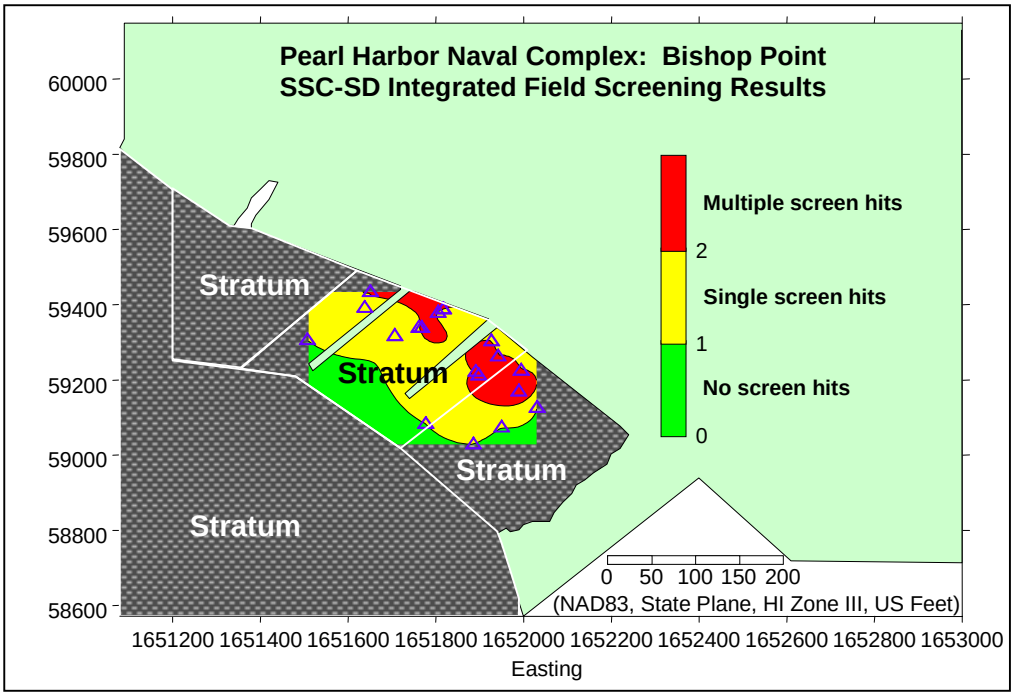
GUIDE FOR USING RAPID SEDIMENT CHARACTERIZATION METHODS IN ECOLOGICAL RISK ASSESSMENTS



Prepared for

Naval Facilities Engineering Service Center
1100 23rd Avenue (Code 411)
Port Hueneme, CA 93043

and

Space and Naval Warfare Systems Center
San Diego D36
53475 Strothe Road
San Diego, CA 92152

by

Battelle
. . . Putting Technology To Work

505 King Avenue
Columbus, Ohio 43201-2693

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Acronyms and Abbreviations

ASTM	American Society for Testing and Materials
BTEX	benzene, toluene, ethylbenzene, and xylenes
COC	contaminant of concern
DDT	dichlorodiphenyltrichloroethane
ERA	ecological risk assessment
ER-M	effects range-median
FP	Fundamental Parameter
HPS	Hunters Point Shipyard
IC	inhibition concentration
MS	Microsoft®
NAS	Naval Air Station
NFESC	Naval Facilities Engineering Service Center
PAH	polycyclic aromatic hydrocarbon
PC	personal computer
PCB	polychlorinated biphenyl
PHNC	Pearl Harbor Naval Center
PMT	photomultiplier tube
ppb	parts per billion
ppm	parts per million
QA	quality assurance
QC	quality control
RSC	rapid sediment characterization
RSCT	rapid sediment characterization tools
RSD	relative standard deviation
SOP	standard operating procedure
SSC	Space and Naval Warfare Systems Center
SSCS	site-specific calibration standards
U.S. EPA	United States Environmental Protection Agency
UVF	ultraviolet fluorescence spectrometry
XRF	x-ray fluorescence spectrometry

1. Introduction

1.1 Background

As part of the process of remediating contaminated sites, the Navy conducts ecological risk assessments (ERAs) to evaluate the likelihood that exposure to environmental stressors, such a chemical contaminants, will have adverse ecological effects. The results of ERAs provide the Navy with information for decision making about remedial actions at Navy sites.

Navy policy requires that "Ecological Risk Assessments conducted for the Navy be scientifically based, defensible, and done in a manner that is cost-effective and protective of human health and the environment" (Chief of Naval Operations, 1999). Sampling programs are focused primarily on identifying potential contaminant sources and on delineating potential areas of contamination. Navy policy further requires that sampling programs use *advanced chemical and biological screening methods*, data quality objectives, and statistical procedures to minimize needed sampling.

Because traditional sampling and analysis methods for marine ecosystems do not always provide the information needed for decision making in a timely and cost-effective manner, rapid characterization methods have been developed to speed up site characterization and to reduce its cost. These advanced screening methods can detect contaminants in many different environmental media, including air, water, soil, and sediment.

This guide provides information on four analytical methods for quick and cost-effective characterization of **contaminated sediments** at **marine sites**:

- ❑ X-ray fluorescence spectrometry (XRF) – used to determine concentrations of metals
- ❑ Ultraviolet fluorescence spectrometry (UVF) – used to determine concentrations of polycyclic aromatic hydrocarbons (PAHs)
- ❑ Immunoassay techniques – used to determine concentrations of polychlorinated biphenyls (PCBs), pesticides, and PAHs
- ❑ QwikSed bioassay – uses marine microorganisms (dinoflagellates) to detect toxicity arising from organic and inorganic contaminants.

All four of these rapid sediment characterization (RSC) methods can be used to generate semi-quantitative results on site within minutes or hours, rather than the several days or weeks that other methods require. Also, most of the RSC methods can be used to perform on-site analyses on 10 or more samples per unit per day, which reduces the number of samples needed for analysis at an off-site laboratory.

1.2 Overview of RSC Methods

Rapid characterization methods are real-time or near-real-time screening methods to rapidly delineate the extent of contamination as well as to determine the physical characteristics and/or biological effects of the contaminants The instruments used are

field-portable analytical tools that provide measurements of chemical, physical, or biological parameters.

The application and use of rapid characterization methods are discussed in detail in *Field Analytical and Site Characterization Technologies—Summary of Applications* (United States Environmental Protection Agency [U.S. EPA], 1997) and *Field Analytical Measurement Technologies, Applications, and Selection* (California Military Environmental Coordination Committee, 1996). In addition, the online *Field Analytic Technologies Encyclopedia* provides information about field methods that can be used for characterizing contaminated soil and groundwater, for monitoring the progress of remedial actions, and for confirming sampling and analysis for site close out (U.S. EPA, 2000).

Although not all of the rapid characterization methods discussed in these handbooks are applicable to marine sediments, the four RSC methods discussed in this guide (and shown in Table 1-1) have been demonstrated to be effective at contaminated Navy marine sediment sites.

TABLE 1-1. RSC methods tested in marine sediments by the Navy

Measurement Type	Analytical Technique	Analytes
Chemical	XRF	Metals
	UVF	PAHs
	Immunoassay	PCBs, pesticides, PAHs
Biological	QwikSed bioassay	Organics and inorganics

1.3 Applicability of RSC Methods

The cost-effective collection of data at contaminated marine sites is often hindered by the heterogeneity and complexity of marine ecosystems. Implementing RSC methods at different stages during a site investigation can help focus sampling requirements and facilitate achieving timely and cost-effective results.

In particular, RSC methods can be effectively implemented during exploratory site investigations as well as during investigations specific to ERAs (see Figure 1-1). For exploratory site investigations, RSC methods can be used as field screening tools to detect general classes of contaminants in marine sediments, so that major types are not overlooked, to examine trends in contaminant types and distributions, and to define zones of contamination. For ERA purposes, RSC methods can be used by field investigators to concentrate sampling of marine sediments where contaminants are known to be present.

1.4 Benefits and Limitations of RSC Methods

The major benefits of using RSC methods during ERAs are that the methods provide rapid results at a reduced cost compared to standard laboratory analyses, and can improve sampling in general by reducing uncertainty at several steps in the assessment process while avoiding the cost of repeated or multiple resamplings. However, because RSC methods involve less rigorous sample preparation than standard laboratory analysis, the

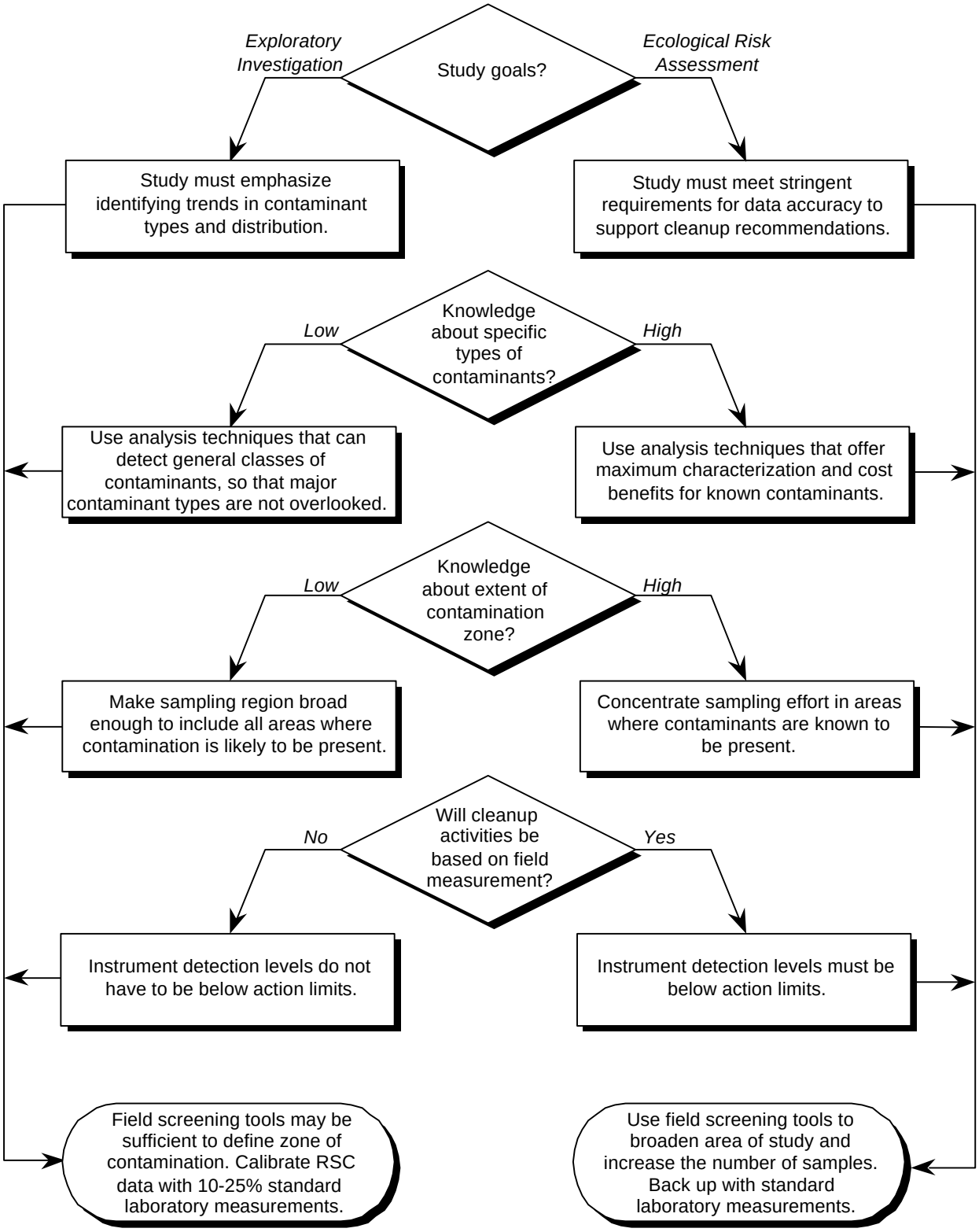

FIGURE 1-1. Guidance for using RSC methods

data generated using the methods are not equivalent to those generated by standard laboratory analyses. In general, data generated using standard analytical methods (such as U.S. EPA-approved reference methods [i.e., SW-846]) are classified as "definitive data," whereas data generated using rapid, less precise methods of analysis (such as RSC methods) are classified as "screening data." Screening data are semi-quantitative at best, and generally are not contaminant-specific. (Metals analysis by XRF is element-specific, but semi-quantitative nonetheless).

Overall, depending on data quality requirements, a well-designed RSC protocol paired with laboratory validation can provide data of sufficient quality (i.e., screening data with definitive confirmation) for ERA decision making. Table 1-2 compares the benefits and limitations of RSC methods with standard laboratory analysis methods.

TABLE 1-2. Comparison of RSC and standard laboratory methods

	RSC Methods	Standard Laboratory Methods
Benefits	Rapid results can guide sampling locations Potential for high data density for mapping Reduced cost per sample	Quantitative, with high accuracy Often can remove interferences
Limitations	Often nonspecific for organic contaminants Semi-quantitative Matrix sensitive	Often blind sampling Delayed results Costly

1.5 Reporting Results

Because data produced using RSC methods are not equivalent to those generated by standard laboratory analyses, the data must be reported with appropriate references to the techniques used. Three options are available for reporting RSC data in a clear manner for ease of interpretation. A first option is to flag data in spreadsheets and reports as having been generated by nonstandard methods, and/or references or qualifiers can be included in text form to address any potential offsets from standard analyses.

A second option is to use site-specific calibration of RSC analyses and to report only corrected, calibrated data. This option provides results that are more easily interpolated between or contoured with standard data. However, site-specific calibration requires a higher level of effort at a site, such as sending more samples off-site for laboratory analysis, and may reduce the time- and cost-effectiveness of using RSC tools.

A third option, one which is particularly suited to analyses that generate only qualitative data, is to report only detection findings instead of reporting concentration values. This option can be used when RSC measurements are intended to identify the presence or absence of target analytes, but may have little relationship to the true concentrations of the analytes. Using this option, samples can still be ranked by concentration, or ranges of concentrations can be reported.

1.6 Meeting Regulatory Requirements

RSC tools can streamline ERA site investigations by delineating areas of concern, filling in information gaps, and assuring that expensive, certified laboratory analyses provide the

most cost-effective results possible. However, RSC methods and tools are not subject to the same quality assurance (QA) and quality control (QC) protocols as are standard laboratory methods, so the approach for managing and interpreting RSC data must addressed up front with regulators and stakeholders. Historically, regulators and the user community have accepted RSC results *as part* of the analytical results for decision making in ERAs, but not as stand-alone data, so these results should be balanced with and supplemented by standard, certified laboratory analyses.

1.7 References

California Military Environmental Coordination Committee. 1996. *Field Analytical Measurement Technologies, Applications, and Selection*. Prepared by the Chemical Data Quality/Cost Reduction Process Action Team. April. Available at: http://www.epa.gov/region09/qa/measure-technol.pdf.

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U.S. EPA, see United States Environmental Protection Agency.

2. Description and Use of RSC Methods

This section describes the four RSC methods developed for quickly characterizing contaminated sediments at marine sites: XRF to detect metals; UVF to detect PAHs; immunoassay to detect PCBs, pesticides, and other organics; and QwikSed bioassay using dinoflagellates for toxicity testing. Each of these methods is performed on site, and produces semi-quantitative results within a few minutes or hours. Also, most of these methods enable a user to analyze 10 or more samples per unit per day.

2.1 Overview of RSC Methods

X-Ray Fluorescence Spectrometry. XRF measures the fluorescence spectrum of x-rays emitted when metal atoms are excited by an x-ray source. The energy of the emitted x-rays identifies the metals in a sample, and the intensity of the emitted x-rays indicates their concentration.

XRF can identify a wide range of elements from sulfur through uranium, encompassing typical elements found in soils and sediments. Field-portable XRF instruments provide near-real-time measurements with minimal sample handling, and thus allow for extensive semi-quantitative analysis on site. Benchtop XRF instruments, although not field-portable, can be used in the laboratory for rapid semi-quantitative analysis of samples as well as for quantitative analyses. In both cases, reasonably low detection limits usually can be achieved.

XRF instruments can be calibrated using any one of three methods: internally, empirically, or using the Compton normalization method. Internal calibration uses the fundamental parameters determined by the manufacturer. Empirical calibration uses site-specific calibration standards. The Compton normalization method is based on the analysis of a certified standard and normalization for a peak. Detection limits are different for each element. For common metals such as lead, zinc, and copper, detection limits using a field-portable XRF unit typically range from 50 to 150 parts per million (ppm). Lower detection limits can be achieved using laboratory, benchtop XRF systems (e.g., Cu and Zn: 20 ppm; Pb: 10 ppm, in wet sediments).

Ultraviolet Fluorescence Spectrometry. UVF measures the fluorescence generated following ultraviolet excitation of organic solvent extracts of sediments. In general, UVF can be used to measure fluorescent organics, especially PAHs. However, care must be exercised to minimize interference by naturally fluorescing compounds (such as humics).

Because fluorescence measurements are matrix sensitive, they must be made on solvent extracts rather than directly on wet, solid sediment samples in order to achieve detection limits appropriate for benchmark criteria. Solvent extraction requires additional time for sample extract analysis. Thus, although fluorescence is a near-real-time measurement, the total time for analysis may be up to one half hour.

For PAHs, detection limits using UVF range from 1 to 5 ppm total solid phase.

Immunoassay. The immunoassay technique can be used for the identification and quantification of organic compounds (e.g., PCBs, PAHs, pesticides). Immunoassays use antibodies that have been developed to bind with a target compound or class of compounds.

Concentrations of analytes are identified through the use of a sensitive colorimetric reaction. The determination of the target analyte's presence is made by comparing the color developed by a sample of unknown concentration with the color formed by the standard containing the analyte at a known concentration. The concentration of the analyte is determined by the intensity of color in the sample and is measured through use of a spectrophotometer. Immunoassay kits are relatively quick and simple to use. Detection limits can vary depending upon the dilution series used. For example, the detection limit for PCBs in sediments ranges from 50 to 500 parts per billion (ppb).

QwikSed Bioassay. QwikSed bioassays measure the inhibition of light emitted by marine bioluminescent dinoflagellates exposed to test solutions of effluents, elutriates, or sediment porewaters. Bioavailable contaminants or other environmental stressors are indicated by decreases in light output relative to controls.

QwikSed bioassays can be used to evaluate sediment toxicity. The bioassays can measure responses within 24 hours of test setup and can be conducted for a standard four-day acute test or a seven-day chronic test. If contaminated sediments require cleanup, QwikSed bioassays also can be used to assess toxicity reduction. The *1998 Annual Book of ASTM Standards (*American Society for Testing and Materials [ASTM], 1998) includes a "Standard Guide for Conducting Toxicity Tests with Bioluminescent

2.2 Detailed Descriptions of RSC Methods

This section describes the four RSC methods in greater detail. For each method, it provides information on the technology, discusses the performance factors and the advantages and limitations of use, describes instrumentation and calibration, and lists vendors. Standard operating procedures (SOPs) for the methods and instruments are provided in Appendix B.

2.2.1 X-Ray Fluorescence Spectrometry

XRF is a method for detecting metals and nonmetallic elements in soil and sediment. Generally, XRF can be used to detect and quantify elements from sulfur through uranium, and can detect and measure up to 35 elements simultaneously. Some of the primary elements of environmental concern that XRF can identify are arsenic, barium, cadmium, chromium, copper, lead, selenium, silver, and zinc. Action levels for some elements (such as arsenic or cadmium) may be lower than the detection limits of XRF. XRF analyses are not intended to replace more rigorous laboratory analyses for regulatory purposes. Rather, XRF provides near-real-time data that can be used to produce a contaminant distribution map independently of time-consuming and costly laboratory analysis. XRF analysis allows better delineation of contaminant distribution by providing higher data density in a time- and cost-effective manner.

XRF analysis involves using the photoelectric effect to analyze samples. In these analyses, a sample is exposed to an x-ray source that has an excitation energy similar to, but greater than, the binding energy of the inner-shell electrons of the elements in the sample. Some of the source x-rays are scattered, but a portion are absorbed by the elements in the sample. Because of their higher energy, the absorbed rays cause ejection of inner-shell electrons, and the electron vacancies that result are filled by electrons which cascade in from outer electron shells. However, because outer-shell electrons

possess higher energy states than the inner-shell electrons they are replacing, the outer-shell electrons give off energy as they cascade in (see Figure 2-1). The energy is given off in the form of x-rays, and the phenomenon is referred to as x-ray fluorescence.

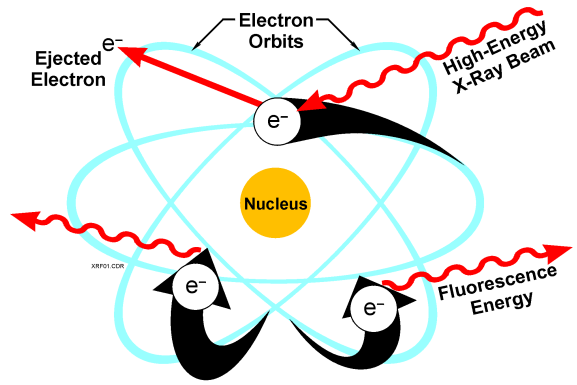

FIGURE 2-1. Physics of XRF technology

Because every element has a different electron shell configuration, each element emits a unique x-ray at a set energy level or wavelength that is characteristic of that element. The elements present in a sample can be identified by observing the energy levels of the characteristic x-rays. The intensity of the x-rays is proportional to the concentration and can be used in quantitative analysis. That is, qualitative analysis is performed by observing the energy of the characteristic x-rays, and quantitative analysis is performed by measuring the intensity of the x-rays.

System components. An XRF system has two basic components, an x-ray source and a detector. The source irradiates the sample to produce characteristic x-rays. The detector measures both the energy of the characteristic x-rays that are emitted and their intensity. There are two types of XRF units: benchtop units that use an x-ray tube and field-portable XRF analyzers that use up to four radioisotopes as sources of x-rays. Instruments that use x-ray tubes as sources are typically not used in the field because of the larger power requirements for the x-ray tube and the added weight of the instrument. Field-portable instruments typically use one or more of the following radioisotopes: cadmium-109; curium-224; americium-241; and iron-55. The field-portable units typically weigh less than 30 pounds and can be operated for 8 to 10 hours using battery power. A comparison of minimum detection limits for field-portable and desktop XRF instruments is presented in Table 2-1.

TABLE 2-1. Minimum detection limits for XRF analysis of sediment

Metal	Field-Portable (mg/kg)	Benchtop (mg/kg)
Arsenic	50[a]	10
Barium	20[b]	100
Cadmium	140[b]	10
Chromium	150[c]	40
Copper	100[a]	20
Lead	30[a]	10
Selenium	50[a]	10
Silver	100[b]	10
Zinc	80[a]	20

(a) Cadmium-109 source.
(b) Americium-241 source.
(c) Iron-55 source.

Most XRF instruments have software capable of storing all analytical results and spectra. The results are displayed in parts per million and can be downloaded to a personal computer (PC), which can provide a hard copy printout. Some instruments also may provide software to prepare results or spectra for importing into a spreadsheet. Operators of XRF instruments usually can be trained in one or two days.

Modes of analysis (in situ and intrusive modes). XRF analyses can be conducted in either the in situ or the intrusive mode; some XRF instruments can operate in both modes of analysis, whereas others are designed to operate in only one mode. As many as 150 samples per day can be analyzed in situ, and a throughput rate of 50 to 100 samples per day typically can be achieved through intrusive analysis. Because little or no sample preparation is required using XRF, and samples can be analyzed in less than five minutes, sample throughput is enhanced and time and money are saved.

An *in situ* analysis refers to the rapid screening of sediments in place. Naturally, this can only be done in the absence of any water covering the sediment, such as dry stream beds and shoreline exposure during low tide. In situ analyses typically are completed in times of 30 to 60 seconds per sample. Heterogeneity of the samples is sometimes a concern, so multiple measurements should be taken in a small area and the values averaged to determine the concentrations of metals.

For *intrusive* operation, a sample is collected, prepared, placed in a cup, and analyzed. Intrusive analysis is used to ensure greater precision when lower detection limits are needed. To increase the sensitivity of the instrument, intrusive analyses commonly involve longer analysis times (up to 200 seconds per sample). Also, more extensive sample preparation often is needed to reduce heterogeneity among samples. The intrusive mode of analysis is the primary type used for sediment samples.

An advantage of XRF analytical techniques is that samples are not destroyed during preparation or analysis. Therefore, it is possible to perform repeated analyses on a single sample as well as to send the same sample for confirmatory analysis, so that comparability studies can be performed. Samples also can be archived for later use as standards. Because no solvents or acids are used for sample extraction, no waste is generated.

Factors affecting performance. XRF performance is affected primarily by operating conditions and matrix interferences. Variation in sample preparation and measurement technique by operators can affect XRF results. These effects can be controlled by using the same personnel to prepare samples and operate the instruments throughout a sampling regime or by carefully training an alternate analyst.

Sample analysis time affects sample throughput as well as precision and detection limits. Increasing analysis time by a factor of four improves precision by a factor of two and improves detection limits by 50%. However, it is impractical, in terms of sample throughput, to extend sample analysis times beyond 600 to 800 seconds. Analysis time can be controlled by careful and consistent adherence to SOPs.

Physical matrix effects result from variations in the physical character of samples such as particle size, uniformity, homogeneity, and surface conditions. Coarse-grained sediments or nuggets of contaminated material may preclude a representative sample and adversely affect analysis results, typically by underreporting the target element. Preparation consistency minimizes variation in analytical results. Anomalies in measurement geometry,

sample surface morphology, moisture content, sample grain size, and matrix should be documented.

Fundamental differences between the way XRF and standard "wet chemical" analyses treat and measure a sample limit the degree of direct comparison of results. In standard analyses, a sample is either partially or completely digested, and the extract is cleaned and analyzed. Digestion of a sample allows for analysis of all extractable metal in a sample. XRF analyzes only the metals near the sample surface. If a highly contaminated or pure metal particle is in a sample but not near the surface, XRF will reflect the concentration in the exposed cross section only, whereas digestion will reflect the total concentration in the sample.

Moisture content may also affect the accuracy of sediment analyses. Differences in the way XRF and extractive analyses are carried out make XRF more sensitive than standard methods to moisture content methods.

Instrument calibration. Calibration procedures vary among XRF instruments according to the manufacturers of the instrument and the use of the data. Generally, however, three types of calibration procedures exist for XRF instruments: fundamental parameters calibration; empirical calibration; and the Compton peak ratio or normalization method.

The fundamental parameters calibration is a "standardless" calibration. It relies on the physics of the spectrometer's response to pure elements in order to set the calibration. Built-in mathematical algorithms are used to adjust the calibration for analysis of samples and to compensate for the effects of the matrix. Fundamental parameters calibration is performed by the manufacturer, but the analyst can adjust the calibration curves (slope and intercept) on the basis of results of analyses of check samples.

In an empirical calibration, a number of actual samples, such as site-specific calibration standards (SSCS), are used, and the instrument's measurements of the concentrations of known analytes in the samples are measured. Empirical calibration is effective because the samples used closely match the sample matrix. SSCS should contain all the analytes of interest and interfering analytes. Manufacturers recommend that 10 to 20 calibration samples be used to generate a calibration curve.

The Compton normalization method incorporates elements of both empirical and fundamental parameters calibration. A single, well-characterized standard is analyzed, and the data are normalized for the Compton peak. The Compton peak is produced from incoherent backscattering of x-ray radiation from the excitation source and is present in the spectrum of every sample. The intensity of the Compton peak changes as various matrices affect the way in which source radiation is scattered. For that reason, normalizing to the Compton peak can reduce problems with matrix effects that vary among samples.

