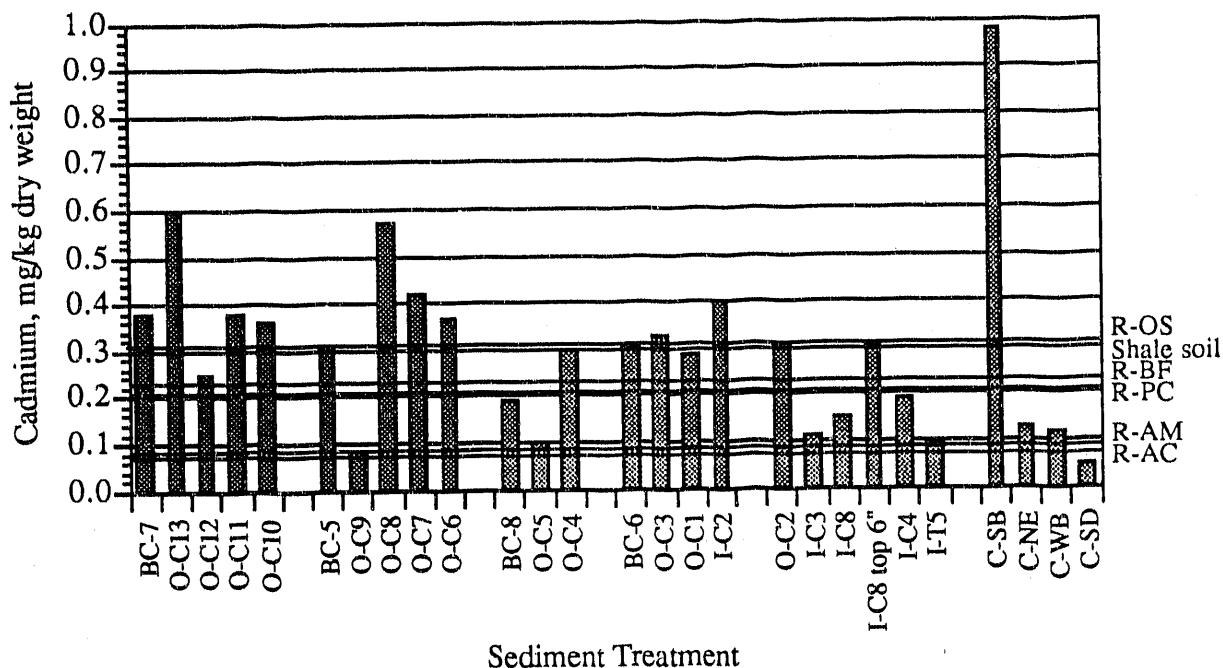


FIGURE 3.25. Concentration of Cadmium in Sediments, Oakland Harbor Phase III B (Reference sediment R-PF has a Cd value of 2.48 mg/kg.)

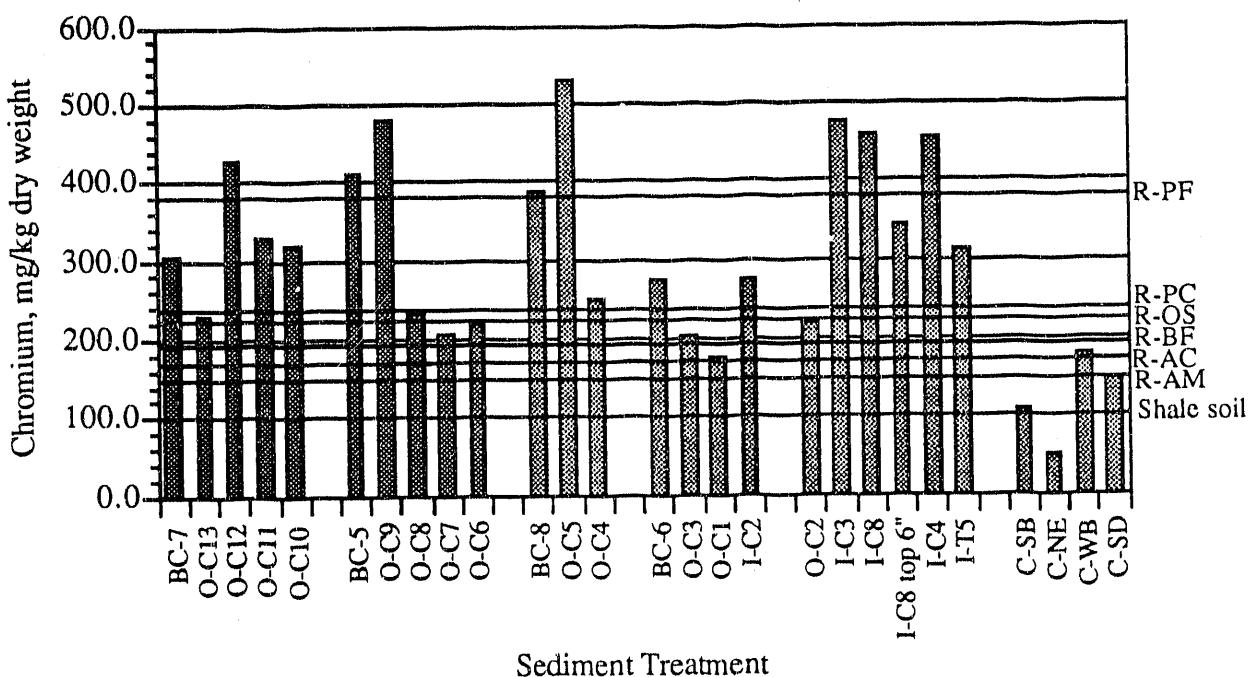


FIGURE 3.26. Concentration of Chromium in Sediments, Oakland Harbor Phase III B

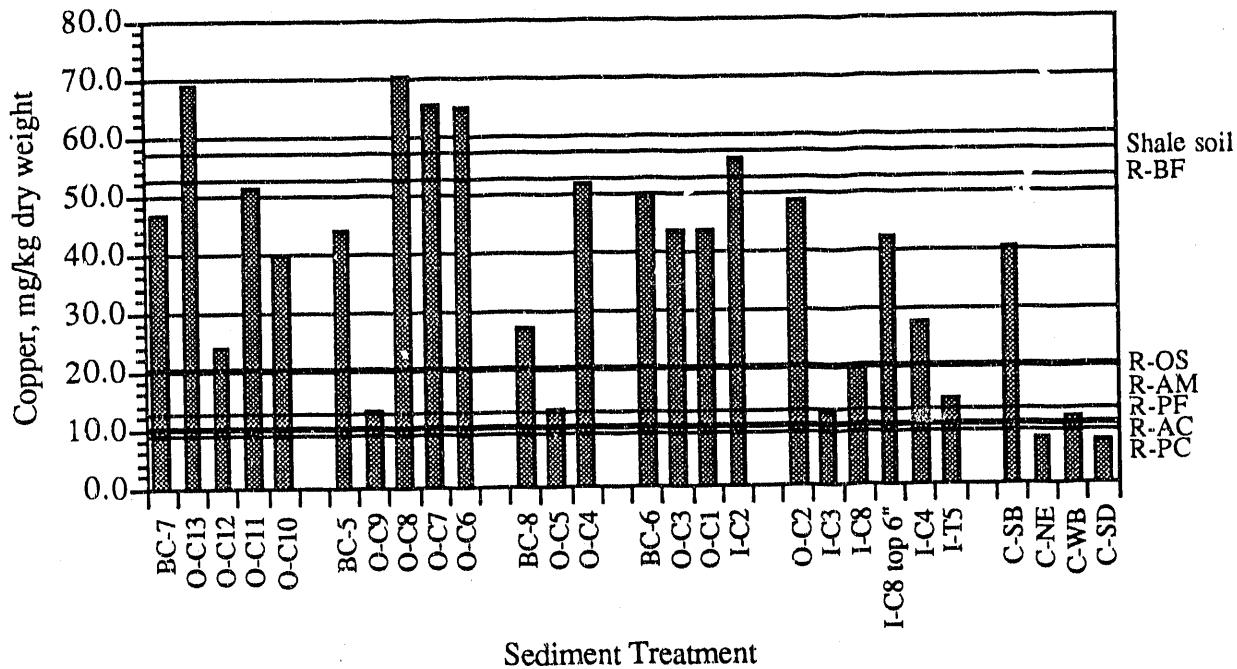


FIGURE 3.27. Concentration of Copper in Sediments, Oakland Harbor Phase III B

(57 mg/kg) (Krauskopf 1967). All four control and six reference sediments had Cu concentrations lower than the shale soil concentration, ranging from 7.0 mg/kg in C-SD to 52.6 mg/kg in R-BF.

Concentrations of mercury (Hg) are presented graphically in Figure 3.28. Concentrations of Hg ranged from 0.045 mg/kg in I-T5 to 2.204 mg/kg in O-C8, a 49-fold difference. Reference and control sediments contained Hg concentrations covering most of this range with values that ranged from 0.057 mg/kg in C-SD to 1.8 mg/kg in C-SB. Six Oakland Harbor sediment treatments had concentrations below the typical concentration of Hg in shale soil of 0.4 mg/kg (Krauskopf 1967). All the control and reference sediments but R-BF and C-SB had Hg concentrations lower than typical shale soil. The Hg concentration at R-BF was approximately four times higher than shale soil.

Nickel (Ni) concentrations in sediment treatments (Figure 3.29) ranged from 35.7 mg/kg in I-C3 to 115.7 mg/kg in O-C6. Reference and control sediments had Ni concentrations covering most of this range with values ranging from 24.4 mg/kg in C-NE to 107.9 mg/kg in R-BF. Eleven sediment treatments and one reference sediment (R-BF) exceeded the shale soil Ni concentration of 95 mg/kg.

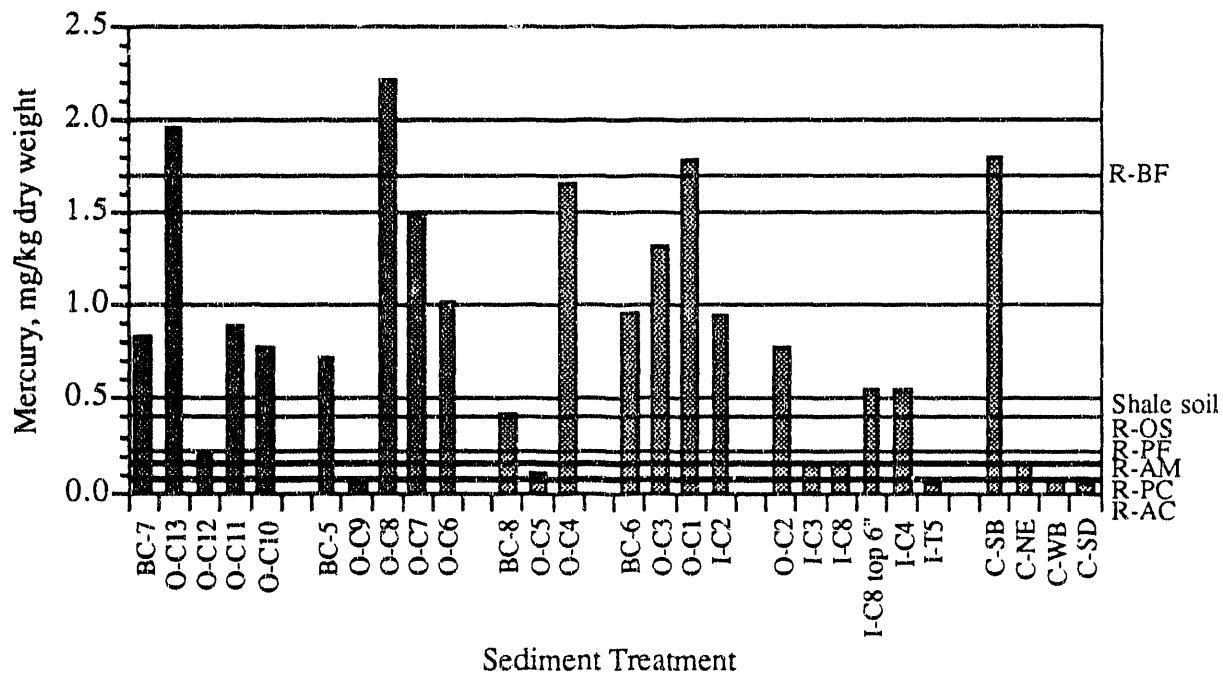


FIGURE 3.28. Concentration of Mercury in Sediments, Oakland Harbor Phase III B

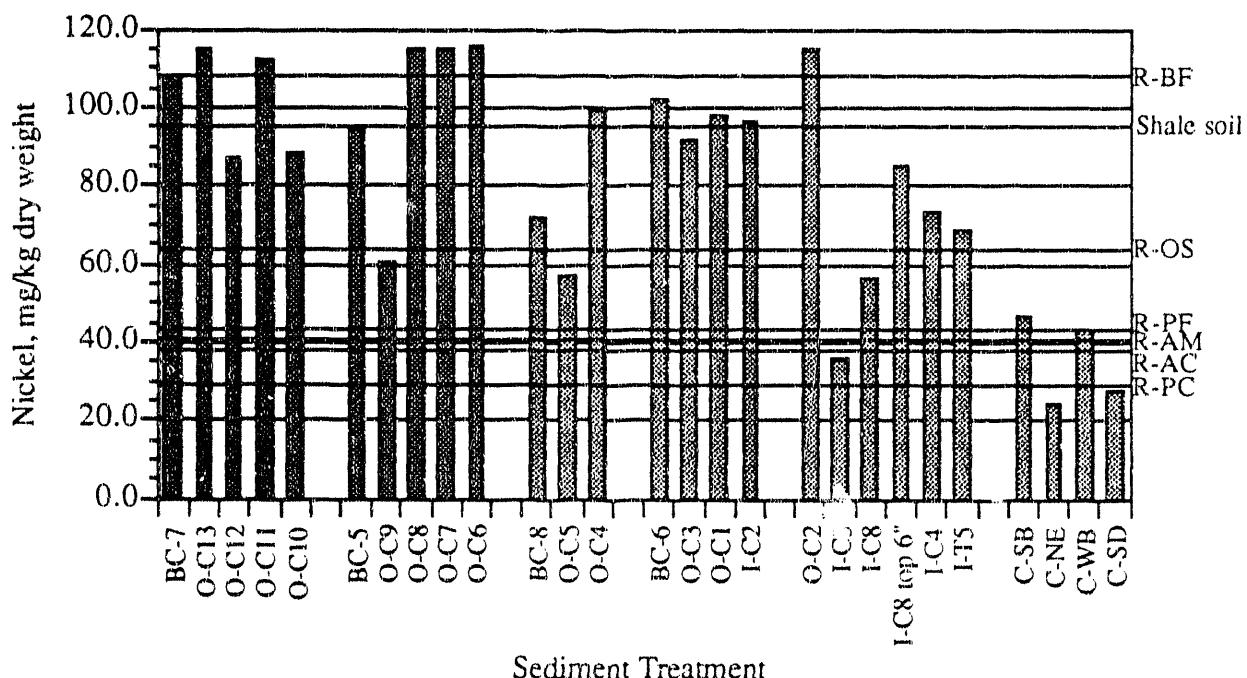


FIGURE 3.29. Concentration of Nickel in Sediments, Oakland Harbor Phase III B

Lead (Pb) concentrations in sediment treatments (Figure 3.30) ranged from 5.3 mg/kg in I-T5 to 49.8 mg/kg in O-C7. Lead concentrations for reference and control sediments covered most of this range with values ranging from 4.2 mg/kg in C-SD to 31.8 mg/kg in R-BF. Twelve sediment treatments and one reference sediment had Pb concentrations exceeding the typical shale soil concentration of 20 mg/kg. The four sediment treatments with the highest Pb concentrations were O-C7 (49.8 mg/kg), O-C13 (48.0 mg/kg), O-C6 (47.5 mg/kg), and O-C8 (47.1 mg/kg).

Selenium (Se) was detected in only 16 of the 33 sediment treatments (Figure 3.31). Concentrations of detected levels of Se ranged from 0.12 mg/kg in BC-7, O-C12, and O-C10, to 0.8 mg/kg in C-SB. Only C-SB had a Se concentration exceeding the typical shale soil concentration of 0.60 mg/kg (Krauskopf 1967).

