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Selenium Fractionation and Cycling in the Intertidal Zone of the Carquinez Strait

Draft Annual Report
October 1, 1994 through September 30, 1995

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H.S. Mountford, T.M. Johnson, L. Tsao,
J. Oldfather, A.F. Haxo, and T.C. Sears
Earth Sciences Division

November 1995

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1 Introduction

Research aimed at gaining a better understanding of selenium cycling in marshes and mudflats of the Carquinez Strait is being performed by scientists from Lawrence Berkeley National Laboratory and collaborators from the University of California at Davis. This work was initiated in the fall of 1994 and is scheduled to continue through the fall of 1996. This report summarizes the results of the effort to date.

Interest in selenium cycling in the San Francisco Bay was prompted by the discovery of elevated selenium concentrations in various components of the food web (summarized in SFBRWQCB, 1992). Of particular concern are levels of selenium in diving ducks. Although no adverse effects have been observed, selenium tissue levels in diving ducks of up to 209 ppm* are comparable and sometimes exceed levels found in bird populations at Kesterson Reservoir (SWRCB, 1990). Even though selenium concentrations in water (0.1 to 0.3 ppb; Cutter, 1989) and sediment (0.2 to 0.5 ppm; Johns et. al., 1988) are relatively low when compared with Kesterson Reservoir, the potential for biomagnification, especially through algae and bottom feeders, is apparently large. The most likely major source of selenium for diving ducks are bivalves. Selenium concentrations in filter-feeding clams in the San Francisco Bay range from 2.8 to 5.2 ppm (Anderlini et. al. 1975). Because clams ingest both suspended and bottom sediment, and any organic suspended particulates, understanding the role of sediment in the selenium cycle is critically important. Past studies of selenium speciation and fractionation have shown that a large percentage of selenium in sediments is in either reduced or highly immobile form (Weres et. al., 1989; Tokunaga et. al., 1991; Zawislanski and Zavarin, in press). Uptake efficiency of selenium by clams is determined by the form ingested (Luoma et. al., 1992). For example, the most oxidized form of selenium, selenate, is also the form most readily taken up by plants (Gissel-Nielsen and Bisbjerg, 1970). On the other hand, elemental selenium is very insoluble and not easily assimilated by animals (Mayland, 1994). All

* All sediment and tissue concentrations are on a dry weight basis.

of this leads to a very important conclusion: the total selenium concentration in sediments is less biologically important than the concentrations of the more readily available forms.

The primary purpose of this study is to define routes and rates of selenium transformations in the water-sediment system as pertinent to biological cycling. This is going to be accomplished by testing cycling hypotheses (Chapter 2) in both the field and lab settings. Two field sites have been chosen for this study and are described along with preliminary field activities in Chapter 3. Along the way, a number of methods for selenium fractionation and analysis are being perfected. The development of these methods comprised the major part of the research effort over the last year. These include: sequential extraction procedures, parts-per-trillion range analytical methods, selenium purification for isotope analysis, and the isotope analysis itself. Method development is described in Chapters 4, 5, and 6. Results, including selenium concentrations in sediment and water, fractionation in sediments, and spatial distribution as related to other chemical and physical parameters comprise Chapter 7. A summary and synthesis of findings are presented in Chapter 8.

2 Selenium Chemistry in Wetlands -- An Overview

Selenium cycling in wetlands has been investigated in the past with respect to Kesterson Reservoir sediments. Much less work has been done to elucidate the Se cycle in tidal marsh sediments, such as those of the San Francisco Bay. Some aspects of this cycle are known while others are hypothesized. The following is an overview of what is understood and what is surmised about Se transformations in this environment.

Selenium (Se) inputs to the Carquinez Strait consist of riverine-Se and refinery-Se. The input of Se from the rivers, mostly the San Joaquin, into the Delta, consists of roughly 60% dissolved selenate, 15% each of dissolved selenite and organically-associated-Se (O-Se), 5% of suspended-particulate matter (SPM) selenite, and 5% of SPM-O-Se, at a total annual load of roughly 1000 kg. Oil refineries contribute about 2500 kg Se per year, primarily as selenite. Both riverine and refinery selenium enter the sediment system. There are three primary routes for Se incorporation: 1) deposition of SPM and associated Se, 2) uptake of dissolved and suspended Se by benthic organisms, and 3) the reduction of soluble Se in interstitial waters within shallow sediments. In terms of the total contribution of Se to the sediment system, SPM deposition is likely more important. Only limited data exists on the fractionation of Se on SPM, primarily distinguishing between "organic" and inorganic Se (Cutter and Bruland, 1984). One may assume that SPM-Se is largely adsorbed selenite. SPM-Se contribution to the sediment system can be quantified to a certain degree, because approximately 50% of SPM is deposited in the Bay. This means that on average approximately 300 kg of adsorbed Se is deposited every year with SPM. However, it is not known how much of the dissolved species, including selenite, selenate, and O-Se is incorporated into the sediment. There are four potential mechanisms for such incorporation:

1) *In-situ adsorption.* Approximately 60% of the selenite in the water column is in dissolved form. Upon encountering shallow sediments, it could become adsorbed either onto organic or inorganic particles, thereby becoming immobilized. The degree to which this happens is not known and will depend to a large extent on the availability of adsorption sites, the pH of the interstitial waters, and contact time.

2) *In-situ reduction.* Under conditions of fluctuating tides and moisture, both reduction and oxidation of Se in shallow sediments could occur. However, past experience shows that reduction kinetics are far more rapid than oxidation kinetics (Tokunaga, et. al., 1994). This is especially true in organic-rich environments. Therefore, net selenium reduction and immobilization would occur more readily in the marsh environment, rather than the mudflat. There is no data on reduction or oxidation rates under these conditions. If dissolved selenate is effectively reduced, it could comprise a major fraction of sediment Se.