Instruments and Vendors. Several XRF instruments are commercially available. Instrument design and accessories vary. Typical XRF instrumentation is shown in Figure 2-2. A specific license is required to operate some XRF instruments. Licensing entails attending a radiation safety course, completing the necessary paperwork, and paying a fee for the license.

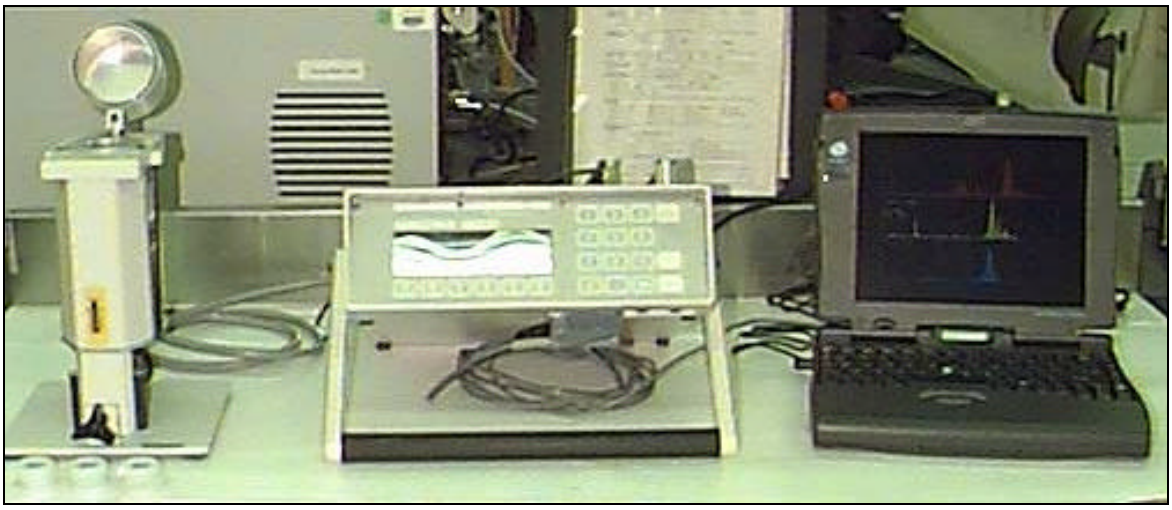

FIGURE 2-2. Example of field-portable XRF instrumentation (KevexSpectrace TN 2000 shown in center)

Field-Portable XRF Instruments

- ***KevexSpectrace TN 9000***. The TN 9000 is a commercially available instrument that can use up to three radioactive sources and a mercuric iodide semiconductor detector for the analysis of metals in sediment and soil. It is field-portable, weighs less than 20 pounds, and can be battery-powered for up to 8 hours. Based on stored information for each application, the analyzer software automatically selects sources and measurement times for each sample.

  The TN 9000 can conduct in situ measurements or measure samples in cups. A layer of 0.2-mil polypropylene XRF film can be mounted on the surface probe to minimize contamination while conducting in situ measurements. Using plastic films thicker than 0.2-mils may interfere with the measurement of light elements (i.e., those with low atomic numbers), and may affect the fundamental parameters calibration of other element concentrations. Additionally, certain films (such as ordinary plastic bags) may contain significant levels of target element contamination.

- ***Metorex X-MET 920 and 920-MP.*** The Metorex X-MET 920 is designed to produce quantitative data on metals in soils, sludges, and other solids. It consists of a laptop computer, an electronics unit, and a surface analysis probe system. In the 920-MP ("mini-portable") model the electronics are housed in a rugged, weatherproof, self-contained case that weighs about 5 pounds and can be battery-powered for up to 8 hours.

  The probe system is designed to house one excitation source and a gas-filled proportional counter detector. It weighs about 3 pounds and is specifically designed for in situ analysis, but can be adapted for measurement of samples in cups. The single excitation source of the X-MET 920 limits the number of metals that can be quantified to about six. However, additional probe units can be purchased to extend the capabilities of the instrument.

The X-MET 920 is operated and calibrated using "X-MET" software to analyze samples with an empirical calibration. Training and field experience are necessary to successfully derive empirical calibration curves and to operate "X-MET" software.

- ***Niton XL-702 and -721 Spectrum Analyzers.*** The Niton XL Spectrum Analyzer (see Figure 2-3) originally was designed to produce quantitative data for lead in painted surfaces. The analyzers are handheld instruments that weigh less than 3 pounds each and can be battery-powered for up to 8 hours. The XL-702 uses a single radioactive source, cadmium-109, and a silicon pin-diode detector for the analysis of metals using a 60-second count time. The single radioactive source limits the number of analytes that can be detected to about six. The XL-722 is a dual source (cadmium-109 and americium-241) bulk sample analyzer that can detect a greater number of analytes. The XL Spectrum Analyzers are calibrated using the Compton normalization method to quantitate metals. They can conduct in situ measurements or measure samples in cups.

FIGURE 2-3. Example of handheld XRF device (Niton XL-700 series instrument shown)

Benchtop XRF Instruments

QuanX EDXRF System: The QuanX system provides a programmable x-ray tube source with automated filtering for optimized excitation. The high-resolution lithium-drifted silicon x-ray detectors provide low detection limits, few spectral interferences, and accuracy over a wide range of concentrations. The system is available with a choice of detector cooling configurations: electrically cooled or liquid nitrogen cooled.

X-ray tube excitation provides excellent sensitivity across the full 1-37 KeV (i.e., sodium through uranium) analytical range. Optimum sensitivity for a particular element is achieved by selecting an x-ray tube voltage and primary beam filter, which produce a band of excitation radiation just above the absorption edge of the target element. This approach has the dual effect of improving sensitivity and reducing background.

Detection limits depend on the element and the sample matrix. From sodium through calcium detection limits range from a few hundred ppm to 10 ppm. From titanium through uranium, detection limits as low as 2 ppm are achieved. Analytical precision depends on the element(s) of interest the concentration, and the counting time. For most metals at 100 ppm in an organic matrix, precision of 3% relative standard deviation (RSD) can be achieved with counting times of 10 minutes or less. Long-term stability of the system is better than 1% relative, which permits operation without restandardization for weeks or months at a time.

Several algorithms are available for analysis of measured intensities, including linear and quadratic curves, an empirical matrix correction which uses a suite of type standards, and fundamental parameters matrix correction. The software is designed to operate with minimal operator intervention once an analytical method has been established. Automation of analytical parameters such as voltage, current, and atmosphere (i.e., air, vacuum, or helium) allow total software control of the system so that even with the tremendous flexibility of the system, operation is initiated with only two keystrokes.

Shimadzu EDX-700/800 System: The Shimadzu EDX-700/800 is a compact, desktop XRF unit that can analyze solid and liquid samples with minimal preparation. The sample chamber can be evacuated to enhance x-ray detection, or flooded with helium (for moist solid or liquid samples). Model 700 is designed for determining elements as light as sodium (Z=11) and as heavy as uranium (Z=92). Model 800 is designed for measuring elements between carbon (Z=6) and uranium (inclusive). The EDX-700 can accommodate samples as large as 30 cm $\times$ 15 cm. The standard sample analysis area for both instruments is 10 mm; however, smaller areas can be examined with an additional aperture mask. Fully automated analysis can be set up using a programming function.

The software system runs in Microsoft® (MS) Windows 95, 98, and NT, and provides qualitative and quantitative analysis of measured data. The software includes an integrated fundamental parameters program for standardless operation. Alternatively, the software system can build a calibration curve based on user-provided standards. Bulk determination of oxides and metals also can be performed by the software. Another useful feature is sample-to-sample comparison, in which spectrum matching software allows one to compare a sample spectrum with that of a registered standard library.

X-ray signal detection is performed using a Si(Li) detector that can be thermally cycled any number of times. A 3-L internal dewar holds liquid nitrogen sufficient for 2-3 days of operation. An external dewar is also available for longer hold times. Also, the units contain a set of five primary filters (Zr, Al, Ti, Ni, and polymer) for increased signal to noise ratio.

The SOP for XRF is provided in Appendix B.

2.2.2 Ultraviolet Fluorescence Spectrometry

UVF is a standard analytical method that can be used in the field to measure the location and relative extent of PAHs in many different matrices, including marine sediment. The method yields qualitative and semi-quantitative results, making it appropriate for preliminary assessments of contaminant distribution. In addition, the high sensitivity and ease of operation of field fluorometers make UVF a good choice for field screening. PAHs require only ultraviolet light excitation to fluoresce visible light (compared to metals which require x-rays to induce fluorescence in the x-ray spectrum).

Figure 2-4 is a schematic of a UVF system. In this method, light is passed through an excitation filter that transmits a chosen range of excitation wavelengths. As the light passes through the sample, it causes the sample to fluoresce at a level proportional to the concentration of fluorescent contaminant molecules in the sample. (Excitation wavelengths are chosen so that the contaminant under investigation absorbs it strongly, and any other interfering fluorescent materials that may be present absorb the light weakly.)

The light emitted from the molecules then passes through an optical emission filter to reach the detector, where the contaminant data are read by the user. Like the selection of excitation wavelengths, filters also are chosen so that they respond as much as possible to the light emitted by the contaminant under study, and as little as possible to the emission of any interfering fluorescent materials that may be present.

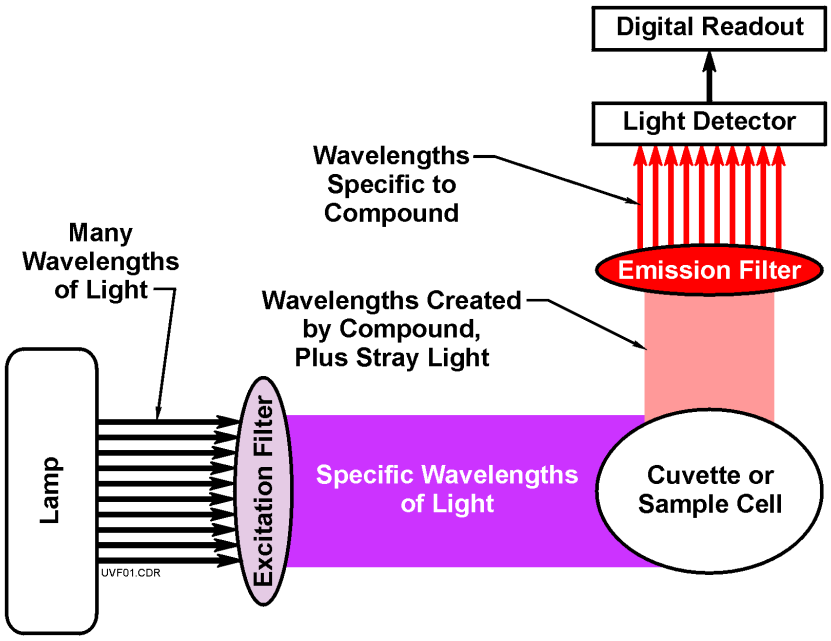

FIGURE 2-4. Schematic of a UVF System

Factors affecting performance. Because fluorescence measurements are matrix-sensitive, they must be made on solvent extracts rather than directly on wet, solid sediment samples. Solvent extraction techniques allow UVF instruments to achieve detection limits for PAH compounds which are appropriate for marine sediment benchmarks and

typical of levels in many marine sediments. Solvent extraction requires additional time for sample extract analysis (although fluorescence is a near-real-time measurement, the total time for analysis may be up to 30 minutes). However, using solvent extraction improves detection limits by several orders of magnitude. Method sensitivity can vary depending on sediment matrix, extraction solvent, excitation and emission wavelengths, and specific PAHs present. For PAHs, detection limits using UVF range from 1 to 5 mg/kg total solid phase.

Instrument calibration. UVF measurements can be calibrated using any one of three methods: (1) samples can be split and direct correlations made between UVF readings and standard laboratory results; (2) historic site-specific data can be used to determine relative ratios of PAHs; and (3) surrogate calibration can be performed using standard reference material.

Instruments and vendors. The Turner Model AU-10 Digital Filter Fluorometer with standard optical package is suitable for total PAH screening in water quality analysis. The manufacturer's optical package is specifically designed to measure fluorescence by heavier weight PAHs. The detector system has a high gain, low noise photomultiplier tube (PMT) with detection wavelength of 400 to 650 nanometers. Figure 2-5 is a photograph of the Turner UVF instrumentation.

FIGURE 2-5. Turner UVF Fluorometer
(Courtesy of Turner Designs, Sunnyvale, CA)

SiteLAB offers a portable UVF instrument (Model UVF-3100A) that can be fitted with optical filters for detection of total petroleum hydrocarbons and BTEX compounds (i.e., benzene, toluene, ethylbenzene, and xylenes). This instrument uses certified calibrations standards, so results correlate directly to laboratory data, and fewer samples must be sent for confirmatory analysis.

SOPs for the Turner fluorometer and for the SiteLAB instrument, as well as for generic soil and sediment sampling, are provided in Appendix B.

2.2.3 Immunoassay

Immunoassays are analytical methods that can provide real-time field analysis of a wide variety of environmental contaminants rapidly and for low cost. Immunoassays can be used effectively in hazardous waste remediation to delineate the extent of contamination and to ascertain that cleanup activities have been successfully completed. For example, they can be used to analyze for petroleum hydrocarbons in fuel oil spills, PCBs originating from electrical transformer fluids, and explosives found at old munitions sites. Also, they are commonly used to analyze for pesticide, insecticide, and herbicide contamination. Examples of analytes and typical detection limits for immunoassays are shown in Table 2-2.

TABLE 2-2. Analytes and limits of detection for immunoassay methods

Analyte	Limit of Detection in Sediment (mg/kg)
BTEX	0.9 to 2.5
TPH	5 to 25
PAHs	0.2 to 1
Carcinogenic PAHs (CaPAHs)	10
Polychlorinated biphenyls (PCBs)	0.5 to 1
Trinitrotoluene (TNT)	0.25 to 1
Royal Demolition Explosive (RDX)	0.5 to 1
Pentachlorophenol (PCP)	0.1 to 0.5
Chlordane	0.1 to 0.5
Lindane	0.1 to 0.5
DDT	0.1 to 0.5
2,4-D	0.1 to 0.5
Silvex	0.1 to 0.5
Carboxoamide pesticides	0.1 to 0.5
Cyclodiene pesticides	0.1 to 0.5
Organochlorine pesticides	0.1 to 0.5
Organophosphorus pesticides	0.1 to 0.5
Phenol pesticides	0.1 to 0.5

The most common environmental immunoassay is the enzyme-linked immunosorbent assay. This method uses antibodies (i.e., proteins produced by mammalian immune systems) and enzyme conjugates (i.e., enzymes that bind with contaminants of concern [COCs]) to detect and quantify target compounds in field samples. The predominant enzyme used in enzyme conjugation for immunoassays is horseradish peroxidase. In this method, the enzyme portion of an enzyme conjugate serves as a catalyst to change a colorless compound to a measurable colored product that can be detected using a fluorometer. As COCs leave the enzyme conjugates to bind to the antibody sites, the amount of enzyme conjugate available to catalyze the color reaction gradually decreases. (Figure 2-6 is a schematic of how the COC part of an enzyme conjugate binds with antibody sites.) In effect, the amount of contaminant present in the sample is proportional to and inverse of the color intensity detected by a fluorometer.

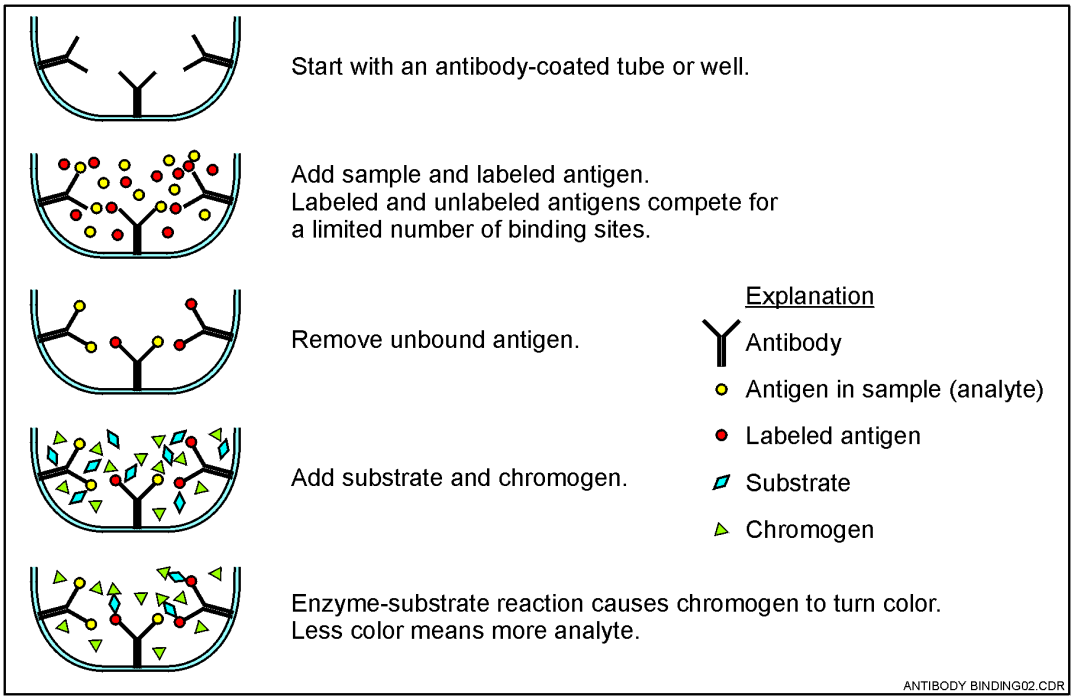

FIGURE 2-6. Schematic of COC-enzyme binding (Source: U.S. EPA, 1996)

Factors affecting performance. Sites with a single contaminant, or only one type or chemical class of contamination, are the sites most suited for the immunoassay method. Immunoassays may not be applicable to sites contaminated with complex mixtures of chemicals because interferences may arise. Immunoassays are not applicable to sites with unknown site conditions and contaminants or to sites that do not have established cleanup criteria.

Instruments and vendors. Almost all environmental immunoassays use a variation of the general procedure illustrated in Figure 2-6. Vendor-specific aspects of this method include how and where the engineered antibody is attached to the solid phase, how the sample and reagents are mixed and in what order, and how the analytical measurement (colorimetric determination) is performed. Regardless of vendor, the solid phase, antibody, enzyme conjugate, and color agents are manufactured so that results are highly reproducible.

SOPs for the following vendor immunoassays are provided in Appendix B:

- ❑ RaPID Assay System for PCB Analysis (Strategic Diagnostics, Inc.)
- ❑ EnviroGard PCB Test Kit (Strategic Diagnostics, Inc.)
- ❑ D TECH PCB Test Kit (Strategic Diagnostics, Inc.)
- ❑ PCB Immunoassay Kit (Hach Company)
- ❑ PCB In Soil Tube Assay (EnviroLogix, Inc.).

2.2.4 QwikSed Bioassay

The QwikSed bioassay is a rapid and cost-effective bioassay system for conducting contaminated sediment toxicity tests. Toxicity is measured as a reduction in light emitted from a bioluminescent dinoflagellate such as *Gonyaulux polyedra* or *Ceratocorys horrida* (see Figure 2-7) following exposure to a toxicant. Figure 2-8 shows a schematic of the bioassay procedure. A measurable reduction or inhibition in bioluminescence is an adverse effect and suggests the presence of bioavailable contaminants and other stressors. The toxic response is usually measured within 24 hours from the start of the test and can be conducted for a 4-day acute test or a 7- to 11-day chronic test. The endpoint used to measure light reduction is the inhibition concentration 50 (IC50), a 50 percent reduction in light output when compared to controls.

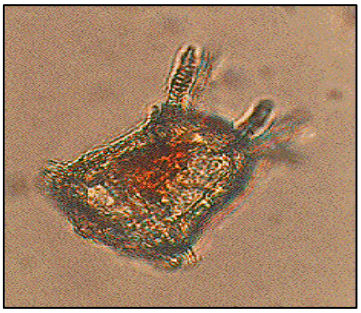

FIGURE 2-7. Dinoflagellate *Ceratocorys horrida*

The QwikSed bioassay can be used for RSC prior to conducting other, more expensive toxicity tests. It can be set up quickly and easily, and can be performed by personnel with minimal training in toxicity testing. Testing can be done in less than one hour per day, and initial assessments of toxicity can be made within 24 hours.

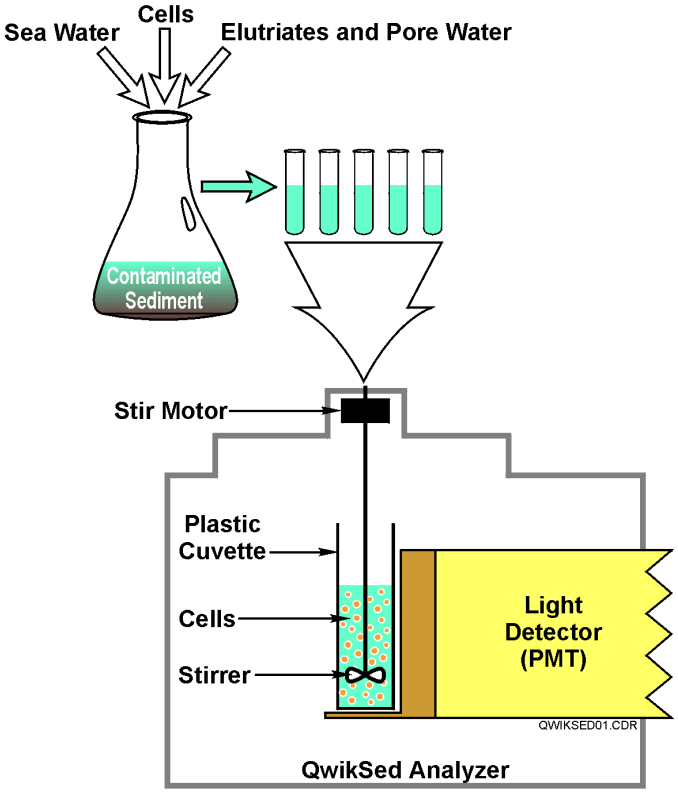

FIGURE 2-8. Schematic of the QwikSed bioassay

QwikSed bioassays can evaluate both acute and sublethal chronic effects from exposure to a variety of toxicants. The dinoflagellates in the QwikSed bioassay are at least as sensitive to organic and inorganic toxicants as mysid shrimp, silverside fish, chain diatoms, and sea urchins.

The QwikSed bioassay has been effective for testing the potential toxicity of storm drain discharges, leachates of ship hull coatings, and industrial discharges as well as marine sediments.

Instruments and vendors. The QwikSed bioassay (see Figure 2-9) is licensed to Sealite Instruments, Inc., of Fort Lauderdale, FL. The cost of the QwikSed analyzer and supporting software is about $15,000. The analyzer includes built-in software that automatically stores the raw data and calculates the mean, the standard deviation, the coefficient of variation, the percent of control, and the estimated IC50.

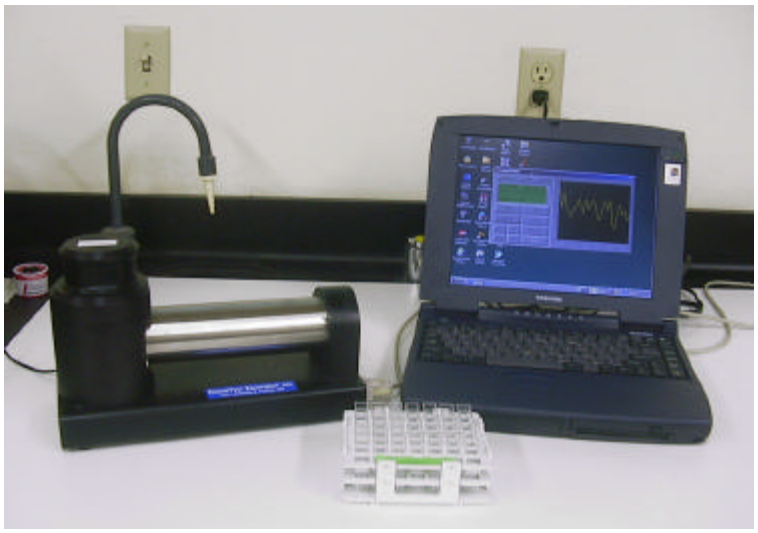

FIGURE 2-9. QwikSed bioassay instrumentation

The QwikSed bioassay analyzer can be connected either to a printer through a serial port on the back of the analyzer or to a computer for further manipulation or graphing the data. The data from the QwikSed bioassay can be correlated with more conventional toxicity test such as amphipod and sea urchin development.

The SOP for a QwikSed bioassay is provided in Appendix B.

2.3 Selecting an RSC Method

To determine if RSC methods are appropriate for use at a given site, site-specific project goals and parameters as defined by data quality objectives should be considered (see Figure 1-1). First, the contaminants or criteria that will be used in decision making must be detectable using RSC methods. Even if screening methods are not available for all COCs, RSC methods may still be appropriate.

Because classes of contaminants tend to co-associate, parameters that are more easily measured can act as proxies for a suite of contaminants, to guide sampling and to interpolate between samples when a full suite of analyses is undertaken. In all cases, RSC methods should be supplemented with samples for which thorough standard laboratory analyses are carried out.

Once it is determined that RSC methods can be used effectively at a given site, then the appropriate method(s) for the site must be selected. Figure 2-10 is a decision tree for selecting among the four RSC methods discussed in this guide. It considers whether there are known contaminants at the site, and, if so, what the characteristics of these contaminants are. An appropriate RSC method then is selected based on the outcome of the decision process.

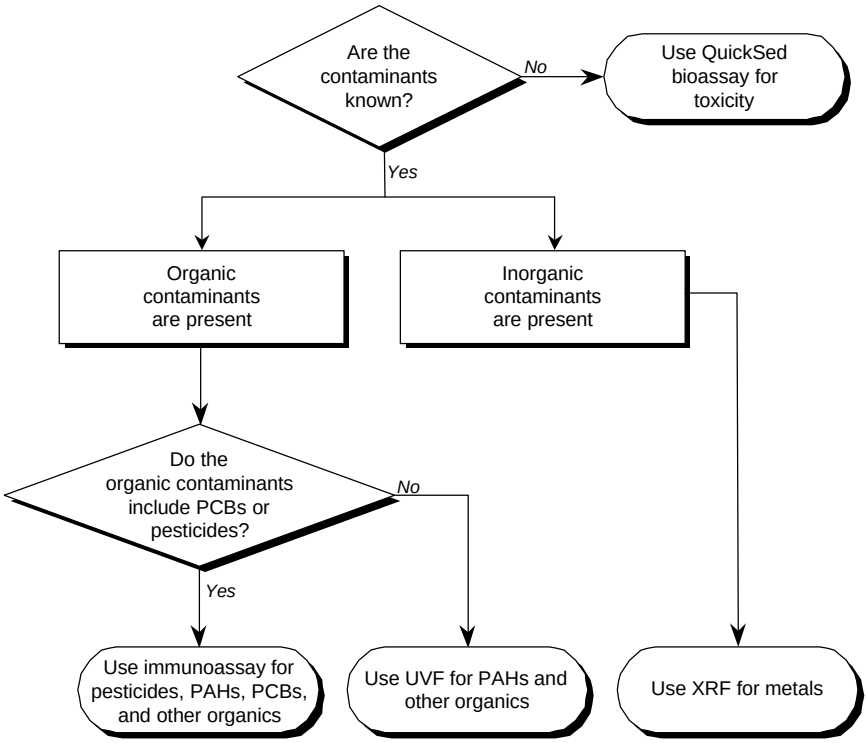

FIGURE 2-10. Decision tree for selection of RSC method

2.4 References

American Society for Testing and Materials. 1998. "Standard Guide for Conducting Toxicity Tests with Bioluminescent Dinoflagellates." *Annual Book of ASTM Standards*. ASTM E 1924-97.