Concentrations of zinc (Zn) in sediment treatments ranged from 31.6 mg/kg in I-C3 to 175.0 mg/kg in O-C8 (Figure 3.32), a fivefold difference. The Zn concentration in the control and reference sediments covered most of this range with values ranging from 20.3 mg/kg in C-NE to 143.4 mg/kg in R-BF. Fifteen sediment treatments, two reference sediments, and one control sediment had Zn concentrations exceeding the shale soil concentration of 80 mg/kg (Krauskopf 1967). The four sediment treatments with the highest Zn concentrations were O-C8 (175.0 mg/kg), O-C7 (170.2 mg/kg), O-C6 (167.1 mg/kg), and O-C13 (165.0 mg/kg).

3.3.5 Butyltin

Results of the mono-, di-, and tributyltin analyses are shown graphically in Figures 3.33, 3.34, and 3.35, respectively. Complete results, quality control data, and a quality control summary are contained in Volume 2 of this report, Appendix C, Tables C.22 and C.23. Quality Control criteria were met for butyltin analyses. Monobutyltin (MBT) concentrations ranged from undetected at 1 $\mu\text{g}/\text{kg}$ in eight sediment treatments to 4.8 $\mu\text{g}/\text{kg}$ in O-C6. Two control and four reference sediments had no detected MBT. The highest MBT sediment concentrations were at OC-6, followed by OC-8, OC-11, OC-13, and C-SB. Dibutyltin (DBT) concentrations ranged from undetected above 1 $\mu\text{g}/\text{kg}$ in five sediment treatments to 9.9 $\mu\text{g}/\text{kg}$ in O-C7. Two control and two reference sediments had undetected concentrations of 1 $\mu\text{g}/\text{kg}$. The highest DBT sediment concentrations were at Station OC-7, followed by OC-6, OC-13, and OC-8. Tributyltin (TBT) concentrations ranged from undetected at 1 $\mu\text{g}/\text{kg}$ in five sediment treatments to 9.5 $\mu\text{g}/\text{kg}$ in the top 6 in. of sediment from I-C8. Three control and four reference sediments had undetected concentrations at 1 $\mu\text{g}/\text{kg}$. The highest TBT sediment concentrations in Oakland Outer Harbor were at Station OC-13 followed by OC-10, and OC-8. No butyltins were detected in the control sediment (C-NE). Only MBT was detected in C-SD and R-PF, and only TBT was detected in R-AM. Only DBT was detected in C-WB, R-AC, R-OS, and R-PC.

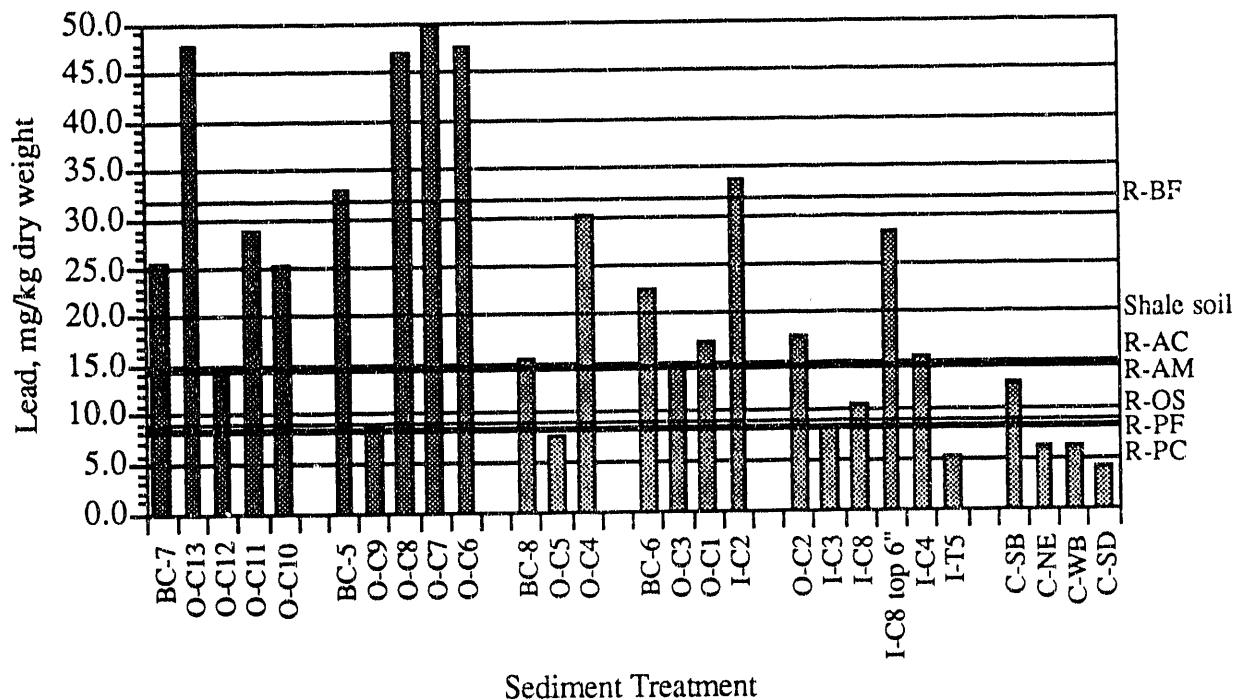


FIGURE 3.30. Concentration of Lead in Sediments, Oakland Harbor Phase III B

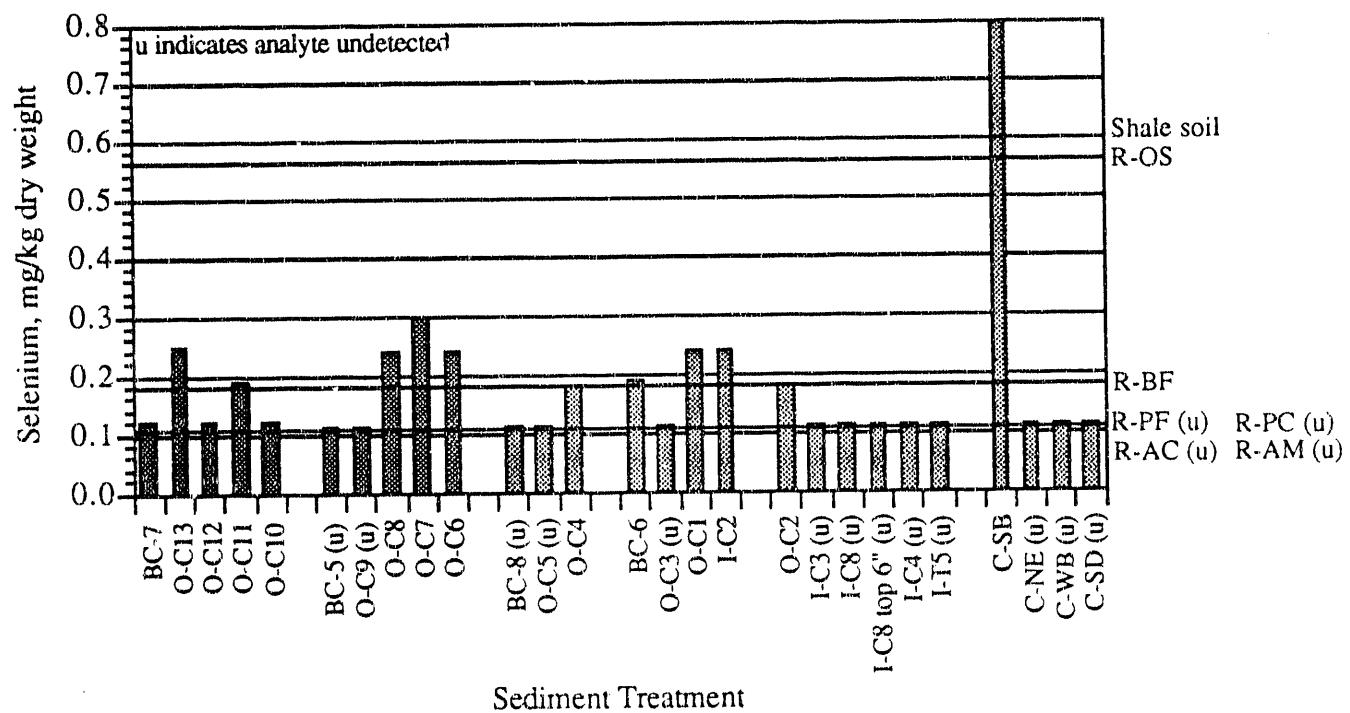


FIGURE 3.31. Concentration of Selenium in Sediments, Oakland Harbor Phase III B

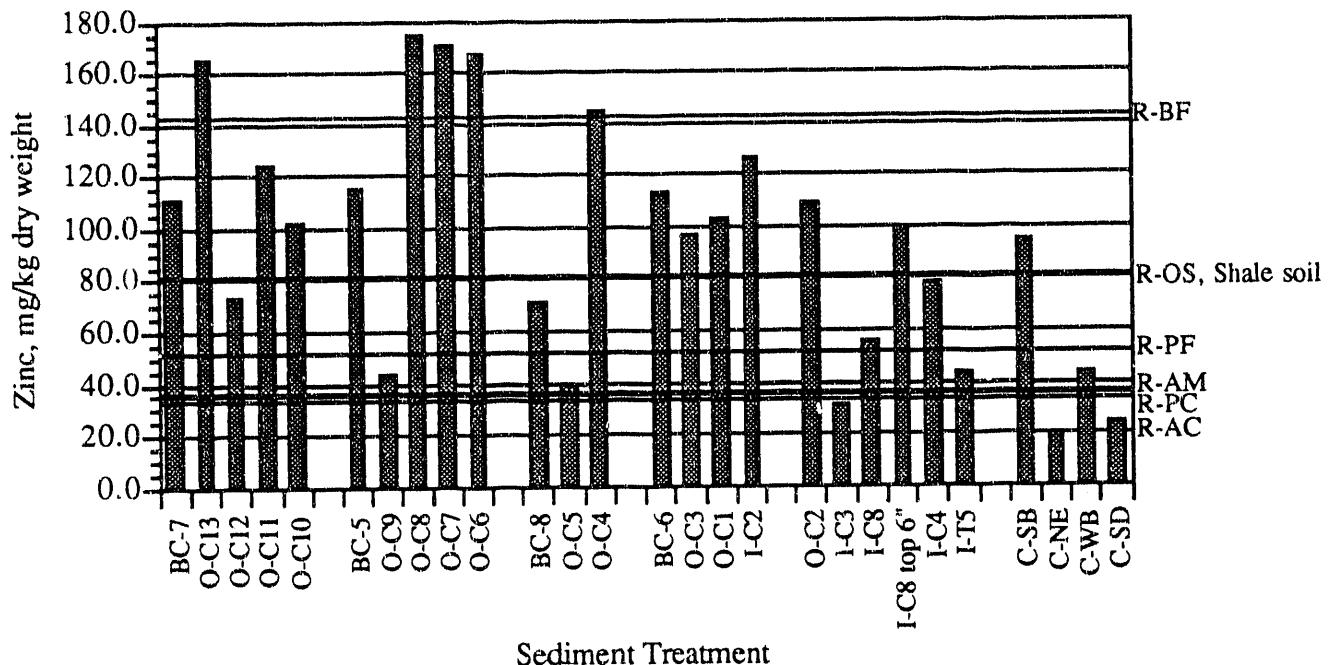


FIGURE 3.32. Concentration of Zinc in Sediments, Oakland Harbor Phase III B

3.4 TOXICOLOGICAL TESTING RESULTS

Solid-phase toxicity tests were conducted to evaluate 18 Oakland Harbor sediment treatments, 6 reference sediment treatments, and 4 control sediment treatments. Solid-phase toxicity testing consisted of a 10-day flow-through solid-phase test with the polychaete *N. caecoides*, a 28-day flow-through solid-phase test with *N. caecoides* and the benthose clam *M. nasuta*, a 10-day static solid-phase test with the amphipod *R. abronius*, and a 10-day flow-through solid-phase test with the juvenile speckled sanddab *C. stigmaeus*. The data results from each solid-phase test were evaluated to ensure that they were validated by at least 90% survival in the native control sediment and that test conditions were maintained within acceptable ranges (Section 2.4). Once validated, the data were evaluated by ANOVA and Dunn's Test to determine if significant differences occurred between treatments (Section 2.5.2).

The SPP toxicity tests were conducted using three species of sensitive marine organisms: the mysid shrimp *H. sculpta*, juvenile speckled sanddab *C. stigmaeus*, and larvae of the mussel *M. edulis*. These tests evaluated the SPP toxicity of treatments BC-5, BC-6, BC-7, BC-8, R-BF, R-PF, and R-AM. Four concentrations were tested: 0% (seawater), 10%, 50%, and 100% SPP. The SPP preparation is described in Section 2.2.4 and toxicological testing procedures are found in

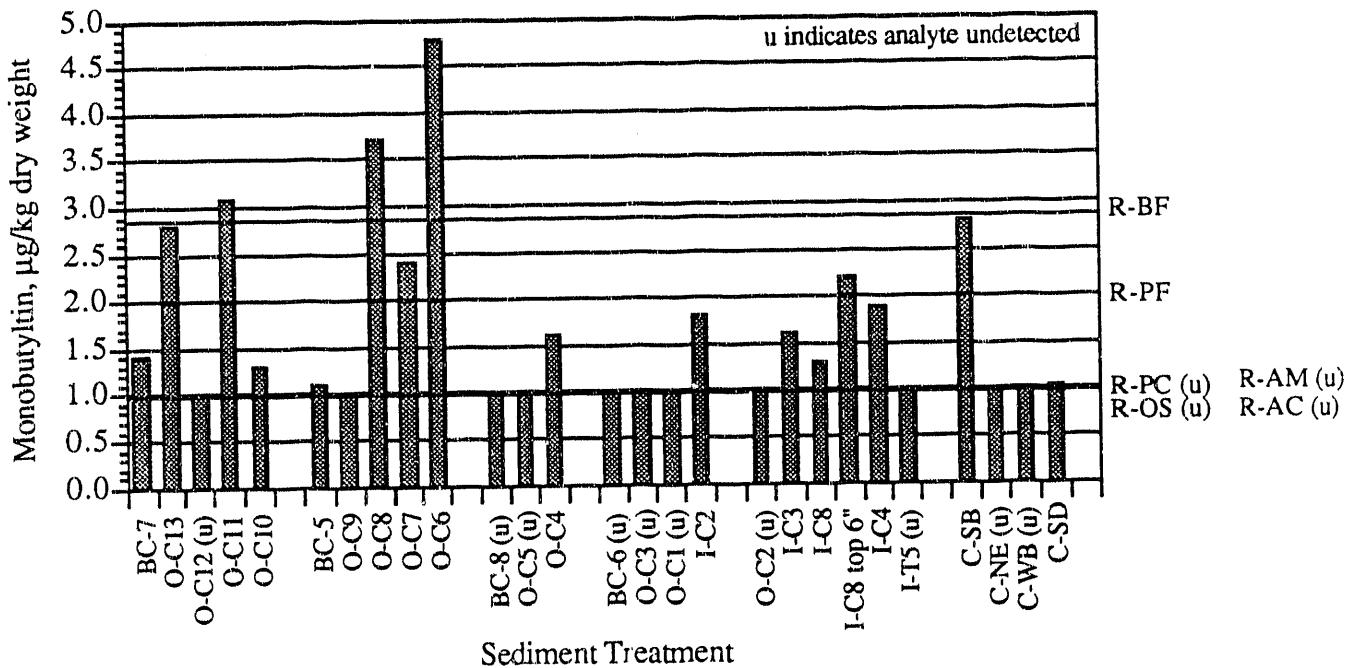


FIGURE 3.33. Concentration for Monobutyltin in Sediments, Oakland Harbor Phase III B