3) *Plant uptake.* Plants which come in contact with Se-containing interstitial water will take up Se as part of the soil solution. Such uptake can be estimated based on generic water uptake rates for various species and the concentration of dissolved Se. Plants can do one of three things with Se: (i) volatilize it in methylated form, thereby removing it from the system (Terry and Zayed, 1994), (ii) assimilate it into amino acids, thereby converting it into organo-Se compounds (Gissel-Nielsen, 1979), or (iii) accumulate it in inorganic form (Gissel-Nielsen, 1987). Gissel-Nielsen's work has shown that most of the Se is converted to amino acid form. Therefore, upon the incorporation of plant material into the sediment or soil, Se will become part of the organo-Se pool, the availability of which to clams is not well established.

4) *Plankton uptake/animal uptake.* These can draw upon all dissolved Se species, and generally convert them to organic forms. Similarly to plants, their contribution of Se to the sediment system is related to their life cycle. The relative contribution of total Se from plankton and benthic organisms is not well established but can be estimated based on their biomass.

2.1 Sulfur as a Selenium Analog

Subsequent to incorporation into the sediment system, Se undergoes a number of chemical transformations. The environmental behavior of Se is commonly compared to that of sulfur (S), because of their similar chemistry. There is a substantial body of research on S cycling in marshes. Several points relevant to S (and Se) cycling may be made. Oxidation-reduction cycles of S are both seasonal and tidal (Casey and Lasaga, 1987; Luther and Church, 1988) and controlled by the relative elevation, i.e., average degree of water saturation (Howes, et. al., 1981). Therefore, within a relatively small area, such as a 1 acre tidal marsh, there will be redox state variability not only normal to shore but also chaotically distributed throughout the site, due to local depressions which may be ponded far more frequently than the surrounding areas. Another factor which controls the redox state of sediments is texture. Clayey sediments retain more moisture and drain more slowly than sandy soils. Therefore, subsequent to inundation, a clayey sediment may remain very near complete saturation in between tides, while a sandy sediment may lose on the order of 75% of its interstitial water to draining. Each part of a wetland will exhibit an oxic layer at the sediment surface, where periodic re-oxidation occurs (even if the net trend is that of reduction). The thickness of this oxic layer will also be determined by the frequency with which the area is inundated. Lord and Church (1983) found that in a marsh which is on average inundated only during the highest tide of the month, the oxic layer was about 10 cm deep. On the other hand, sediments near the intertidal/subtidal boundary are always inundated and the presence of free oxygen is not expected. The cycling of S under these oxic/anoxic conditions results in the reduction of sulfate to sulfide and the eventual mineralization as pyrite (FeS_2) (Lord and Church, 1983). Depth profiles show sulfide to be completely dominant below a certain depth, which may range from around 5 cm to as much as 30 cm, and is controlled by the factors described above (Luther and Church, 1988).

Although in many ways chemically similar, Se behavior in soils and sediments differs from that of S, primarily because of the common presence of selenite (SeO_3^{2-}) which sorbs strongly onto iron oxides (Hamdy and Gissel-Nielsen, 1977), clay minerals (Bar-Yosef and Meek, 1987), and soil organic matter (Yläranta, 1983). Although according to thermodynamic calculations selenite and selenate should not occur under the same pH-pE conditions (Neal et. al., 1987), they commonly occur

simultaneously in nature. This has to do with kinetic limitations of selenite oxidation and, to a lesser extent, selenate reduction, which is microbially mediated. Selenate does not sorb significantly (Neal and Sposito, 1989). This results in soil solutions heavily dominated by selenate (Tokunaga et. al., 1994) and has important implications for Se cycling in the intertidal environment, especially considering Se speciation in the refinery vs. riverine inputs.

Another difference is the ecologically important transformation to elemental Se (Se^0). The presence of Se^0 in shallow soils as a result of selenate and selenite reduction has been shown (Weres et. al., 1989; Tokunaga et. al., 1991). Elemental Se is generally considered unavailable to biological organisms and its oxidation kinetics are very slow (Zawislanski and Zavarin, in press). Following the current understanding of Se reduction, Se^0 should occur in areas which remain water-logged or have been water-logged for an extended amount of time at one time or another. This clearly applies to the anoxic zone underlying marshes and mudflats. Velinsky and Cutter (1990) found Se^0 to comprise about 30% to 80% of the total Se in a number of East Coast marsh sediments. These same sediments contained as much as 70% of the Se in some sort of organic association, although the O-Se was determined by difference and is therefore not very reliable. In contrast to S cycling, iron selenide (or ferroselite, $FeSe_2$, the pyrite analog), is less common. Velinsky and Cutter (1990) found it to comprise between 0 and 26% of total Se in the same sediments, but none was detected in sediments shallower than 10 cm. Therefore, it appears that pyrite-Se is only important in sediments which have undergone some early diagenesis or mineralization of O-Se.

It is not clear how tidal cycles affect selenium speciation. Given that selenium reduction is more rapid than oxidation, very little re-oxidation may take place during low tide. Seasonal effects may be even more difficult to quantify. There are three factors that influence Se speciation in the surface sediments: 1) the relative amount of time the sediments are submerged, 2) sediment texture, and 3) organic matter content and plant/microbial activity. There may be a significant correlation between Se speciation and distance from shore. Soils farther inland will be submerged less and may therefore be less reduced. On the other hand, sediments closer to the marsh will be more organic, enhancing biologically-mediated reduction. Biological activity within the sediments may largely control selenium reduction. It is not clear to what degree Se

is reduced far away from the shore, in the subtidal zone, since the organic content of those sediments is rather low.

Uptake by benthic organisms affects the Se cycle. Living organisms convert inorganic Se, primarily selenite, to organic form. Subsequent to their death, organism decay will lead to organo-Se release to the sediment system. The rate of Se mineralization may be limited by sediment burial rates.