ASTM, see American Society for Testing and Materials.

United States Environmental Protection Agency. 1996. *Region I, EPA-New England: Immunoassay Guidelines for Planning Environmental Projects.* October.

U.S. EPA, see United States Environmental Protection Agency.

3. Case Studies

This section contains case study descriptions of three contaminated marine sites where RSC tools were used to identify types of contaminants and delineate their distributions. These case studies are intended to demonstrate the usefulness of RSC tools during actual site investigations. Although each of the study areas is unique in terms of past usage and contamination history, investigators were able to deploy RSC tools readily at each site in order to quickly produce screening data which were used to augment existing site data. In all three cases, the increased spatial coverage made possible by RSC tools was sufficient to satisfy the objectives of the site investigations.

3.1 Hunters Point Shipyard

3.1.1 Background

Hunters Point Shipyard (HPS) is a former Naval facility located on a peninsula southeast of San Francisco, CA. HPS comprises about 955 acres, with approximately 400 acres of offshore sediments. From 1945 to 1974, the Navy used HPS predominantly for ship repair and maintenance. HPS was deactivated in 1974 and remained relatively unused until 1976, when it was leased to a private ship repair company. In 1986, the Navy resumed occupancy of HPS. The base was closed in 1991.

Historical activities at HPS resulted in the release of chemicals to offshore sediments. A feasibility study (TtEMI and LFR, 1998) showed high- and low-volume contaminant footprints based on two different decision flow processes. The high-volume footprint was based on a more conservative set of criteria. The low-volume footprint was initially based on effects range-median (ER-M) values and bioaccumulation criteria for PCBs and dichlorodiphenyltrichloroethane (DDT). The low-volume contaminant footprint consisted of the five areas of HPS shown in Figure 3-1.

Based on an evaluation of existing sediment chemistry and bioassay data, the Navy and the regulatory authorities agreed that the low-volume footprint would be the focus of remedial activities. However, a review of previous investigations indicated that existing sediment chemistry data were insufficient to reliably determine the boundaries of the five low-volume footprint areas and contaminant distribution within each area. As a result, RSC methods were used to more clearly define the extent of sediments that pose an unacceptable risk to the environment.

Because previous sampling at HPS focused on delineating contamination associated with specific outfalls, the available data did not provide sufficient spatial coverage of the five areas within the low-volume footprint. However, because of the size of the five areas and the number of samples required to adequately delineate spatial variability, a comprehensive sampling program was not feasible. Therefore, a sediment screening survey using RSC methods was conducted to help refine the sampling design for a more comprehensive field and laboratory study. The objectives of the screening survey were as follows:

- ❑ Provide data to support mapping of concentrations of contaminants in sediments
- ❑ Confirm areas of higher and lower concentrations of contaminants
- ❑ Provide data to support designs for analytical sampling.

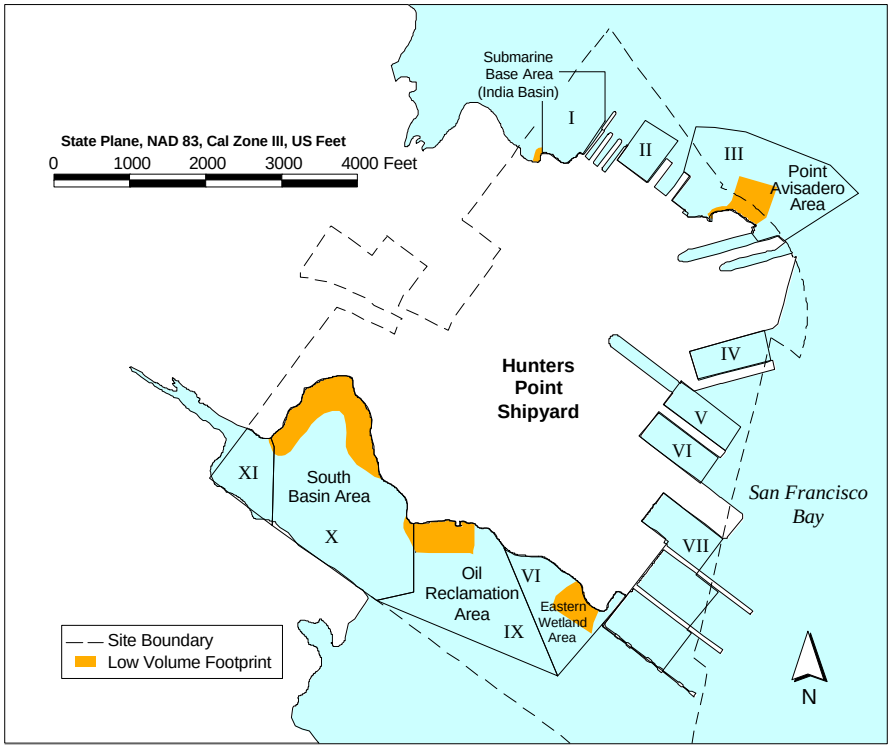

FIGURE 3-1. Location map of low-volume contaminant footprint at HPS

3.1.2 Methods and Results

In April 2000, the Navy collected 95 surface sediment samples in a systematic grid pattern designed to represent each of the five areas in the low-volume footprint of HPS. The sediment samples were shipped ovenight to laboratory facilities at SSC San Diego for analysis. The samples also were analyzed using a benchtop XRF to identify concentrations of lead, copper, zinc, and chromium; and immunoassay to identify PCBs. In addition, confirmatory samples were obtained from 10 of the 95 sediment sampling locations; these samples were analyzed using standard laboratory procedures.

The screening survey and laboratory results were combined with historical data collected at other Navy sites in San Francisco Bay, and correlations were identified to show the relationships between the data. The screening data were adjusted based on the relationships previously established in the historical data sets (Figure 3-2). Following these adjustments, the screening data were combined with other historical data and plotted to evaluate the spatial distribution of contaminants in each of the five areas. By augmenting the historical data with the new screening results, excellent spatial coverage of each of the areas was obtained. Figures 3-3 through 3-7 show the spatial distributions of copper, lead, zinc, chromium, and PCBs, respectively, in the form of bubble plots.

Review of the spatial analysis of historical and screening data at HPS revealed that contaminant distributions within the low-volume footprint were structured spatially. High values were found together rather than randomly distributed. This finding confirmed that sediments in near shore areas were potential source terms and that these areas might be from a different “population” than sediments farther away from shore.

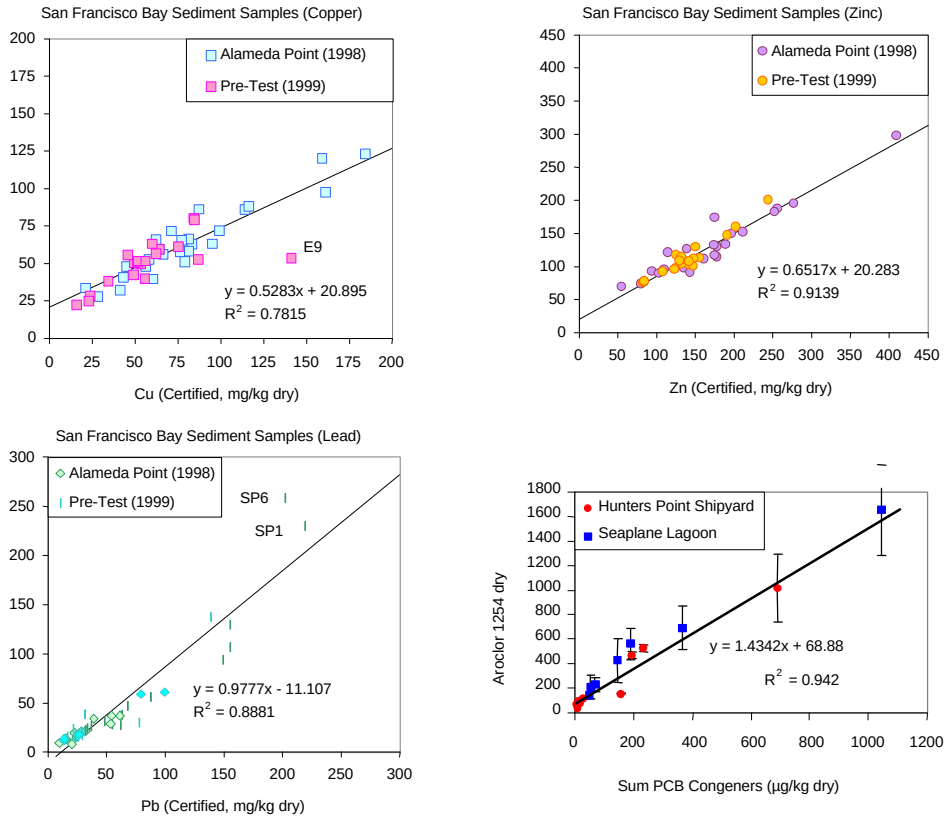

FIGURE 3-2. RSC historical data sets from San Francisco Bay

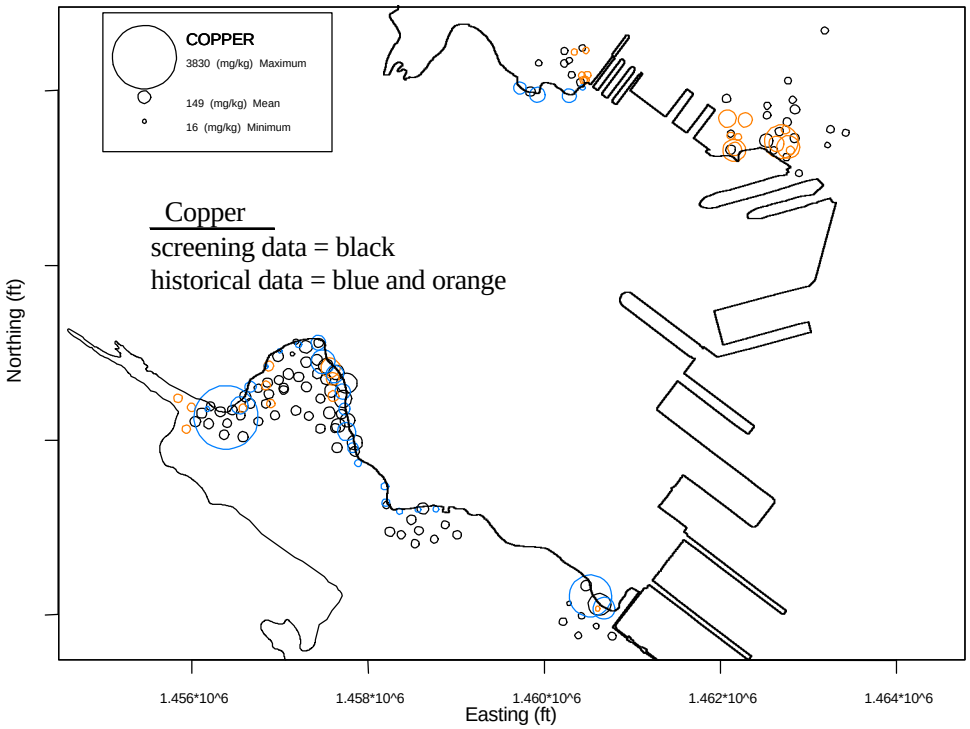

FIGURE 3-3. Bubble plot for copper from historical and screening data at HPS

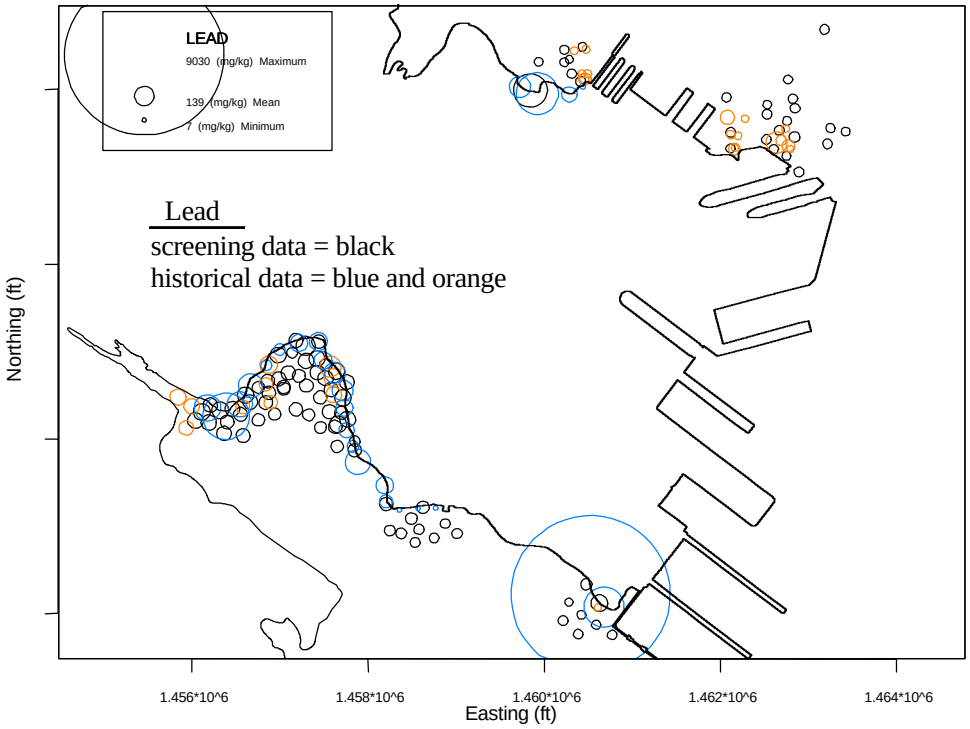

FIGURE 3-4. Bubble plot for lead from historical and screening data at HPS

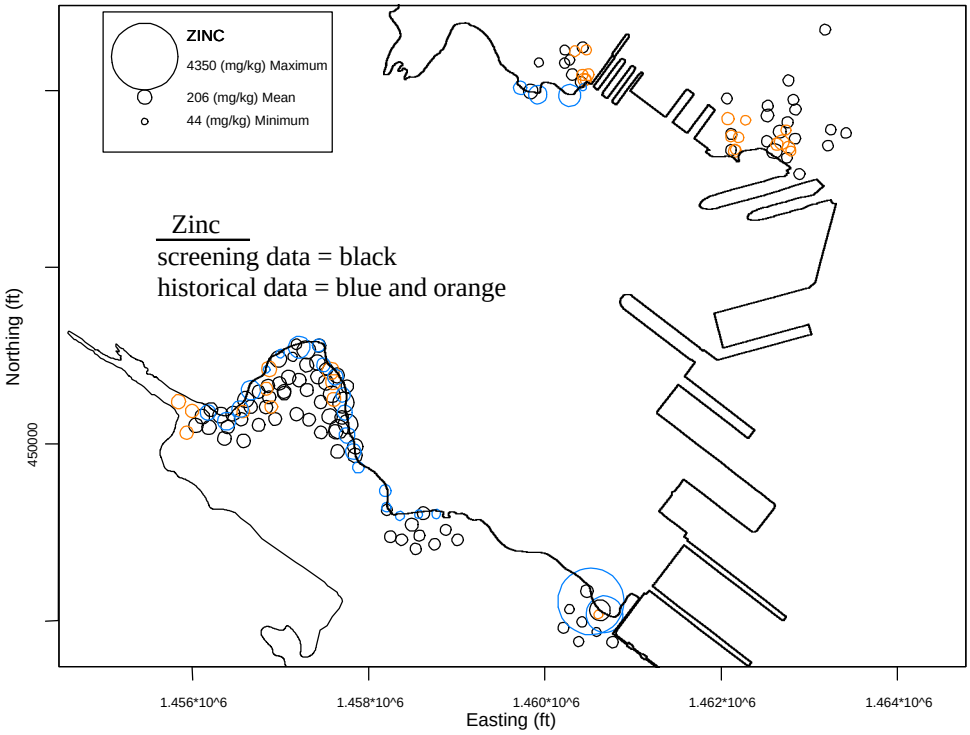

FIGURE 3-5. Bubble plot for zinc from historical and screening data at HPS

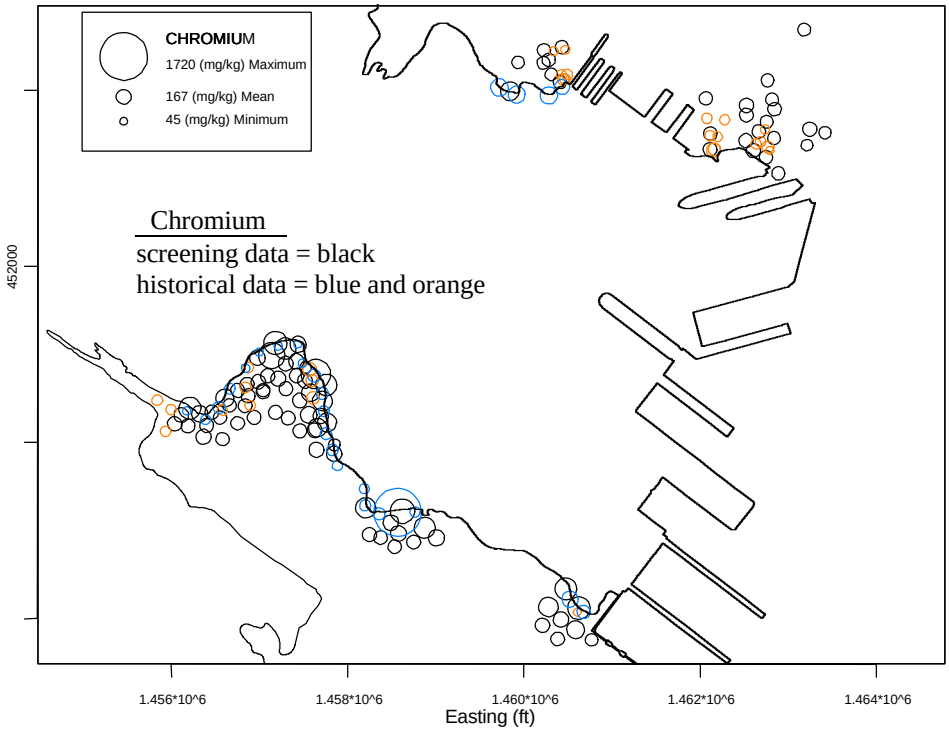

FIGURE 3-6. Bubble plot for chromium from historical and screening data at HPS

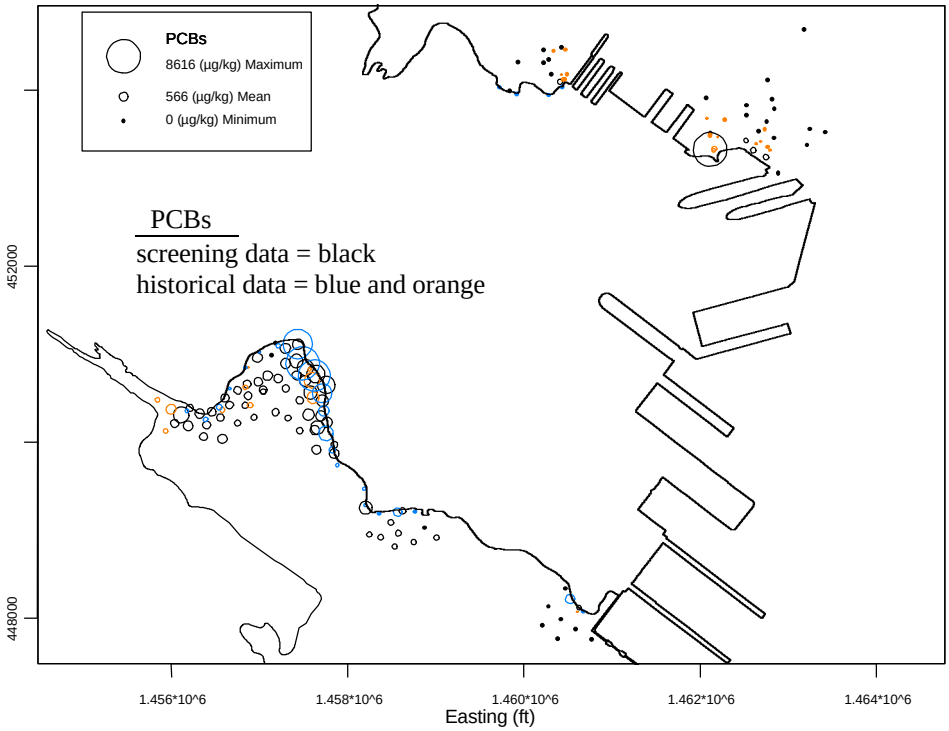

FIGURE 3-7. Bubble plot for PCBs from historical and screening data at HPS

Because of the implications of these results, the subsequent sample design was set up to identify spatial strata based on chemical concentration within each of the five areas. This new sampling strategy was designed to ensure that a full range of chemical concentrations was sampled.

Thus, RSC methods provided a cost-effective means to obtain the necessary spatial coverage to determine the existence of contamination contours, so that a focused sampling design could be developed.

3.2 Alameda Point

3.2.1 Background

Alameda Point is located on Alameda Island, at the western end of the City of Alameda in Alameda County, CA. Alameda Island lies along the eastern side of San Francisco Bay and is adjacent to the City of Oakland (Figure 3-8). Alameda Island was the location of Naval Air Station (NAS) Alameda, until the closing of the NAS in 1997. The rectangular-shaped base was about 2 miles long and 1 mile wide, and occupied about 1,526 acres of land and 1,108 submarine acres (2,634 total acres). The majority of Alameda Point is land that was created by hydraulically filling existing tidelands, marshlands, and sloughs; the majority of the fill was dredged material from many areas, including Oakland Inner Harbor (Ecology and Environmental, Inc., 1983).

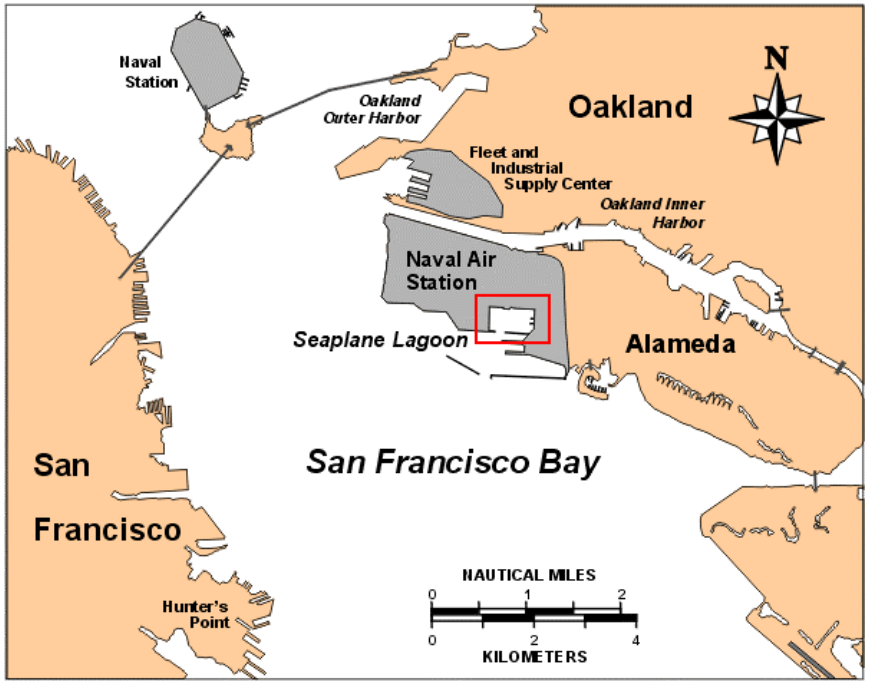

FIGURE 3-8. Site map of Alameda Harbor including Seaplane Lagoon

In 1997, RSC techniques (XRF and UVF) were used to delineate the extent of PAH and metals contamination at the Pier Area inside Seaplane Lagoon. Then, in 1998, an expanded investigation was initiated using a combination of field RSC techniques and

laboratory analyses to investigate the extent of contamination in deep water and lagoon sediments. The projects focused on Seaplane Lagoon, which is 110 acres in size and is located at the southeastern corner of the former NAS, and on areas near the deep-water piers located on the south side of the lagoon. Sea walls surround most of the lagoon, inhibiting the natural flushing processes of bay tides. A breakwater extending from Pier No. 1 forms the southern wall of the lagoon. The entrance to the lagoon is through an 800-ft-long opening in the breakwater. The depth of the lagoon varies from small beach surfaces to a depth of 15 ft. Outside the Seaplane Lagoon are berths for deep draft ships (Piers 1, 2, and 3). These berths are protected by an outer breakwater and have periodic maintenance dredging. No regular dredging program has ever existed at the Seaplane Lagoon, and sediment accumulation is evident in many areas of the lagoon.

Industrial wastewater generated at former NAS Alameda before 1974 was discharged directly to the storm drains. The storm drains, in turn, discharged to the Seaplane Lagoon and other offshore areas. Wastewater discharged in the lagoon from 1940 through 1975 was reported to contain heavy metals, solvents, paints, detergents, acids, caustics, mercury, and oil and grease (Ecology and Environment, Inc., 1983). Ship wastewater, which may have contained solvents, chromium, waste oil, and fuel, was also released into the lagoon (Ecology and Environment, Inc., 1983). Between 1972 and 1975, the industrial waste collection system was rerouted to discharge to the East Bay Municipal Utilities District wastewater system. Other chemicals may have entered the lagoon due to tidal action sweeping ship wastewater—possibly containing solvents, chromium, waste oil, and fuel—from the berthing area into the lagoon. Today, sources of sediment contamination may be caused by current berthing practices or historical activities at Piers 1, 2, and 3.

3.2.2 Methods and Results

Pre-Demonstration Study

For the 1997 (i.e., pre-demonstration) study, 31 sediment samples were collected from the Pier Area inside Seaplane Lagoon. These samples were analyzed on site using two RSC methods: XRF and UVF. Fifteen (15) of these samples were subsampled, properly preserved, and sent to a State of California-certified laboratory for confirmatory analyses.

Figures 3-9 through 3-11 show the metals results from the XRF screening of the pre-demonstration samples. In this case, the data were not adjusted using site-specific calibration procedures. Rather, the results were plotted to simply identify regions of increasing levels of contamination. As can be seen in the figures, the sediments near the Pier 1 quay wall appear to be more heavily impacted by zinc, lead, and copper than are the other regions.

Figure 3-12 shows the results from pre-demonstration screening data for PAH contamination in deep-water pier sediments at Alameda Point. These data were adjusted using site-specific calibration procedures. The screening study indicated that elevated concentrations of PAH compounds are predominantly located in the outfall areas along the quay wall. These observations, in particular, led regulatory sampling in 1998 to focus on the high PAH areas.