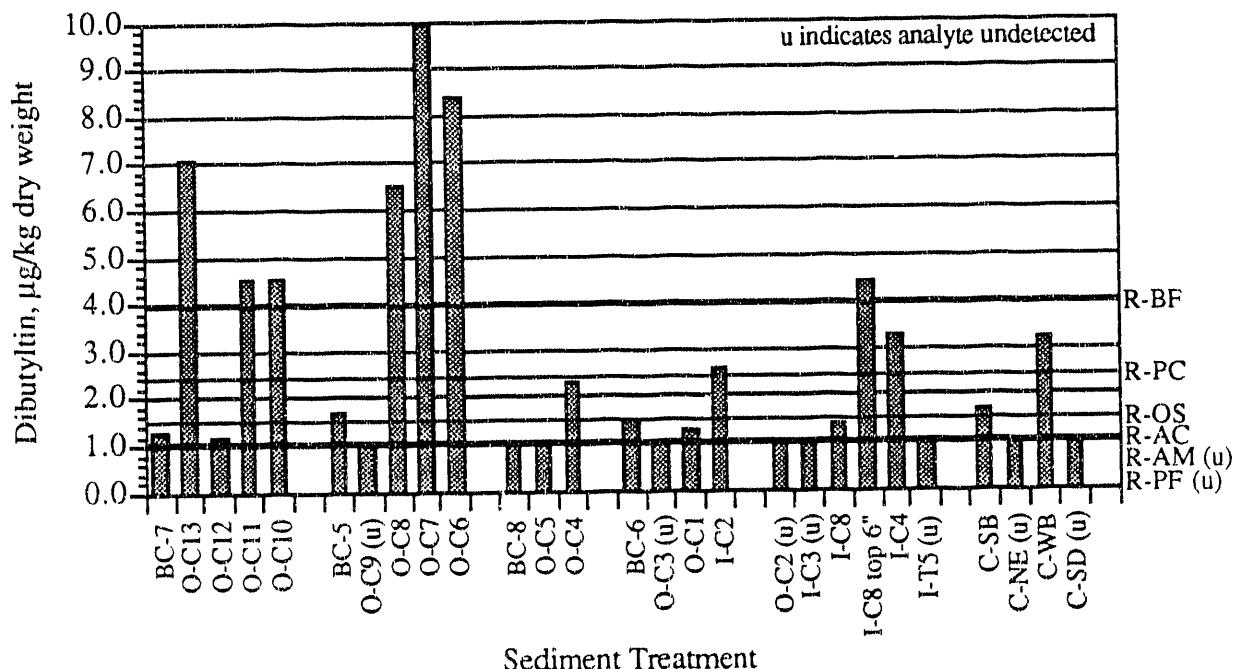


FIGURE 3.34. Concentration of Dibutyltin in Sediments, Oakland Harbor Phase III B

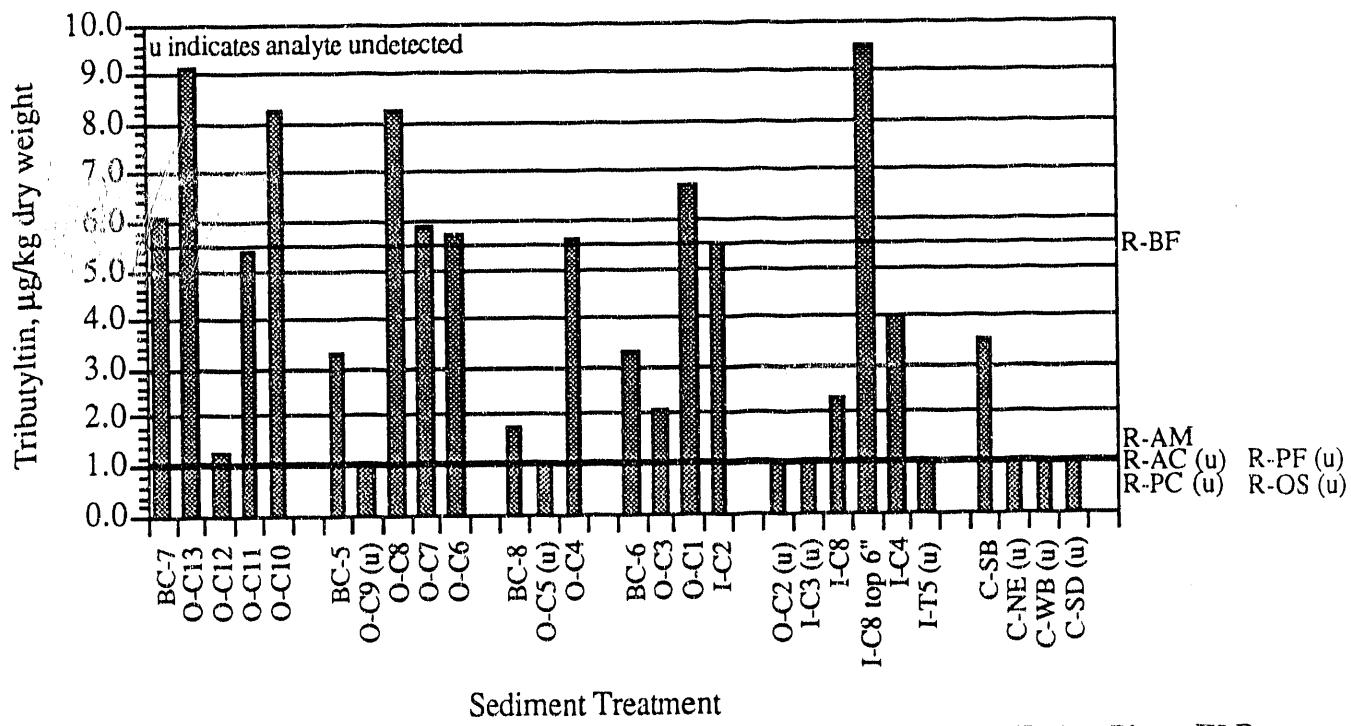


FIGURE 3.35. Concentration of Tributyltin in Sediments, Oakland Harbor Phase III B

Section 2.4. Control survival and appropriate water quality data resulting from each SPP test were first evaluated to determine whether the test was valid. The data for the 0% (control) and 100% SPP treatment were then statistically compared with a two-sample t-test. If the result was significant ($\alpha = 0.05$), and at least 50% reduction in survival relative to control was noted, an LC50 or EC50 estimate was made using a trimmed Spearman-Karber model.

3.4.1 10-Day Flow-Through Solid-Phase Test with *N. caecoides*

The *N. caecoides* 10-day solid-phase test was validated by 100% mean survival in the control sediment treatment C-NE (Table 3.5); ANOVA results are presented in Table 3.6. Water quality data showed that most measured parameters remained within the target ranges set for the test, except for a few aquaria that had some temperature, salinity, and flow rate measurements that were out of target ranges. These fluctuations did not appear to affect the outcome of the test. Results of the *N. caecoides* 10-day solid-phase test, including water quality and observation data, are presented in Volume 2, Appendix D.

Table 3.5 shows that mean survival of *N. caecoides* ranged from a low of 82% in O-C7 to 100% in I-C3, R-AC, and C-NE. Mean survival in four test treatments was at least 10% lower than in the control treatment C-NE. In no case was survival lower than 80% or 10% below the validation level for control survival of 90%. ANOVA on arcsine square-root (Table 3.6)

TABLE 3.5. Results of the 10-Day Flow-Through Solid-Phase Test with *N. caecoides*

<u>Sediment Treatment</u>	<u>Mean Percent Survival</u>	<u>Statistical Grouping</u>
O-C7	82.0	A
O-C13	85.0	AB
O-C1	87.0	ABC
O-C11	86.0	ABC
O-C8	90.0	ABCD
O-C12	91.0	ABCD
I-C8	93.0	ABCD
R-PC	92.0	ABCD
O-C6	93.0	ABCD
I-T5	93.0	ABCD
I-C4	95.0	ABCD
O-C10	93.0	ABCD
O-C4	95.0	ABCD
R-BF	96.0	ABCD
O-C3	95.0	ABCD
O-C2	98.0	BCD
R-PF	98.0	BCD
I-C2	98.0	BCD
O-C5	98.0	BCD
R-AM	99.0	CD
R-OS	99.0	CD
O-C9	99.0	CD
I-C3	100.0	D
R-AC	100.0	D
C-NE	100.0	NA ^(a)
C-SB	95.0	NA

(a) Not applicable; not included in statistical analysis.

TABLE 3.6. ANOVA Results for the 10-Day Flow-Through Solid-Phase Test with *N. caecoides*

<u>Source of Variation</u>	<u>Sum of Squares</u>	<u>d.f.</u>	<u>Mean Square</u>	<u>F-Ratio</u>	<u>Significance Level</u>
Between Groups	1.738	23	0.076	3.648	0.0001
Within Groups	1.989	96	0.021		

transformations of proportion surviving identified significant differences between treatments. Further comparison using Dunn's Test ($\alpha = 0.10$) revealed that survival in some sediment treatments was significantly different and had at least 10% lower survival relative to survival in reference sediments. Sediment treatment O-C7 was significantly different from R-PF, R-AC, R-AM, and R-OS. Sediment treatment O-C13 was significantly different from R-AC, R-AM and R-OS. Treatments O-C1 and O-C11 were significantly different from R-AC.

3.4.2 28-Day Flow-Through Solid-Phase Test with *N. caecoides* and *M. nasuta*

The results of the *N. caecoides* and *M. nasuta* 28-day bioaccumulation tests are summarized in Table 3.7. The tests were validated for both solid-phase tests by *M. nasuta* survival of 99.2% in C-SB and by *N. caecoides* survival of 97.3% in C-NE. The water quality data (Volume 2, Appendix E, Table E.5) show that most measured parameters remained within the target ranges. There were a few test containers that had a few measurements for salinity and flow rates that were out of range, but these fluctuations did not appear to affect the outcome of the test. *M. nasuta* survival ranged from 88.8% in O-C6 to 100% in I-C3 and O-C1. *N. caecoides* survival ranged from 84.7% in O-C12 to 99.3% in R-AM. The low mortality to both species observed in all treatments resulted in adequate tissue mass to conduct chemical analyses on the organisms from each replicate.

3.4.3 10-Day Static Solid-Phase Test with *R. abronius*

The 10-day *R. abronius* solid-phase test was validated by a 98% mean survival in the control sediment treatment C-WB (Table 3.8). ANOVA results are presented in Table 3.9. Water quality data (Volume 2, Appendix F, Table F.3) showed that most measured parameters remained within the target ranges set for the test. A few test containers had measurements that were out of range but did not seem to affect the validity of the test.

Table 3.8 shows that mean survival of *R. abronius* ranged from 47% in O-C1 to 100% in R-PC. Statistical analyses using ANOVA (Table 3.9) and Dunn's Test ($\alpha = 0.10$) show that a significant difference occurred between the test sediment and the reference sediments. Five treatments had greater than 20% difference in survival from one or more references. These sediment treatments included OC-1, IC-2, OC-2, OC-12, and OC-9.

The results of the *R. abronius* reference toxicant test using a cadmium standard were analyzed using the Spearman-Karber method (Section 2.5.3). The LC50 was estimated to be 0.83 mg/L, meaning that a 50% decrease in survival could be expected. This LC50 is lower than those estimated during Oakland Harbor Phase III A (1.22 mg/L Cd) and Oakland Harbor Phase III 38-

TABLE 3.7. Results of the 28-Day Flow-Through Solid-Phase Test With *M. nasuta* and *N. caecoides*

<u>Sediment Treatment</u>	<i>M. nasuta</i> <u>Mean Percent Survival</u>	<i>N. caecoides</i> <u>Mean Percent Survival</u>
O-C13	96.0	86.0
O-C12	96.0	84.7
O-C11	97.6	88.7
O-C10	96.0	90.7
O-C9	96.8	93.3
O-C8	96.8	88.0
O-C7	97.6	92.0
O-C6	88.8	93.3
O-C5	96.8	92.7
O-C4	97.6	93.3
O-C3	98.4	92.0
O-C2	99.2	88.6
O-C1	100.0	91.8
I-C2	99.2	93.3
I-C3	100.0	98.0
I-C8	96.8	86.7
I-C4	97.6	92.7
I-T5	98.4	92.7
R-AC	98.4	94.7
R-AM	99.2	99.3
R-BF	96.8	94.7
R-OS	96.0	92.7
R-PC	94.4	98.0
R-PF	97.6	98.0
C-NE	98.4	97.3
C-SB	99.2	93.3

Foot (1.80 mg/L Cd) testing, indicating that the *R. abronius* used for Phase III B testing were more sensitive to Cd.

3.4.4 10-Day Flow-Through Solid-Phase Test with *C. stigmaeus*

The 10-day *C. stigmaeus* solid-phase test was validated by 100% mean survival in the control sediment treatment C-SD (Table 3.10). Water quality data (Volume 2, Appendix G, Table G.3) showed that most measured parameters remained within the target ranges set for the test, except for a few aquaria with low flow rates.

The mean survival of *C. stigmaeus* ranged from 94% to 100%. Statistical analyses using ANOVA (Table 3.11) and Dunn's Test ($\alpha = 0.10$) showed that no significant difference occurred between test treatments and reference treatments.

TABLE 3.8. Results of the 10-Day Static Solid-Phase Test with *R. abronius*

<u>Treatment</u>	<u>Mean Percent Survival</u>	<u>Statistical Grouping</u>
OC-1	47.0	A
IC-2	59.0	AB
OC-2	67.0	ABC
OC-12	68.0	ABCD
OC-9	70.0	ABCDE
R-BF	80.0	BCDEF
R-OS	83.0	BCDEFG
OC-4	84.0	BCDEFG
OC-5	85.0	BCDEFG
OC-3	81.0	BCDEFG
OC-8	88.0	BCDEFGH
OC-7	86.0	BCDEFGH
IC-8	89.0	CDEFGH
OC-10	92.0	CDEFGH
OC-11	93.0	DEFGH
OC-6	93.0	DEFGH
OC-13	94.0	EFGH
IC-3	96.0	FGH
R-PF	96.0	FGH
R-AM	98.0	GH
R-AC	99.0	GH
R-PC	100.0	H
C-WB	98.0	NA ^(a)
C-SB	92.0	NA

(a) Not applicable; not included in statistical analysis.

TABLE 3.9. ANOVA Results for the 10-Day Static Solid-Phase Test with *R. abronius*

<u>Source of Variation</u>	<u>Sum of Squares</u>	<u>d.f.</u>	<u>Mean Square</u>	<u>F-Ratio</u>	<u>Significance Level</u>
Between Groups	4.804	21	0.229	9.045	0.0001
Within Groups	2.225	88	0.025		

TABLE 3.10. Results of the 10-Day Flow-Through Solid-Phase Test with *C. stigmaeus*

<u>Sediment Treatment</u>	<u>Mean Percent Survival</u>	<u>Statistical Grouping</u>
O-C13	94.0	A
O-C6	94.0	A
I-C3	96.0	A
O-C10	96.0	A
I-C8	97.5	A
O-C1	98.0	A
O-C11	98.0	A
I-C2	98.0	A
O-C4	98.0	A
O-C5	100.0	A
O-C3	100.0	A
O-C2	100.0	A
O-C8	100.0	A
O-C7	100.0	A
O-C12	100.0	A
O-C9	100.0	A
R-AC	100.0	A
R-AM	100.0	A
R-BF	100.0	A
R-OS	100.0	A
R-PC	100.0	A
R-PF	100.0	A
C-SB	100.0	NA ^(a)
C-SD	100.0	NA

(a) Not applicable; not included in statistical analysis.

TABLE 3.11. ANOVA Results for the 10-Day Flow-Through Solid-Phase Test with *C. stigmaeus*

<u>Source of Variation</u>	<u>Sum of Squares</u>	<u>d.f.</u>	<u>Mean Square</u>	<u>F-Ratio</u>	<u>Significance Level</u>
Between Groups	0.433	21	0.021	1.982	0.0146
Within Groups	0.906	87	0.010		

3.4.5 96-h Static Suspended-Particulate-Phase Test with *C. stigmaeus*

The 96-h *C. stigmaeus* SPP test was validated by 100% mean survival in the control (0% SPP) treatments (Table 3.12). Water quality data (Volume 2, Appendix H, Table H.3) show that all measured parameters remained within the targeted range during the test. A mean proportion surviving of 100% was found in all SPP treatments at all concentrations except for treatments BC-6 and R-AM 100% SPP, both of which averaged 97% survival. Table 3.13 presents the t-test comparisons of control treatments to 100% SPP for each treatment, and shows that there were no significant differences within treatments and that LC50 values could not be calculated.

3.4.6 96-h Static Suspended-Particulate-Phase Test with *H. sculpta*

The 96-h *H. sculpta* SPP test was validated by 90% to 97% mean survival in the control (0% SPP) treatments (Table 3.14). T-test and LC50 results are presented in Table 3.15. Water quality data (Volume 2, Appendix I, Table I.3) show that all measured parameters remained within range during the test. Survival in the 100% SPP remained at or above 67%. Table 3.15 shows that all treatments except BC-5 produced statistically significant differences in survival between the control and 100% SPP dilutions. However, survival remained above 50% in all 100% SPP treatments, thereby producing no calculable LC50 values.