Chemical transformations are overprinted by the physical redistribution of sediments. Sedimentation rates are the net result of deposition and resuspension, which occur on time scales ranging from diurnal tidal fluctuations to seasonal flow fluctuations. The sedimentation rate will obviously affect the amount of selenium deposited and also potentially affect the rate of selenium immobilization. Depending on site-specific conditions, it is likely that SPM-Se will be largely selenite, while resuspended-particulate material-Se will have been further reduced. On the other hand, resuspension can facilitate oxidation of SPM-Se thus altering the speciation of Se in sediments.

2.2 The Selenium Cycle -- A Preliminary Hypothesis

Based on the chemical and physical relationships described above, the following hypothetical Se cycling model may be considered.

- Se enters the intertidal zone primarily as dissolved selenite, dissolved selenate, and selenite adsorbed onto SPM. The relative significance of organic Se is not known.

- SPM-Se is deposited with SPM. The deposition rate is dependent on distance from shore and the energy of the system. However, a generalization may be made that more SPM is deposited in the subtidal zone than in the mudflat than in the lower marsh than in the upper marsh. Therefore, Se concentration in mudflat sediments is controlled more so by Se concentration on SPM than it is in the marsh.

- Dissolved selenite sorbs strongly onto organic-rich, peat-like sediments of the marsh, and less readily to mudflat sediments, mostly because of the larger density of

available sites. Dissolved selenate will not be sorbed but will be reduced in organic-rich areas, such as the lower marsh. The relative percentage of selenate which will become immobilized in this fashion is site-specific.

- An unknown percentage of dissolved and suspended Se enters the biological system directly through clams and other benthic organisms. This leads to conversion to organo-Se and the eventual incorporation into the sediment system.

- Very little Se is transported deeper into the sediment profile because the marsh and especially the mudflats are at or very near full water saturation at all times. Therefore, the hydraulic gradient is very slight. This may not be the case during major storm events, when the hydraulic head is significantly greater.

- Water (including SPM) leaving the marsh and the mudflat between high and low tide is depleted with respect to Se but the difference may not be measurable.

- Any selenite and selenate remaining in the sediments will be subject to microbially-mediated reduction. The reduction of selenate to selenite is rapid, on the order of hours to days, depending on the level of microbial activity. The rate of selenite reduction to Se^0 is slower but has not been well defined. Once again, it will be a function of sediment redox status and microbial activity. Mudflat sediments are generally reduced most of the time. Therefore, they should be dominated by Se^0. In contrast, near-surface upper marsh sediments are seldom inundated and oxidized Se forms will be found in higher concentrations.

- Se which remains dissolved will be taken up by marsh plants. Although Se concentration data for plants in the Carquinez Strait are not widely available, studies from other areas generally show marsh plants to contain between 0.5 and 5 ppm Se (Hothem and Ohlendorf, 1989; Saiki and Lowe, 1987). In areas with highly Se-contaminated waters, these same plants may accumulate up to 100 ppm Se (Schuler, et. al., 1990). Given that the annual primary productivity for marsh vascular plants in the Bay ranges from 500 to 1500 g m^{-2} yr^{-1} (Josselyn, 1983), anywhere between 0.25 and 7.5 mg of Se per m^2 could be taken up by plants each year. (This compares with around 1.5 mg Se m^{-2} yr^{-1} which is brought in with suspended sediment.)

- Sediment deposition in the estuarine environment occurs at an average rate of about 1-2 mm/yr, although some areas may experience much higher rates, up to 5 mm/yr (L. Wells, personal communication). Burial of Se-containing sediments will result in the eventual reduction and mineralization of Se. With increasing depth, Se^0 and pyrite-Se should become more dominant; at some depth, perhaps on the order of 10's of meters, organic matter will become completely mineralized and all Se forms will be inorganic. Whether or not early burial, down to depths of a few centimeters, will significantly change Se speciation remains to be seen.

- As plants decay, and become part of the litter, organo-Se becomes part of the shallow sediment system. The decay rate of litter will determine the rate of Se mineralization.

- Sediment re-suspension and erosion will occur from time to time. These processes will affect unvegetated areas much more, i.e., mudflats much more than marshes. Therefore, a more complete and less disturbed record of Se deposition is available in marsh sediments.

3 Field Activities

Field activities of the last year were focused on the selection and preliminary characterization of field sites. Two sites were chosen and are described below. In addition, a summary of field sampling is provided.

3.1 Site Selection

Two sites were selected in the Carquinez Strait area (Figure 3.1). The primary criterion for this selection was the presence of an undisturbed intertidal zone, including a well developed mudflat and marsh. The sites had to be accessible but not used by the public for hiking, fishing, etc. The sites needed to be downstream of the local refineries. A third, upstream, control site has not yet been selected. The difficulty in selecting such a site lies in the fact that most upstream areas which fit our criteria are not significantly influenced by tides and, therefore, may not be comparable. Several potential candidates for this site have been selected and will be further investigated.