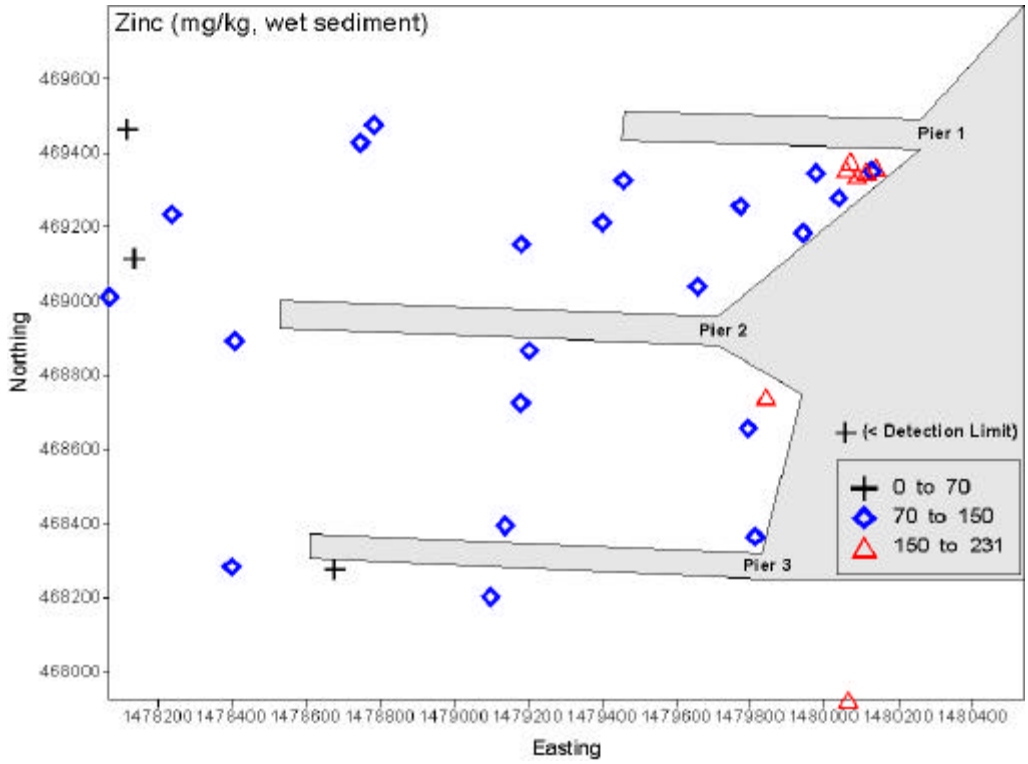

FIGURE 3-9. Pre-demonstration screening results for zinc using XRF

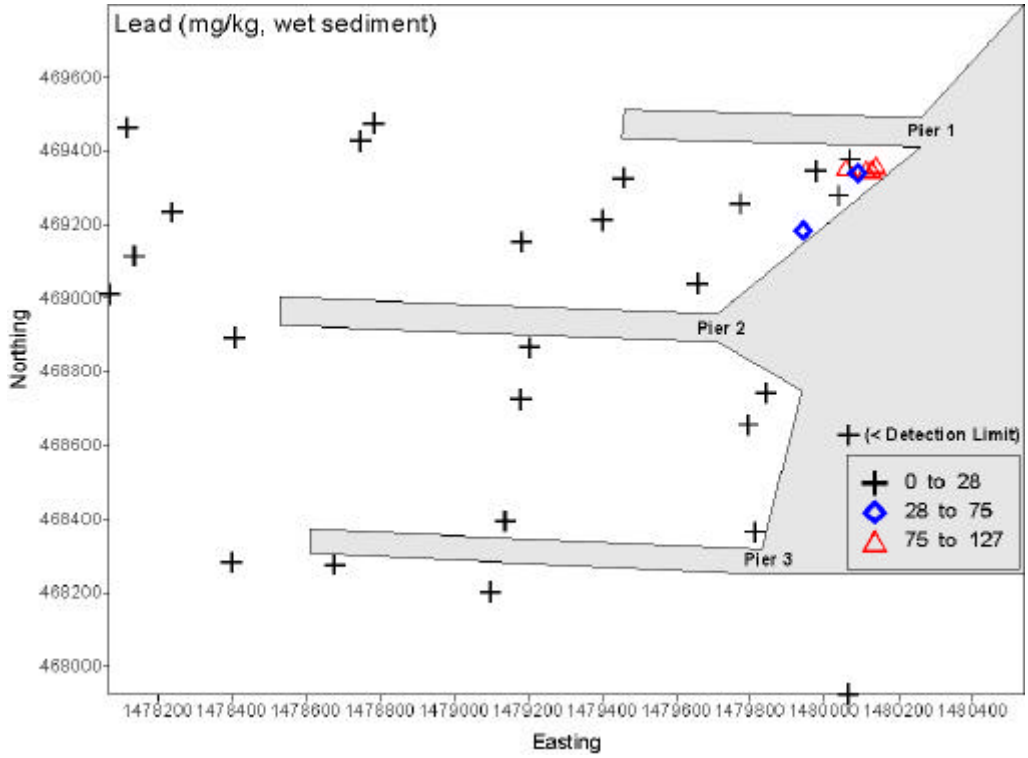

FIGURE 3-10. Pre-demonstration screening results for lead using XRF

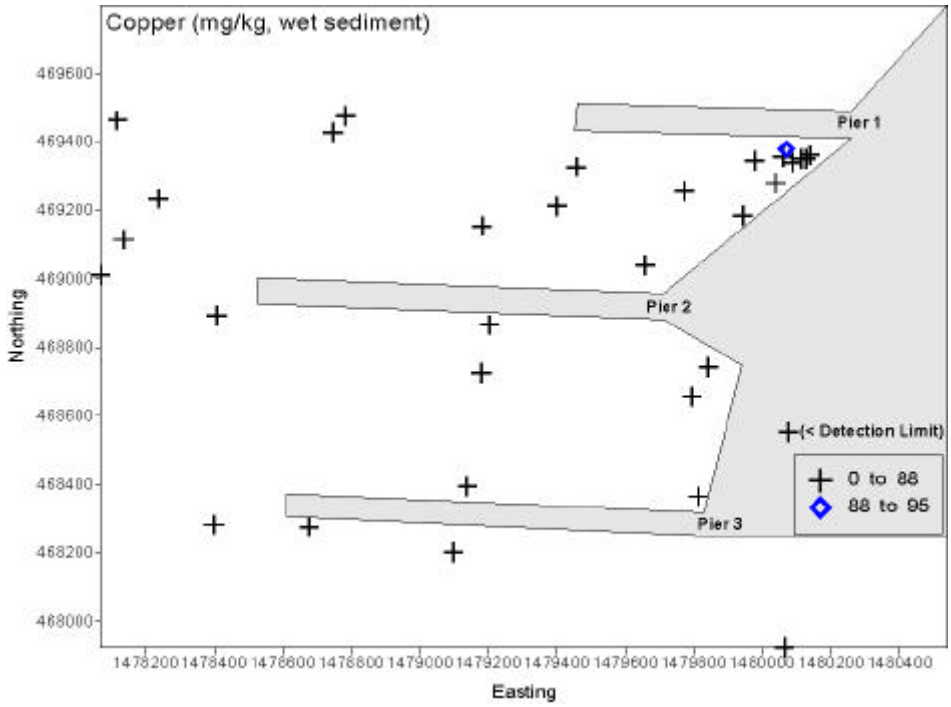

FIGURE 3-11. Pre-demonstration screening results for copper using XRF

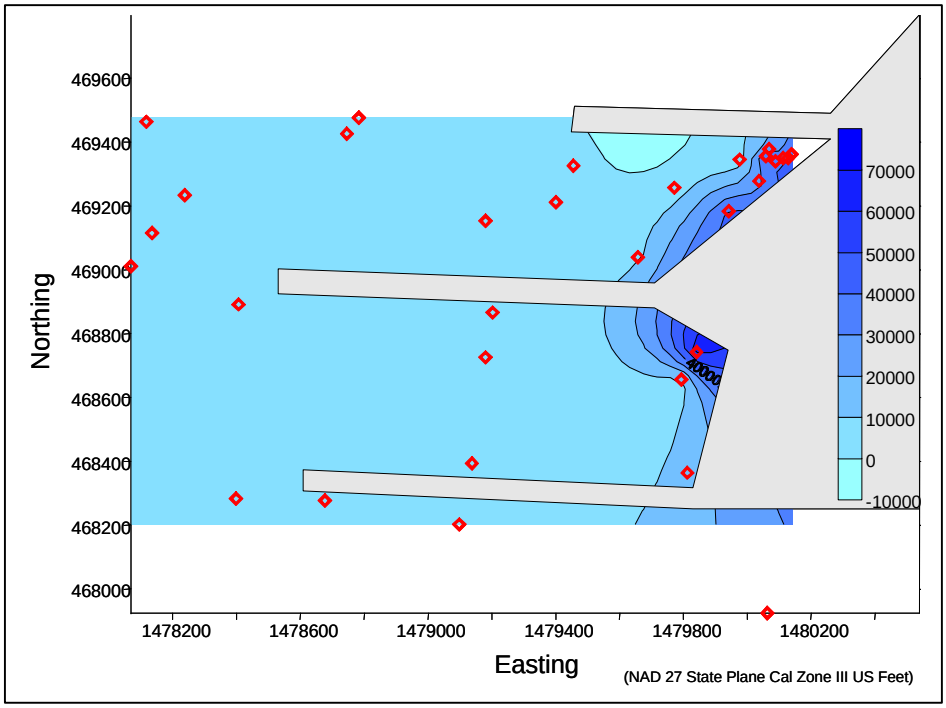

FIGURE 3-12. Pre-demonstration screening results for PAHs (ppb) using XRF

Overall, results of pre-demonstration sampling clearly delineated the extent of contamination at the Pier Area, and were used to help design 1998 *Baseline Ecological Risk Assessment* sampling plan (PRC, 1998).

Demonstration Study

For the 1998 (i.e., demonstration) project, a total of 29 sediment samples were collected from three offshore areas of concern at Alameda Point: Seaplane Lagoon, the Pier Area, and the Beach Area. Each sample was split mechanically; subsamples from each of the 29 samples were analyzed using the suite of RSC techniques, and some subsamples were properly preserved and sent to a State of California-certified laboratory for confirmatory analyses. The RSC demonstration was completed simultaneously with the regulatory sampling in order to provide "real-time" feedback to ensure that samples were collected over the full chemical range at the site.

In the metals analysis portion of the study, correlations were sought between the field XRF results and laboratory confirmations for zinc, lead, and copper. Field XRF results included results from samples collected from the Pier Area (pre-demonstration) and results from samples collected from the lagoon (demonstration). Due to time constraints, the remaining samples collected during the demonstration (Pier Area and Beach Area) were analyzed upon return to the laboratory. Linear regression calculations were performed to find the coefficients of determination (R^2), slopes, and intercepts for all three sets of data. The R^2 result measures the goodness of fit and can range between one (or −1), in which case the data sets are exactly correlated (or inversely correlated) with one another, and zero, in which case there is no correlation between the two sets of data (i.e., random distribution).

Both physical and chemical matrix interference effects were expected with samples from Alameda Point. The anticipated physical matrix effects included moisture and sample heterogeneity. The samples collected from Alameda Point ranged in percent moisture from 30% to 70%. The sediment particle size ranged from coarse, sandy to fine-grained. Sediment samples from certain locations (e.g., corners of Seaplane Lagoon, and near the piers) contained chunks of foreign material including wood from pier pilings, paint chips, and unidentified particles. The coarse-grained material in most other samples appeared to be primarily shell hash and mineral material. Chemical matrix effects were encountered which were caused by elevated concentrations of iron (~ 5%), which absorb (attenuate) copper x-rays, thereby reducing the intensity of copper measured by the detector. Although not all types of interferences can be easily corrected, the use of Fundamental Parameter (FP) coefficients can correct for both physical and chemical matrix effects to some degree. Standardless FP calibration was used throughout the demonstration.

In general, zinc and lead data obtained by field XRF measurements correlated well with laboratory data; typical coefficients of determination ranged from 0.7 to 0.8. Figure 3-13 shows the results for zinc measurements graphically, in which circles represent samples collected for the pre-demonstration in the Pier Area and squares represent samples collected during the demonstration in Seaplane Lagoon. For zinc, R^2 was approximately 0.71, based on regression of 26 data points. Figure 3-14 shows the results for lead measurements during the same investigations. In the case of lead the R^2 value was 0.81 and the sensitivity of the field instrument was closer to that of the laboratory method, as indicated by the line slope of ~ 1. An important feature of the XRF measurements,

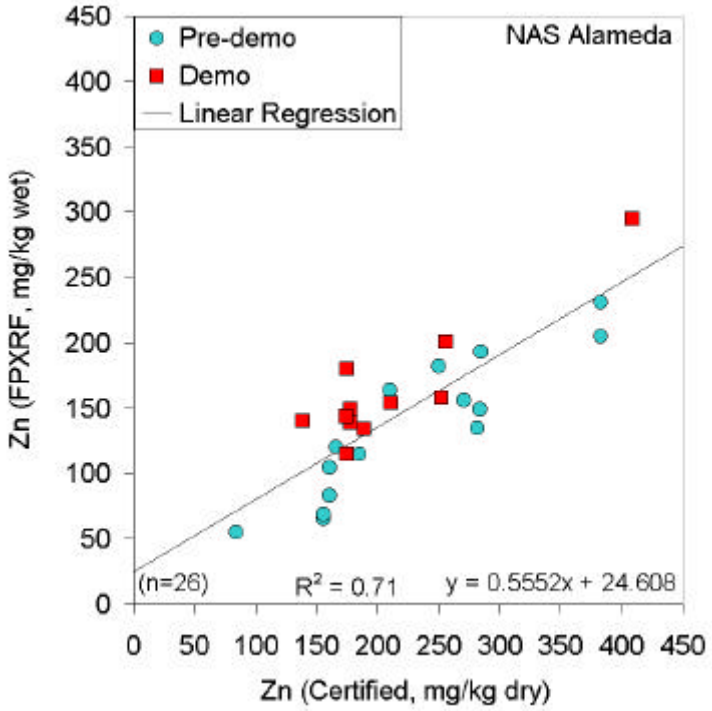

FIGURE 3-13. Pre-demonstration and demonstration results for zinc using XRF, plotted against results from standard methods

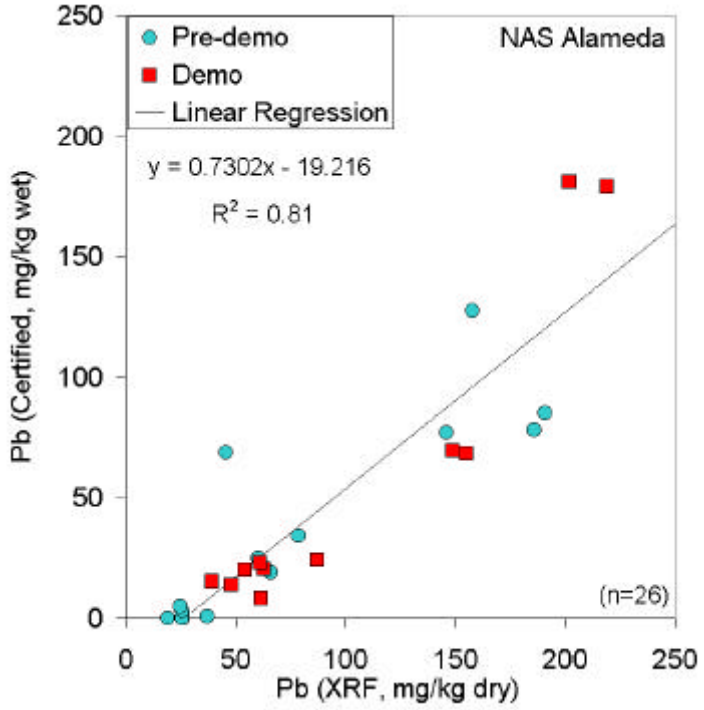

FIGURE 3-14. Pre-demonstration and demonstration results for lead using XRF, plotted against results from standard methods

illustrated by these two examples, is that even though these data were collected at different areas and during two different deployments, there appears to be little deviation in instrument response. In general, when field XRF measurements are taken continuously over a long period, say more than six months, the only adjustment needed is to increase the analysis time to correct the instrument for x-ray source decay.

It can be observed from Figure 3-13 that the field XRF underpredicts the zinc concentration throughout most of the measurement range. Underprediction by field XRF, compared to laboratory methods, is commonly observed for metals. However, underprediction is not always severe, as shown by the data for lead in Figure 3-14, which shows an improved relationship between the field XRF and certified laboratory measurements. Some differences between the field and laboratory methods are expected because wet samples have a higher density than dry samples. However, another reason is lower sensitivity of field instruments for most elements. Nevertheless, the good correlation between field and laboratory results implies that field instruments can be calibrated with certified laboratory results using slope and intercept parameters. Therefore, simple linear regression of data sets allows the field instrument to produce accurate results.

Figure 3-15 shows field XRF and laboratory results for lead during the demonstration study, where contamination is predominant in the corners of the lagoon. It can be seen that the same concentration ranges of lead were found by the field XRF and laboratory methods.

Results of copper analyses were less satisfactory, as a majority of the samples had certified copper values below the field XRF detection limit. Thus, while these data can be analyzed, they should be examined with caution, as the proportion of samples that are appropriate for the instrument's capabilities are limited.

Other activities at Alameda Point included analysis of PCBs in sediments. Figure 3-16 shows a comparison between of certified laboratory measurements of PCB congeners and immunoassay test results for Aroclor 1254. It can be seen that the fit is quite good, with R^2 equal to 0.942.

Similar comparisons between field and laboratory methods were made for PAHs and aquatic toxicity. A correlation between PAH measurements by UVF and laboratory methods is shown in Figure 3-17. In addition to two locations at Alameda Point, Figure 3-17 contains data for Bishop Point, which is discussed in Section 3.3. It can be seen that the sensitivity of UVF is very specific to the type of PAHs present in the sediment. However, within each sediment area, the correlation tends to be quite good (R^2 values range from 0.7 to 0.9).

Results for PAH measurements are shown in Figure 3-18 and results for aquatic toxicity tests are shown in Figure 3-19. There is general agreement between all types of analyses at Alameda Point that contaminated sediments are confined to the corners of the lagoon and the Pier Area, which appears to have been impacted by creosote-pier pilings along the quay wall.

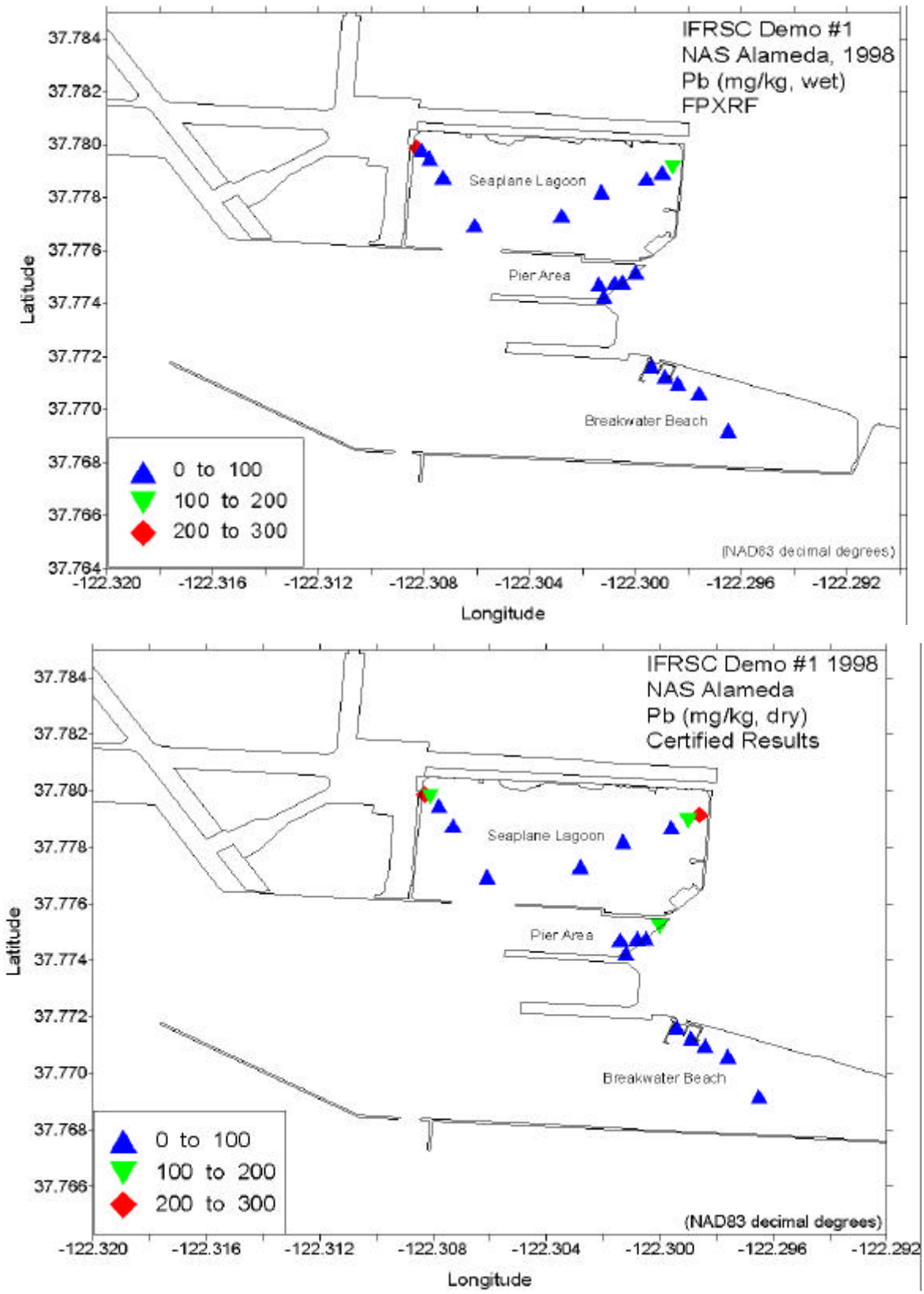

FIGURE 3-15. Comparison of demonstration results for lead using XRF (top) and certified laboratory (bottom)

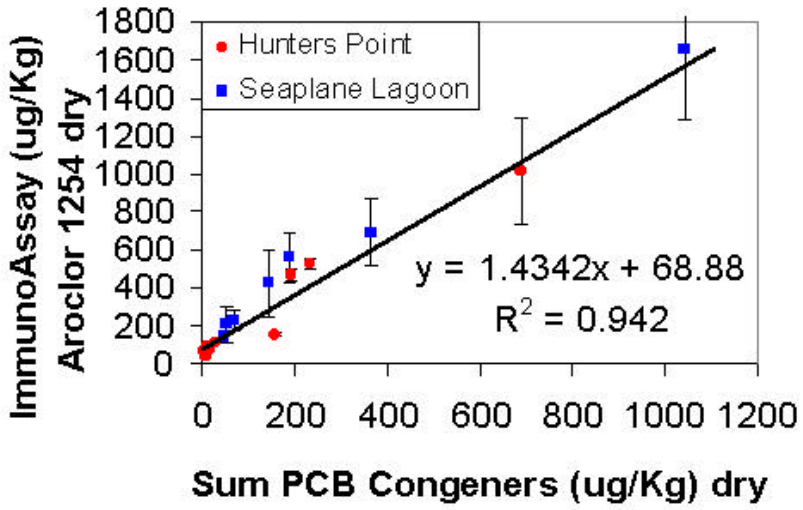

FIGURE 3-16. Demonstration results for PCBs using field immunoassay method, plotted against results from certified laboratory

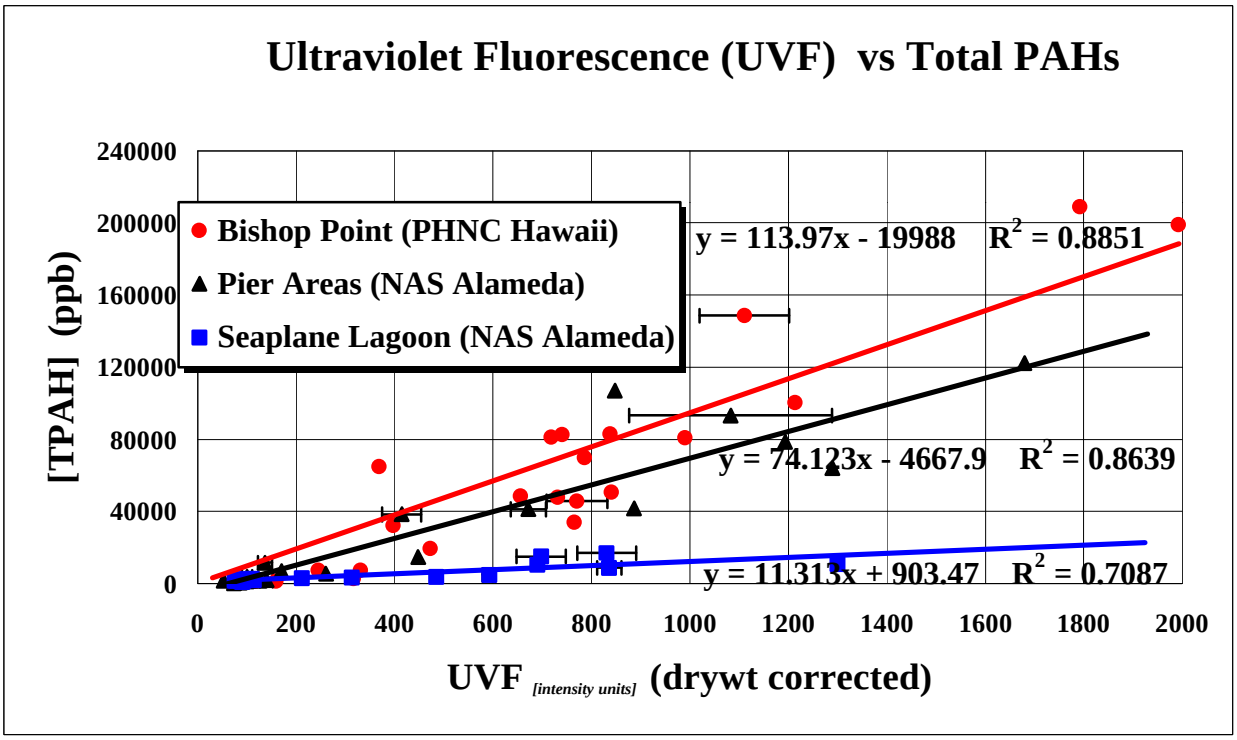

FIGURE 3-17. Demonstration results for PAHs using UVF, plotted against results from certified laboratory