The *H. sculpta* reference toxicant test using a zinc chloride ($ZnCl_2$) was analyzed using the trimmed Spearman-Karber Method (Section 2.5.3). The LC50 was estimated to be 0.39 mg/L of Zn, meaning that a 50% decrease in survival could be expected at this concentration. The *H. sculpta* tested during Phase III B appeared to be more sensitive to Zn than those tested in other programs at MSL in which LC50s of >0.5 mg/L Zn was estimated.

3.4.7 48-h Static Suspended-Particulate-Phase Test with Larval *M. edulis*

The 48-h *M. edulis* SPP test was validated by 87.6% to 99.6% survival in the control (0% SPP) treatments (Table 3.16). Water quality data (Volume 2, Appendix J, Table J.3) showed that all measured parameters remained within range during the test. The results presented in Table 3.16 display the mean percent survival and the mean percent normal development for all treatments. The mean percent of normal larvae development was generally close to the percent survival, with the exception of treatment BC-7. Table 3.17 presents the results of two sample t-tests, comparing the 0% and 100% survivals for each treatment. This table shows that there were no significant differences in survival between the 0% and 100% SPP concentrations in any of the SPP treatments. Table 3.18 presents the results of two sample t-tests, comparing the proportion normal between the 0% and 100% dilutions for each treatment. BC-7 is the only SPP treatment

TABLE 3.12. Results of the 96-h Static Suspended-Particulate-Phase Test with *C. stigmaeus*

<u>Sediment Treatment</u>	<u>% SPP Concentration</u>	<u>Mean Percent Survival</u>
BC-5	0	100
BC-5	10	100
BC-5	50	100
BC-5	100	100
BC-6	0	100
BC-6	10	100
BC-6	50	100
BC-6	100	97
BC-7	0	100
BC-7	10	100
BC-7	50	100
BC-7	100	100
BC-8	0	100
BC-8	10	100
BC-8	50	100
BC-8	100	100
R-AM	0	100
R-AM	10	100
R-AM	50	100
R-AM	100	97
R-BF	0	100
R-BF	10	100
R-BF	50	100
R-BF	100	100
R-PF	0	100
R-PF	10	100
R-PF	50	100
R-PF	100	100

TABLE 3.13. T-Test and LC50 Determinations for the 96-h Static Suspended-Particulate-Phase Test with *C. stigmaeus*

<u>Sediment Treatment</u>	<u>Table t-value</u>	<u>d.f.</u>	<u>Calculated t-value</u>	<u>Significance^(a)</u>	<u>LC50 as Percent SPP</u>
BC-5	2.776	4	NA ^(b)	NS ^(c)	>100 ^(d)
BC-6	2.776	4	1.000	NS	>100
BC-7	2.776	4	NA	NS	>100
BC-8	2.776	4	NA	NS	>100
R-AM	2.776	4	1.000	NS	>100
R-BF	2.776	4	NA	NS	>100
R-PF	2.776	4	NA	NS	>100

(a) $\alpha = 0.05$ for two sample t-test comparisons of 0 and 100% SPP.

(b) Test not applicable because of zero reduction in survival.

(c) No significant difference in response between 0 and 100% SPP.

(d) Test organism survival was > 50% in all concentrations.

that significantly reduced the proportion of larvae with normal development. An EC50 value could only be calculated for treatment BC-7, and indicated that 87.6% of SPP was required to reduce the proportion of larvae with normal development in the test to 50% relative to the 0% control.

A reference toxicant test was also conducted using *M. edulis* larvae. An LC50 value of 26.0 mg/L of Cu reduced the percent survival of larvae to 50% compared to controls, as calculated by the trimmed Spearman-Karber method. Using the same method, an EC50 value of 9.37 mg/L Cu was determined based on the reduction of normal larval development in the Cu solutions compared to controls. This EC50 value is within the range of EC50s estimated during other tests at MSL using *M. edulis* larvae, indicating that the larvae tested were of comparable sensitivity to Cu.

3.5 TISSUE BIOACCUMULATION

Contaminants of concern were measured in the tissues of *M. nasuta* and *N. caecoides* after the 28-day exposure to test, reference, and control sediment treatments. These contaminants were PAHs, pesticides, PCBs, metals, and butyltins. The tissue chemistry results and the statistical analyses performed using Dunn's Test for comparison of all means are summarized in the following sections. Dunn's Test is a conservative model that uses an experiment-wise error rate, and allows comparison of all possible treatment combinations (Section 2.5). Information about

TABLE 3.14. Results of the 96-h Static Suspended-Particulate-Phase Test with *H. sculpta*

<u>Sediment Treatment</u>	<u>% SPP Concentration</u>	<u>Mean Percent Survival</u>
BC-5	0	90
BC-5	10	87
BC-5	50	90
BC-5	100	70
BC-6	0	93
BC-6	10	77
BC-6	50	70
BC-6	100	73
BC-7	0	93
BC-7	10	93
BC-7	50	73
BC-7	100	67
BC-8	0	97
BC-8	10	90
BC-8	50	83
BC-8	100	77
R-AM	0	90
R-AM	10	83
R-AM	50	70
R-AM	100	70
R-BF	0	90
R-BF	10	93
R-BF	50	83
R-BF	100	67
R-PF	0	93
R-PF	10	90
R-PF	50	83
R-PF	100	70

TABLE 3.15. T-Test and LC50 Determinations for the 96-h Static Suspended-Particulate-Phase Test with *H. sculpta*

<u>Sediment Treatment</u>	<u>Table t-value</u>	<u>d.f.</u>	<u>Calculated t-value</u>	<u>Significance^(a)</u>	<u>LC50 as Percent SPP</u>
BC-5	2.776	4	2.450	NS ^(b)	>100 ^(c)
BC-6	2.776	4	4.243	S ^(d)	>100
BC-7	2.776	4	5.657	S	>100
BC-8	2.776	4	4.243	S	>100
R-AM	2.776	4	NC ^(e)	S	>100
R-BF	2.776	4	3.500	S	>100
R-PF	2.776	4	3.500	S	>100

(a) $\alpha = 0.05$ for two sample t-test comparison of 0 and 100% SPP.

(b) No significant difference in response between 0 and 100% SPP.

(c) Test organism survival was >50% in all concentrations.

(d) Significant difference in response between 0 and 100% SPP.

(e) t-value is not calculable because of zero variance; survival difference is assumed significant at $\alpha = 0.05$.

treatments that are significantly different from reference is preserved while additional information about significant differences from other treatments is provided. During this analysis, test treatments were omitted from the statistical comparison if all replicate tissue values for a compound were undetected. Reference treatment replicates were never omitted, even if compounds were undetected. For undetected values in reference treatments and in test treatments with both detected and undetected values, the detection limit value was used in the analysis. Data from stations I-C4 and I-T5 were not included in the statistical analysis of *M. nasuta* tissue bioaccumulation because that analysis was completed during the Oakland Harbor III A Project.

3.5.1 Polynuclear Aromatic Hydrocarbon Bioaccumulation in *M. nasuta*

Complete *M. nasuta* tissue chemistry data in both wet and dry weight PAH concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix K, Tables K.1 through K.9. Eight of the 16 PAH compounds were detected in *M. nasuta* tissues. The mean concentrations of detected PAHs ($\mu\text{g}/\text{kg}$ dry weight) are presented in Table 3.19. Statistical comparisons were performed only on those PAH compounds that were detected.

The results of Dunn's Test are summarized in Table 3.20. This table shows that relative to reference treatments, four PAH compounds, naphthalene, fluoranthene, pyrene, and benzo(b)fluoranthene, were elevated in *M. nasuta* tissues from at least one of the treatments that was tested. Of those four, all except naphthalene are high molecular weight PAHs (HPAH).

TABLE 3.16. Results of the 48-h Static Suspended-Particulate-Phase Test with *M. edulis*

<u>Sediment Treatment</u>	<u>% SPP Concentration</u>	<u>Mean Percent Survival</u>	<u>Mean Percent Normal</u>
BC-5	0	92.8	89.5
BC-5	10	99.0	97.3
BC-5	50	91.5	90.3
BC-5	100	90.1	74.4
BC-6	0	95.4	90.9
BC-6	10	94.0	94.0
BC-6	50	88.4	87.2
BC-6	100	89.3	88.9
BC-7	0	88.5	87.5
BC-7	10	94.7	93.9
BC-7	50	93.4	91.5
BC-7	100	68.8	32.5
BC-8	0	87.6	86.0
BC-8	10	92.0	90.8
BC-8	50	87.1	86.9
BC-8	100	93.6	92.0
R-AM	0	92.8	90.4
R-AM	10	87.0	83.3
R-AM	50	88.6	87.9
R-AM	100	92.0	90.4
R-BF	0	95.1	89.4
R-BF	10	95.4	91.4
R-BF	50	82.7	82.0
R-BF	100	85.4	83.2
R-PF	0	99.6	98.3
R-PF	10	96.4	95.5
R-PF	50	91.0	89.8
R-PF	100	96.7	95.6

TABLE 3.17. T-Test and LC50 Determinations for the the 48-h Static Suspended-Particulate-Phase Test Based on Percent Survival with *M. edulis*

<u>Sediment Treatment</u>	<u>Table t-value</u>	<u>d.f.</u>	<u>Calculated t-value</u>	<u>Significance^(a)</u>	<u>LC50 as Percent SPP</u>
BC-5	2.776	4	0.577	NS ^(b)	>100 ^(c)
BC-6	2.776	4	2.057	NS	>100
BC-7	2.776	4	1.987	NS	>100
BC-8	2.776	4	NA ^(d)	NS	>100
R-AM	2.776	4	0.158	NS	>100
R-BF	2.776	4	0.998	NS	>100
R-PF	2.776	4	0.861	NS	>100

(a) $\alpha = 0.05$ for two sample t-test comparison of 0 and 100% SPP.

(b) No significant difference in response between 0 and 100% SPP.

(c) Test organism survival was > 50% in all concentrations.

(d) Test not applicable due to no reduction in survival.

TABLE 3.18. T-Test and EC50 Determinations for the 48-h Static Suspended-Particulate-Phase Test Based on Proportion Normal with *M. edulis*

<u>Sediment Treatment</u>	<u>Table t-value</u>	<u>d.f.</u>	<u>Calculated t-value</u>	<u>Significance^(a)</u>	<u>EC50 as Percent SPP</u>
BC-5	2.776	4	1.620	NS ^(b)	>100 ^(c)
BC-6	2.776	4	0.597	NS	>100
BC-7	2.776	4	13.773	SI ^(d)	87.6
BC-8	2.776	4	NA ^(e)	NS	>100
R-AM	2.776	4	NA ^(e)	NS	>100
R-BF	2.776	4	0.663	NS	>100
R-PF	2.776	4	0.813	NS	>100

(a) $\alpha = 0.05$ for two sample t-test comparison of 0 and 100% SPP.

(b) No significant difference in response between 0 and 100% SPP.

(c) Normal development was >50% in all concentrations.

(d) Significant difference in response between 0 and 100% SPP.

(e) Test not applicable because of no reduction in normal larval development.

TABLE 3.19. Mean PAH Concentrations in the Tissues of *M. nasuta* ($\mu\text{g}/\text{kg}$ dry weight)

Sediment Treatment	Naphtha-lene	Phenanthrene	Benzo(a) Fluoranthene	Pyrene	Anthra-cene	Chrysene	Benzo(b) Fluoranthene	Benzo(a) pyrene
O-C13	70 U ^(a)	70 U	170	591	70 U	81	83	70
O-C12	73 U	73 U	73 U	129	73 U	73 U	73 U	73 U
O-C11	82	69 U	123	350	74	108	69	69 U
O-C10	157	80	134	284	83	106	80	76 U
O-C9	181	76 U	101	95	76 U	87	76 U	76 U
O-C8	110	74 U	163	336	81	96	78	74 U
O-C7	145 U	73 U	125	226	73 U	74	74	73 U
O-C6	159	84	129	228	74 U	74 U	74 U	77
O-C5	166	98	89	114	80 U	80 U	80 U	80 U
O-C4	132 U	66 U	95	132	66 U	66 U	66 U	66 U
O-C3	189	69 U	69 U	72	69 U	69 U	69 U	69 U
O-C1	198	71 U	71 U	71 U	71 U	71 U	71 U	71 U
I-C2	140 U	70 U	70 U	179	70 U	70 U	70 U	70 U
O-C2	138 U	69 U	69 U	69 U	69 U	69 U	69 U	69 U
I-C3	128 U	64 U	64 U	76	64 U	64 U	64 U	64 U
I-C4	75 U	75 U	85	144	75 U	75 U	75 U	75 U
I-C8	135 U	67 U	75	407	67 U	74	125	78
I-T5	78	72 U	139	133	72 U	72 U	72 U	72 U
R-AC	76 U	76 U	76 U	139	76 U	98	76 U	90
R-AM	65 U	86	149	233	65 U	73	65 U	65 U
R-BF	97 U	97 U	97 U	97 U	97 U	97 U	97 U	97 U
R-OS	69 U	69 U	69 U	69 U	69 U	75	69 U	72
R-PC	69 U	69 U	69 U	69 U	69 U	69 U	69 U	69 U
R-PF	77 U	77 U	77 U	77 U	77 U	77 U	77 U	77 U
C-SB	74 U	74 U	74 U	74 U	74 U	74 U	74 U	74 U
C-NE	74 U	74 U	74 U	74 U	74 U	74 U	74 U	74 U

(a) Undetected in all replicates; value is mean of dry weight detection limits.

Pyrene was detected most frequently and at the highest mean levels of any PAH in the test treatments, ranging from 72 $\mu\text{g}/\text{kg}$ in O-C3 to 591 $\mu\text{g}/\text{kg}$ in O-C13. Pyrene levels in seven treatments were significant relative to all three offshore reference treatments, while five treatments had significant levels of pyrene relative to in-bay references R-BF and R-AC. *M. nasuta* exposed to the Alcatraz Island Enviros reference R-AM had a mean pyrene level of 233 $\mu\text{g}/\text{kg}$; only the 591 $\mu\text{g}/\text{kg}$ pyrene at O-C13 was significantly greater. Phenanthrene, benzo(a)anthracene, chrysene, and benzo(a)pyrene showed no significant elevation above reference in *M. nasuta* tissues from any of the treatments tested. PAHs did not accumulate in significant levels relative to any reference treatment in *M. nasuta* exposed to sediment from O-C12, O-C4, I-C2, O-C2, and I-C3.

TABLE 3.20. PAHs in Tissues of *M. nasuta* That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means (bold numbers are treatment mean concentrations in $\mu\text{g}/\text{kg}$ dry weight)

Sediment Treatment and Mean Tissue Concentration ($\mu\text{g}/\text{kg}$ dry weight)	R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Naphthalene</u>	76 U ^(a)	65 U	97 U	69 U	69 U	77 U
O-C10	157	S ^(b)	S	- ^(c)	S	S
O-C9	181	S	S	S	S	S
O-C6	159	S	S	S	S	S
O-C5	166	S	S	S	S	S
O-C3	189	S	S	S	S	S
O-C1	198	S	S	S	S	S
<u>Fluoranthene</u>	76 U	149	97 U	69 U	69 U	77 U
O-C13	170	S	-	S	S	S
O-C8	163	S	-	S	S	S
<u>Pyrene</u>	139	233	97 U	69 U	69 U	77 U
O-C13	591	S	S	S	S	S
O-C11	350	S	-	S	S	S
O-C10	284	S	-	S	S	S
O-C8	336	S	-	S	S	S
O-C7	226	-	-	S	S	S
O-C6	228	-	-	S	S	S
I-C8	407	S	-	S	S	S
<u>Chrysene</u>	98	73	97 U	75	69 U	77 U
None are significantly different						
<u>Benzo(b)fluoranthene</u>	76 U	65 U	97 U	69 U	69 U	77 U
I-C8	125	S	S	-	S	S
<u>Phenanthrene</u>	76 U	86	97 U	69 U	69 U	77 U
None are significantly different						
<u>Benzo(a)anthracene</u>	76 U	65 U	97 U	69 U	69 U	77 U
None are significantly different						
<u>Benzo(a)pyrene</u>	76 U	65 U	97 U	72	69 U	77 U
None are significantly different						

(a) Undetected in all replicates; value is mean of detection limits.

(b) Significant ($\alpha = 0.1$).

(c) Not Significant ($\alpha = 0.1$).