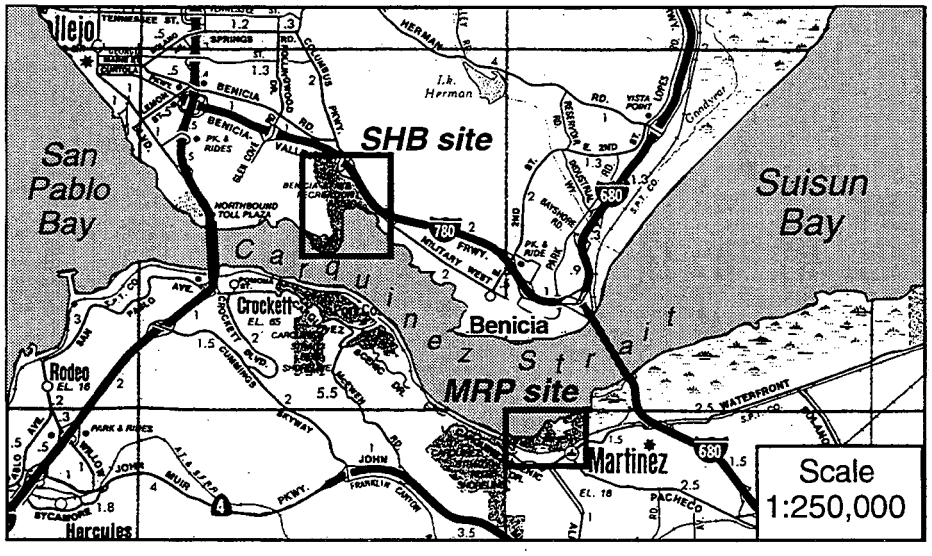

Figure 3.1 Location of field sites within the Carquinez Strait.

3.1.2 The Martinez Regional Shoreline Park Site

The Martinez Regional Park site ("MRP site") is located in the mudflats and marsh roughly 300 to 400 m west of the Martinez Marina (Figure 3.2). This area falls under East Bay Regional Park District jurisdiction and the appropriate permits have been obtained for performing field research. The specific site from which samples were taken is some 20 meters east of Arroyo Del Hambre, a creek which flows through the city of Martinez and cuts through the marsh. Creek influence on sedimentation in the area was inadequately estimated, and some of the sediments sampled may have come from the creek. This conclusion was drawn subsequent to a fortuitous airplane fly-over during which the sedimentation patterns around the mouth of the creek became apparent. As a result of this, future sampling will take place at least 50 m west of the creek. Nonetheless, the physical description of the site is valid and representative.

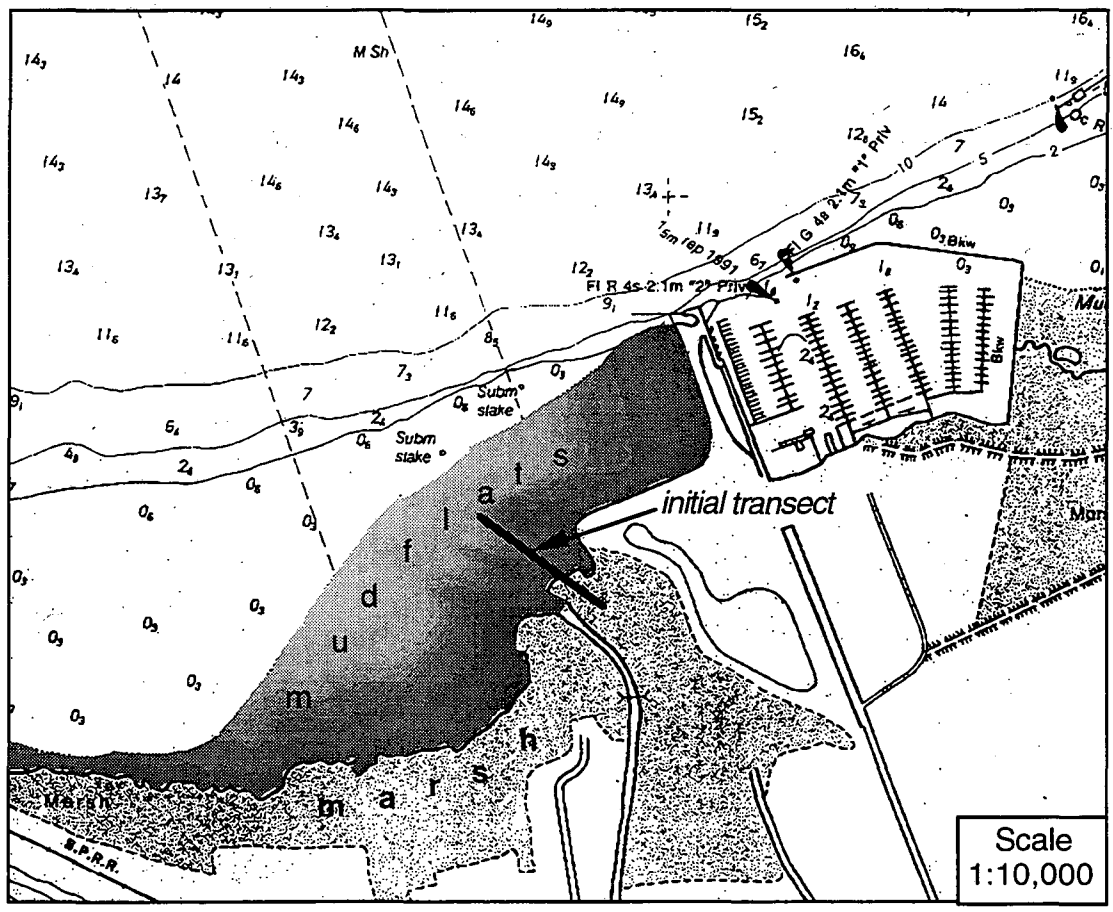

Figure 3.2 Close-up view of MRP site and sample location.

The MRP site is a relatively undisturbed shoreline surrounded by largely developed areas to the east and south. To the west it is bounded by a developed shoreline and the cliffs of the Carquinez Strait Regional Park. The area to the east is a popular marina and the shoreline there is rip-rap reinforced. According to Ogden Beeman et. al. (1992), the area immediately surrounding the marina, and probably including the site, experienced significant sedimentation between 1955 and 1990 (0 to 6 ft). The areas west of the marina, and possibly including the western edge of the site, experienced very modest net erosion over the same period of time (0 to 1 ft). The MRP site is a relatively high-energy shoreline because of its location on the Strait and its proximity to shipping channels. Strong winds out of the northwest produce high-energy waves on a regular basis, and passing ships, primarily refinery-related, can cause foot-high waves on occasion.