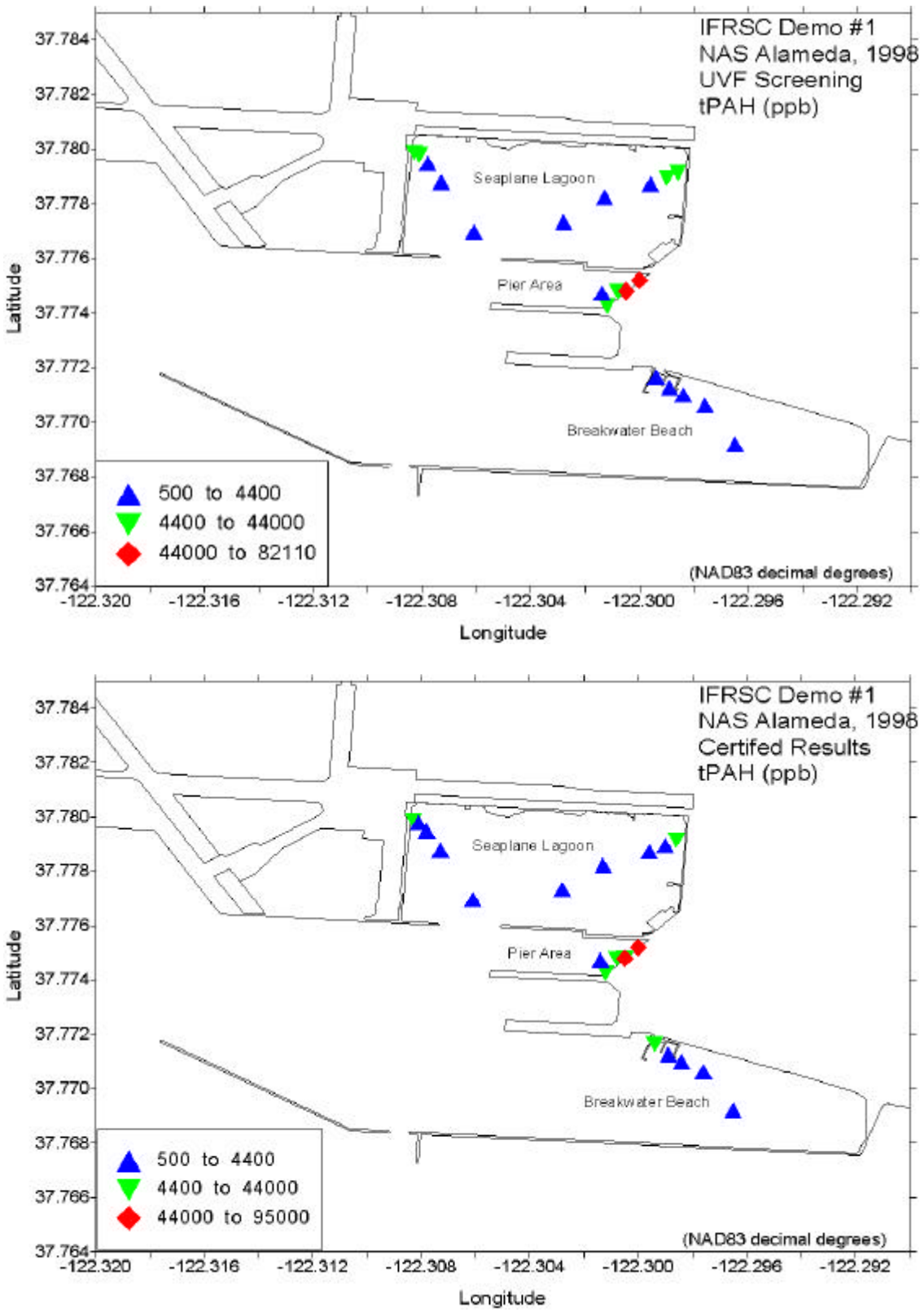

FIGURE 3-18. Comparison of demonstration results for total PAHs using field RSC methods (top) and certified laboratory (bottom)

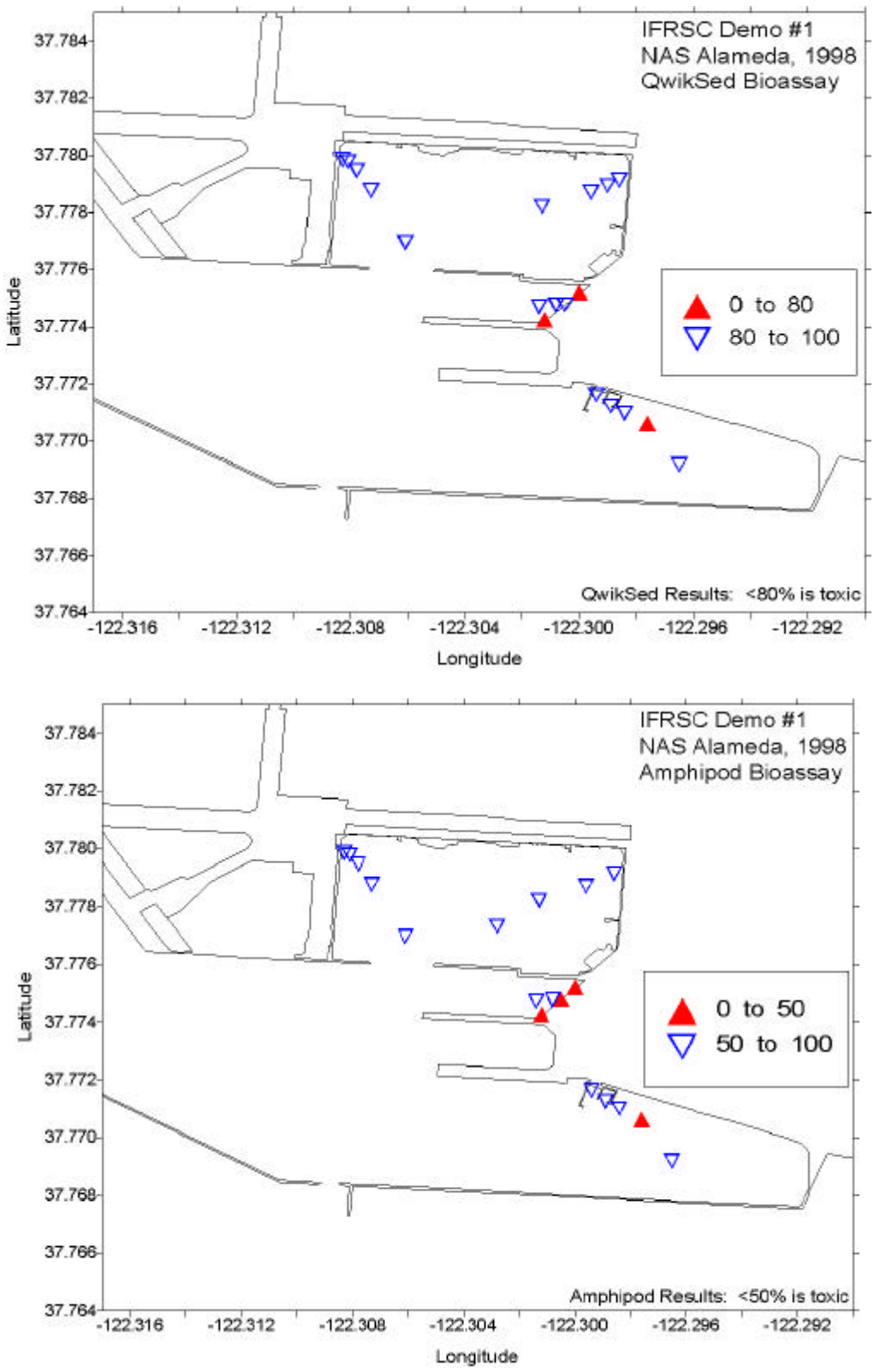

FIGURE 3-19. Comparison of demonstration results from aquatic toxicity measurements using field RSC methods (top) and certified laboratory results (bottom)

3.3 Pearl Harbor Naval Center

3.3.1 Background

Pearl Harbor is a large, complex natural estuary located on the south coast of Oahu in the Hawaiian Islands (Figure 3-20). Most of the harbor itself lies within the Pearl Harbor Naval Center (PHNC). PHNC is located in the southern portion of the Ewa plain, approximately 5.8 miles northwest of downtown Honolulu. Pearl Harbor contains 2,024 hectares (5,000 acres) of surface water area and 58 kilometers (36 miles) of shoreline. Through the influence of drainage, the Pearl Harbor estuary receives runoff from approximately 28,502 hectares (70,400 acres) of upland habitat comprising the watershed for much of the southern portion of Oahu.

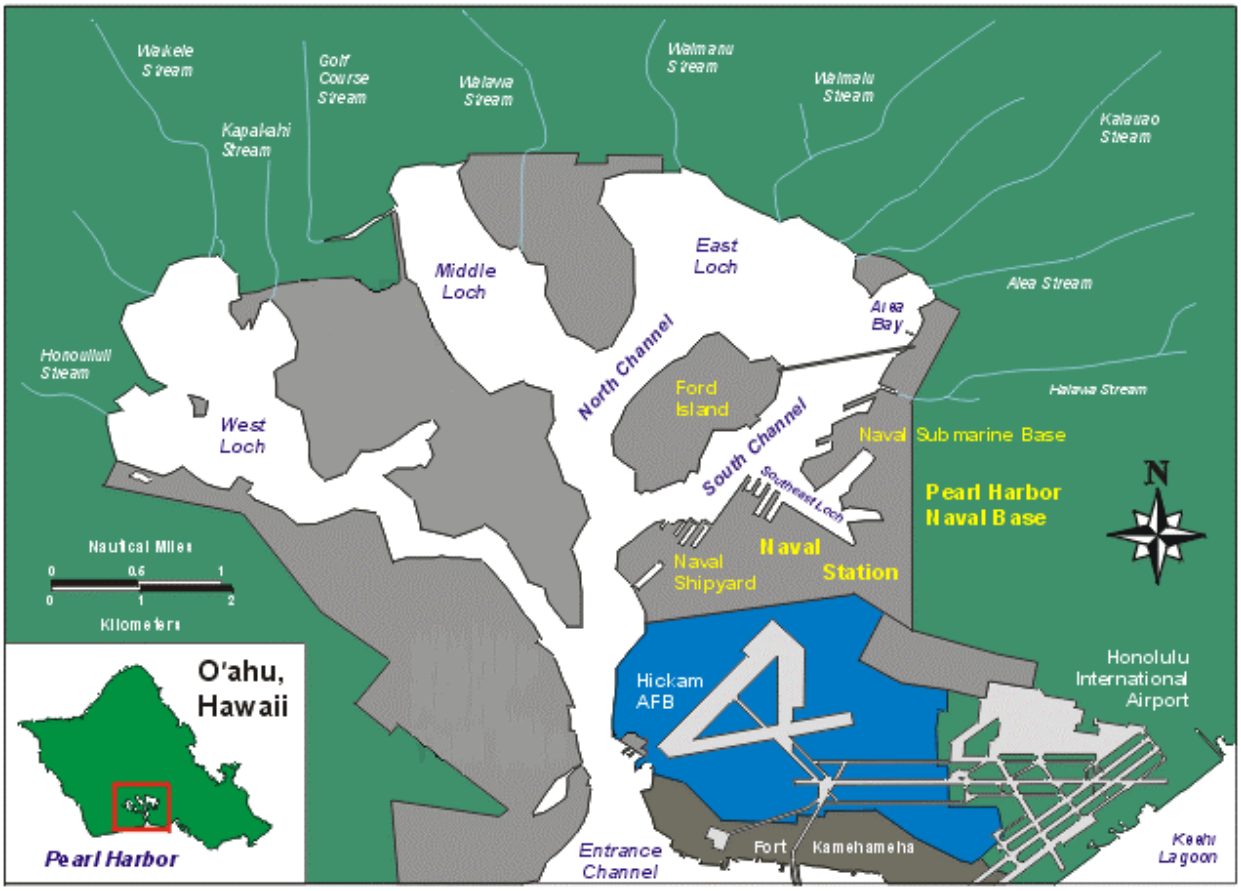

FIGURE 3-20. Site map of Pearl Harbor Naval Center

The air attack by the Japanese Imperial Navy against the U.S. Fleet in Pearl Harbor on December 7, 1941, sank or severely damaged 21 U.S. Navy warships (Lenihan, 1989; U.S. Navy, 1989). Chemical evidence (i.e., elevated concentrations of copper, lead, and zinc) of this period remains detectable in buried Middle Loch sediments that have not been disturbed by dredging activities (Ashwood and Olsen, 1988). The bombing attack also resulted in about six times more lead input to this estuarine area than the total

combined lead input from sewage disposal and naval maintenance operations during the succeeding 45 years (Ashwood and Olsen, 1988).

RSC technologies were tested in two areas at PHNC, based on a review of available contaminant distribution data. These included the Middle Loch and Bishop Point areas. The Middle Loch sediments are very rich in fine-grained sediment (75–90%), although TOC values are relatively low (2.0-3.8%). The Bishop Point area has less fines (41–56%), yet the TOC values are higher (4–6%) than in the Middle Loch area. Bishop Point is a small pier area (approximately 3 acres) that is thought to be very heterogeneous, with bottom substrate ranging from hard coral to soft mud. The pier area is in constant use by ships. In contrast, the Middle Loch area is much larger and consists of homogeneously distributed fine-grained mud. The Middle Loch is regularly dredged to maintain a draft of 20 ft and is currently used to store a "mothballed" fleet of ships.

The contaminants of concern differ in each of these two areas. Metals concentrations in the Middle Loch area are reported to be elevated, whereas they are very low at Bishop Point. However, PAH concentrations at Bishop Point are elevated; typically they range from 20–40 ppm (total) PAH. PAHs do not appear to be elevated in the Middle Loch area. Neither the Middle Loch nor Bishop Point sediments contain appreciable levels of pesticides, PCBs, or TBTs.

3.3.2 Methods and Results

At Bishop Point, the project sampling design consisted of collecting single sediment samples from designated areas. The basis for this approach was that the result from each sample was representative of the designated sampling area. RSC screening methods were used to determine whether single sample results actually were representative and to confirm heterogeneity of types and levels of contaminants for some sampling areas. Using results from multiple RSC analyses, certain sampling areas were highlighted for additional consideration.

Figures 3-21 to 3-24 show results of the screening demonstration study at the Bishop Point location in Pearl Harbor. According to Figure 3-21, elevated total PAH concentrations in Strata 2 and 3 appear to be associated with the inboard region near quay walls and piers. Figure 3-22 shows elevated zinc concentrations in Strata 2 and 3 which also appear to be associated with the inboard region, but possibly associated with ships. These profiles suggest that the sources for PAHs and zinc may have been different.

Figure 3-23 shows results of QwikSed bioassay testing. Bioassay screening results can provide evidence for the existence of contaminants, similar to chemical screening tools, but they can also flag effects of other contaminants, or synergistic effects from multiple types of contaminants. Thus, the combination of biological and chemical tools increase the likelihood of a meaningful assessment. For example, historical data showed that one sample between two piers in Stratum 2 contained high levels of contaminants and depressed amphipod survival rates. However, the screening data indicated that contamination is not widespread, but rather exists only in sediments close to the quay wall. By integrating the different screening results it is possible to develop a "weight of evidence" scenario similar to that used with standard laboratory data to differentiate areas of concern. Figure 3-24 shows several areas with multiple "hits" from the several screening techniques. Hits are defined by zinc concentrations above 250 ppm; total PAH exceeding

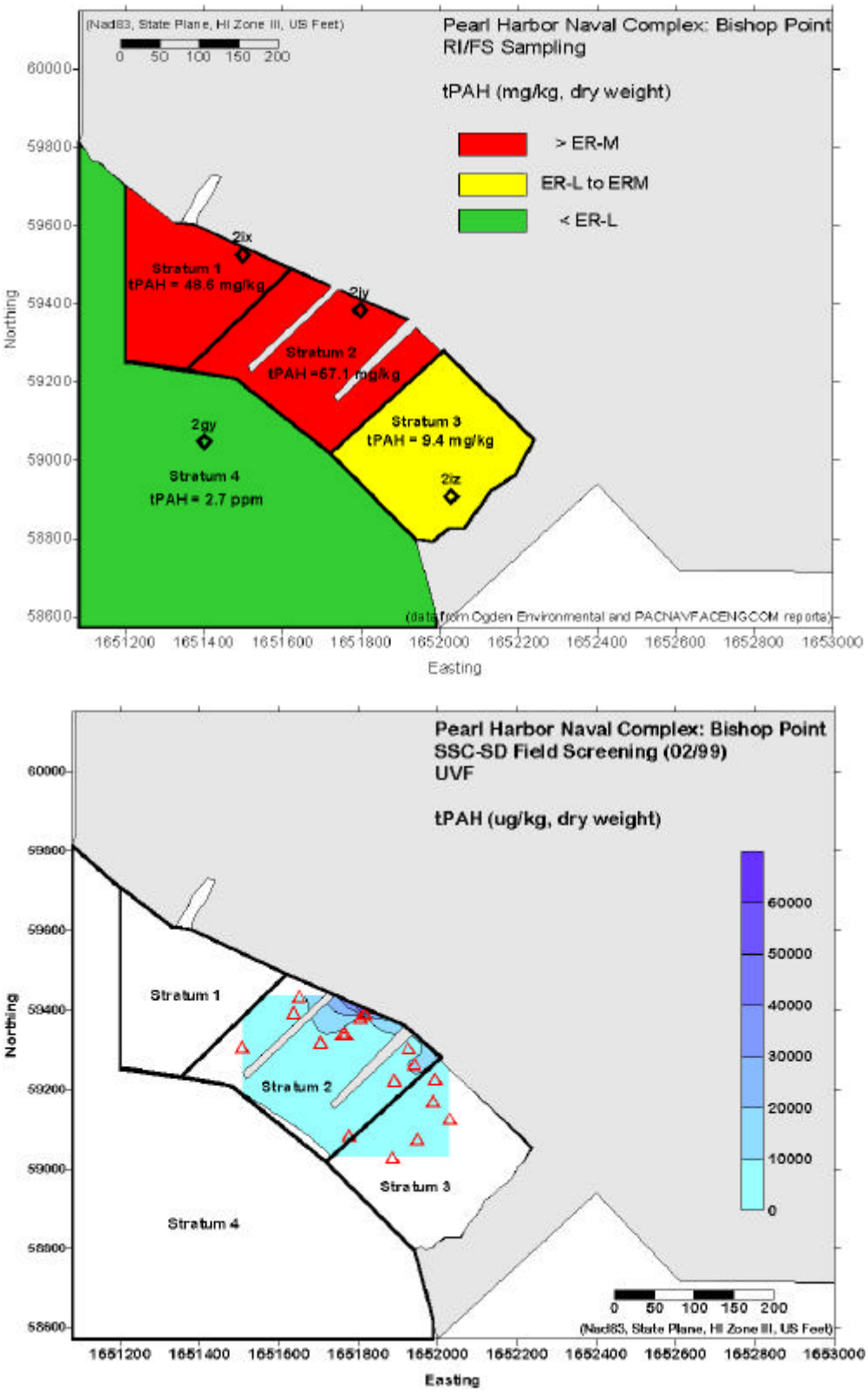

FIGURE 3-21. Comparison of regulatory sampling results (top) and UVF screening results for total PAHs (bottom) at Bishop Point, Pearl Harbor

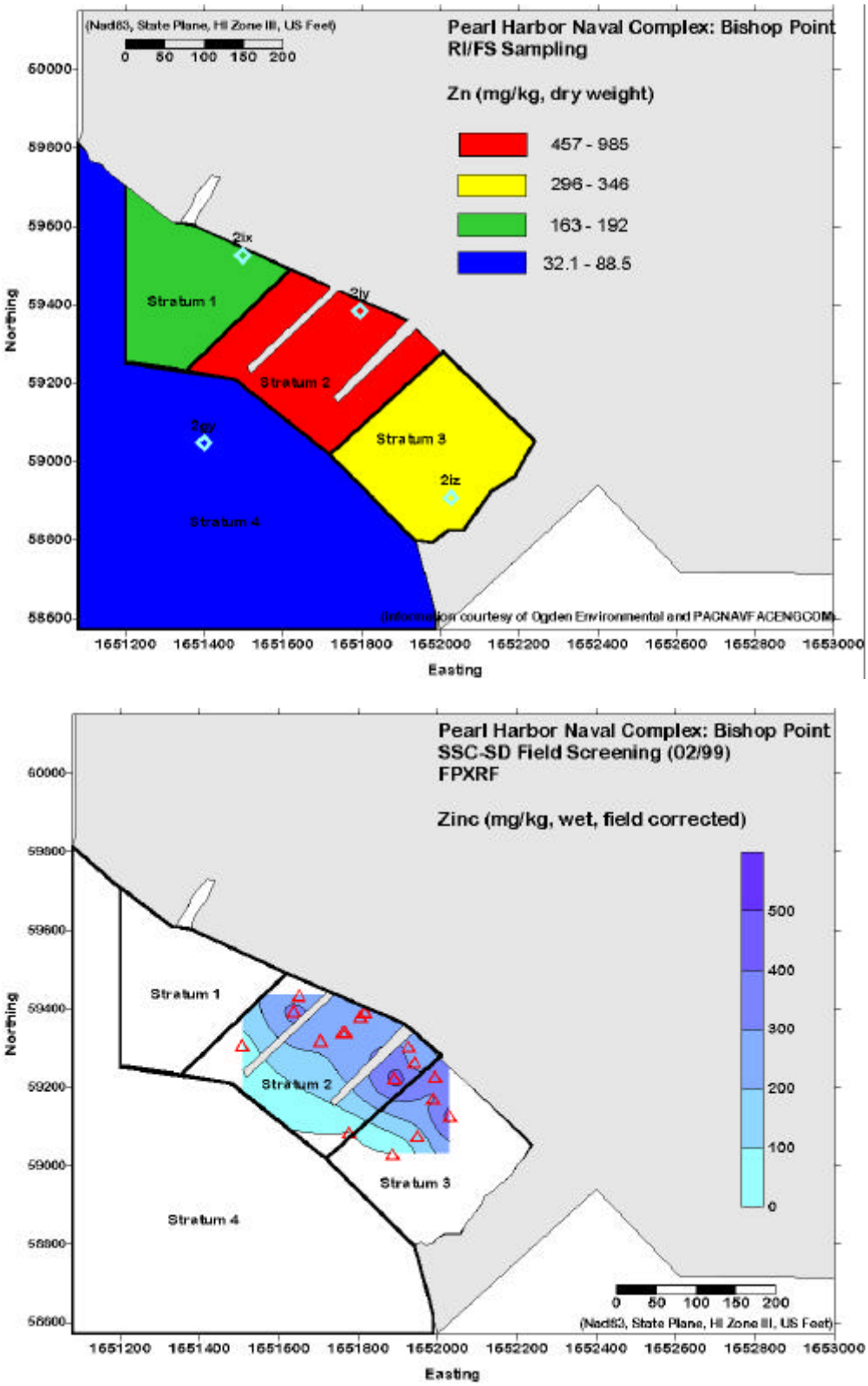

FIGURE 3-22. Comparison of regulatory sampling results (top) and UVF screening results for zinc (bottom) at Bishop Point, Pearl Harbor

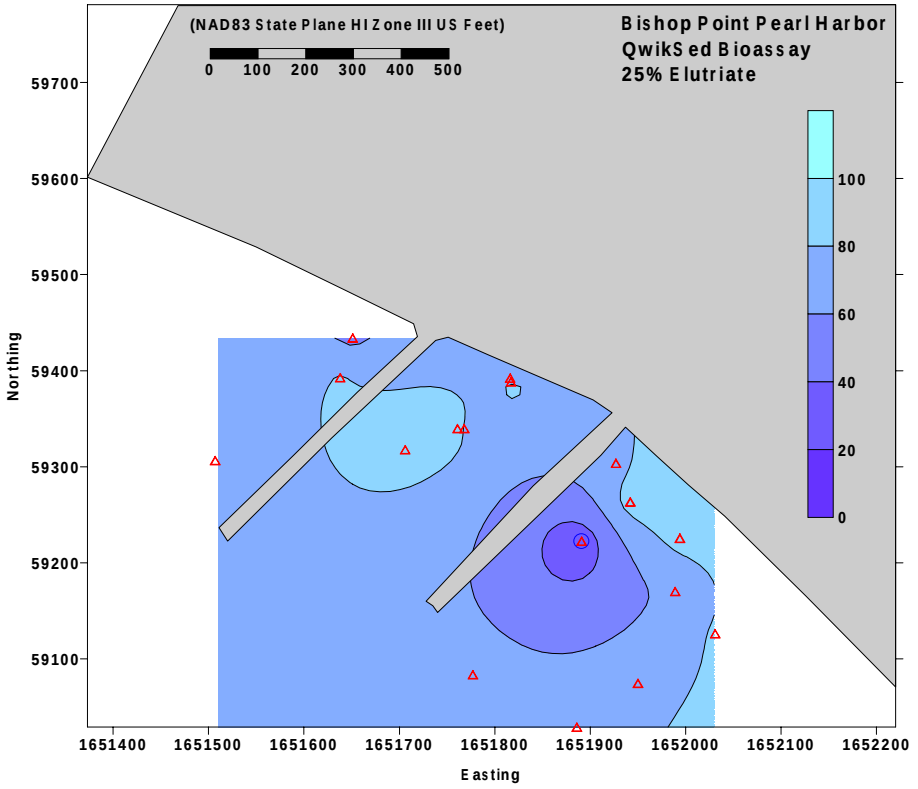

FIGURE 3-23. Results of bioassay screening using the QwikSed bioassay at Bishop Point, Pearl Harbor

Effects-Range Low (ER-L) (Long et al, 1995); and QwikSed bioassay less than 80% of control for the 25% elutriate.

The procedure used at Bishop Point could be used to prioritize areas for additional work or discussions with the regulators about the existing regulatory data. The contouring of these screening data is preliminary, and the heterogeneity suggests that very different results are possible if the initial assumptions had been different. This conclusion supports the use of this type of screening method to assess area heterogeneity and to support discussions with regulators about how much data are required within a stratum to make a regulatory decision. For other areas of Pearl Harbor (for example Middle Loch), data are much more homogeneous and single samples within strata might provide sufficient confidence to reach a decision.

3.4 References for Pearl Harbor

Ashwood, T.L., and C.R. Olsen. 1988. "Pearl Harbor Bombing Attack: A Contamination Legacy Revealed in the Sedimentary Record." *Marine Pollution Bulletin, 19*: 68-71.

Ecology and Environment, Inc. 1983. "Initial Assessment Study, Naval Air Station, Alameda, California," Prepared for Navy Assessment and Control of Installation Pollutants Department, Naval Energy and Environmental Support Activity, Port Hueneme, CA.

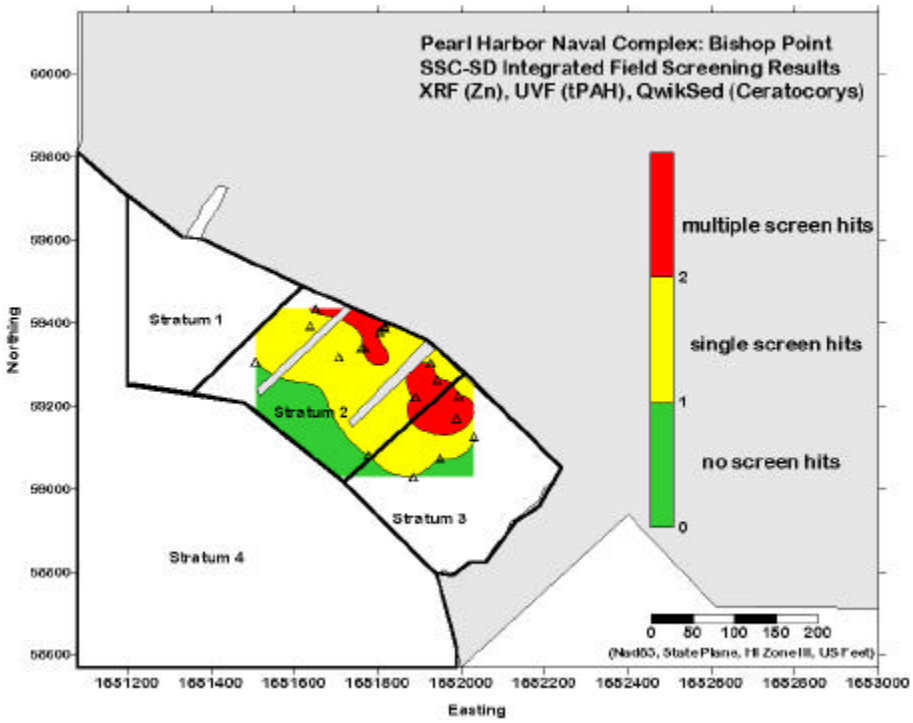

FIGURE 3-24. Integrated results from RSC methods at Bishop Point, Pearl Harbor

Lenihan, D.J. (Ed.). 1989. "Submerged Cultural Resources Study: USS Arizona Memorial and pearl Harbor National Historic Landmark." NPS Professional Paper #23. Prepared for the National Park Service, Southwest Cultural Resources Center, Santa Fe, NM.

PRC, see PRC Environmental Management, Inc.

PRC Environmental Management, Inc. 1998. *Ecological Risk Assessment Work Plan and Field Sampling and Analysis Plan, Preliminary Draft: Breakwater Beach, Pier Area, and Seaplane Lagoon, Alameda Point, Alameda, California.* August 1.

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TtEMI and LFR, see Tetra Tech EM, Inc. and Levine-Fricke-Recon, Inc.