Results of ANOVA and Dunn's Test comparing mean tissue concentrations and statistical groupings for individual compounds are presented in Volume 2, Appendix M, Tables M.1 through M.16. Though the results of the statistical tests were summarized in Table 3.20, the tables in Appendix M show the relationship of *M. nasuta* tissue PAH levels in each reference treatment to each other as well as to the test treatments.

3.5.2 Pesticide and PCB Bioaccumulation in *M. nasuta*

Complete *M. nasuta* tissue chemistry data in both wet and dry weight pesticide and PCB concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix K, Tables K.10 through K.19. Quality control criteria were met for all *M. nasuta* tissue pesticide and PCB analyses. Chlorinated pesticide and PCB analysis of *M. nasuta* tissue show that only one sample had a detected PCB and one sample had a detected pesticide value. Tissue sample O-C7 Rep 2 had a value of 167 µg/kg of aroclor 1254, and sample I-C2 Rep 2 had a value of 15 µg/kg of Aldrin. Both values were within the range of dry weight detection limits (undetected values) for all the samples, therefore, statistical comparisons were not performed.

3.5.3 Metals Bioaccumulation in *M. nasuta*

Complete *M. nasuta* tissue chemistry data in both wet and dry weight metals concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix K, Tables K.20 through K.22. Mean metals concentrations in the tissues of *M. nasuta*, expressed in mg/kg dry weight, are presented in Table 3.21. This table also shows the ratio of the highest concentration to the lowest for all treatments, to indicate the range of mean tissue values.

The results of Dunn's Test are summarized in Table 3.22. This table shows that all metals except As and Cd were accumulated in *M. nasuta* tissues from one or more sediment treatments relative to one or more reference treatments. Metals that showed significant *M. nasuta* bioaccumulation from several treatments relative to three or more reference treatments were Ag, Cr, Cu, Hg, and Pb. Arsenic and Cd showed no significant difference among sediment treatments when compared to reference sites, while Ni, Se, and Zn were elevated in tissues from only one treatment (O-C13, I-C8, and O-C5, respectively). The stations with significant metals bioaccumulation in *M. nasuta* tissues were O-C3, O-C1, I-C2, O-C2, I-C3, and I-C8. *M. nasuta* tissues from treatments O-C11, O-C9, and O-C7 showed no significant bioaccumulation of any metals relative to the six reference stations.

The results of individual ANOVA and Dunn's Tests with each metal are presented in Volume 2, Appendix M, Tables M.17 through M.36. These tables show *M. nasuta* metals levels

TABLE 3.21. Mean Metals Concentrations in Tissues of *M. nasuta* (mg/kg dry weight)

<u>Sediment Treatment</u>	<u>Ag</u>	<u>As</u>	<u>Cd</u>	<u>Cr</u>	<u>Cu</u>	<u>Hg</u>	<u>Ni</u>	<u>Pb</u>	<u>Se</u>	<u>Zn</u>
O-C13	0.289	19.0	0.23	1.93	17.3	0.052	9.54	1.82	1.40	90.4
O-C12	0.483	21.8	0.31	3.13	27.0	0.066	5.31	2.43	1.88	104.1
O-C11	0.274	16.8	0.19	1.67	14.5	0.045	2.70	1.30	1.47	70.8
O-C10	0.385	18.3	0.23	2.23	18.2	0.061	3.50	2.15	1.35	87.9
O-C9	0.411	18.5	0.26	1.97	17.9	0.056	3.61	1.46	1.51	92.6
O-C8	0.765	21.4	0.25	2.02	28.6	0.074	3.92	4.47	1.86	96.5
O-C7	0.443	18.5	0.21	1.66	20.2	0.063	4.62	2.45	1.63	88.8
O-C6	0.304	18.7	0.22	1.86	22.6	0.058	3.78	1.90	1.59	85.9
O-C5	0.793	22.1	0.33	2.01	24.9	0.072	4.77	3.42	1.41	113.0
O-C4	0.352	20.7	0.27	2.17	16.6	0.069	3.95	2.06	1.30	95.7
O-C3	1.208	22.6	0.33	1.85	36.4	0.142	3.91	4.27	1.65	82.6
O-C1	1.232	22.9	0.31	1.97	41.1	0.411	4.73	4.11	1.78	97.5
I-C2	1.238	20.9	0.37	1.81	42.1	0.231	2.92	4.25	1.94	112.5
O-C2	1.212	21.9	0.29	2.03	39.2	0.089	4.39	5.17	1.90	91.7
I-C3	1.446	23.1	0.38	1.34	46.7	0.147	3.70	4.12	2.00	102.3
I-C4	0.503	19.5	0.27	2.98	26.5	0.073	5.15	2.28	1.73	106.6
I-C8	1.400	23.3	0.33	1.92	48.0	0.165	3.71	4.26	2.30	104.7
I-T5	0.236	14.8	0.21	1.71	12.2	0.045	3.77	0.85	1.17	78.0
R-AC	0.972	23.7	0.30	1.13	31.1	0.083	2.57	3.49	1.92	122.5
R-AM	0.438	17.8	0.23	1.12	14.0	0.054	2.47	1.44	1.22	83.7
R-BF	0.407	21.9	0.25	1.79	14.5	0.059	4.04	1.61	1.49	90.3
R-OS	0.980	22.4	0.25	2.24	32.1	0.085	9.33	2.85	2.08	104.2
R-PC	0.458	19.6	0.24	1.09	21.8	0.063	2.69	2.23	1.95	91.6
R-PF	0.396	19.6	0.26	1.98	15.3	0.062	2.98	1.24	1.38	93.0
C-SB	0.564	20.7	0.26	1.66	23.9	0.073	3.94	2.17	1.66	91.0
C-NE	0.513	21.5	0.22	1.04	14.0	0.055	2.21	1.08	1.22	95.6
Ratio of Highest Concentration to Lowest	6	1.6	2	3	3.9	9.1	4.3	6	2	1.7

3.5.4 Butyltin Bioaccumulation in *M. nasuta*

Complete *M. nasuta* tissue chemistry data in both wet and dry weight butyltin concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix K, Tables K.23 and K.24. Mean butyltin concentrations (µg/kg dry weight) in *M. nasuta* tissues exposed to reference, control, and test treatments are summarized in Table 3.23. Tributyltin concentrations ranged from 10.4 µg/kg (R-PC) to 23.4 µg/kg (O-C9). Dibutyltin concentrations ranged from 4.9 µg/kg (I-C4) to 13.2 µg/kg (C-NE). Monobutyltin concentrations ranged from 2.2 µg/kg (O-C12) to 19 µg/kg (R-PF).

TABLE 3.22. Metals in Tissues of *M. nasuta* That Are Significantly Different From Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means (bold numbers are treatment mean concentrations in mg/kg dry weight)

Sediment Treatment and Mean Tissue Concentration (mg/kg dry weight)	R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Silver (Ag)</u>	0.972	0.438	0.407	0.980	0.458	0.396
O-C3	1.208	-(a)	-	S ^(b)	-	S
O-C1	1.232	-	-	S	-	S
I-C2	1.238	-	-	S	-	S
O-C2	1.212	-	-	S	-	S
I-C3	1.446	-	S	S	-	S
I-C8	1.400	-	S	S	-	S
<u>Arsenic (As)</u>	23.7	17.8	21.9	22.4	19.6	19.6
None are significantly different						
<u>Cadmium (Cd)</u>	0.30	0.23	0.25	0.25	0.24	0.26
None are significantly different						
<u>Chromium (Cr)</u>	1.13	1.12	1.79	2.24	1.09	1.98
O-C13	1.93	-	-	-	S	-
O-C12	3.13	S	S	-	S	-
O-C10	2.23	S	S	-	S	-
O-C8	2.02	S	S	-	S	-
O-C6	1.86	-	-	-	S	-
O-C5	2.01	S	S	-	S	-
O-C4	2.17	S	S	-	S	-
O-C3	1.85	-	-	-	S	-
O-C1	1.97	-	-	-	S	-
O-C2	2.03	S	S	-	S	-
I-C8	1.92	-	-	-	S	-
<u>Copper (Cu)</u>	31.1	14.0	14.5	32.1	21.8	15.3
O-C3	36.4	-	S	S	-	S
O-C1	41.1	-	S	S	-	S
I-C2	42.1	-	S	S	-	S
O-C2	39.2	-	S	S	-	S
I-C3	46.7	-	S	S	-	S
I-C8	48.0	-	S	S	-	S

TABLE 3.22. (contd)

Sediment Treatment and Mean Tissue Concentration (mg/kg dry weight)		R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Mercury (Hg)</u>	0.083	0.054	0.059	0.085	0.063	0.062	
O-C3	0.142	-	S	S	-	S	S
O-C1	0.411	S	S	S	S	S	S
I-C2	0.231	S	S	S	S	S	S
I-C3	0.147	-	S	S	-	S	S
I-C8	0.165	S	S	S	S	S	S
<u>Nickel (Ni)</u>	2.57	2.47	4.04	9.33	2.69	2.98	
O-C13	9.54	S	S	-	-	S	-
<u>Lead (Pb)</u>	3.49	1.44	1.61	2.85	2.23	1.24	
O-C8	4.47	-	S	-	-	-	S
O-C3	4.27	-	S	S	-	-	S
O-C1	4.11	-	S	-	-	-	S
I-C2	4.25	-	S	S	-	-	S
O-C2	5.17	-	S	S	-	S	S
I-C3	4.12	-	S	-	-	-	S
I-C8	4.26	-	S	S	-	-	S
<u>Selenium (Se)</u>	1.92	1.22	1.49	2.08	1.95	1.38	
I-C8	2.30	-	S	-	-	-	-
<u>Zinc (Zn)</u>	122.5	83.7	90.3	104.2	91.6	93.0	
O-C5	113.0	-	S	-	-	-	-

(a) Not significant ($\alpha = 0.1$).

(b) Significant ($\alpha = 0.1$).

Table 3.24 summarizes the results of Dunn's Test for butyltin concentrations in *M. nasuta*. Tributyltin was significantly greater in test sediments O-C11, O-C10, O-C9, O-C8, O-C7, O-C5, O-C4, O-C1, and I-C8 relative to reference stations R-AC, R-OS, R-PC and R-PF. There were no significant elevations of dibutyltin and monobutyltin in test treatments when compared to reference treatments. The ANOVA and Dunn's Test results for comparison of mean butyltins in *M. nasuta* tissues are presented in Volume 2, Appendix M, Tables M.37 through M.42. These tables show the relationship of reference treatments to each other as well as to test treatments.

TABLE 3.23. Mean Butyltin Concentrations in Tissues of *M. nasuta* (µg/kg dry weight)

<u>Sediment Treatment</u>	<u>Tributyltin</u>	<u>Dibutyltin</u>	<u>Monobutyltin</u>
O-C13	11.8	6.9	2.5
O-C12	12.4	6.0	2.2
O-C11	20.3	11.4	3.3
O-C10	22.3	10.5	3.2
O-C9	23.4	9.8	5.9
O-C8	18.0	9.0	7.2
O-C7	16.7	9.5	9.5
O-C6	13.8	7.7	2.8
O-C5	16.7	9.1	3.3
O-C4	17.5	10.9	6.1
O-C3	11.0	8.4	5.4
O-C1	15.9	6.8	4.2
I-C2	14.9	7.8	3.9
O-C2	13.3	12.6	4.5
I-C3	11.1	7.1	3.1
I-C4	14.1	4.9	2.5
I-C8	18.4	8.5	4.7
I-T5	14.8	8.6	2.7
R-AC	14.7	7.2	4.1
R-AM	18.4	10.3	5.2
R-BF	19.9	12.9	5.2
R-OS	11.9	16.0	3.5
R-PC	10.4	8.3	2.7
R-PF	15.3	9.3	19.0
C-SB	14.1	7.8	3.3
C-NE	17.5	13.2	3.5

3.5.5 Polynuclear Aromatic Hydrocarbons Bioaccumulation in *N. caecoides*

Complete *N. caecoides* tissue chemistry data in both wet and dry weight PAH concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix L, Tables L.1 through L.9. Quality control criteria were met for all *N. caecoides* PAH analyses. Twelve of the 16 PAH compounds were detected in *N. caecoides* tissue samples. The mean concentrations (µg/kg dry weight) of PAHs present in the tissues of *N. caecoides* are presented in Table 3.25. Statistical comparisons were performed only on those PAH compounds that were detected.

The results of Dunn's Test are summarized in Table 3.26. This table shows that only three PAHs, naphthalene, fluoranthene, and pyrene were significantly elevated in *N. caecoides* tissues from at least one test treatment relative to one or more reference treatments. Naphthalene is a low molecular weight PAH, while pyrene and fluoranthene are HPAHs. Pyrene showed the

TABLE 3.24. Butyltins in Tissues of *M. nasuta* That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means (bold numbers are treatment mean concentrations in $\mu\text{g}/\text{kg}$ dry weight)

Sediment Treatment and Mean Tissue Concentration ($\mu\text{g}/\text{kg}$ dry weight)	R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
Tributyltin	14.7	18.4	19.9	11.9	10.4	15.3
O-C11	20.3	-(a)	-	S(b)	S	-
O-C10	22.3	-	-	S	S	-
O-C9	23.4	S	-	S	S	S
O-C8	18.0	-	-	S	S	-
O-C7	16.7	-	-	-	S	-
O-C5	16.7	-	-	-	S	-
O-C4	17.5	-	-	-	S	-
O-C1	15.9	-	-	-	S	-
I-C8	18.4	-	-	S	S	-
Dibutyltin	7.2	10.3	12.9	16.0	8.3	9.3
None are significantly different						
Monobutyltin	4.1	5.2	5.2	3.5	2.7	19.0
None are significantly different						

(a) Not significant ($\alpha = 0.1$).

(b) Significant ($\alpha = 0.1$).

highest incidence of bioaccumulation in *N. caecoides*, bioaccumulating from five treatments relative to at least four reference treatments (Table 3.26). *N. caecoides* exposed to sediment treatments O-C12, O-C9, O-C7, O-C6, O-C5, O-C4, O-C3, O-C1, I-C2, I-C3, I-C4 and I-T5 showed no significant bioaccumulation of any PAH.

Results of ANOVA and Dunn's Tests comparing mean *N. caecoides* tissue concentrations and statistical groupings for individual PAHs are presented in Volume 2, Appendix N, Tables N.1 through N.14. These tables show the relationship of *N. caecoides* tissue levels in each reference treatment to each other as well as to the test treatments.

3.5.6 Pesticide and PCB Bioaccumulation in *N. caecoides*

Complete *N. caecoides* tissue chemistry data in both wet and dry weight pesticide and PCB concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix L, Tables L.10 through L.19. Chlorinated pesticide and PCB analysis of *N. caecoides* tissue showed that PCBs were undetected in all the samples and that only two samples had detected

TABLE 3.25. Mean PAH Concentrations in Tissues of *N. coccoides* (µg/kg dry weight)

PHASE II B

(a) Undetected in all replicates; value is mean of dry weight detection limits.

TABLE 3.26. PAHs in Tissues of *N. caecoides* That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means (bold numbers are treatment mean concentrations in $\mu\text{g}/\text{kg}$ dry weight)

Sediment Treatment and Mean Tissue Concentration ($\mu\text{g}/\text{kg}$ dry weight)		R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Naphthalene</u>		185	302 U	310 U	167 U	166 U	340 U
O-C2	379	S	-(a)	-	S(b)	S	-
I-C8	289	-	-	-	S	-	-
<u>Acenaphthene</u>		58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different							
<u>Fluorene</u>		58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different							
<u>Phenanthrene</u>		58 U	69	62 U	63 U	55 U	68 U
None are significantly different							
<u>Anthracene</u>		58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different							
<u>Fluoranthene</u>		58 U	60 U	62 U	63 U	55 U	68 U
O-C13	208	S	S	S	S	S	-
I-C8	162	-	-	-	-	S	-
<u>Pyrene</u>		159	109	62 U	63 U	55 U	68 U
O-C13	580	S	S	S	S	S	S
O-C11	307	-	S	S	S	S	S
O-C10	229	-	-	S	S	S	S
O-C8	225	-	-	S	S	S	S
I-C8	205	-	-	S	S	S	S
<u>Benzo(a)anthracene</u>		58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different							
<u>Chrysene</u>		58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different							

TABLE 3.26. (contd)

Sediment Treatment and Mean Tissue Concentration ($\mu\text{g}/\text{kg}$ dry weight)	R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Benzo(b)fluoranthene</u>	58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different						
<u>Benzo(k)fluoranthene</u>	58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different						
<u>Benzo(a)pyrene</u>	58 U	60 U	62 U	63 U	55 U	68 U
None are significantly different						
(a) Not significant ($\alpha = 0.1$).						
(b) Significant ($\alpha = 0.1$).						

pesticide values. Both values were well within the range of dry weight detection limits (undetected values) for all the samples, therefore statistical comparisons were not performed. Those two samples were I-T5 Rep 3 (62 $\mu\text{g}/\text{kg}$ of Beta-BHC) and R-BF Rep 5 (77 $\mu\text{g}/\text{kg}$ of Beta-BHC).