At extremely low tide, approximately 160 m of mudflats are exposed. They are covered seasonally by a thin film of light brown diatoms. The mudflats are sandy and can be easily traversed and sampled. The 2 cm surface layer has a coarse silt to fine sand texture and is low in organic matter (OM). The underlying layers, 2-10 and 10-20 cm, have varying textures that are dependent on the sedimentation history of the specific spot. Because this area is open to waves and tidal flow, deposited layers are interrupted and heterogeneous in texture. Therefore, generalizations about the three-dimensional physical description of the sediment profile cannot be made. The interface between the mudflats and the marsh is well defined by a distinct rise in elevation and the appearance of plants. This interface will be used as a reference (point 0) for the purpose of identifying sample locations and site descriptions at all sites. In the marsh, sediments are of finer texture and more homogeneous. At the surface, dead plant material forms a litter layer between 2 and 5 cm in depth. Below the accumulated plant material is a dark, fine textured soil that has higher OM concentrations than in the mudflats.

The interface between marsh and mudflat is sparsely vegetated by cordgrass (*Spartina foliosa*), up to 1 m tall. About 2 or 3 m inland, cordgrass is intermixed with California bulrush (*Scirpus californicus*), which grows up to 2 m tall. About 10 m into the marsh, cordgrass is no longer found and the plant community is dominated by bulrush and cattails (*Typha latifolia*). Here the bulrush can grow to nearly 3 m tall while the cattails are generally 2 m tall. The density of plants here increases to as many

as 100 per m^2. Mixed in with the bulrush and cattails are occasional patches of higher elevation with a variety of more upland plants, including saltgrass (*Distichlis spicata*). This marsh composition continues inland for about 200 m.

3.1.3 The Southampton Bay Site

The Southampton Bay site ("SHB site") is located in the southwest quadrant of the Benicia State Recreation Area, northeast of Dillon Point (Figure 3.3). This area is part of the California State Park system and the appropriate permits have been obtained for performing field research.

The SHB site is within a system of marshes, upland areas, and very extensive mudflats. The shoreline is undisturbed except for ship wrecks which are submerged and not visible. There are hiking trails and picnic areas in the vicinity. The specific site was chosen because of its proximity to the main park road. Hills both west and east of the site are undeveloped and grass-covered. Dillon Pt., to the southwest, is a rocky cliff popular for fishing. According to Ogden Beeman et. al. (1992), the part of Southampton Bay which contains the site experienced moderate sedimentation between 1955 and 1990 (0 to 3 ft). The area between the site and Dillon Pt. experienced very modest net erosion over that same period (0 to 1 ft). The SHB shoreline is a relatively low-energy regime, probably because it is nestled within the bay.

At extremely low tide, over 200 m of mudflats are exposed. The surface sediments of the mudflats, from 0-2 cm, are lighter in color than the lower layers, which are black. They are covered seasonally by a film of light brown diatoms, and more recently (September) by a layer of green algae. The 0-2 cm layer is light brown color and very loose. From 2-20 cm the sediment is very fine, dominated by clays, and reduced carbon. Overall, the mudflats are fine-textured and remain near saturation even at lowest tide. This makes walking on the mudflats nearly impossible and as a result, specialized equipment or boards are required. Much like the MRP site, the interface between the mudflats and the marsh is well defined by a distinct rise in elevation and the appearance of plants. Marsh sediments have less color differentiation in the upper 2 cm, and the sediments contain higher concentrations of reduced carbon throughout the profile, making the color of all the sediments darker. The surface of the marsh has a poorly defined layer of decomposing plant tissue. There are numerous channels and

irregularities in the surface of the lower marsh, some with relief on the order of 0.25 to 0.5 m.