U.S. Navy. 1989. "Case Studies of Selected Harbor Clearance Operations, NAVSEA Technical Manual SO300-BE-MAN-010." Published by the direction of the Commander, Naval Sea Systems Command, Pearl Harbor, HI.

4. Cost Assessment

This section discusses the cost factors that affect the total cost of analyzing sediment samples using RSC methods. Cost data can be either obtained from instrument vendors or collected from the literature. In general, several site-specific factors can affect the cost of using RSC methods at a given site, including the number of samples processed in a given period of time. Where possible, these cost factors are addressed so that users can independently complete site-specific economic analyses. Therefore, some assumptions must be made before these data can be used in cost calculations.

4.1 Cost Factors

Two types of factors can affect the cost of RSC analyses: equipment costs and labor costs. *Equipment* costs include mobilization and demobilization expenses, the cost of reagents and other consumable supplies needed for the analyses, and rental fees and/or cost of equipment purchases. Equipment mobilization and demobilization costs are those associated with shipping equipment and supplies to and from a site. Supplies include consumable reagents as well as preparation and assay materials required for RSC methods. Purchase and rental costs for the RSC equipment (discussed in Section 2), along with the cost of supplies, are listed in Table 4-1.

Labor costs include travel, per diem, and on-site labor expenses. Travel involves the expense for an analyst to travel to a site, and typically includes airfare (or other transportation) and rental car fees (approximate range is $250-1,500 per trip). Per diem expenses include food, lodging, and incidentals (approximately $75 per day). Labor expenses can include preparing and managing the samples, performing the analyses, and reporting the results (if an analyst performs all of this work, the estimated cost is $86 per hour).

4.2 Estimating Costs

To assist in estimating total and per-sample costs, a spreadsheet tool called the *Cost Estimating Program for Using Rapid Sediment Characterization Tools* (RSCT) was developed to guide the user through the process of cost estimation. This program allows the user to choose default parameters or to modify these parameters to suit specific applications. RSCT was developed using MS Visual Basic programming language. It runs on the MS Excel for Windows 95/NT 4.0 platform. MS Excel 7.0 or higher is required to run RSCT.

To begin cost estimations, start the MS Excel program. Within MS Excel file menu, select the “Open” submenu to access the Open dialog box. Select the file “RSCT.xls” either by typing in the file name and location or by browsing the file hierarchy. Once the RSCT.xls file has been selected, clicking on the “Open” button will make the opening screen appear, as shown in Figure 4-1. Selecting the “Enable Macros” button will run the program.

Once the program file is open, the main screen will be displayed, as shown in Figure 4-2. On that screen are four command buttons labeled with the names of each of the four RSC methods. Selecting and clicking on one of these buttons displays a user form, such as the XRF screen shown in Figure 4-3. The user form allows the user to select a vendor and an instrument model; to check options for rental or purchase of equipment; and to input the number of samples, analysis time, and cost data associated with the analyses. Default values for labor rate, travel, per diem, and car rental costs are input automatically by the program, but can be modified by the user.

TABLE 4-1. RSC cost information

RSC Technology	Instrument Vendor	Instrument Model	Purchase Price	Monthly Rental Cost	Cost of Supplies per Sample[f]	Sample Throughput per Day
XRF Benchtop	KevexSpectrace	QuanX	$73,500	N/A	$2	25-50
	Shimadzu	EDX-700	$85,200	N/A	$2	25-50
XRF Field Portable	KevexSpectrace	TN 9000	$48,000	$6,000[a]	$2	50-100
	Metorex	X-MET 920	$29,380	$3,000	$2	50-100
	Metorex	X-MET 920 MP[g]	$35,380	$3,500	$2	50-100
	Niton	XL-702	$29,000	$4,060	$2	50-100
	Niton	XL-722	$38,000	$5,320	$2	50-100
UVF	Turner	10-AU	$11,140	$1,765[b]	$3	20-40
	SiteLab	UVG-3100A	$12,000	$3,600[c]	$3	20-40
Immunoassay for PCB Analysis	Strategic Diagnostics	RaPID Assay	$4,700	$800[d]	$24	40-82
	Strategic Diagnostics	EnviroGard Kit	$315	$800[d]	$30	24-48
	Strategic Diagnostics	D Tech Kit	$300[e]	N/A	$36	24-48
	Hach	PCB Kit	$995	N/A	$35	24-48
	EnviroLogix	Soil Tube Assay	$425	N/A	$18	24-48
Bioassay	Sealite Instruments	Qwiklite	$15,000	N/A	$10	6-10

N/A indicates that information in this block is not available

(a) Alternate lease rate for KevexSpectrace TN 9000 is $3,500 for 2 weeks

(b) Alternate lease rate for Turner 10-AU is $1,265 for 2 weeks

(c) Alternate lease rate for SiteLab UVG-3100A is $900 for one week

(d) Alternate lease rates for Strategic Diagnostics Accessory Kits are $450 per week and $175 per day

(e) Price refers to Strategic Diagnostics DTECHtor reflectometer to read results

(f) XRF supplies consist of sample cups, Mylar film, gloves, mixing rods; UVF supplies consist of hexane solvent, glassware, gloves, mixing rods; immunoassay supplies consist of methanol solvent, gloves, micro-liter pipettes, timer, photometer; other reagents, tubes, and vials are provided with kit assemblies; bioassay supplies consist of dinoflagellate culture, sea salt, gloves, glassware, cuvettes, micro-liter pipettes.

(g) Mini-portable, battery operated unit.

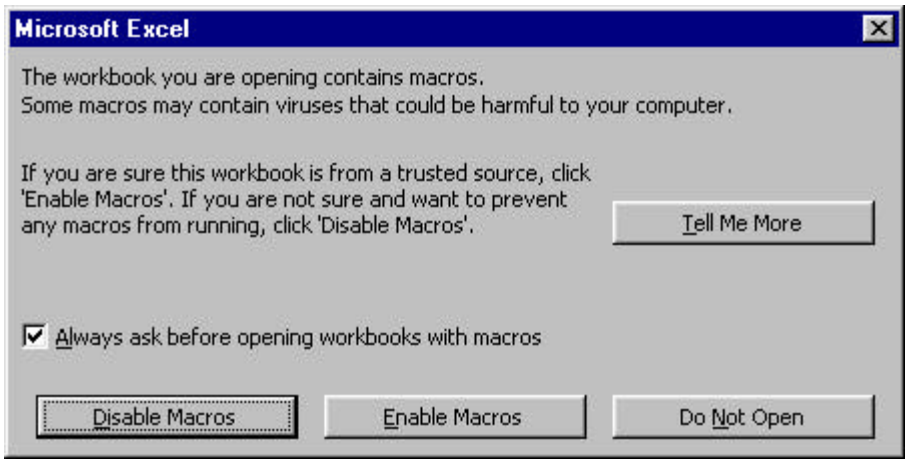

FIGURE 4-1. RSCT opening screen

After the form is completed, clicking on the "Finish" button will generate a cost summary sheet, as shown in Figure 4-4. The cost summary sheet includes a list of all user inputs, and a table summarizing the unit cost, quantity, and total cost for each cost component. The total cost and average cost per sample also are presented.

Two buttons, labeled "Main" and "Back," allow the user to return to the main menu or to the user form, respectively. The user then can modify the cost estimate either directly on the spreadsheet or through the user form. It is recommended that the user give each RSCT.xls file a unique file name and save each file individually.

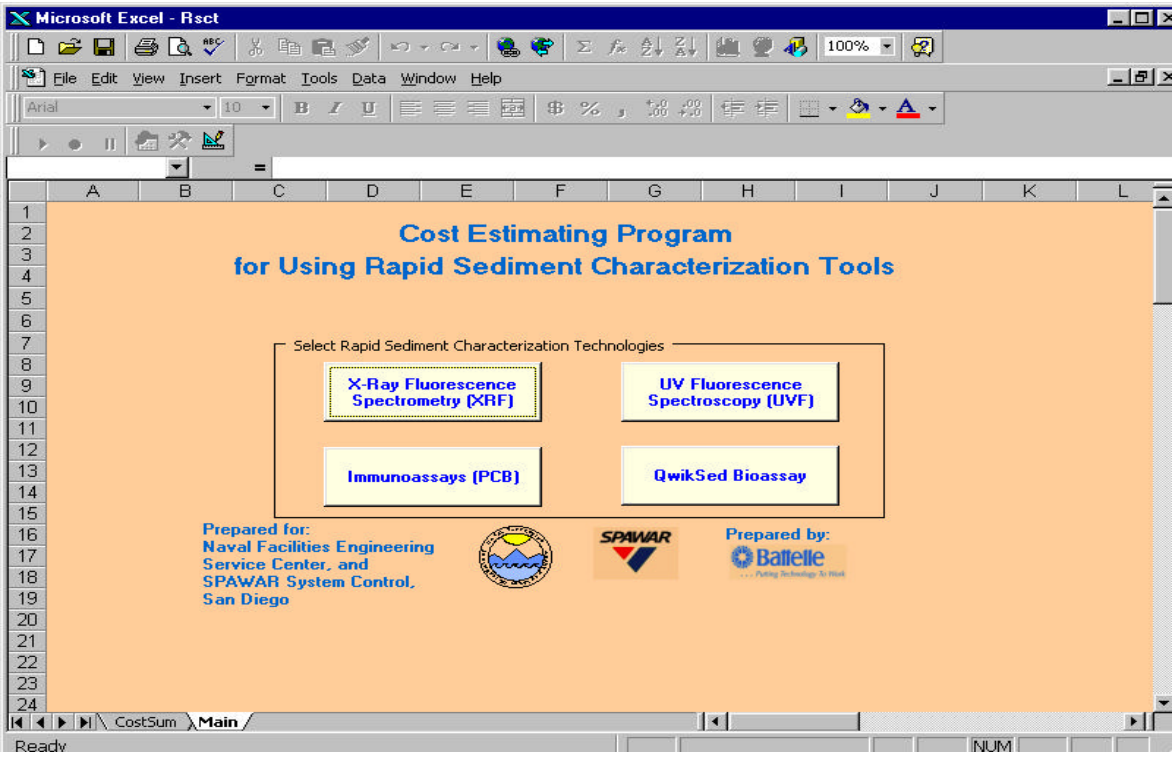

FIGURE 4-2. RSCT main screen

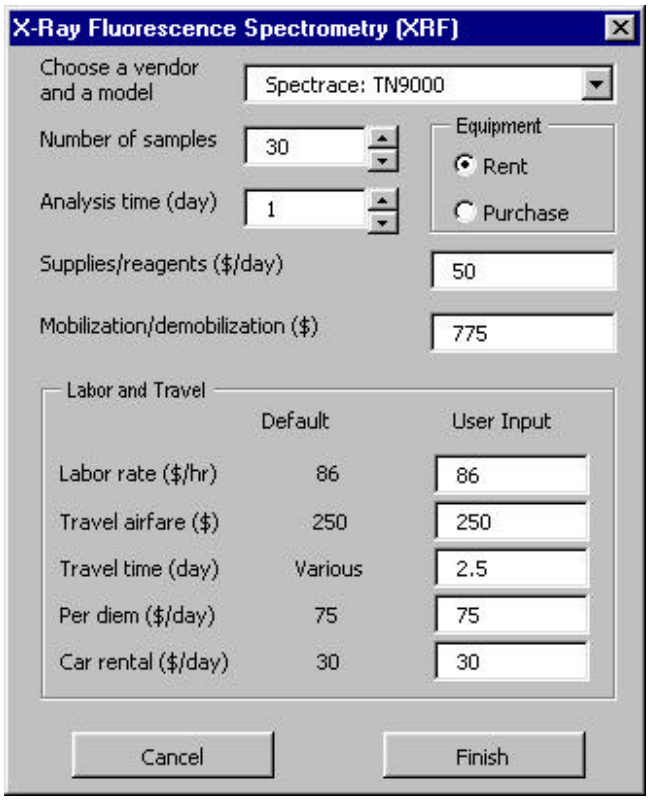

FIGURE 4-3. Example RSCT user form for XRF

Cost Summary

Technology	X-Ray Fluorescence Spectrometry (XRF)
Vendor and Model	Spectrace: TN9000
Number of samples	30
Analysis time (day)	1
Supplies/reagents ($/day)	50
Mobilization/demobilization	775
Own/rent	Rent
Labor rate ($/hr)	86
Travel airfare ($)	250
Travel time (day)	2.5
Per diem ($/day)	75
Car rental ($/day)	30

Main

Back

Cost Category	Sub-Category	Unit	Unit Cost	Quantity	Total
Equipment	Mobilization/demobilization	LS	$775	1	$775
	Equipment rental	2 WKS	$3,600	1	$3,600
	Supplies/reagents	EACH	$50	1	$50
	Sum				$4,425
Labor	Labor cost	HR	$86	9	$774
	Travel airfare	LS	$250	1	$250
	Per diem	DAY	$75	2.5	$188
	Car rental	DAY	$30	2.5	$75
	Sum				$1,287
Total					$5,712
Cost per sample					$190

FIGURE 4-4. Example RSCT cost summary sheet for XRF

5. Additional References

Readers interested in more information on specific methodologies and analytical instruments may find references to publications in this section.

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United States Environmental Protection Agency. 1998. *Environmental Technology Verification Report, Immunoassay Kit, Strategic Diagnostics Inc., D TECH PCB Test Kit*, EPA/600/R-98/112, EPA, Washington, D.C., August.

Appendix A
Points of Contact

Naval Facilities Engineering Service Center (NFESC)

Naval Facilities Engineering Service Center
1100 23rd Avenue
Port Hueneme, CA 93043
Tel: (805) 982-2631

Space and Naval Warfare Systems Center (SSC)

Space and Naval Warfare System Center
53475 Stothe Rd.
San Diego, CA 92152
Tel: (619) 553-1395

Space and Naval Warfare System Center
53475 Stothe Rd.
San Diego, CA 92152
Tel: (619) 553-2798

Space and Naval Warfare System Center
53475 Stothe Rd.
San Diego, CA 92152
Tel: (619) 553-6240

Appendix B
Standard Operating Procedures

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SOP for Field-Portable X-Ray Fluorescence Spectrometry

This SOP contains guidelines for the operation of the field-portable x-ray fluorescence spectrometry (XRF) instrument for elemental analysis of sediment samples. It does not replace the manufacturer's standard operating instructions. The operating instructions contain additional information for optimizing instrument performance. Also, see the references listed at the end of this SOP for published reports and product performance evaluations.

Description

The field-portable XRF instrument is a line-voltage or battery-powered unit that detects metals and other elements in sediment or other materials. It generally can detect and quantify elements of atomic number 16 or greater. Some of the primary elements of environmental concern that XRF can identify are arsenic, barium, cadmium, chromium, copper, lead, mercury, selenium, silver, and zinc. It should be pointed out that XRF is typically not a suitable analytical technique for measuring mercury in marine sediment samples due to the lack of sensitivity of XRF instrument detection limits for mercury. Field-portable XRF units cannot detect "light" elements, such as lithium, beryllium, sodium, magnesium, aluminum, silicon, and phosphorus. (Some benchtop XRF instruments can detect elements as light as carbon.). XRF is most valuable as a rapid field screening procedure. See Section 2.2.1 of the main document for more information on the technology.

Theory of Operation

Energy dispersive XRF is a nondestructive analytical technique used to determine the chemical composition of a sample. In an XRF analysis, primary x-rays emitted from an x-ray tube, or a sealed radioisotope source, are used to irradiate a sample. Up to three sources may be used to irradiate the sample. During interaction with the sample, source x-rays may either undergo scattering or absorption by sample atoms in a process known as the photoelectric effect. This phenomenon originates when incident radiation knocks an electron out of the atom's innermost shell creating a vacancy. The atom is excited and releases its surplus energy almost instantly by filling the vacancy with an electron from one of the higher energy shells. This rearrangement of electrons is associated with the emission of x-rays characteristic of the given atom. The process is referred to as emission of fluorescent x-rays.

Method Summary

Most XRF instruments are menu-driven by software either built into the units or through interconnection with a personal computer (PC).

To begin the analysis, the sample is placed in front of the probe window of the XRF instrument. The sample then is exposed to primary radiation from the source. Fluorescent and backscattered x-rays from the sample enter through the detector window and are converted into electrical pulses in the detector. The detector in XRF instruments is usually either a solid-state detector or a gas-filled proportional counter. Within the detector, energies of the characteristic x-rays are converted into a train of electrical pulses, the amplitudes of which are linearly proportional to the energy of the x-rays. An electronic multichannel analyzer (MCA) measures the pulse amplitudes, which is the basis of qualitative x-ray analysis. The number of counts at a given energy per unit of time represents the element concentration in a sample and is the basis for quantitative analysis.

The user selects the count time for each source. The count time is the length of time the sample is actually exposed to each radioisotope source. Shorter count times (30 seconds) generally are used for initial

screening and hot spot delineation, and longer measurement times (up to 300 seconds) typically are used for higher precision and accuracy.

Interferences and Possible Problems

A number of factors can interfere with the detection and quantification of elements in a sample. Careful preparation and sample design can prevent or reduce some of these factors. Generally, instrument precision is the least significant source of error in XRF analysis. User or application error is more significant and varies with each site and method used. Common sources of user or application error are listed below.

- **Sample Bias.** The samples collected must be representative of the site or area under investigation. Contaminant concentrations can vary throughout a site, and more than one type of matrix may exist.

- **Physical Matrix Interference Effects.** Variations in the physical characteristics of a sample, such as particle size, uniformity, homogeneity, and surface condition, can affect analysis results. To minimize physical matrix interference effects, homogenize sediment samples thoroughly before analysis. Grind and sieve all sediment samples to a uniform particle size. The sample's heterogeneity generally has the greatest effect on comparability with confirmatory samples.

- **Chemical Matrix Interference Effects.** Differences in the concentrations of interfering elements cause chemical matrix effects, which occur as either spectral interferences (peak overlaps) or as x-ray absorption and enhancement phenomena. Both effects are common in sediments contaminated with heavy metals. Correct chemical matrix effects mathematically using Fundamental Parameter (FP) coefficients, or compensate for them using site-specific calibration standards (SSCS), which contain all the elements present on-site that can cause interference.

- **Moisture Content.** A strong negative bias in FPXRF results can occur when the moisture content of the sediment sample is over 20 percent. Depending on the project and data quality objective (DQO) goals, sample can be analyzed wet or dry. Drying and grinding of samples will improve accuracy. However, if rapid delineation of hot spots is required, samples can be analyzed without the additional sample processing measures. If drying samples is required, samples can be partially or completely dried in a convection or toaster oven before analysis. Do not use a microwave oven because (1) microwave drying can increase the variability between the XRF data and the confirmatory analysis; (2) high levels of metals in the sample can cause arcing in a microwave oven; (3) slag can form in the sample; and (4) the plastic containers holding the sample sometimes melt.

- **Detector Resolution Effects.** If certain x-ray lines from different elements are present in a sample and are very close in energy, they can produce a severely overlapped spectrum. The degree to which a detector can resolve the two different peaks depends on the energy resolution of the detector. If the energy difference between the two peaks (in electron volts) is less than the resolution of the detector, then the detector will not be able to fully resolve the peaks. Most XRF instruments use overlap factors to correct for these spectral overlaps.

- **Reference Analysis.** Because XRF measures the total concentration of an element, the reference method should use a total digestion procedure for sample preparation. This will ensure the greatest comparability between XRF and the reference method. The critical factor is that

the digestion procedure and analytical reference method should meet the data quality objectives (DQOs) of the project and match the method used for confirmation analysis.

- **Sample Placement.** Inconsistent positioning of samples in front of the probe window can cause errors because the x-ray signal decreases as the distance from the radioactive source increases. Maintain a consistent distance between the sample and the window to minimize this effect. For best results, place the window of the probe in direct contact with the sample.

- **Ambient Temperature Changes.** Temperature changes in the area surrounding the XRF instrument can affect the gain of the amplifiers and produce instrument drift. Most field-portable XRF instruments have a built-in automatic gain control. If the automatic gain control is allowed to make periodic adjustments, the instrument will compensate for the influence of temperature changes on its energy scale. If an error message appears, follow the manufacturer's procedures for troubleshooting the problem.

Safety

Radiation safety for each specific instrument is found in the operator's manual. Personnel should receive radiation training and instruction in the safe operation of the field-portable XRF instrument before performing analyses. An appointed radiation safety officer should instruct all personnel, maintain inspection records, and monitor x-ray equipment at regular intervals.

Compliance with local, state, and national regulations concerning the use of radiation-producing equipment and radioactive materials is required. Licenses for radioactive materials are of two types:

1. General license, which is usually provided by the manufacturer for receiving, acquiring, owning, possessing, using, and transferring radioactive material incorporated in a device or equipment, and
2. Specific license, which is issued to named persons for the operation of radioactive instruments as required by local state agencies.

Copies of the radioactive material licenses and leak tests should be kept with the instrument at all times.

X-ray tubes do not require radioactive material licenses or leak tests, but do require approvals and licenses, which vary from state to state. Fail-safe x-ray warning lights should illuminate whenever an x-ray tube is energized, and a log of times and operating conditions should be kept. An often overlooked hazard associated with x-ray tubes is the danger of electrical shock from the high voltage supply.

Instrument operators should wear thermal luminescent detector (TLD) badges or rings to monitor radiation exposure. The best ways to prevent radiation exposure are distance and shielding.

Reagents and Standards

Some or all of the following reagents and standards are used for calibration and quality control, depending upon the requirements of the instrument and the project's DQOs.

Pure Element Standards
Each pure, single-element standard produces a strong characteristic x-ray peak of the element of interest only. If required for the instrument, the manufacturer will supply a set of pure element standards for the most common analytes. The standards are used to set the region of interest (ROI) for each element and also can be used as energy calibration and resolution check samples.

Site-Specific Calibration Standards (SSCS)
Instruments using FP or similar mathematical models to minimize matrix effects may not require SSCS. Site-specific calibration standards are collected, prepared, and analyzed if empirical calibration is necessary or if the FP calibration model is to be optimized. At least 10 samples, representative of the site's matrix, are collected. These samples must span the concentration ranges of the analytes of interest and the interfering elements. Each sample is homogenized, dried, and passed through a sieve before analysis.

Blank Samples
"Clean" quartz (silicon dioxide) matrix is used as a blank to monitor cross-contamination and laboratory-introduced contaminants or interferences. Blanks must not contain any analytes at concentrations above the method detection limits (MDLs).

Standard Reference Materials (SRMs)
Standard reference materials are standards containing certified amounts of metals and are used to check the accuracy and performance of field-portable XRF analyses. SRMs can be obtained from the National Institute of Standards and Technology (NIST), the U.S. Geological Survey (USGS), the Canadian National Research Council, or the national Bureau of Standards in foreign nations. Pertinent SRMs for field-portable XRF analysis include

- NIST 2704, Buffalo River Sediment
- NIST 2709, San Joaquin Soil
- NIST 2710 and 2711, Montana Soil
- CRNC PACS-1 and PACS-2 Marine Sediment.

These SRMs contain sediment or soil from actual sites that has been analyzed using independent inorganic analytical methods by many different laboratories.

Calibration

Three calibration methods, which are discussed in the following paragraphs, are used to calibrate field-portable XRF instruments. The methods vary depending upon the particular instrument and whether the data collected will be used for screening or for definitive analysis. Follow the calibration procedures outlined in the operator's manual for each specific instrument.

Fundamental Parameters Calibration
The FP calibration is a "standardless" calibration. Rather than calibrating a unit's calibration curve by measuring its response to standards that contain analytes of known concentrations, FP calibration relies on the known physics of the spectrometer's response to pure elements to set the calibration. Built-in mathematical algorithms adjust the calibration for analysis of sediment samples and compensate for the effects of the sediment matrix. The FP calibration is performed by the manufacturer, but the analyst can adjust the calibration curves (slope and y-intercept) based on the results of field analyses of check samples, such as standard reference materials.

Empirical Calibration
For an empirical calibration, the field-portable XRF instrument measures the concentrations of known analytes in a number of actual samples, such as site-specific calibration standards. Empirical calibration is effective because the samples used closely match the sample matrix. SSCSs are well-prepared samples collected from the site of interest in which the concentrations of analytes have been determined by inductively coupled plasma (ICP), atomic absorption (AA), or other methods approved by the U.S. Environmental Protection Agency (U.S. EPA). The standards should contain all the analytes of interest, as well

as interfering analytes. Manufacturers recommend using 10 to 20 calibration samples to generate a calibration curve.

Compton Normalization
The Compton normalization method incorporates elements of both empirical and FP calibration. A single, well-characterized standard, such as an SRM or an SSCS, is analyzed, and the data are normalized for the Compton peak. The Compton peak is produced from incoherent backscattering of x-ray radiation from the excitation source and is present in the spectrum of every sample. The intensity of the Compton peak changes as various matrices affect the way in which source radiation is scattered. For that reason, normalizing to the Compton peak can reduce problems with matrix effects that vary among samples.

Quality Control

The amount and type of quality control (QC) will depend on the project's data quality objectives and the pical QC program would include the following:

Quality Control	Purpose
An energy calibration check sample at least twice daily	Tests FP calibrations. Determines whether the instrument is operating within resolution and stability tolerances.
An instrument blank for every 20 environmental samples	Verifies that there is no contamination in the spectrometer or on the probe window.
A method blank for every 20 samples	Monitors sampling and analysis methods for laboratory-introduced contaminants.
A calibration verification check sample for every 20 samples	Checks the accuracy of the instrument. Assesses stability and consistency of the analysis of the target analytes.
A precision sample for every 20 environmental samples.	Checks the instrument's ability to produce the same results for several measurements of the same sample.
A confirmatory sample for every 10 environmental samples	Compares the analysis done on site by XRF with the analysis of the same sample material done off-site by a laboratory.

Equipment and Supplies

Item	Note
XRF spectrometer	Required
Spare battery	A battery charger also may be useful
Polyethylene sample cups	31 to 40 mm in diameter with collar, or equivalent (appropriate for field-portable XRF instrument)
X-ray window film	Mylar™, Kapton™, Spectrolene™, polypropylene, or equivalent; 2.5 to 6.0 µm thick
Mortar and pestle	Glass, agate, or aluminum oxide, for grinding consolidated sediment samples
Containers	Glass or plastic, to store samples
Stainless steel trowel	For collecting sediment samples and smoothing sediment surfaces
Plastic bags	For collection and homogenization of sediment samples
Drying oven	Standard convection or toaster oven, for drying sediment samples
Sieves (dried and ground samples only)	60-mesh (0.25-mm), stainless-steel, nylon, or equivalent; for preparing sediment samples

Sample Collection

Eight-ounce samples are collected in either glass or plastic containers or plastic zip-lock bags. Samples can be stored at room temperature and have an indefinite shelf life. Samples for mercury analysis have a six-month holding time (note: this technique is typically not considered for measurement of Hg in marine sediments).

To maintain sample integrity, implement chain-of-custody procedures at the time of sampling by documenting the following:

- All sample locations and associated field sample identification numbers
- All quality control samples taken
- The transfer of field samples from the field sampler to the field chemist to the fixed laboratory.

Sample Preparation

Samples are prepared for analysis using either the *in situ method* or the *intrusive method*. The in situ method involves analyzing the sediment in its original location. Although not a standard technique for analysis of marine sediment samples, this mode can be used under certain circumstances and with extreme care to the equipment. For in situ analysis, the probe window is placed in direct contact with the surface to be analyzed. This method requires that the sediment is above water and is not saturated with water. For example, this method could be used to analyze sediment along outflow areas or shoreline sediments during periods of low tide.