3.5.7 Metals Bioaccumulation in *N. caecoides*

Complete *N. caecoides* tissue chemistry data in both wet and dry weight metals concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix L, Tables L.20 through L.22. Mean metals concentrations in the tissues of *N. caecoides*, expressed in mg/kg dry weight, are presented in Table 3.27. This table also shows the ratio of the highest concentration to the lowest for all treatments, to indicate the range of mean tissue values. The mean concentrations of most metals varied by a factor of less than 2.5. Four metals had larger concentration ranges in *N. caecoides* tissues: Ag varied by a factor of 15, Cr by a factor of 23.5, Hg by a factor of 6.5, and Pb by a factor of 29.5. These factors of variation were generally the result of a few high values (Table 3.27), rather than truly variable mean values. It should be noted that *N. caecoides* metals data were developed from no more than three tissue replicates because of the very limited tissue mass available for analysis. The original five replicates from each treatment were composited, then analyzed in triplicate (except for O-C13, R-OS, and C-SB, which were analyzed in duplicate). The variability between replicates is lost by compositing. This becomes important in the statistical comparisons, where low variance leads to more confidence about a mean and its difference from another mean.

Table 3.27. Mean Metals Concentrations in the Tissues of *N. caecoides* (µg/kg dry weight)

Sediment Treatment	<u>Ag</u>	<u>As</u>	<u>Cd</u>	<u>Cr</u>	<u>Cu</u>	<u>Hg</u>	<u>Ni</u>	<u>Pb</u>	<u>Se</u>	<u>Zn</u>
O-C13	0.046	27.3	1.85	0.471	15.3	0.029	4.4	1.38	1.66	163.3
O-C12	0.041	21.3	1.96	0.454	13.2	0.041	1.9	1.03	1.84	178.8
O-C11	0.042	26.5	2.03	0.485	14.5	0.035	2.3	1.29	1.79	165.4
O-C10	0.085	28.3	1.74	1.413	14.8	0.042	2.1	2.41	1.73	174.5
O-C9	0.064	18.3	1.85	0.390	11.4	0.060	2.6	0.35	1.78	170.2
O-C8	0.033	31.8	1.77	2.939	12.6	0.049	2.3	1.06	2.06	171.9
O-C7	0.028	28.4	1.75	0.313	11.0	0.048	2.5	1.25	2.02	172.2
O-C6	0.048	27.8	2.07	0.347	13.6	0.029	2.4	1.14	2.01	176.5
O-C5	0.083	15.6	1.45	0.332	13.2	0.060	3.3	0.20	2.02	154.8
O-C4	0.209	23.8	1.74	3.950	13.7	0.188	3.7	6.00	1.95	168.9
O-C3	0.017	19.0	1.32	0.168	9.7	0.088	3.4	0.36	1.65	160.4
O-C1	0.014	24.3	1.18	0.288	7.8	0.046	4.0	0.45	1.56	169.6
I-C2	0.024	19.2	1.27	0.453	9.1	0.057	3.0	0.89	1.80	163.4
O-C2	0.019	18.3	1.21	0.243	10.9	0.042	2.9	0.52	1.74	152.3
I-C3	0.034	16.3	1.10	0.918	10.7	0.050	2.9	0.38	1.74	159.3
I-C4	0.043	24.8	1.89	0.235	13.8	0.046	2.0 U ^(a)	1.08	1.74	166.8
I-C8	0.021	17.7	1.36	0.404	9.2	0.050	3.4	0.45	1.67	160.8
I-T5	0.083	17.9	2.10	0.465	14.7	0.041	3.8	0.53	1.51	166.2
R-AC	0.081	17.3	1.47	0.309	15.1	0.051	2.8	0.77	1.74	174.6
R-AM	0.079	18.9	1.36	0.362	13.3	0.063	2.8	0.63	1.95	191.5
R-BF	0.044	25.6	1.52	0.526	14.7	0.070	2.4	1.29	2.00	195.9
R-OS	0.035	16.2	1.56	0.212	10.5	0.064	2.5	0.54	1.83	162.6
R-PC	0.042	17.0	1.47	1.501	13.6	0.067	2.2	0.84	1.67	169.4
R-PF	0.037	19.5	1.35	0.337	11.9	0.072	2.5	0.55	2.02	168.9
C-SB	0.033	20.0	1.38	0.226	12.5	0.047	2.3	0.56	1.77	156.1
C-NE	0.049	21.1	1.46	0.246	13.1	0.109	2.2	0.53	1.89	182.3
Ratio of Highest Concentration to Lowest	15.0	2.0	1.9	23.5	2.0	6.5	2.3	29.5	1.4	1.3

(a) Undetected in all replicates; value is mean of dry weight detection limits.

The results of the Dunn Test are summarized in Table 3.28. This table shows that all metals except Ni and Se were accumulated in *N. caecoides* tissues from one or more sediment treatments relative to one or more reference treatments. Copper was accumulated in seven treatments relative to R-OS and in one treatment relative to R-PF, while Zn was accumulated in only one treatment relative to R-OS. Metals that showed significant *N. caecoides* bioaccumulation from several treatments relative to three or more reference treatments were Ag, As, Cd, Cr, Hg, and Pb (Table 3.28). Inspection of replicate data for As, Cd, and Cu revealed extremely low variability between

TABLE 3.28. Metals in Tissues of *N. caecoides* That Are Significantly Different from Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means (bold numbers are treatment mean concentrations in mg/kg dry weight)

Sediment Treatment and Mean Tissue Concentration (mg/kg dry weight)	R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Silver (Ag)</u>	0.081	0.079	0.044	0.035	0.042	0.037
O-C10 0.085	- ^(a)	-	S ^(b)	S	S	S
O-C9 0.064	-	-	S	S	S	S
O-C5 0.083	-	-	S	S	S	S
O-C4 0.209	S	S	S	S	S	S
I-T5 0.083	-	-	S	S	S	S
<u>Arsenic (As)</u>	17.3	18.9	25.6	16.2	17.0	19.5
O-C13 27.3	S	S	-	S	S	S
O-C12 21.3	S	S	-	S	S	-
O-C11 26.5	S	S	-	S	S	S
O-C10 28.3	S	S	S	S	S	S
O-C9 18.3	-	-	-	S	-	-
O-C8 31.8	S	S	S	S	S	S
O-C7 28.4	S	S	S	S	S	S
O-C6 27.8	S	S	-	S	S	S
O-C4 23.8	S	S	-	S	S	S
O-C3 19.0	-	-	-	S	S	-
O-C1 24.3	S	S	-	S	S	S
I-C2 19.2	S	-	-	S	S	-
O-C2 18.3	-	-	-	S	-	-
I-C4 24.8	S	S	-	S	S	S
<u>Cadmium (Cd)</u>	1.47	1.36	1.52	1.56	1.47	1.35
O-C13 1.85	S	S	S	-	S	S
O-C12 1.96	S	S	S	S	S	S
O-C11 2.03	S	S	S	S	S	S
O-C10 1.74	-	S	-	-	-	S
O-C9 1.85	S	S	S	-	S	S
O-C8 1.77	-	S	-	-	S	S
O-C7 1.75	-	S	-	-	-	S
O-C6 2.07	S	S	S	S	S	S
O-C4 1.74	-	S	-	-	-	S
I-C4 1.89	S	S	S	S	S	S
I-T5 2.10	S	S	S	S	S	S
<u>Chromium (Cr)</u>	0.309	0.362	0.526	0.212	1.501	0.337
O-C10 1.413	-	-	-	S	-	-
O-C4 3.950	S	S	S	S	-	S

TABLE 3.28. (contd)

Sediment Treatment and Mean Tissue Concentration (mg/kg dry weight)	R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Copper (Cu)</u>	15.1	13.3	14.7	10.5	13.6	11.9
O-C13	15.3	-	-	S	-	S
O-C11	14.5	-	-	S	-	-
O-C10	14.8	-	-	S	-	-
O-C6	13.6	-	-	S	-	-
O-C4	13.7	-	-	S	-	-
I-C4	13.8	-	-	S	-	-
I-T5	14.7	-	-	S	-	-
<u>Mercury (Hg)</u>	0.051	0.063	0.070	0.064	0.067	0.072
O-C4	0.188	S	S	S	S	S
O-C3	0.088	S	S	-	S	-
<u>Nickel (Ni)</u>	2.8	2.8	2.4	2.5	2.2	2.5
None are significantly different						
<u>Lead (Pb)</u>	0.77	0.63	1.29	0.54	0.84	0.55
O-C13	1.38	S	S	-	S	-
O-C11	1.29	-	S	-	S	-
O-C10	2.41	S	S	-	S	S
O-C8	1.06	-	-	S	-	S
O-C7	1.25	-	S	-	S	-
O-C6	1.14	-	-	S	-	S
O-C4	6.00	S	S	S	S	S
I-C4	1.08	-	-	-	S	-
<u>Selenium (Se)</u>	1.74	1.95	2.00	1.83	1.67	2.02
None are significantly different						
<u>Zinc (Zn)</u>	174.6	191.5	195.9	162.6	169.4	168.9
O-C12	178.8	-	-	S	-	-

(a) Not significant ($\alpha = 0.1$).(b) Significant ($\alpha = 0.1$).

replicates of each treatment, resulting in statistically significant differences with only minor differences in mean tissue concentrations (Volume 2, Appendix L, Table L.20).

Sediment from stations I-C8 and I-C3 showed no significant bioaccumulation into *N. caecoides* tissues for any metal relative to the six reference treatments. Only two treatments, O-C3 and O-C4, had mean Hg levels greater than reference. In O-C4, Hg levels were significantly greater than all six references. In O-C3, Hg levels were significantly greater than the offshore reference treatments R-AC, R-AM, and R-OS. Significant metals bioaccumulation was most consistently observed among *N. caecoides* exposed to sediment from treatment O-C4, where Ag, Hg, and Pb were elevated relative to all reference treatments and Cr was elevated relative to all but R-PC. Most determinations of significant metals bioaccumulation in *N. caecoides* were relative to two reference treatments, R-OS and R-PF.

Mean tissue concentrations are shown with the ANOVA and Dunn's Test results for each metal in Volume 2, Appendix N, Tables N.15 through N.32. These tables show *N. caecoides* metals levels in the reference treatments relative to each other and to the test treatments. The statistical groupings show treatments that were similar as well as those that were different.

3.5.8 Butyltin Bioaccumulation in *N. caecoides*

Complete *N. caecoides* tissue chemistry data in both wet and dry weight butyltin concentrations, quality control data, and quality control summaries are contained in Volume 2, Appendix L, Tables L.23 and L.24. Mean butyltin concentrations ($\mu\text{g}/\text{kg}$ dry weight) in *N. caecoides* tissues exposed to test, reference, and control treatments are summarized in Table 3.29. Tributyltin was detected in nine treatments (seven test, two reference), dibutyltin was detected in nine treatments (five test, three reference, one control), and monobutyltin was detected in only four treatments (two test, one reference, one control). The three butyltin species were never detected together in any one treatment.

Table 3.30 presents the results of Dunn's Test for butyltin concentrations in *N. caecoides*. This table shows that no test sediments had significantly higher concentrations of butyltins compared to any of the reference sediments. The ANOVA and Dunn's Test results for comparison of mean butyltins in *N. caecoides* tissues are presented in Volume 2, Appendix N, Tables N.35 through N.40. These tables show how the reference treatments compare to each other as well as to test treatments.

TABLE 3.29. Mean Butyltins Concentrations in Tissues of *N. caecoides* ($\mu\text{g}/\text{kg}$ dry weight)

<u>Sediment Treatment</u>	<u>Tributyltin</u>	<u>Dibutyltin</u>	<u>Monobutyltin</u>
O-C13	121.5 U ^(a)	60.0	53.1
O-C12	86.5 UJ ^(b)	41.1 UJ	43.3 U
O-C11	837.1	62.1	39.1 UJ
O-C10	56.9	46.6 U	42.4 U
O-C9	119.9	47.5 U	43.2 U
O-C8	117.2 U	55.3 U	47.4 UJ
O-C7	100.6 U	47.5 U	43.2 U
O-C6	97.7 U	46.0 U	41.9 U
O-C5	107.8 U	50.8 U	46.2 U
O-C4	92.2 U	89.9	39.6 U
O-C3	138.2	93.4	49.0 U
O-C1	79.1	55.5 U	50.5 U
I-C2	114.1 U	96.1	51.8
O-C2	120.7 U	56.9 U	51.8 U
I-C3	115.0 U	54.2 U	49.3 U
I-C4	98.5	59.6 U	54.2 U
I-C8	119.9 U	56.5 U	51.4 U
I-T5	87.8	43.7 UJ	34.3 UJ
R-AC	128.4	52.0 U	47.3 U
R-AM	114.9 U	164.3	49.3 U
R-BF	117.8 U	159.5	50.5 U
R-OS	154.2	62.1 U	57.5
R-PC	65.7 UJ	48.3 U	43.9 U
R-PF	128.0 U	140.2	54.9 U
C-SB	119.8 U	56.5 U	52.6
C-NE	106.9 U	135.4	45.8 U

(a) Undetected in all replicates; concentration is mean of dry weight detection limits.

(b) Undetected or detected below method detection limits in all replicates; concentration is mean of dry weight detection limits and detected values.

TABLE 3.30. Butyltins in Tissues of *N. caecoides* That Are Significantly Different From Reference Tissue Concentrations Using Dunn's Test for Comparison of All Means (bold numbers are treatment mean concentrations in $\mu\text{g}/\text{kg}$ dry weight)

Sediment Treatment and Mean Tissue Concentration ($\mu\text{g}/\text{kg}$ dry weight)	R-AC	R-AM	R-BF	R-OS	R-PC	R-PF
<u>Tributyltin</u>	128.4	114.9 U	117.8 U	154.2	65.7 U	128.0 U
None are significantly different						
<u>Dibutyltin</u>	52.0 U	164.3	159.5	62.1 U	48.3 U	140.2
None are significantly different						
<u>Monobutyltin</u>	47.3 U	49.3 U	50.5 U	57.5	43.9 U	54.9 U
None are significantly different						

4.0 DISCUSSION AND CONCLUSIONS

4.1 GEOLOGIC EVALUATIONS

Geologic evaluations were performed on sediment cores that represent the material proposed for dredging. This evaluation permits determination of the proportion of Older Bay Mud (OBM) and Younger Bay Mud (YBM) present within dredging prisms and allows USACE to estimate the quantity of material that may be suitable or unsuitable for unconfined open-water disposal. Mudline elevations ranged from -26.6 to -41.1 ft MLLW, resulting in core lengths of 2.9 to 17.4 ft (Table 4.1). Table 4.1 shows that at most locations, YBM was the predominant material from the mudline to -44 ft MLLW. The exceptions were at stations O-C12 and O-C5, where the OBM depth was equal to or greater than the YBM depth. The YBM was generally composed of clay or silty clay, although at stations O-C7 and I-C2 the YBM was predominantly silt. The OBM was identified at depths ranging from -39.7 to -43.6 ft MLLW at seven sites, where it was usually sand (O-C11, O-C10, O-C5, O-C3) or clay (O-C13, O-C12, O-C4).

4.2 SEDIMENT CHEMISTRY

According to the Draft Implementation Manual, sediment chemistry results are intended to provide information about chemicals that could potentially cause toxicity or be bioaccumulated. Sediment chemistry results are to be used in conjunction with toxicity tests and bioaccumulation results in order to evaluate appropriate disposal options. In general, Oakland Outer Harbor sediments had relatively low contaminant levels. Where contaminant levels were higher they were often associated with a higher proportion of fine-grained sediment (Younger Bay Mud) and higher levels of other conventional parameters (TOC, TVS, oil and grease, and TPH).