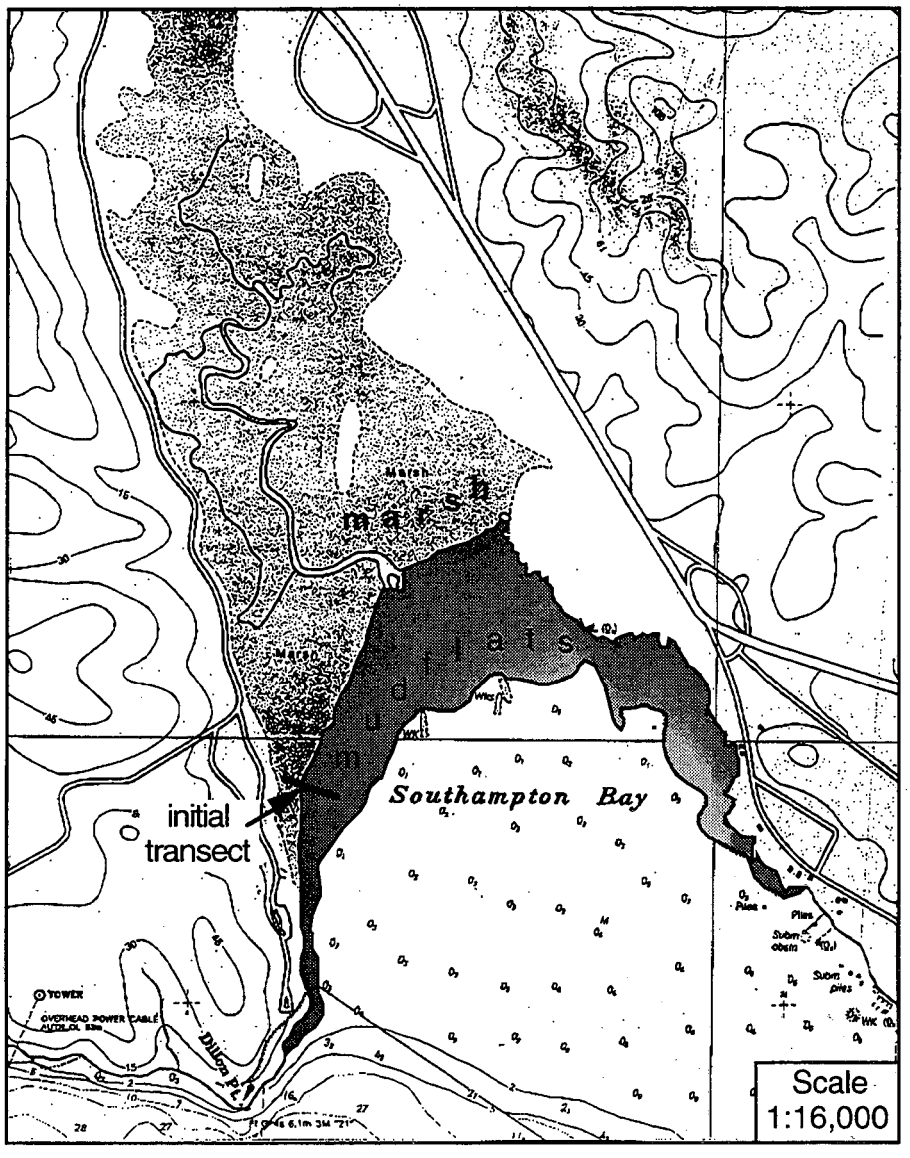

Figure 3.3 Close-up view of SHB site and sample location.

The interface between marsh and mudflat is sparsely vegetated with cordgrass, up to 1 m tall. About 3 m inland, cordgrass is intermixed with two *Scirpus* species: California bulrush (*S. californicus*, up to 2.5 m tall), and alkali bulrush (*S. robustus*, up to 2 m tall). Also within this zone, but less common, are cattails, generally no taller than 1.5 m. The density of plants here is somewhat smaller than at the MRP site.

About 25 m into the marsh, a transition to shorter plants occurs, a zone dominated by alkali bulrush (here up to 1 m tall), with less common California bulrush and pickleweed (*Salicornia virginica* or *S. subterminalis* or both). About 30 to 35 m into the marsh, there is a transition to what will be referred to as the "upper marsh." This zone is dominated by a mixture of pickleweed and saltgrass. At 35 m and beyond, saltgrass is dominant. The upper marsh continues to a distance of approximately 90 m from the mudflat/marsh interface. Beyond 90 m, the elevation rises abruptly to about 5 m above marsh level.

3.2 Field Sampling

Field sampling has taken place several times, generally during periods of low tide. Because many low tides occur at night and because the amount of time around the low tide during which the mudflats are exposed may be no longer than 2 hours, sampling opportunities are limited. The SHB site was sampled on 3/15/95 (only marsh samples, high tide period), 6/16/95 (low tide = -1.2 m), and 8/8/95 (low tide = -0.8 m). The MRP site was sampled on 5/17/95 (low tide = -1.6 m), 7/12/95 (low tide = -1.5 m) and again on 8/8/95. Except for the samples taken on 8/8/95, a hand-auger was used to collect the sample, except for the surface interval of 2 cm which was scraped off using a spatula. Samples were placed and sealed in plastic freezer bags. Upon the return to the lab, which generally occurred within 1 hour, samples were either immediately processed or frozen/refrigerated for future use. On 8/8/95, samples were taken using a prototype acrylic piston sampler. This tool proved effective in mudflats but less so in the marsh, as it was impeded by the presence of plant roots. Samples obtained using this sampler were sealed and frozen upon arrival at the laboratory. They were processed in an anaerobic chamber to test the potential for Se oxidation during routine sample handling. The results of this test were not available as of the writing of this report.

Currently, an improved sampling tool is being engineered. It will allow for sampling of both soft mudflat sediments and root-containing marsh sediments. The tool will be used to perform comprehensive sampling along transects at both sites. A field safety plan has been written and approved by the Earth Sciences Division Safety Officer.

4 Methods For Se Extraction From Sediments

Characterization of field sites with respect to Se speciation requires the development of reliable and precise techniques. Understanding the dynamics of Se cycling in the San Francisco Bay begins with an evaluation of the state of the sediments and determination of dominant Se forms. Identification and quantification of Se fractions will allow us to begin to determine whether Se in sediments is readily available on anion exchange sites as selenate and selenite, or in a more recalcitrant form such as in its elemental state, associated with refractory compounds such as humic acids and humins.

Analyses have been developed to distinguish between various selenium fractions in sediments and SPM to speciate the oxidation states of Se under different conditions. A result of this fractionation has been the further classification of the Se fractions such as *inorganic* (metal or free selenide, elemental, selenite, and selenate) and *organic* (selenide in amino acids, humic and fulvic acids, methyl-selenium, and selenite and/or selenate adsorbed to OM) into their associated species. It is impossible to identify and quantify Se in all its different forms and oxidation states because of the limitations of extraction techniques to exclusively remove individual fractions. Certain extraction techniques result in the transformation of a limited amount of one fraction into another (e.g. amino acid Se being transformed into inorganic Se by hydrolysis). More species-specific analytical methods, such as x-ray absorption spectroscopy (Pickering et. al., 1994), require higher Se concentrations and will be used in method development, but cannot be used in the direct analysis of Bay sediments. Therefore, the purpose of this report is to delineate the Se fractions that the techniques chosen for this study can identify, and define what the limitations of each technique are.