In Situ Sample Preparation

Action	Notes
1. Remove any foreign objects from sample before beginning analysis.	The user's judgment can help eliminate sampling bias.
2. Cover probe window with additional piece of x-ray window film to protect probe window from debris and contamination.	A larger piece of x-ray window film can be taped over the probe window prior to analysis. It should be changed between each analysis
3. Place the sample in direct contact with the probe window.	Consistent sample placement can improve instrument precision.

The intrusive method is the method normally practiced for marine sediments, and involves collecting samples using an appropriate sampling device. Preparation of the sample usually includes homogenization, drying, grinding, and passing the sample through a sieve. Some or all of these steps are necessary depending on the data quality objectives of the project; performing all four steps ensures the best quality data. The prepared sample is then placed in a sample cup, which is positioned on top of the probe window inside a protective cover.

Intrusive Sample Preparation

Action	Notes
1. Remove surface debris, such as rocks, pebbles, and vegetation. Not feasible for submersed sediments.	The user's judgment can help eliminate sampling bias.
2. Collect sediment sample according to sampling design.	This produces a sample of approximately 375 grams, which fills an 8-ounce jar.

Action	Notes
3. Homogenize the sample by mixing thoroughly in a beaker or similar container. A wet sample, or one high in clay content, can be kneaded in a plastic bag.	A sample can be homogenized before or after drying. To monitor homogenization, add sodium fluorescein dye to samples kneaded in a plastic bag. After kneading the sample, expose the bag to ultraviolet light to see how well the dye is distributed throughout the sample. Continue kneading the sample if the dye is not evenly distributed. This process usually requires three to five minutes per sample.
4. Place 20 to 50 grams of the homogenized sample in a container suitable for drying.	Drying is recommended if the sample contains more than 20 percent moisture.
5. Dry the homogenized sample. Standard drying procedure requires drying at 103°C for 16 hours. However, depending on data quality objectives and analysis conditions, drying can be performed in a toaster oven or convection oven for 2 to 4 hours at a temperature less than 150°C.	Microwave drying is **not** recommended for any samples. Note: Air drying is recommended for samples that contain mercury, because heat can volatilize mercury.
6. Grind the homogenized, dried sample with a mortar and pestle.	Grinding normally takes about 10 minutes. Continue grinding until at least 90 percent of the original sample passes through the sieve.
7. Pass the ground sample through a 60-mesh sieve to achieve uniform particle size.	
8. Fill a 31-mm polyethylene sample cup (or equivalent) at least one-half to three-quarters full with an aliquot of the sieved sample.	Consistent sample preparation can improve instrument precision.
9. Cover the sample cup with 2.5-µm Mylar film (or equivalent). Make sure the film is free of wrinkles.	
10. Save the rest of the sample in a labeled jar for possible confirmation analysis.	–
11. Clean all sample preparation equipment thoroughly so that no cross contamination can be detected, based on the MDL of the procedure or the DQOs of the analysis.	–

Analysis Procedure

XRF instruments vary in design and operation. Some instruments can operate in both in situ and intrusive analysis modes while others operate in only one mode. Consult the manufacturer's manual before operating any XRF instrument.

1. Allow the instrument to warm up for 15 to 30 minutes before analysis of samples to alleviate drift or energy calibration problems.

2. Perform any necessary instrument performance checks, calibrations, and quality control.

3. Position the sample in front of the plastic film measurement window of the probe as follows.

   In situ method: Place the probe window in direct contact with the surface to be analyzed.

   Intrusive method: Rotate the probe so the window faces up. Put a protective sample cover over the window. Place the sample cup on top of the window inside the protective sample cover.

4. Depress the trigger or start button to expose the sample to the source radiation. If the instrument has more than one radioisotope source, the instrument's source turret will rotate to expose the sample to the next radiation source.

   The MCA processes the data from the sample. The concentrations of target analytes are usually shown in parts per million on a liquid crystal display (LCD). Field-portable XRF instruments can store spectra and from 100 to 500 sets of numerical analytical results, which can be viewed later, downloaded into a computer, or printed.

5. Submit the same sample that was analyzed by field-portable XRF to a fixed laboratory for confirmatory analysis.

Instrument Vendors and Models

Several XRF instruments are available commercially. See Section 2.2.1 of the main document for a description of specific instruments identified below.

Instrument Vendor	Instrument Model
KevexSpectrace	QuanX
KevexSpectrace	TN 9000
Shimadzu	EDX-700/800
Metorex	X-MET 920/920-MP
Niton	XL Spectrum Analyzers 702/722

Reference

Office of Solid Waste, United States Environmental Protection Agency. 1986. Test Methods for Evaluating Solid Waste, Volume 1A-Laboratory Manual, Physical/Chemical Methods, Document Control Number 955-001-00000-1,SW846, 3rd ed. Method 6200: Field Portable X-Ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment.

SOP for Ultraviolet Fluorescence Spectroscopy

This SOP contains guidelines for using ultraviolet fluorescence spectroscopy (UVF) for analysis of organic compounds in marine sediments. It does not replace the manufacturer's operating instructions for any instrument. The operating instructions contain additional information for optimizing instrument performance.

Description

UVF is a rapid, highly sensitive method that is used for the semi-quantitative field screening of polycyclic aromatic hydrocarbons (PAHs) and other organic compounds. Because each fluorescent organic compound produces its own unique excitation and emission spectrum, UVF is highly specific to each fluorescent compound.

Theory of Operation

Fluorescent compounds absorb light at one wavelength (producing an excitation spectrum) and emit it at a longer wavelength (producing an emission spectrum). Instruments used for fluorometric analysis contain the following four basic components:

- Source of excitation energy
- Sample cuvette
- Detector to measure photoluminescence
- Pair of filters or monochromators for selecting the excitation and emission wavelengths.

The fluorometer produces the wavelength of light necessary to excite the organic compound and then selectively transmits the emitted light to a detector. The detector measures the amount of emitted light, which is proportional to the concentration of the organic compound. Most field fluorometers use optical filters to provide specific excitation and emission wavelengths. The filter design allows for lower cost and greater ease of handling compared to spectrofluorometric instruments, which use an excitation monochromator instead of a filter. To measure different substances, most filter fluorometers allow the user to mechanically change to different optical filter configurations.

Method Summary

A sample is collected using a method appropriate for marine sediment. The sample is thoroughly mixed and treated with a solvent to extract the organic compounds. The fluorescence of the solvent extract is measured using a fluorometer, which has been calibrated with standards. The concentration of the organic compounds is determined using linear regression.

Interferences and Possible Problems

The following factors can cause inaccurate fluorescent readings.

- **Linearity.** Fluorescence is not always linearly related to analyte concentration. For example, samples with a high concentration of an analyte may have a low fluorescence because the light necessary for excitation cannot penetrate the sample. Other factors that affect linearity include the sample's chemical makeup, the cuvette size, and other instrument factors. To check unknown samples for linearity, dilute them by a factor, such as 1:10. Compare the

reading of the diluted sample to the reading of the undiluted sample. If the sample is linear, the value of the diluted sample should be less than the value of the undiluted sample by the same factor as the dilution. Also, check linearity when changing the optical filters or the cuvette size.

- **Turbidity.** The turbidity of the sample can affect fluorescence as follows:

  – Light-reflective particles scatter light and increase fluorescence values.
  – Light-absorbing particles decrease fluorescence values.
  – Particles that do not absorb light have no effect on fluorescence values, unless the turbidity is so great that the emitted light cannot penetrate the sample.

  To reduce the turbidity, filter the samples. To cancel out the effect of turbidity, use the same procedures and matrix water for blanks, standards, and samples.

- **Temperature.** A rise in temperature causes fluorescence to decrease. Analyze standards, blanks, and samples at the same temperature. Some UVF instruments will automatically compensate for temperature changes.

- **Sample Bubbles.** Bubbles in the sample can cause fluctuations in the readings. Use good laboratory technique to avoid the introduction of bubbles into the sample. Take readings once the bubbles dissipate or settle.

- **pH.** pH can affect fluorescence. To minimize the effect of pH, prepare the blank, standards, and samples with the same liquid, such as the solvent used for extraction. Adjusting the pH before analysis can sometimes reduce spectral interferences.

- **Cuvette Size (Path Length).** Selecting a smaller diameter cuvette can improve the detection limits (as the cuvette diameter decreases, the detection limits increase) and the range of concentrations that can be measured by the analysis.

- **Light Exposure.** Because many fluorescent molecules are sensitive to light, exposing samples to light can cause a reduction in fluorescence. If the analyte of interest is light sensitive, protect the sample, as well as any light-sensitive standards, from light.

Calibration and Standards

The conditions under which the fluorometer is calibrated must closely match the conditions under which the samples are analyzed. If the optics or filters are changed, recalibrate the instrument.

Techniques for calibrating filter fluorometers vary. One common method uses a blank and a standard for calibration. The blank, which is usually water or the solvent used for extraction, does not contain any of the substance to be analyzed and is used to zero the instrument. The extraction solvent is also used to make the calibration standard, which contains known amounts of the substances to be analyzed. Dilutions of the standard (for example, 20x, 40x, 60x, and 100x) are read on the fluorometer, and the results are used to create a calibration curve.

Changes in temperature and/or exposure to light can cause some standards to deteriorate, resulting in inaccurate readings. The accuracy of the standards is a critical factor in measuring fluorescence. Follow laboratory procedures meticulously when preparing standards. Store standards carefully to prevent

deterioration, or use freshly prepared standards for calibration. Commercially prepared standards are also available.

Secondary standards can be used once the primary calibration has been completed. A secondary standard contains a substance that fluoresces at a wavelength that is the same, or almost the same, as that of the analyte of interest. The secondary standard is read against the primary standard and is then used, instead of the primary standard, for calibration and as a check on the accuracy of the instrument's readings. A secondary standard is useful when the primary standard is unstable.

Equipment and Reagents

The following items may be needed for UVF, depending on the procedure chosen for analysis:

- Digital balance
- 50 mL polypropylene test tubes, or an extraction jar
- Sonicating water bath
- Wrist action shaker
- Centrifuge, or syringe with filter
- Pipettes
- Cuvettes
- Fluorometer
- Standards
- Anhydrous sodium sulfate
- Solvent for extraction, such as *n*-hexane or methanol.

Analysis Procedure

The following steps are general guidelines for the analysis of a sample by UVF. Refer to the manufacturer's manual for specific instructions on the operation of the instrument.

1. Perform any necessary instrument checks, calibrations, and quality control.
2. Weigh an appropriate amount of sample.
3. Treat the sample with anhydrous sodium sulfate to remove water, if necessary.
4. Add the correct amount of the appropriate extraction solvent to the sample.
5. Seal with a leak-proof cap.
6. Shake the sample as directed.
7. Centrifuge, or filter, the sample.
8. Store the supernatant or filtrate in sealed foil-lined scintillation vials at 4°C, if unable to analyze immediately.
9. Measure the fluorescence of the supernatant, or filtrate, using the fluorometer.
10. Use linear regression to determine the concentration of the organic compound(s).

Instrument Vendors and Models

Several UVF instruments are available commercially. See Section 2.2.2 of the main document for a description of specific instruments identified below.

Instrument Vendor	Instrument Model
Turner Designs	10-AU-005-CE Field Fluorometer
SiteLAB	UVF-3100A

References

Lakowicz, J.R. 1983. Principles of Fluorescence Spectroscopy, Plenum Press, New York.

Guilbault, G.G. 1990. Practical Fluorescence, 2nd ed., Marcel Dekker, Inc., New York.

A useful introduction to fluorometry is available online at
http://www.fluorometer.com/applications/998_0050/998_0050.htm

SOP for Immunoassay Techniques

This SOP contains guidelines for using immunoassay techniques for analysis of organic compounds in marine sediment. It does not replace the vendor's instructions included in each immunoassay kit. The operating instructions contain additional information for optimizing instrument performance. Also, see the references listed at the end of this SOP for published reports and product performance evaluations.

Description

Immunoassay is an analytical technique that uses an antibody molecule as a binding agent in the detection and quantification of substances in a sample. It is useful for the separation, detection, and quantification of both organic and inorganic analytes in a wide variety of environmental and waste matrices. Commercially available immunoassay kits are cost effective, rapid, and simple to use with the appropriate training. The kits work well in both laboratory and field settings and allow an operator to analyze a number of samples simultaneously within a short time period. Results are available as soon as the tests are completed and can assist in the on-site management of personnel and equipment and the data management activities of the laboratory. Immunoassay is best used for sites that have a single contaminant, or one type or chemical class of contaminant. It is not recommended for sites with unknown site conditions and contaminants, or for sites that do not have established cleanup criteria.

The most common immunoassay method for environmental analysis, Enzyme Linked Immunosorbent Assay (ELISA), uses antibodies and enzyme conjugates to detect and quantify contaminants of concern (COCs).

Method Summary

Immunoassay products vary in format and chemistry. The characteristics of each product are described in the vendor's package insert. This summary provides a general description of the ELISA method.

An enzyme is chemically linked to a COC molecule to create a labeled COC reagent known as a conjugate. The conjugate is mixed with an extract of the native sample, which contains the COC. A portion of the mixture is applied to a surface to which an antibody specific for the COC is attached. The native COC and the COC-enzyme conjugate compete for a limited number of antibody sites. After a period of time, the solution is washed away. What remains is either COC-antibody complexes or enzyme-COC-antibody complexes attached to the test surface. The proportion of the two complexes is determined by the amount of native COC in the original sample. The enzyme present on the test surface is used to catalyze a color change reaction in a solution added to the test surface. The amount of color development is inversely proportional to the concentration of the COC. In other words, a sample with intense color development will have a low concentration of the COC. A sample with little color development will have a high COC concentration.

Kit Information

Environmental immunossay kits are engineered to detect a single target compound, or one or more structurally similar target compounds within a chemical class, depending upon the following factors:

- The compounds present in the chemical class
- The molecular size of the target compound(s)
- The specificity of the engineered antibody.

The effectiveness of each immunoassay kit for sample analysis will depend upon:

- The various product mixtures present in the sample,
- The kit's sensitivity to the target compound and structurally similar compounds, and
- The presence of interferences in the sample.

Most vendors have designed their environmental immunoassay kits for use in both field and laboratory settings. All of the available field kits can be used by a fixed or field laboratory as a screening tool prior to sample preparation and/or instrumental analysis.

Field kits are differentiated from laboratory-based kits by the number of samples analyzed per batch. The sequence of standard, blank, samples, and QC samples—followed by the standard and blank set again—constitutes a batch sequence in both settings. Only the number of samples between the standard and blank sets changes. Field kits recommend performing fewer samples (4-6) between standard and blank sets, whereas laboratories will set up banks made up of several batches or sequences of up to ten samples each (possibly 40 samples at one time).

Each immunoassay kit is designed to function within a particular detection and/or calibration range, depending on whether the kit produces quantitative, semiquantitative, or qualitative data. The kit detection limits must be lower than the project Action Levels.

Immunoassay kits are usually more sensitive than is needed for most environmental studies, which generally requires dilution of the sample extracts to bring the COC concentrations into the kit's detection/calibration range. Vendors' instruction guides usually detail step-by-step procedures for performing their specific assays on soil (sediment) matrices. Several vendors have simplified this process by developing a formula to calculate the required dilution factor. Others have ready-to-use dilution kits available to simplify immunoassay use.

Interferences and Possible Problems

The following factors can affect the results of immunoassay analyses, which must be performed in a very consistent manner to ensure the production of usable data. Immunoassay methods also are affected by kit storage and operating circumstances, field conditions, and sample matrix characteristics.

- **Vendor's Instructions.** The vendor of each immunoassay kit includes specific procedures, which are engineered and validated for that particular product. Do not use one vendor's procedures with another vendor's kit.

- **Storage Conditions.** Most immunoassay kits require storage at 2 to 8°C. Bring the kit to ambient temperature just before use.

- **Shelf Life.** The antibody, enzyme conjugate, and color reagents are biological media and have a limited shelf life. The vendor must identify the maximum length of time the reagents will produce usable results. Many vendors put an expiration date on each kit. Do not use a kit past its expiration date.

- **Operating Temperature Range.** The operating temperature range of an immunoassay kit is one of the most important criteria for generating precise and accurate data. Operate the kit within the vendor's recommended temperature range. Do not use the kit at temperatures that will inhibit or increase the recommended processing times. If there are large temperature

fluctuations in the field, make sure that all field samples, standards, blanks, and QC samples are analyzed at the same relative temperature conditions. Inaccurate results can occur if samples are analyzed during the day under normal temperatures (60 to 80°F) and then later in the day as temperatures drop (40°F). The data generated at 60°F will not be comparable to the data generated at 40° F using the calibration curves and QC samples analyzed at 60° F. Also, temperatures below –40° or –50° F will cause false negatives by interfering with the reaction times for incubation and color development. In very cold climates, operate the immunoassay kit in a heated enclosure or field trailer.

- **Operational Consistency.** Analyze all samples, standards, blanks, and quality control samples under the same operating conditions. The sequence and timing of reagent additions, sample additions, and washing procedures is critical to the proper use of each immunoassay kit. Reagent additions between samples, etc., must be performed rapidly, precisely, and consistently once the immunochemical reaction has started so that each sample will incubate with the same reagent volume for the same time period. Because the timing of these assays is so critical, most vendors of field kits recommend small batch sizes. Any deviation from the vendor's prescribed procedure can affect the results within and between batches. Also, user training is critical to consistently accurate and precise immunoassay results. Immunoassays require proficiency in sampling, weighing, pipetting, sample dilution, and colorimetric measurement. Each vendor offers product-specific training. Personnel should attend the vendor's training course and practice with the kit before going out in the field.

- **Sediment Characteristics.** The physical characteristics of some types of sediment, mainly the particle size and the organic content, can affect the adsorption and retention of organic compounds, especially chlorinated organics. Sediments containing increasing amounts of silt, clay, and organic content are much more difficult to quantitatively extract. Sediment pH and cation exchange capacity can also affect extraction. Some organic compounds may be in the salt form and, therefore, will have poor extraction efficiencies. Highly colored sediments, or sediments that cause highly colored solutions upon extraction, may interfere with the color development stage of the assay. Sediment samples with >30% moisture may require further water removal techniques, such as decanting, filtration, air drying, or oven drying. Note that some PAH compounds are volatile and may evaporate if the sample is heated. Immunoassay may not be the best technique to use on samples with more than 70% moisture.

- **Extraction Solvent.** Most immunoassay kits use methanol as the extraction solvent for sediments and solids because it is completely soluble in water, does not break down the antibody or enzyme conjugate, and does not inhibit reactions between the antibody and the COC. However, methanol may not efficiently extract COCs from sediments and solids that contain large quantities of water (>30%). Water dilutes the methanol and limits its solubilizing properties, especially for higher molecular weight organic compounds. In situations where the COC is less soluble in methanol, enhance the extraction step by heating gently, shaking for a longer period of time, or by using sonication.

- **False Results.** The engineering of the antibody/COC along with the enzyme conjugate controls the selectivity of the immunoassay kit to particular target compounds and nontarget compounds. Nontarget compounds that are structurally similar to the COC may bind with the antibody present, producing false positive results. These "cross reactive" nontarget analytes compete for the finite number of antibody binding sites, which affects color development. In addition, interferences caused by the testing of incompatible matrices may increase the number of false positive or false negative results. Immunoassay products contain

sample-processing technology that has been developed and validated for use with specified matrices. Each product designates the intended sample matrices.

Kit Standardization and Quality Control (QC)

Most vendors design their kits for use in one of the following modes:

- Quantitative – produces results from a specified lower detection limit to a linear upper limit
- Semiquantitative –produces results either (1) above or below a specified detection limit (Action Level or Go/No Go test) or (2) between an upper and lower range
- Qualitative – detects the presence or absence of a specific COC.

Most environmental immunoassay kits are used in the quantitative or semi-quantitative mode. For the data from these analyses to be considered usable, quality control procedures must be performed at the correct frequency. The QC must also meet the criteria specified in the pre-approved Quality Assurance Project Plan. In addition, immunoassay results for a representative number of samples (10% minimum) must be confirmed through the use of split samples. Split samples are collected throughout the entire sampling and analysis episode. They are prepared and analyzed using conventional full protocol analytical methods performed in a fixed laboratory or a field laboratory (mobile or transportable) setting. The split sample results obtained using both analytical methods must not deviate from the criteria specified in the Quality Assurance Project Plan.

To develop the QC requirements for a project, the analyst should consult the vendor's kit instructions, which contain recommended QC requirements. Key QC elements for immunoassay analyses include process calibration, the analysis of continuing calibration checks, blanks, duplicates, and performance evaluation samples. Documentation that all QC elements were performed and met project requirements is essential. The documentation must include the kit lot number, the kit expiration date, and the temperature at which the tests were performed.

Samples can be analyzed once the project QC criteria have been met. If QC objectives were not met, the analyst must implement and document the appropriate corrective actions. Samples run after the last in-control QC sample must be prepared and/or analyzed again.

- **Calibration.** Calibration using standards of known concentrations is performed to determine the sensitivity and detection/calibration range for the immunoassay kit.

  — Semiquantitative kits in the Action Level test mode use one calibrator – a standard that contains the target compound at the detection limit.
  — Semiquantitative kits in the detection range mode use two calibrators to define a detection range (i.e., a 1 ppm standard and a 10 ppm standard).
  — Quantitative kits are calibrated using multiple calibrators to create a calibration curve. Usually, one calibrator is a zero point.

  When using semiquantitative and quantitative kits, continuing calibration checks are necessary to evaluate the calibration stability and accuracy for each batch. At the beginning of each batch of samples multiple standard initial calibrations are performed. In the field setting, bracket every 4 to 6 samples with a continuing calibration standard. The vendor's kit instructions usually define how many samples can be successfully analyzed between standards. If samples are from

different areas of the site, or temperature or weather conditions change, perform full calibrations before and after each batch.

The absorbance of the continuing calibration standard should not vary more than 20% from the absorbance of that standard in the initial calibration. If the continuing calibration standard is not within 20%, perform a full calibration, and retest all samples run prior to the noncompliant standard.

- **Blanks.** Blanks represent the highest absorbance of color and indicate the absence of the COC. Blanks are analyzed to evaluate the presence of contaminants originating from sampling and analysis activities. Equipment blanks assess the effectiveness of equipment decontamination procedures performed in the field. Reagent blanks, which are included with every batch or a chosen sequence of samples, evaluate the purity and reactivity of reagents used in the immunoassay kits. They also help the analyst determine the kit's response when no target contaminants are present. Blanks should not show contamination above the kit's detection limit.

  If contamination is found in the reagents or the equipment rinsates, the analyst must determine the cause and eliminate the contamination. Do not analyze samples until the blanks meet the vendor's recommended acceptance criteria.

- **Duplicates.** Field duplicates measure the precision of the immunoassay test as well as the sample homogeneity. Analyze duplicates at a frequency of 1 per 10 samples, or 1 per batch of samples prepared, whichever is greater. Perform duplicates at a greater frequency when samples are less homogeneous.

- **Performance Evaluation (PE) Samples.** Performance evaluation (PE) samples are analyzed to evaluate qualitative and quantitative accuracy for each immunoassay kit batch. PE samples should contain the target compound at or near the project Action Level and should be tested under the same conditions as the calibrations, blanks, and field samples.

  Depending on the batch size of the individual analysis episode, run a PE sample at least once in 20 samples or once per day, whichever is greater. If multiple sets or batches are analyzed under the same conditions during one day, one PE sample per day is recommended. Analyze the PE sample more frequently if there are changes in field conditions (temperature and relative humidity) during the sampling episode. Poor PE sample results may indicate incomplete sample extraction, operation of the immunoassay kit outside its required operating temperature range, or inconsistent timing of reagent additions and performance of batch processes.

Equipment and Reagents

Immunoassay methods include sample processing and immunoassay components. The immunochemical reagents and sample processing components are specific to each manufacturer.

The following table lists examples of the equipment and reagents that are typically supplied by the vendor for immunoassay analysis and sample preparation, depending on the particular kit used. The table also indicates other items necessary for analysis that may not be supplied by the vendor.

Examples of Equipment and Reagents for Immunoassay Analysis

Supplied with Immunoassay Component	Supplied with Sample Processing Component	Other Items Not Necessarily Supplied by Kits
• Antibody-coated test tubes or antibody-coupled paramagnetic particles • Standards • Controls • Enzyme conjugate • Color solution • Washing solution • Stopping solution	• Sediment collection device • Filter units/caps • Extract collection vials • Chain-of-custody container labels • Portable Styrofoam tube holder • Extraction solution • Extract diluent • 25-µL precision pipette with tips • Weigh boats • Wooden spatulas • 20-cc syringe with coupler	• Digital balance • Precision pipettes and tips • Combos-syringes • Positive displacement pipette • Vortex mixer • Test tube racks • Methanol • Distilled water • Wash bottle • Test tube rack • Magnetic separation rack • Timer • Permanent marking pen • Lab coat, gloves, and goggles • Photometer

Sample Preparation

Testing solid waste by immunoassay requires the production of a particulate-free leachate, using a solvent that allows the reproducible extraction and recovery of the target analytes. This solvent also must be compatible with the antibody/enzyme conjugate of the immunoassay system used. Effective extraction is accomplished using buffers, detergents, and solvents, together or in combination. Filtration of particulate matter may be integrated into the immunoassay test or completed as a separate step within the protocol.

In general, immunoassay sample preparation for sediment includes the following steps:

- Sample measurement by weight
- Introduction of the extractant
- Extraction of the sample
- Filtration of the extract
- Pipetting sample extract into the immunoassay container.

Procedural Notes

The recognition characteristics, sensitivity, detection ranges(s), effective operating temperature, interferences, and cross-reactivity of the immunoassay will depend on the product being used. Methods available from different manufacturers for the same compound and application may have significantly different performance characteristics.

The analysis procedure, which includes pipetting, incubation, and color development, usually takes 25 to 45 minutes per sample batch (or per sample if only one sample is being analyzed). The exact analysis time depends on the specific requirements of each vendor's kit and the COCs being analyzed. The timing sequences for each vendor's kit control the number of samples that can be accurately and precisely analyzed in a single batch. Approximately 35 to 200 samples/person/day can be processed using immunoassay kits, depending on the COC tested, the extent of sample preparation, and the experience of the analyst. To ensure accurate results, the analyst must do all of the following:

- Use the test products before the specified expiration date,
- Use reagents only with the test products for which they are designated
- Use the test products within their specified storage temperature and operating temperature limits.

Analysis Procedure

The vendor supplies the specific procedure for each immunoassay test product in the package insert. Follow the manufacturer's instructions for the test product being used. Critical factors in immunoassay include the timing of each step and the order in which the samples and reagents are added. Refer to the manufacturer's instructions for the specific timing of each step and the correct sequence for adding samples and reagents.

General steps are listed below for assays using antibody-coated test tubes:

1. Collect and prepare the sample.
2. Prepare standards and controls as directed.
3. Add the sample, blank, standards, and controls to appropriately labeled antibody-coated test tubes.
4. Add the enzyme conjugate to the test tubes.
5. Mix as directed.
6. Incubate. Refer to the package insert for the correct incubation time and temperature.
7. Add the wash solution to the test tubes. Follow the washing procedure in the package insert.
8. Add color reagent to the test tubes.
9. Incubate as directed.
10. Add the stopping solution.
11. Measure the optical density of the test tube contents using a photometer at the appropriate setting. The tubes must be read within a specified time period after the addition of the stopping solution.

The following general steps are for assays that use paramagnetic particles with specific antibodies attached:

1. Collect and prepare the sample.
2. Prepare standards and controls as directed.
3. Pipette the sample, standards, and controls into test tubes.
4. Add the enzyme conjugate.
5. Add the antibody.
6. Mix as directed. Avoid foaming.
7. Incubate. Refer to the package insert for the correct incubation time and temperature.
8. Separate in a magnetic rack for the length of time indicated by the manufacturer.
9. Decant and gently blot.
10. Remove tubes from the magnetic rack.
11. Add the washing solution and mix.
12. Separate in a magnetic rack for the specified time.
13. Decant and gently blot
14. Repeat steps 10-13.
15. Remove the tubes from the magnetic rack.
16. Add the color solution to the test tubes.
17. Incubate as directed by the manufacturer.