In Section 3.3, contaminant concentrations in Phase III B test treatments were graphically depicted and compared to the six reference treatments. Test treatments O-C7 and O-C8 had metal and organic contaminant loads that were consistently higher than all references. Test treatments O-C6, O-C13, and I-C2 were also consistently higher than reference except when comparing the metals load to R-BF. The anthropogenic metals silver, copper, mercury, lead, and to some extent zinc, appeared to be elevated in those test treatments relative to all reference except R-BF. Of the organic contaminants, chlorinated pesticides were not detected and PCBs were present at very low levels. Butyltins were detected in sediments at very low concentrations; nearly all values were below the target detection limit for the project. Both low and high molecular weight PAH compounds were detected in most sediment samples. However, the achieved detection limits were

TABLE 4.1. Summary of Geologic Descriptions

<u>Sediment Treatment</u>	<u>MLLW Depth (ft)</u>	<u>Sediment Thickness, ft</u>		<u>Physical Description (to -44 ft)</u>
		<u>YBM^(a)</u>	<u>OBM^(b)</u>	
O-C13	34.4	6.1	3.5	YBM is silty clay, OBM is clay
O-C12	39.0	1.0	4.0	YBM and OBM are clay
O-C11	35.6	7.4	1.0	YBM is clay, OBM is sand
O-C10	38.3	3.0	2.7	YBM is silty clay, OBM is sand
O-C9	41.1	2.9	0.0	Upper 1.0 ft silty sand, lower sand
O-C8	37.7	6.3	0.0	Silty clay to clayey silt
O-C7	27.6	16.4	0.0	Silt
O-C6	29.4	14.6	0.0	Silty clay to clayey silt
O-C5	38.2	1.5	4.3	YBM is clay, OBM is sand
O-C4	36.3	7.3	0.4	Clay with shell fragments
O-C3	33.4	8.1	2.5	YBM is silty clay, OBM is sand
O-C1	38.1	5.9	0.0	Clay, worm burrows present
I-C2	37.2	6.8	0.0	Silt, worm tubes present
O-C2	26.6	17.4	0.0	Clay, gas voids, shells near bottom
I-C3	35.7	8.3	0.0	Upper 1.5 ft silt, lower sandy silt
I-C8	37.7	6.3	0.0	Upper 2.0 ft silt, lower sand

(a) Younger Bay Mud.

(b) Older Bay Mud.

substantially lower than the target detection limits, and the total PAH concentrations in the sediment were relatively low. Only four treatments had total PAH loads greater than 1 part per million (1000 $\mu\text{g/kg}$) dry weight: O-C13, O-C4, I-C8 top 6 in., and the Alcatraz Environs reference R-AM. Treatments that approached 1 part per million total PAH were O-C6, O-C7, O-C8, I-C2, and BC-6 (a composite of cores O-C1, O-C3, and I-C2).

4.3 TOXICOLOGY AND BIOACCUMULATION

Toxicology and bioaccumulation results are of primary importance in the characterization of sediment proposed for dredging. The Oakland Harbor Phase III B solid phase test treatments that produced significant acute toxicity or bioaccumulation relative to the six reference treatments are

discussed in this section. For the purposes of this discussion, "significant" acute toxicity means that the solid phase test material does not comply with the benthic bioassay criteria for ocean disposal [CFR40, Section 227.13(c)]. By examining the results of the toxicity and bioaccumulation evaluations of test treatments compared to each reference, USACE will be better able to assess disposal options for Oakland Outer Harbor dredged material. The results of the SPP tests are not included in this discussion because none of the sediment composites tested resulted in any acute toxicity.

Although the results of these comparisons vary, depending on the choice of reference treatment, a number of observations were consistent between reference comparisons. First, acute toxicity was noted only in two of the five solid-phase tests: the 10-day *R. abronius* test and the 10-day *N. caecoides* test. No significant acute toxicity was noted in the 10-day *C. stigmaeus* test. Second, only three PAHs figured prominently in *M. nasuta* and *N. caecoides* bioaccumulation, regardless of which reference the test treatments were compared to. Bioaccumulated PAHs included the LPAH naphthalene and the HPAHs fluoranthene and pyrene. Though several other HPAHs were detected, they were not significantly bioaccumulated in test organisms exposed to test sediment relative to any reference.

Another observation that was consistent regardless of reference comparison was the difference in metals bioaccumulation between the two species tested. In *M. nasuta*, metals bioaccumulation was due primarily to the presence of Ag, Cu, Hg, and Pb while metals bioaccumulation in *N. caecoides* was due primarily to the presence Ag, As, Cd, and Pb. Bioaccumulation results for metals, especially As and Cd, in the tissues of *N. caecoides* seemed to be influenced primarily by the compositing of the tissue from a treatment into three rather than five replicates. This procedure was necessary to provide adequate tissue mass for analysis, but it resulted in relatively low variance between replicates. The low variance between *N. caecoides* replicate values resulted in numerous significant differences between test and reference treatments, especially when treatments had similar mean values. In the following summaries, bold type is used to distinguish metals with mean treatment values more than twice the mean reference value. The bioaccumulation of the other listed metals by *N. caecoides* in test treatments relative to reference may be overestimated and should be viewed carefully when evaluating the data for unconfined open-water disposal. Mean concentrations of metals in *N. caecoides* tissues are listed in Table 3.27; concentrations of metals in each *N. caecoides* sample are listed in Volume 2, Appendix L, Tables L.20 and L.21 (wet weight).

4.3.1 Comparison of Test Treatments to the Reference Sediment R-AC

Table 4.2 summarizes the results of acute toxicity and bioaccumulation tests for each test treatment relative to the reference R-AC (Alcatraz Island Disposal Site). This table shows that significant acute toxicity to at least one species was observed in 8 of 16 test treatments relative to R-AC: O-C13, O-12, O-C11, O-C9, O-C7, O-C1, I-C2, and O-C2. Treatment O-C1 was acutely toxic to both *N. caecoides* and *R. abronius*. The results of the 28-day *M. nasuta* exposure indicated significant bioaccumulation of PAHs in all test treatments except O-C12, O-C7, O-C4, I-C2, O-C2, and I-C3. *M. nasuta* significantly bioaccumulated tributyltin only in test treatment O-C9, but the mean TBT value was less than twice that of R-AC. *M. nasuta* bioaccumulated at least one metal from most test treatments; however, only Ni in O-C13, Cr in O-C12, and Hg in O-C1 and I-C2 were more than twice the respective levels in R-AC (see Table 3.22). *N. caecoides* exposures showed only two cases of PAH bioaccumulation (O-C13 and O-C2), no butyltin bioaccumulation, and numerous incidences of metals bioaccumulation. Metals bioaccumulation was probably related to the compositing procedure employed as there were relatively few cases where the treatment mean was more than twice the reference mean. One test treatment, I-C3, produced no incidences of acute toxicity or bioaccumulation relative to R-AC; Phase III A treatments I-C4 and I-T5 showed bioaccumulation of only one or two metals in the tissues of *N. caecoides*. Pesticides and PCBs were not bioaccumulated by either *M. nasuta* or *N. caecoides*. A total of 58 toxicological and bioaccumulation hits were observed relative to R-AC, where a hit is defined as a significant occurrence of test organism mortality or significant bioaccumulation of LPAH, HPAH, a butyltin, and any of the 10 metals measured. This was the second lowest total number of hits among the six references compared in this study.

4.3.2 Comparison of Test Treatments to the Reference Sediment R-AM

Table 4.3 summarizes the results of acute toxicity and bioaccumulation tests for each test treatment relative to the reference R-AM (Alcatraz Island Environs). Significant acute toxicity was noted in 7 of 16 test treatments relative to R-AM: O-C13, O-C12, O-C9, O-C7, O-C1, I-C2, and O-C2. The *M. nasuta* bioaccumulation exposures showed elevated PAH in half of the test treatments, no evidence of butyltin bioaccumulation, and metals bioaccumulation in all test treatments except O-C11, O-C9, O-C7, and O-C6. In most treatments, Cr, Zn, and Se levels were not more than twice the reference levels. The results of the *N. caecoides* exposures showed PAH bioaccumulation only in test treatments O-C13 and O-C11. *N. caecoides* showed numerous incidences of metals bioaccumulation; again, relatively few treatment means were more than twice the reference mean. Pesticides, PCBs, and butyltins were not bioaccumulated by either *M. nasuta*

PHASE III B

TABLE 4.2. Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Alcatraz Disposal Site Reference R-AC (bold italic type indicates *N. caecoides* treatment mean value is more than twice the reference mean value for metal)

Sediment Treatment	Acute Toxicity	<i>M. nasuta</i> Bioaccumulation				<i>N. caecoides</i> Bioaccumulation			
		PAH	Pest/PCB	Metals	Butyltins	PAH	Pest/PCB	Metals	Butyltins
O-C13	Nep 10 ^(a)	HPAH ^(b)	- ^(c)	Ni	-	HPAH	-	As ^(d) , Cd ^(d) , Pb	-
O-C12	Rhe 10 ^(c)	-	Cr	-	-	-	-	As, Cd	-
O-C11	Nep 10	HPAH	-	-	-	-	-	As, Cd	-
O-C10	-	LPAH ^(f) , HPAH	-	Cr	-	-	-	As, Pb	-
O-C9	Rhe 10	LPAH	-	-	TBT	-	-	Cd	-
O-C8	-	HPAH	-	Cr	-	-	-	As	-
O-C7	Nep 10	-	-	-	-	-	-	As	-
O-C6	-	LPAH	-	Cr	-	-	-	As, Cd	-
O-C5	-	LPAH	-	Cr	-	-	-	-	-
O-C4	-	-	-	Cr	-	-	-	Ag, As, Cr, Hg, Pb	-
O-C3	-	LPAH	-	-	-	-	-	Hg	-
O-C1	Nep 10, Rhe 10	LPAH	-	Hg	-	-	-	As	-
I-C2	Rhe 10	-	-	Hg	-	-	-	As	-
O-C2	Rhe 10	-	-	Cr	-	-	-	-	-
I-C3	-	-	-	-	-	-	-	-	-
I-C8	-	HPAH	-	Hg	-	-	-	-	-
I-C4	NA ^(g)	NA	NA	NA	NA	NA	NA	As, Cd	-
I-T5	NA	NA	NA	NA	NA	NA	NA	Cd	-

(a) Nep 10 is 10-day flow-through test with *N. caecoides*.

(b) HPAH is high molecular weight PAHs fluoranthene and/or pyrene.

(c) No significant difference from reference.

(d) Arsenic and cadmium bioaccumulation probably overestimated as a result of *N. caecoides* tissue compositing.

(e) Rhe 10 is 10-day static test with *R. abronius*.

(f) LPAH is low molecular weight PAH naphthalene.

(g) Not applicable, not tested.

TABLE 4.3: Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Alcatraz Island Environ Reference R-AM (bold italic type indicates *N. caecoides* treatment mean value is more than twice the reference mean value for metal)

PHASE III E

Sediment Treatment	Acute Toxicity	<i>M. nebulosa</i> Bioaccumulation				<i>N. caecoides</i> Bioaccumulation			
		PAH		Metals		PAH		Metals	
		Pes/PCB	PAH	Metals	Butylins	Pes/PCB	PAH	Metals	Butylins
O-C13	Nep 10 ^(a)	HPAH ^(b)	-	Ni	-	HPAH	-	As ^(d) , Cd ^(d) , Pb	-
O-C12	Rhc 10 ^(c)	-	-	Cr	-	-	-	As, Cd	-
O-C11	-	-	-	Cr	-	HPAH	-	As, Cd, Pb	-
O-C10	-	LPAH ^(f)	-	-	-	-	-	As, Cd, Pb	-
O-C9	Rhe 10	LPAH	-	-	-	-	-	Cd	-
O-C8	-	-	-	Cr, Pb	-	-	-	As, Cd, Cr	-
O-C7	Nep 10	-	-	-	-	-	-	As, Cd, Pb	-
O-C6	-	LPAH	-	-	-	-	-	As, Cd	-
O-C5	-	LPAH	-	-	-	-	-	-	-
O-C4	-	-	-	Cr, Zn	-	-	-	-	-
O-C3	-	LPAH	-	Cr	-	-	-	-	-
O-C1	Rhc 10	LPAH	-	Cu, Hg, Pb	-	-	-	-	-
I-C2	Rhe 10	-	-	Cu, Hg, Pb	-	-	-	-	-
O-C2	Rhe 10	-	-	Cu, Hg, Pb	-	-	-	-	-
I-C3	-	-	-	Cr, Cu, Pb	-	-	-	-	-
I-C8	-	-	-	Ag, Cu, Hg, Pb	-	-	-	-	-
I-C4	NA ^(g)	HPAH	-	Ag, Cu, Hg, Pb, Se	-	-	-	-	-
I-T5	NA	NA	NA	NA	NA	NA	NA	As, Cd	-
								Cd	-

(a) Nep 10 is 10-day flow-through test with *N. caecoides*.

(b) HPAH is high molecular weight PAHs fluoranthene and/or pyrene.

(c) No significant difference from reference.

(d) Arsenic and cadmium bioaccumulation probably overestimated as a result of *N. caecoides* tissue compositing.

(e) Rhe 10 is 10-day static test with *R. abronius*.

(f) LPAH is low molecular weight PAH naphthalene.

(g) Not applicable; not tested.

or *N. caecoides*. Test treatments O-C8, O-C4 and I-C3 showed no evidence of acute toxicity and bioaccumulation of metals only. *M. nasuta* and *N. caecoides* accumulated metals from different sets of test treatments. The reason for this difference is not known, although it may be related to the compositing procedure used to produce adequate *N. caecoides* tissue mass for analysis. Comparison of test treatment acute toxicity and bioaccumulation to the reference R-AM produced a total of 76 hits, the third highest number of hits among the six references compared.

4.3.3 Comparison of Test Treatments to the Reference Sediment R-BF

Table 4.4 summarizes the results of acute toxicity and bioaccumulation tests for each test treatment relative to the reference R-BF (Bay Farm Borrow Area). This table shows that there was significant acute toxicity only in O-C1 relative to R-BF. The *M. nasuta* exposures showed significant bioaccumulation of PAHs in most test treatments. *M. nasuta* accumulated at least three metals from six test treatments O-C3, O-C1, I-C2, O-C2, I-C3, and I-C8. Pesticides, PCBs, and butyltins were not bioaccumulated by *M. nasuta*. Examination of *N. caecoides* bioaccumulation results in Table 4.4 shows significant bioaccumulation of PAHs in test treatments O-C13, O-C11, O-C10, O-C8, and I-C8. Pesticides, PCBs, and butyltins were not bioaccumulated. At least one metal was bioaccumulated in most test treatments, but only in O-C4 were they more than twice the levels of R-BF. Test treatments O-C12, O-C7, O-C6, O-C4, I-C2, O-C2, and I-C3 showed no acute toxicity, and bioaccumulation of metals only. Comparison of metals bioaccumulation between the two species again showed that *M. nasuta* and *N. caecoides* accumulated metals from different sets of test treatments. Comparison of test treatments to the reference R-BF produced 54 hits, the lowest number of hits among the six references.