4.1 Inorganic Se Extraction

4.1.1 Selenides and Elemental Se

Inorganic Se as selenide can be found in ferroselite ($FeSe_2$), achavalite (FeSe), clausthalite (PbSe), and in chalcopyrite ($CuFeS_2$), and pyrite (FeS_2) substituting for sulfide. Elemental Se can be found in a variety of structural forms, with the two most common being gray or black Se and red Se. Identifying inorganic selenide, and distinguishing it from elemental selenium is often difficult. Velinsky and Cutter (1990) developed methods for the distinction of elemental and pyrite-selenium using sodium sulfite and Cr (II) solutions respectively. These methods are adaptations of those used for the extraction and quantification of sulfur in marine sediments. Interference from elemental selenium is problematic, and therefore the pyrite extraction must be done after elemental Se has been removed by a sulfite extraction.

Elemental Se Analysis

1 M sodium sulfite buffered at pH 7 is used to dissolve elemental Se for analysis, and the solution is analyzed using the HG-AAS method. The technique is 91 ± 8.6 % effective at removing an elemental Se spike from a sediment matrix when the pH is adjusted to pH 7. 0.3 M sodium sulfide can also be used for extraction of elemental Se, where an alkaline pH is maintained to diminish the hazard of the formation and liberation of hydrogen sulfide gas. Interference is observed in both methods from the desorption of organic Se, and selenate and selenite, but at pH 7 only 11% of selenate and selenite is released (88% at pH 9). The problem of selenate + selenite interference can be reduced by extracting this fraction prior to sulfite extractions (see selenate-selenite extraction methods).

The chemistry of this extraction is as follows:

$$\text{Sulfite}_{(aq)} + \text{Se}_o \text{ ----> } \text{Se-Sulfite}_{(aq)}.$$

Sulfite reacts with the elemental selenium forming a seleno-sulfite complex which solublizes the selenium. Because of the difficulty of completely removing OM,

interference from organic Se cannot be completely eliminated, but after removal of selenate and selenite, interferences should be minimal.

Pyrite-Se (Inorganic Selenide) Analysis

After extraction of selenate and selenite, as well as elemental Se, pyrite-Se is removed using concentrated HCl and acidic Cr (II). This is done using a stripping apparatus in which the residual solids are placed and reacted for 25 minutes. The hydrogen selenide is trapped in a liquid nitrogen trap and after reaction warmed and passed through a Porapak-PS chromatographic column to prevent interference from hydrogen sulfide. The sample is then analyzed using the AAS method.

Once elemental Se, selenate, and selenite are removed, this method has few other interferences (OM interference should be small). It is not a quantitative method, in that it only extracts 81±12% of the pyrite-Se present (Velinsky and Cutter, 1990), but it does provide a useful estimate of pyrite and sulfide-associated Se.

4.1.2 Carbonate Selenium

Selenium has been found to co-precipitate with carbonates as an impurity, and extraction techniques developed by Goldberg and Glaubig (1988), Lipton (1991) and others have attempted to address this fraction in soils of the Central Valley. Bay sediments can contain organisms which accumulate carbonate to form exoskeletons, and Se can be trapped in these structures. In addition, Se can adsorb and then become occluded on the surface of carbonates as carbon monoxide is evolved by microbial processes and then precipitated in salt-rich basic waters. No measurements of carbonate Se have been reported in the literature for estuarine sediments and marsh soils.

The method for removing carbonate Se uses an acetic acid and sodium acetate (pH 5) extracting solution which dissolves the carbonates and liberates carbon monoxide. Soils are treated with the extracting solution for 1 to 5 hours (5 hr is the time recommended by the soil handbook of chemical analysis). A 24 hr phosphate extraction is used to remove adsorbed selenite dissolved out of the carbonate.

Analysis of the efficiency of this method has not been documented. Given the efficiency of the technique to remove carbonates, it is assumed to be very effective at carbonate removal.

4.1.3 Selenate and Selenite

Selenate and selenite are free in the interstitial water, occluded in precipitates, and adsorbed onto mineral surfaces. Extraction of selenite in particular is difficult because of its strong affinity for mineral surfaces at ambient soil pH (5-7). In salt-marsh sediments, the pH is approximately 8, and adsorption is more limited. Therefore interstitial Se is more important than in soil systems. Selenite extraction involves its replacement with another strong anion such as phosphate, which has the ability to displace selenite at anion exchange sites on mineral surfaces. Most adsorption is attributed to variable charge minerals which have high capacities for anion adsorption (Sposito, 1989). Because the charge of a variable charge minerals changes with pH, anion adsorption is extremely pH sensitive. Therefore, at low pH, below the point of zero charge of many variable charge minerals (oxides and oxyhydroxides of Fe, Mn, and Al principally), the surface charge is predominantly positive, and more able to attract anions. In estuarine marsh soils, where saline water intrusion is common, the pH of the soil typically remains around 8 at the surface, and gradually decreases with increasing depth. Due to the low redox potential, selenite is not stable in lower, marsh soil horizons, because it is assumed to transform into elemental Se over the course of several days to weeks (based on measurements of sulfite transformations in sediments by Casey and Lasaga (1987).

Selenite also adsorbs onto OM and is therefore soluble and easily extracted with a distilled water wash. Some OM adsorbs onto surfaces and can only be removed when displaced by a stronger ligand such as phosphate. In such cases the selenite (and some selenate that becomes adsorbed) is released into solution and can be measured. Organic-rich sediments such as wetland soils, are difficult to analyze for Se because the OM adsorbs Se strongly, and also interferes with HG-AAS measurements. As a result, some phosphate extracts in particular, may not be as efficient with high OM soils as with moderate or low OM soils.

Selenate/Selenite Analysis

Distilled water (DW) extraction, which is used to measure interstitial water Se, is done by a single washing with DW at 1:5 (oven-dry soil to water) ratio, followed by centrifugation and filtration of the supernatant liquid.