18. Add the stopping solution.
19. Place the test tubes in a photometer and read absorbance using a photometer at the appropriate setting. The tubes must be read within a specified time period after the addition of the stopping solution.

Instrument Vendors and Models

Several immunoassay instruments are available commercially. See Section 2.2.3 of the main document for a description of specific instruments identified below.

Instrument Vendor	Instrument Model
Strategic Diagnostics	RaPID Assay System
Strategic Diagnostics	EnviroGard PCB Test Kit
Strategic Diagnostics	D TECH PCB Test Kit
Hach	PCB in Soil, Pocket Colorimeter
EnviroLogix	PCB Soil Tube Assay

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SOP for QwikSed Bioassay

This SOP contains guidelines for using the QwikSed bioassay system, a rapid sediment characterization tool that indicates toxicity of contaminated marine sediment. This SOP does not replace the vendor's instructions which contain additional information for optimizing instrument performance. See the references listed at the end of this SOP for published reports and product information.

Description

The QwikSed bioassay system measures the light output of bioluminescent marine phytoplankton known as **dinoflagellates**. These unicellular, photosynthetic microorganisms, important ecologically as primary marine producers, emit a visible blue light as part of their normal physiological processes. The presence of toxicants inhibits the light production of these microorganisms. Dinoflagellates commonly used for bioassays include *Gonyaulux polyedra, Ceratocorys horrida, Pyrocystis noctiluca,* and *Pyrocystis lunula*. These organisms are found in the coastal waters of most continents and are sensitive to a number of toxicants.

QwikSed bioassays are rapid, easy to set up, and inexpensive compared to more traditional, labor-intensive assays using fish, shrimp, and other invertebrate species. They are used to evaluate and monitor the acute and sublethal chronic effects from exposure to a variety of toxicants. Personnel with minimal training in toxicity testing can use the QwikSed system.

Instrumentation

The QwikSed system consists of a horizontally mounted, two-inch diameter photomultiplier tube (PMT) attached to a test chamber, which is connected to the controller box using a combined power and signal cable. The top of the test chamber is removable and contains a small adjustable-speed motor that drives a stainless steel shaft with a plastic propeller on one end. The controller box displays stirring motor and PMT voltages, PMT counts, time countdown, and cycle status. It has manual and automatic switches for system operation, time settings, start, stop, and reset buttons. In the automatic setting, the high voltage and the stir motor are automatically engaged after the start button is pushed. Neutral density optical filters (ND-1, ND-2, ND-3) can be easily changed to prevent PMT saturation.

Method Summary

Before starting the bioassay, the dinoflagellates are cultured and maintained at approximately 3,000 to 5,000 cells/mL in enriched seawater medium (ESM). An elutriate of the sediment sample is prepared. The elutriate is diluted for testing, usually at concentrations of 100, 50, 25, 12.5, and 6.25%, using ESM and the stock dinoflagellate cell culture. An aliquot of these dilutions is pipetted into optical grade plastic cuvettes for testing. The cuvettes containing the cells are incubated and cycled through a day-night period of 24 hours after the initial setup to allow for dark phase optimization. Dinoflagellates produce the most light three to five hours into their dark (night) phase. Each cuvette is then individually placed into the QwikSed test chamber. The stirring rod is lowered into the cuvette, and the high voltage, timer, counter, and stirrer are engaged. Stirring stimulates the cells to produce bioluminescence which is detected by the photomultiplier tube. The IC50 is calculated using statistical software or by graphically plotting the concentration of the test material against the bioluminescence. The IC50, which is a 50 percent reduction in light output when compared to control cells, is the endpoint of the measurement. The actual testing of all the dilutions of the sample with the QwikSed system requires less than one hour to complete.

Procedural Notes

- The following decisions must be made before starting a bioassay: the length of the test, the type of sample to be tested, the necessary dilutions of the sample, and the number of cells needed in each cuvette.

- The dinoflagellates' response to a toxicant is usually measured within 24 hours from the start of the bioassay. The 24-hour reading serves as a check for the consistent inoculation of the cells in the replicates of the test dilutions and controls. Also, at 24 hours, the analyst can observe the effects of the toxicant on the population of dinoflagellates used in the test. Bioassays are often run for 4 days (acute) or for 7 to 11 days (chronic). For these tests, light-production measurements are taken every day, preferably three to five hours into the organisms' dark phase. The 4-day (96 hour) test is commonly used because effects and trends are usually seen during this time period.

- At least five replicate samples are run at each test dilution to provide confidence in the test results.

- The cell density in each cuvette should be about 300 cells (100 cells/mL). Too many cells will saturate the photomultiplier with light, causing it to lose resolution. Placing neutral density (ND) filters between the cells and the photomultiplier reduces the light levels by factors of ten. The ND-1 filter transmits the most amount of light and the ND-3 filter transmits the least. An ND-2 filter is recommended for most bioassays using Gonyaulux polyedra. To avoid exposing the PMT to light, change the filter in a darkened room.

- Before using cuvettes and glassware for the first time, soak them overnight in 0.45 μm-filtered seawater, and then rinse them in deionized water. After running the bioassay, soak the cuvettes and glassware in a cleaner, such as RBS or Liquinox, followed by a distilled water rinse. Glassware must also be washed with 4 *N* HNO_3. If using disposable cuvettes, simply dispose of them after completing the test. Sterilize any flasks used to maintain the dinoflagellate cell cultures.

- Several dark counts (usually three) are performed at the beginning of the bioassay to check the status of the PMT and to monitor the background electrical noise of the system. A dark count is done without a cuvette in the test chamber and is run for the same length of time as the tests. The dark count is compared to the amount of light detected in the control cuvettes. A dark count of <0.5% when compared to the amount of light detected in the control is desirable. A dark count that is >2% of the light detected in the control indicates that the PMT may have been exposed to light, possibly through a light leak in the test chamber.

Interferences and Possible Problems

- **Ammonia.** High ammonia levels appear to be toxic to dinoflagellates. A protocol describing the procedure to remove ammonia interference is available.

- **pH.** The pH of the media for a bioassay test may range from 7.7 to 8.3. A pH outside of this range can be toxic to the dinoflagellates. Use 1 *N* NaOH or 2 *N* HCL to adjust the pH of the media.

- **Turbidity.** Turbidity in the test dilutions or controls may interfere with the detection of the light emitted by the dinoflagellates. To reduce turbidity, centrifuge the dilutions, and/or filter them through a 0.45-µm filter.

- **Salinity.** When preparing the sediment elutriate, unfiltered site sea water is usually mixed with the sediment sample. Distilled water can be substituted for the site sea water, if necessary, but the salinity of the elutriate must then be adjusted to 29-34 parts per thousand (ppt) by adding American Society for Testing and Materials (ASTM) Sea Salt.

- **Seawater.** Natural seawater does not adequately support the growth of high densities of dinoflagellates. For cultures and test dilutions, enriched seawater medium (ESM) or synthetic dinoflagellate medium (SDM) is preferred. Enriched seawater medium is made from reasonably clean, filtered, natural seawater, or bottled seawater, to which various nutrients and vitamins are added. When preparing ESM, do not use seawater that is suspected of being contaminated. SDM is an artificial saltwater medium that is prepared in the laboratory and does not contain natural seawater.

- **Light.** To prevent light from inhibiting the bioluminescence of the dinoflagellates, protect the dinoflagellates from light exposure during their dark phase and during testing. A red light is permissible in the room where the tests are performed so that the analyst has enough light to complete the test.

- **Temperature.** The optimum temperature for maintaining cultures and testing dinoflagellates is 19 ± 1°C.

- **Safety.** Personnel should wear lab coats and safety glasses, and avoid skin contact with all test materials and solutions. Before starting the bioassay, investigate all necessary safety precautions for handling and disposing of the stock cell solutions and all test materials.

Reagents and Equipment

Reagents	Equipment
• Dinoflagellate cultures • Enriched Seawater Medium (ESM) or Synthetic Dinoflagellate Medium (SDM) • 1 *N* NaOH or 2 *N* HCL • ASTM Sea Salt • Chemicals and reagents for preparing stock standards	• Erlenmeyer flasks • Volumetric flasks • Beakers • Pipettes • Counting chamber or electronic particle counter • Microscope • Cuvettes • Cuvette trays • Incubator • QwikSed bioassay system

Preparation of Enriched Seawater Medium

The following three stock solutions are used to prepare ESM:

- Micronutrient stock solution, which contains trace metals

- Macronutrient stock solution, which contains sodium nitrate and potassium phosphate
- Vitamin stock solution, which contains thiamine, biotin, and vitamin B_{12}.

These stock solutions are made up ahead of time and can be stored indefinitely in the dark at 4°C. Specified amounts of each stock solution are added to filtered seawater or bottled seawater to prepare ESM. The pH is adjusted, if necessary, and the ESM is ready to use. Refer to QwikLite Basics, Version 1.08 (see Lapota and Rosenberger, 1996), for the specific instructions on preparing the stock solutions and ESM.

If the ESM will be used in a bioassay in the next few days, store it at 4°C in the dark, but warm it to 19°C before use.

If the ESM will be used for the dinoflagellate cell cultures, sterilize it by microwaving on high for 25 minutes. Cool to 19°C before use, or store in the dark at 4°C until needed, warming it to 19°C before use.

Cell Cultures

Dinoflagellates for bioassays are available from commercial supply houses, such as East Pacific Culture Collection, University of British Columbia, or Bigelow Marine Laboratory.

Starter cultures of *Gonyaulux polyedra* may take several weeks to achieve the cell density necessary for bioassay testing, even though the organism divides every day. Because *Pyrocystis lunula* divides every four days, it takes longer to achieve adequate cell density.

Dinoflagellate cultures are best maintained in ESM using presoaked 250 mL Erlenmeyer flasks. The cultures are placed under cool white fluorescent bulbs (light intensity of 4,000 lux) on a 12:12 hour (day:night) schedule at 19 ±1°C. For bioassay use, keep the stock cultures at cell concentrations of at least 2,000 cells/mL (preferably about 3,000-5,000 cells/mL). A smaller volume of stock culture is needed to set up the bioassay when the cell density is high. Usually cultures that are 12-20 days old contain the cell density required for bioassays.

When setting up a bioassay, log phase organisms are recommended. To keep the cultures healthy and actively growing, split half of the culture flasks every two to four weeks. To split a culture, swirl the flask to mix it, and then pour half the contents into a sterile, prepared flask. Add an equal volume of ESM. Discard the remaining half flask, or create an additional cell culture by adding an equal volume of ESM to it.

To increase the cell density of the cultures rapidly, pour off the top half of a culture flask into a sterile, prepared flask at the end of the organisms' light phase. The organisms concentrate at the top of the flask during the light phase. This higher density culture can be used in a bioassay right away, or an equal volume of ESM can be added to the flask to maintain the culture.

Estimating Stock Culture Cell Concentration

To estimate the stock culture cell concentration, the cells are counted several times. The counts are done with an electronic particle counter, or manually, using a counting chamber (e.g., a Sedgewick-Rafter cell) and a microscope. To estimate the stock culture cell concentration manually using a counting chamber, complete the following steps:

1. Swirl the stock culture flask to mix the cells.
2. Move the flask side to side and forward and back a few times to evenly distribute the cells in the flask.
3. Pipette a 1 mL aliquot into a small beaker (or volumetric flask).
4. Add 25-mL filtered (0.45-µm) seawater to the beaker to create the diluted cell stock.
5. Mix as in #1 and 2 above.
6. Pipette 1 mL of the diluted cell stock into a counting chamber.
7. Add 1-2 drops of formalin to kill the cells.
8. Allow the cells to settle in the counting chamber.
9. Count the cells under 40× magnification using a compound, dissecting, or inverted microscope.
10. Repeat the counts 4 to 5 times.
11. Average the results to get the average cell count.
12. Multiply the average cell count by 26 (the total volume of the diluted cell stock) to determine the stock culture cell concentration.

Preparation of Sediment Elutriate

This procedure can be used for dredged material or almost any other solid material of concern.

1. Obtain a sample of the sediment to be tested; disaggregate and homogenize if necessary.
2. Combine the sediment sample with unfiltered site sea water (or distilled water) in a 1:4 ratio of sediment to water. If using distilled water, adjust the salinity with ASTM Sea Salt.
3. Stir vigorously for 30 minutes with a magnetic stirrer. Also, stir manually at 10-minute intervals during the 30-minute time period.
4. Allow the mixture to settle for one hour.
5. Siphon off the supernatant carefully so as not to disturb the settled material.
6. Centrifuge the supernatant until the suspension is clear.
7. Filter the supernatant through a 0.45-µm filter for a constant clarity.
8. Prepare the test dilution concentrations of 100, 50, 25, 12.5, and 6.25% using the filtered supernatant, the stock culture cells, and ESM as the makeup water.
9. Prepare a control that does not contain the elutriate. The control contains only stock culture cells and ESM.

Analysis Procedure

The following general steps describe the bioassay procedure. The procedure can be divided into steps that are done before, during, and after the bioassay start day. Refer to the manufacturer's instructions for detailed information on operating the QwikSed system.

Note that reversing the dinoflagellates' normal day-night cycle allows testing to be done during daytime hours. If the organisms are exposed to light from 10 p.m. to 10 a.m., they will be ready for testing by 2 or 3 p.m., which is 3 to 5 hours into their dark phase.

Days Before the Bioassay

1. Prepare enriched seawater medium.
2. Prepare stock cultures of the dinoflagellate.
3. Collect the marine sediment sample.

Day of the Bioassay

1. Determine the stock culture cell concentration.
2. Prepare the elutriate of the sediment sample.
3. Prepare the elutriate test dilutions (concentrations of 100, 50, 25, 12.5, 6.25%) and the control.
4. Prepare the cuvette trays. A set of cuvettes is needed for each 24-hour test period. For example, a 96-hour test would have four sets of cuvettes with one set being read every 24 hours. A cuvette set normally consists of cuvettes for five replicate samples and one control for each concentration of the elutriate test dilutions.
5. Place an aliquot of the test dilutions into the cuvettes.
6. Incubate the cuvette trays at 19°C under cool white fluorescent bulbs (light intensity of 4000 lux) for a 12:12 hour light/dark time period.

Day After the Bioassay (24 hours later)

1. Turn on the red light and then darken the room. Do not shine the red light on any equipment.
2. Allow the QwikSed system to warm up for at least 10 to 15 minutes.
3. Perform dark counts, as directed.
4. Remove the cuvette tray from the incubator. Protect all the cuvettes from light (even the red light), and do not bump the tray or the cuvettes.
5. Place each cuvette into the QwikSed test chamber.
6. Lower the stirring rod into the cuvette.
7. Push the Start button on the QwikSed system. The PMT counts which indicate bioluminescence appear on the LED display.
8. Repeat the above seven steps every 24 hours for the duration of the bioassay to measure the light output of the dinoflagellates. Use the cuvette sets that were prepared on the bioassay start day.

The system's software will automatically print out the raw data and calculate the mean, standard deviation, coefficient of variation, percent of control, and an estimated IC50. Results of the dinoflagellate tests should be calculated based on one measurement of light intensity in each cuvette.

Instrument Vendor

One QwikSed bioassay instrument is available commercially, which is licensed to Sealite Instruments, Inc. See Section 2.2.4 of the main document for more information.

References

ASTM. 1997. "Standard Guide for Conducting Toxicity Tests with Bioluminescent Dinoflagellates." E 1924-97.

Lapota, D, and D. Rosenberger. 1996. "QwikLite Basics," Version 1.08. Naval Command, Control, and Ocean Surveillance Center, February.

Lapota, D., C.H. Liu, D.E. Rosenberger, and J.I. Banu. 1998. "Use of a Rapid Bioluminescent Test (QwikLite): Using Dinoflagellates to Assess Potential Toxicity of Sediment Pore Waters." International Symposium on New Microbiotests for Routine Toxicity Screening and Biomonitoring. 1-3 June, Brno, Czech Republic. http://environ.spawar.navy.mil/Programs/MESO/Newsltr/sd087_r1.pdf

Naval Facilities Engineering Command. 2000. "QwikSed A Bioluminescent Sediment Toxicity Test." TechData Sheet, NFESC TDS-2077-ENV February. http://erb.nfesc.navy.mil/erb_a/restoration/technologies/invest/fld_chem/tds-2077-qwiksed.pdf

QwikLite Bioassay System, product brochure, Sealite Instruments, Inc., Ft. Lauderdale, FL.

Appendix C
Draft Statements of Work

Statement of Work Template

A performance-based Statement of Work (SOW) should clearly define project goals without limiting a contractor's ability to provide creative approaches to implement the project. The document template that follows is designed to support remedial project managers (RPMs) when preparing a performance-based SOW to obtain services for chemical analysis of sediment using on-site application of the rapid sediment characterization (RSC) methods. The following guidelines should be kept in mind when preparing the SOW for a specific project.

(1) Specifically and clearly define the contract goals.

(2) State technical and schedule requirements in terms of desired results.

(3) Completely define the methods needed to meet performance objectives.

(4) Clearly establish deliverables and other reporting requirements.

(5) State the mandatory minimum requirements that meet the Navy's actual needs during the contract period.

This template gives a generic starting point for preparing the SOW. However, to ensure efficient procurement, the RPM must be familiar with the information in the *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments* and apply this information consistent with site-specific needs when modifying the template. Specific features of this document include the following:

(1) Items requiring site-specific input are indicated with [**boldface type**].

(2) Explanatory notes that are not intended to remain in the final text are indicated as *{descriptive text}*.

(3) Add or delete lines from example Tables 1, 2, 3, and 4 in order to build project-specific analyte lists for your site. Sections 2.1 and 2.2.1 of the main RSC document will help guide identification of appropriate detection limits for Tables 2, 3, and 4 only.

Statement of Work
for
Field Analytical Services at [Site Name and Location]

[Date of Preparation]

Purpose

The purpose of this field analytical project is to obtain on-site, rapid turnaround analysis of environmental contaminants in sediment to support characterization of [**site name and location**]. Sample analysis is being conducted to accomplish the following objectives:

{Select items from the following list and/or add new items as applicable to your project.}

- **Preliminary analysis to focus future site characterization plans and provide timely, cost-effective analysis results**
- **Screening to define zones of contamination, locate contamination boundaries, locate hot spots, or identify potential transport pathways and receptors**
- **Exploratory site investigation to identify general classes of contaminant present**
- **Examination of trends in contaminant types and distributions**
- **Studies for technology transfer or environmental model development.**

Scope

The contractor shall provide all labor, supervision, materials, tools, calibration standards, chemicals, and equipment required to perform analysis of sediment samples for the contaminants specified in Table 1. Table 1 also indicates the rapid sediment characterization (RSC) method required for each contaminant analysis.

Samples will be collected from [**sampling location, water depth, bottom conditions, and sediment physical description**]. The contractor shall accept these samples and perform the analyses defined in the Specific Requirements Section in an on-site laboratory. The analysis program will start on [**date**] and be completed by [**date**]. *{Add any site-specific information about the required scheduling of sampling activities (e.g., 9 to 5 on weekdays, or 24-hour / 7-day-per-week coverage.)}*

Supporting Documents

The following supporting documents are applicable to this project:

Battelle. 2001. *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments*. Prepared for Naval Facilities Engineering Service Center and Space and Naval Warfare Systems Center. April 30.

Table 1. Scope of Required Analysis

Analyte	RSC Method
Metals *{e.g., arsenic, barium, cadmium, chromium, lead, selenium, and silver}*	X-ray fluorescence spectrometry
BTEX	Ultraviolet fluorescence spectrometry
Total TPH	
Total PAHs	
BTEX	Immunoassay
TPH	
PAHs	
Carcinogenic PAHs (CaPAHs)	
PCBs	
TNT	
RDX	
Chlordane	
Lindane	
DDT	
2,4-D	
Silvex	
Carboxoamide pesticides	
Cyclodiene pesticides	
Organochlorine pesticides	
Organophosporus pesticides	
Phenol pesticides	
Toxicity	QwikSed

Requirements

This section defines the general and specific requirements for the project.

General Requirements

The contactor shall perform all operations associated with this contract in accordance with all applicable public laws and regulations.

The contractor shall use procedures specified in this Statement of Work in preparation of and analysis of samples.

The contractor shall perform quality assurance calibration and tests and maintain quality assurance records in accordance with written laboratory operating procedures. Laboratory operating procedures shall be available for inspection by Navy personnel. Laboratory quality assurance records shall be available for auditing by Navy personnel.

The contractor shall maintain a chain-of-custody record to account for all samples and supporting records of sampling handling, preparation, and analysis in accordance with written laboratory operating procedures.

The contractor shall perform and document data reduction and reporting in accordance with written laboratory operating procedures.

The contractor shall provide for management of all sample and analysis residuals.

The samples to be analyzed by the contractor are from know or suspected contaminated sites and may contain hazardous inorganic and/or organic materials at high concentration levels. The contractor shall be aware of the potential hazards associated with the handling and analyses of environmental samples containing hazardous contaminants. It is the contractor's responsibility to take all necessary measure to ensure the health and safety of its employees. *{Add a description of any specific warnings or guidance about known contaminants or hazards.}*

Specific Requirements

{Modify this list as appropriate for your site, and use the following example subsections to develop your site-specific SOP. If you will be doing two or more types of analyses on each sample, you may want to combine the tasks descriptions.}

The specific project requirements are defined in the following tasks:

- X-ray fluorescence spectroscopy
- Ultraviolet fluorescence spectroscopy
- Immunoassay
- QwikSed bioassay
- Off-site laboratory confirmatory analyses.

X-Ray Fluorescence Spectroscopy

This task involves providing analysis of metal contaminants using x-ray fluorescence spectroscopy. The analytes of interest and required detection limits are shown in Table 2. Samples will be provided in [**a 250-mL, wide mouth polyethylene jar**] *{describe sample container, and provide information about expected water content of the samples, if known}*. About [**X**] samples per day will be produced over a period of [**Y**] days for a total of [**X × Y**] samples. Analysis shall be performed using Standard Operating Procedures (SOPs) described in Appendix B of *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments*. Analytical results shall be provided within [**time limit**] of receiving the sample. The results shall be ... *{Complete the paragraph with one of the following, using Section 1.5 of the RSC Guide to guide selection}*:

- **... flagged as produced by a non-standard method.**
- **... reported as detection findings without qualitative results.**
- **... corrected using a site-specific calibration; this calibration shall be performed using methods described in Appendix B of *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments.***

Table 2. Required Analytes, Limits of Detection, and Precision

Analyte	Limit of Detection (mg/kg)	Precision (% relative standard deviation)
Barium	100	25
Copper	20	25
Lead	10	25
Zinc	20	25
Etc.		

Ultraviolet Fluorescence Spectroscopy

This task involves providing analysis of organic contaminants using ultraviolet fluorescence spectroscopy. The analytes of interest and required detection limits are shown in Table 3. Samples will be provided in [**a 250-mL, wide mouth polyethylene jar**] *{describe sample container, and provide information about expected water content of the samples, if known}*. About [**X**] samples per day will be produced over a period of [**Y**] days for a total of [**X × Y**] samples. Analysis shall be performed using Standard Operating Procedures (SOPs) described in Appendix B of *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments*. Analytical results shall be provided within [**time limit**] of receiving the sample. The results shall be ... *{Complete the paragraph with one of the following, using Section 1.5 of the RSC Guide to guide selection}*:

- **... flagged as produced by a non-standard method.**
- **... reported as detection findings without qualitative results.**
- **... corrected using a site-specific calibration; this calibration shall be performed using methods described in Appendix B of *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments.***

Table 3. Required Analytes, Limits of Detection, and Precision

Analyte	Limit of Detection (mg/kg)	Precision (% relative standard deviation)
PAHs	5	25
Etc.		

Immunoassay

This task involves providing analysis of organic contaminants using immunoassay methods. The analytes of interest and required detection limits are shown in Table 2. Samples will be provided in [**a 250-mL, wide mouth polyethylene jar**] *{describe sample container, and provide information about expected water content of the samples, if known}*. About [**X**] samples per day will be produced over a period of [**Y**] days for a total of [**X × Y**] samples. Analysis shall be performed using Standard Operating Procedures (SOPs) described in Appendix B of *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments*. Analytical results shall be provided within [**time limit**] of receiving the sample. The results shall be ... *{Complete the paragraph with one of the following, using Section 1.5 of the RSC Guide to guide selection}*:

- **... flagged as produced by a non-standard method.**
- **... reported as detection findings without qualitative results.**
- **... corrected using a site-specific calibration; this calibration shall be performed using methods described in Appendix B of *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments.***

Table 4. Required Analytes, Limits of Detection, and Precision

Analyte	Limit of Detection (mg/kg)	Precision (% relative standard deviation)
BTEX	2	25
TPH	10	25
PAHs	1	25
Carcinogenic PAHs (CaPAHs)	10	25
PCBs	1	25
TNT	1	25
RDX	1	25
Pentachlorophenol (PCP)	0.5	25
Etc.		

QwikSed Bioassay

This task involves providing analysis of acute and sublethal chronic toxicity using the QwikSed bioassay method. Samples will be provided in [**a 250-mL, wide mouth polyethylene jar**] *{describe sample container, and provide information about expected water content of the samples, if known}*. About [**X**] samples per day will be produced over a period of [**Y**] days for a total of [**X × Y**] samples. Analysis shall be performed using Standard Operating Procedures (SOPs) described in Appendix B of *Guide for Using Rapid Sediment Characterization Methods in Ecological Risk Assessments*. Analytical results shall be provided within [**time limit**] of receiving the sample. The results shall be flagged as produced by a non-standard method.

Off-Site Laboratory Confirmatory Analyses

This task involves providing results using standard laboratory analytical methods that can be used to check or calibrate the results produced by RSC methods. The contractor shall split [**X%**] of the sediment samples and ship the duplicate to a laboratory for analysis. The contractor shall select, with Navy approval, the appropriate analytical method(s) to be used *{select the analytical suite that is consistent with your site requirements (e.g., U.S. EPA Contract Laboratory Program, U.S. EPA SW-846, or other standard methods)}*. Shipping containers, shipping conditions, and hold times for analysis shall be consistent with the method requirements. Analytical results shall be provided within [**time limit**] of receiving the sample.

Deliverables

The contractor shall provide the following analytical results data package:

- Sample results in a tabular form reported with a notation indicating whether results are on a wet or dry weight basis
- Data shall be flagged or annotated to indicate the level of calibration used (e.g., produced by nonstandard method, site specific calibration, or detection without quantitation)
- Sample results in electronic format *{specify format required for your data handling needs}*
- Report percent moisture for all samples (except screening samples analyzed without drying)
- Report sample extraction methods and dilution factors (as applicable)
- Return signed copy of the chain of custody forms sent with the samples and any internal or laboratory chain of custody forms
- Report results of required quality assurance calibrations and analyses.

Government Supplied Equipment

Navy will provide access to [**site**] during the period of performance of the project. *{Also describe any site-specific access requirements such as specific hazard training (e.g., unexploded ordnance), security clearance, and/or limitations on time of day when the site is available}.*

Navy will provide a space of [**size**] at [**location**] as a temporary location for the contractors on site laboratory facility. *{If possible, include map showing size and location of facility.}*

Navy will provide access to electrical utilities at [**voltage**] and [**current**]. *{Also specify whether a power drop already exists at the site, or whether the contractor is expected to install a transformer.}*

Points of Contact

{Provide the following information for each point of contact}

- Name
- Title
- Address
- Telephone number
- Fax number
- E-mail address.