4.3.4 Comparison of Test Treatments to the Reference Sediment R-OS

Table 4.5 summarizes the results of acute toxicity and bioaccumulation tests for each test treatment relative to the reference R-OS (Deep Off-shelf). This table shows that three test treatments, O-C13, O-C7 and O-C1, were acutely toxic relative to R-OS. The results of the *M. nasuta* exposures showed bioaccumulation of PAHs in all test treatments except O-C12, O-C4, I-C2, O-C2, and I-C3. TBT was elevated in treatments O-C11, O-C10, O-C9, O-C8, and I-C8, but was never greater than twice the TBT in R-OS. Mercury was elevated in *M. nasuta* exposed in O-C1, I-C2, and I-C8. Examination of *N. caecoides* results shows bioaccumulation of PAHs in only six test treatments, no bioaccumulation of butyltins, and bioaccumulation of metals in all test treatments except I-C3 and I-C8. Again, the numerous instances of metals bioaccumulation in *N. caecoides* may be the result of the tissue compositing strategy, as relatively few mean metal values were more than twice the R-OS mean value. Pesticides and PCBs were not bioaccumulated by

TABLE 4.4. Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Bay Farm Reference R-BF (bold italic type indicates *N. caecoides* treatment mean value is more than twice the reference mean value for metal)

PHASE III B

Sediment Treatment	Acute Toxicity	<i>M. nasuta</i> Bioaccumulation				<i>N. caecoides</i> Bioaccumulation			
		PAH	Pest/PCB	Metals	Butylins	PAH	Pest/PCB	Metals	Butylins
O-C13	- ^(a)	HPAH ^(b)	-	-	-	HPAH	-	Cd ^(c)	-
O-C12	-	-	-	-	-	-	-	Cd	-
O-C11	-	HPAH	-	-	-	HPAH	-	Cd	-
O-C10	-	HPAH	-	-	-	HPAH	-	Ag,As ^(c)	-
O-C9	-	LPAH ^(d)	-	-	-	-	-	Ag,Cd	-
O-C8	-	HPAH	-	-	-	HPAH	-	As	-
O-C7	-	-	-	-	-	-	-	As	-
O-C6	-	-	-	-	-	-	-	Cd	-
O-C5	-	LPAH	-	-	-	-	-	Ag	-
O-C4	-	-	-	-	-	-	-	Ag,Cr,Hg,Pb ^(b)	-
O-C3	-	LPAH	-	-	-	Ag,Cu,Hg,Pb	-	-	-
O-C1	Rhe10 ^(e)	LPAH	-	-	-	Ag,Cu,Hg	-	-	-
I-C2	-	-	-	-	-	Ag,Cu,Hg,Pb	-	-	-
O-C2	-	-	-	-	-	Ag,Cu,Pb	-	-	-
I-C3	-	-	-	-	-	Ag,Cu,Hg	-	-	-
I-C8	-	HPAH	-	-	-	Ag,Cu,Hg,Pb	-	-	-
I-C4	NA ^(f)	NA	NA	NA	NA	NA	-	Cd	-
I-T5	NA	NA	NA	NA	NA	NA	-	Ag,Cd	-

(a) No significant difference from reference.

(b) HPAH is high molecular weight PAHs fluoranthene and/or pyrene.

(c) Arsenic and cadmium bioaccumulation probably overestimated as a result of *N. caecoides* tissue compositing.

(d) LPAH is low molecular weight PAH naphthalene.

(e) Rhe 10 is 10-day static test with *R. abronius*.

(f) Not applicable; not tested.

TABLE 4.5. Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Deep Off-Shelf Reference R-OS (bold italic type indicates *N. caeoides* treatment mean value is more than twice the reference mean value for metal)

(a) Nep 10 is 10-day flow-through test with *N. caeoides*.
 (b) HPAH is high molecular weight PAHs fluoranthene and/or pyrene.
 (c) No significant difference from reference.
 (d) Arsenic and cadmium bioaccumulation probably overestimated as a result of *N. caeoides* tissue compositing.
 (e) LPAH is low molecular weight PAH naphthalene.
 (f) RHE 10 is 10-day static test with *R. abronius*.
 (g) Not applicable; not tested.

either *M. nasuta* or *N. caecoides*. In test treatments O-C12, O-C4, and I-C2, there was no acute toxicity and the only significant bioaccumulation was related to one or more metals. Treatment I-C3 produced no evidence of acute toxicity or bioaccumulation. Comparison of test treatments to the reference R-OS produced a total of 75 hits. This total was the fourth highest number of hits among the six references compared.

4.3.5 Comparison of Test Treatments to the Reference Sediment R-PC

Table 4.6 summarizes the results of acute toxicity and bioaccumulation tests for each test treatment relative to the reference R-PC (Point Reyes Coarse). This table shows that significant acute toxicity was observed in five of the test treatments relative to R-PC: O-C12, O-C9, O-C1, I-C2, and O-C2. The difference in *R. abronius* mortality between R-PC and treatments O-C5, O-C4, and O-C3 was statistically significant but did not exceed 20%. *M. nasuta* exposures indicated significant bioaccumulation of PAHs in all test treatments except O-C12, O-C4, I-C2, O-C2, and I-C3. Tributyltins were bioaccumulated in approximately half the test treatments, though at levels more than twice R-PC in only O-C10 and O-C9. Metals were bioaccumulated in all test treatments except O-C11, O-C9, and O-C7. In many test treatments, the only metal significantly bioaccumulated by *M. nasuta* was Cr, but only in O-C12 and O-C10 were Cr levels more than twice those of R-PC. Examination of *N. caecoides* bioaccumulation results in Table 4.6 shows six instances of PAH bioaccumulation and significant bioaccumulation of metals in all test treatments except O-C2, I-C3, and I-C8. Only Ag in O-C10 and Ag and Hg in O-C4 had levels more than twice those of R-PC. In test treatment I-C3, there was no evidence of acute toxicity and bioaccumulation of only a few metals in the tissues of *M. nasuta*. Pesticides and PCBs were not bioaccumulated by either *M. nasuta* or *N. caecoides*. Comparison of test treatments to the reference R-PC produced 87 hits, the highest number of hits among any of the six references.

4.3.6 Comparison of Test Treatments to the Reference Sediment R-PF

Table 4.7 summarizes the results of acute toxicity and bioaccumulation tests for each test treatment relative to the reference R-PF (Point Reyes Fine). This table shows that six test treatments (O-C12, O-C9, O-C7, O-C1, I-C2, and O-C2) showed acute toxicity relative to R-PF. *M. nasuta* showed significant bioaccumulation of PAHs from all test treatments except O-C12, O-C4, I-C2, O-C2, and I-C3 when compared to R-PF. Tributyltin was bioaccumulated only in treatment O-C9, and metals were bioaccumulated in seven test treatments. *N. caecoides* bioaccumulation results show evidence of PAH accumulation in test treatments O-C13, O-C11, O-C10, O-C8, and I-C8, no evidence of butyltin bioaccumulation, and significant bioaccumulation of metals from most of the test treatments. Once again, the *M. nasuta* and *N. caecoides*

TABLE 4.6. Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Point Reyes Coarse Reference R-PC (bold italic type indicates *N. caecoides* treatment mean value is more than twice the reference mean value for metal)

Sediment Treatment	Acute Toxicity	<i>M. nasuta</i> Bioaccumulation				<i>N. caecoides</i> Bioaccumulation			
		PAH	Pest/PCB	Metals	Butyltins	PAH	Pest/PCB	Metals	Butyltins
O-Cl3	^(a)	HPAH ^(b)	-	Cr,Ni	-	HPAH	-	As(c),Cd(c)	-
O-Cl2	Rhe 10 ^(d)	-	HPAH	Cr	-	-	-	As,Cd	-
O-Cl1	-	HPAH	-	-	TBT	HPAH	-	As,Cd	-
O-C10	-	LPAH ^(e) ,HPAH	-	Cr	-	HPAH	-	Ag,As,Pb	-
O-C9	Rhe 10	LPAH	-	-	TBT	TBT	-	Ag,Cd	-
O-C8	-	HPAH	-	Cr	-	HPAH	-	As,Cd	-
O-C7	-	HPAH	-	-	TBT	TBT	-	As	-
O-C6	-	LPAH,HPAH	-	Cr	-	-	-	As,Cd	-
O-C5	-	LPAH	-	Cr	-	-	-	Ag	-
O-C4	-	-	-	Cr	TBT	-	-	Ag,As,Hg,Pb	-
O-C3	Rhe 10	LPAH	-	Ag,Cr,Hg	-	-	-	As	-
O-C1	Rhe 10	LPAH	-	Ag,Cr,Hg	TBT	-	-	As	-
I-C2	Rhe 10	-	-	Ag,Hg	-	-	-	As	-
O-C2	Rhe 10	-	-	Ag,Cr,Pb	-	-	-	-	-
I-C3	-	-	-	Ag,Cu,Hg	-	-	-	-	-
I-C8	-	HPAH	-	Ag,Cr,Cu,Hg	TBT	-	-	HPAH	-
I-C4	NA ^(f)	NA	NA	NA	NA	NA	-	As,Cd	-
I-T5	NA	NA	NA	NA	NA	NA	-	Ag,Cd	-

(a) No significant difference from reference.

(b) HPAH is high molecular weight PAHs fluoranthene and/or pyrene.

(c) Arsenic and cadmium bioaccumulation probably overestimated as a result of *N. caecoides* tissue compositing.

(d) Rhe 10 is 10-day static test with *R. abronius*.

(e) LPAH is low molecular weight PAH naphthalene.

(f) Not applicable; not tested.

TABLE 4.7. Summary of Significant Acute Toxicity and Bioaccumulation for Test Treatments Relative to the Point Reyes Fine Reference R-PF (bold italic type indicates *N. caecoides* treatment mean value is more than twice the reference mean value for metal)

PHASE III B

Sediment Treatment	Acute Toxicity	<i>M. muscosa</i> Bioaccumulation				<i>N. caecoides</i> Bioaccumulation			
		PAH		Metals		PAH		Metals	
		Pest/PCB	Butyltins	Pest/PCB	Butyltins	Pest/PCB	Butyltins	Pest/PCB	Butyltins
O-C13	^(a)	HPAH ^(b)	-	-	-	HPAH	-	As ^(c) , Cd ^(c) , Cu, Pb	-
O-C12	Rhe 10 ^(d)	-	HPAH	-	-	-	-	Cd	-
O-C11	-	HPAH	-	-	-	HPAH	-	As, Cd, Pb	-
O-C10	-	LPAH ^(e) , HPAH	-	-	-	HPAH	-	Ag, As, Cd, Pb	-
O-C9	Rhe 10	LPAH	-	-	-	TBT	-	Ag, Cd	-
O-C8	-	HPAH	-	Pb	-	HPAH	-	As, Cd, Pb	-
O-C7	Nep 10 ^(f)	HPAH	-	-	-	-	-	As, Cd, Pb	-
O-C6	-	LPAH, HPAH	-	-	-	-	-	As, Cd, Pb	-
O-C5	-	LPAH	-	-	-	-	-	A ^(g)	-
O-C4	-	-	-	-	-	-	-	Ag, As, Cd, Cr, Hg, Pb	-
O-C3	-	LPAH	-	-	-	-	-	-	-
O-C1	Rhe 10	LPAH	-	-	-	-	-	-	-
I-C2	Rhe 10	-	-	-	-	-	-	As	-
O-C2	-	-	-	-	-	-	-	-	-
I-C3	-	-	-	-	-	-	-	-	-
I-C8	NA ^(g)	HPAH	-	-	-	HPAH	-	-	-
I-C4	NA	NA	NA	NA	NA	NA	NA	As, Cd, Pb	-
I-T5	NA	NA	NA	NA	NA	NA	NA	Ag, Cd	-

(a) No significant difference from reference.
 (b) HPAH is high molecular weight PAHs fluoranthene and/or pyrene.
 (c) Arsenic and cadmium bioaccumulation probably overestimated as a result of *N. caecoides* tissue compositing.
 (d) Rhe 10 is 10-day static test with *R. abronius*.
 (e) LPAH is low molecular weight PAH naphthalene.
 (f) Nep 10 is 10-day flow-through test with *N. caecoides*.
 (g) Not applicable; not tested.

accumulated metals from different sets of test treatments. In *M. nasuta*, all treatments with significantly elevated metals had more than twice the levels of R-PF. In *N. caecoides*, relatively few metals were more than twice the levels of R-PF. *M. nasuta* and *N. caecoides* did not bioaccumulate pesticides or PCBs. In test treatments O-C4 and I-C3 no acute toxicity was observed relative to R-PF, and only metals were bioaccumulated. Comparison of test treatments to R-PF produced 85 hits, the second highest number of hits in the six references.

4.4 CONCLUSIONS

The tiered approach to evaluating the potential impacts of ocean disposal of dredged material is presented in the Draft Implementation Manual. (See Figure 3-1 of the manual). This approach consists of a series of activities (tests) and decision modules (determination of compliance) to guide the evaluation of proposed dredged sediment. Because Tier III ocean disposal testing is considered to be the most sensitive, environmentally protective suite of tests conducted on dredged material, the Oakland Phase III B project was conducted under the Tier III guidelines. The results of Phase III B testing are, therefore, discussed and presented in the context of Tier III evaluations, which include water column toxicity, deposited sediment (solid phase) toxicity, and deposited sediment bioaccumulation. Physical and chemical analyses of proposed dredged material were only used in this study to verify sediment grain size and to help explain toxicity and bioaccumulation results. However, the Draft Implementation Manual (EPA/USACE 1990) and the 1991 Implementation Manual (EPA/USACE 1991) do not offer specific guidance for the case of testing and comparing to multiple reference sediments, as was the case for the Phase III B project. Statistical comparisons were conducted in a scientifically sound manner for the multiple reference test design and did not follow the guidance for comparing test sediments to one reference treatment. These analyses are an extremely useful technique for planning and evaluating disposal alternatives for Oakland Harbor Phase III B sediment.

4.4.1 Water Column

Estimates of toxicity in the water column were evaluated by exposing three sensitive marine species (*H. sculpta*, *C. stigmatus*, and *M. edulis*) to the suspended particulate phase of four sediment composites. The sediment composites BC-5, BC-6, BC-7, and BC-8 represented the bulk of Oakland Harbor Phase III B sediments proposed for dredging. Four concentrations of SPP were tested: 0% (seawater), 10%, 50%, and 100%. Determination of compliance for this test involves deciding whether the concentration of dissolved plus suspended contaminants, after allowance for initial mixing, is greater than 0.01 of the acutely toxic concentration beyond the

boundaries of the disposal site within the first 4 h after disposal. Tests of the SPP of the four Oakland Phase III B sediment composites showed no acute toxicity and no calculable LC50. An effective concentration of SPP that resulted in 50% abnormality (EC50) of 87.6% SPP was calculated for BC-7 (composite of O-C13, O-C12, O-C11, and O-C10) in the *M. edulis* test. This determination implies a chronic effect of a relatively high concentration of BC-7 SPP.

4.4.2 Deposited Sediment Toxicity

Deposited sediment toxicity was determined by exposing four species of marine organisms (*M. nasuta*, *N. caecoides*, *C. stigmaeus*, and *R. abronius*) to test sediment treatments in a series of solid-phase tests. Tier III guidelines concerning determination of compliance for deposited sediment toxicity are to evaluate whether the mortality of organisms exposed to test sediments (dredged material) is significantly different than mortality of organisms exposed to reference treatments, and, whether mortality in test treatments exceeds the reference treatment by 20% (*R. abronius*) or 10% (other species). If the mortality of test organisms in test treatments is significantly different than the reference mortality and exceeds the reference by the above percentages, the test material does not comply with the benthic bioassay criteria of the CFR40, Section 227.13(c).

The toxicological tests conducted by MSL showed only two tests (*N. caecoides* and *R. abronius*) in which significant mortality in test treatments was observed when compared to multiple reference sediments using Dunn's Test with $\alpha = 0.10$. The number of test treatments that exceed the acute toxicity criteria depends on the reference to which the test treatments are compared (Tables 4.2 through 4.7). In treatment O-C1, *R. abronius* mortality exceeded the criteria when compared to all references; O-C1 was the only station at which acute toxicity criteria were exceeded in both *R. abronius* and *N. caecoides* tests when compared to R-AC. Comparison of test treatments to the reference R-AC produced the most solid-phase toxicity hits (9); comparison to the reference R-BF produced the least (1). The other four references produced three to seven toxicological hits.

4.4.3 Bioaccumulation

The potential for bioaccumulation of contaminants was evaluated through 28-day solid-phase flow-through tests of *M. nasuta* and *N. caecoides*. The concentrations of contaminants were compared to existing Food and Drug Administration (FDA) limits, and were also compared using Dunn's Test to determine whether statistically significant ($\alpha = 0.10$) levels of contaminants were present relative to reference. Bioaccumulation results showed that significantly elevated levels of contaminants were present in the tissues of *M. nasuta* and *N. caecoides* when compared to the six

references. Comparison of test treatment data to the references R-AC and R-BF produced the lowest total number of elevated levels (49 and 53, respectively); comparison to the reference R-PF and R-PC produced the highest total hits (79 and 82, respectively). When only the metals in *N. caecoides* treatments that were more than twice those of the references are considered, these rankings do not change but the total numbers of bioaccumulation hits decrease to 29 (R-AC), 39 (R-BF), 54 (R-PF), and 57 (R-PC). Again, the significance of the comparisons depends on the reference to which the test treatments are compared.

Further evaluation of the data or of the proposed dredged material may be necessary to select an appropriate disposal alternative. These evaluations may take the form of numerical modeling, case-specific testing, or other management action developed by the USACE District Engineer and USEPA Regional Administrator. The summary results presented in Tables 4.1 through 4.7 should aid in planning for disposal of Oakland Outer Harbor dredged material.

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