Phosphate extraction is used to estimate adsorbed Se. Sediments are shaken in sodium monoprotic phosphate (0.001 M at pH ~ 7.5-8.0) for 24 hr at room temperature. Analysis of the extract is done by HG-AAS. Selenite is the dominant adsorbed species; only about 4% of selenite is removed by water extraction (Tokunaga et al., 1994). More concentrated solutions of phosphate result in the removal of Si, a clear indication of mineral dissolution, and that the extraction is removing some fraction of the structural Se present.

4.2 Organic Selenium

Selenium is associated with organic fractions in sediments as covalently bound selenide (in proteins, amino acids, and humic and fulvic acids), and as adsorbed selenate and selenite. Quantification of organically bound Se (O-Se) is difficult because extraction techniques can destroy Se-C bonds and turn O-Se into inorganic selenide or selenite and selenate (Cutter, personal communication). In addition, complete removal of organic matter is impossible in sediments without complete destruction of the OM into CO_2. Therefore O-Se falls into four categories: protenaceous or amino acid-Se; humic and fulvic acid-Se; and humin or residual O-Se. Dimethylselenide and other volatile species are assumed to be insignificant in these analyses, but measurements will be done to estimate their concentration.

Because of the difficulty of extracting organic-Se from soils, several different techniques have been used to remove OM. Sodium hydroxide digestion (0.002, 0.50, or 1.0 M NaOH at 85° C) is one of the more popular techniques, but it can oxidize OM and alter the chemical composition of the extract. It is also an ineffective extractant for OM in soil because most of the OM remains in the soil after extraction. In an attempt to address this problem, Lipton (1991) developed another technique using sodium hypochlorite digestion to extract O-Se. Anderson (1963) and Lavkulich and Wiens

(1970) demonstrated that hypochlorite is a more efficient technique for OM removal than peroxide, and is less likely to dissolve amorphous oxides associated with OM (Lavkulich and Wiens, 1970). The limitation of this technique is that it is likely to remove significant amounts of elemental Se. Wetland soils typically have low redox potentials and therefore, high proportions of elemental Se. A comprehensive test of the effect of hypochlorite on elemental Se is necessary before this extraction technique can be used with wetland soils. Removal of elemental Se prior to O-Se does not solve the problem because elemental Se removal at pH 7.0 extracts significant amounts of OM.

Serious problems are associated with both methods. Transformation of O-Se to inorganic Se is predicted as a result of hydrolysis of C-Se bonds. No extraction technique has been demonstrated to completely remove OM from sediments without transforming the OM into carbon dioxide. Therefore precise quantification of Se in OM is impossible, and O-Se measures will always be qualitative and dependent on method efficiency. The advantage of the NaOH extraction is that even though it may not remove the more recalcitrant OM fractions, it does not significantly affect other fractions.

Dimethylsulfoxide extraction has been proposed by Hayes (1994), but removal of DMSO from the extract is difficult, and makes further analysis of the mineral surfaces problematic. An additional problem with the DMSO is the introduction of a sulfur compound which can make Se analysis more difficult. Pyrophosphate is often used, but it does not remove a sufficient amount of the OM, and requires a more strongly basic solution.

4.2.1 OM Extraction

Organic matter extraction is done using sodium hydroxide (0.02 M) digestion at 85° C for 2 h with intermittent shaking. Extracts are diluted, acidified, and digested with persulfate to remove OM and prevent interference with Se measurements. Speciation of Se associated with OM is impossible because even at pH 8.0 (Lipton, 1991), selenite will adsorb strongly onto the OM, and low percentages of selenite spikes of these samples are recovered after acidification and removal of insoluble OM (precipitation of large OM molecules occurs at low pH).

4.3 Sequential Extractions

Sequential extractions were run on 10.00 g (or 2.0 g when insufficient sample was available) of dewatered-homogenized soil. Soils and sediments were dewatered by centrifuging 300 g of wet soil at 10,000 rpm for 30 min, and decanting the supernatant liquid for analysis as the interstitial soil water (Ix). Soils were divided into two subsamples: (1) was analyzed for moisture content and then used to determine the total selenium using a total acid digest (TAD) method (see Appendix A); (2) was used for sequential extraction, normalizing to the oven-dry (OD) mass using the theta value (soil moisture content). The series of extractions followed the sequence: distilled water (Dx), phosphate (Px), sodium hydroxide (OHx), acetate (Ax) and phosphate (AxPx), sulfite (Sx), and pyrite (PYRx) (see Appendix A for detailed procedures to all extractions). Dx through Sx extracts were shaken on a reciprocating shaker for the required time, and then centrifuged for 30 min prior to filtration using a Millipore 0.45 μm filter pad and syringe cartridge apparatus. The supernatant solution was refrigerated prior to analysis. Selenate and selenite analysis was done using an HCl boil (see Appendix A). Selenite analysis requires no treatment prior to hydride generation atomic absorption spectrometer (HGAAS) analysis, and organic associated Se is determined by addition of sodium persulfate solution (see Appendix A).

4.3.1 Solid/Solution Ratios

Dx, Px, OHx, and Sx extractions were evaluated to determine the efficiency of extraction and the optimum dilution to facilitate analysis of the Se solution extracted. The Dx extraction was tested at solid/solution ratios of 1:10, 1:5, 1:3, and 1:2 using two MRP samples (3-1 and 3-2) with low Dx values. Based on these tests, 1:1 and 1:2 extraction ratios were selected, but where necessary because of low sample volume, 1:3 ratios were used. The dilution tests with these two sediments showed that even at higher ratios of 1:1 and 1:2, Dx readings were very close to the HG-AAS detection limit (sample MRP 3-1, at dilution ratio 1:1 [Se] = 0.82, 1:2 = 0.70, 1:5 = 0.59; MRP 3-2, 1:1 = 0.76, 1:2 = 0.57, 1:5 = 0.50 ppb). The non-linearity of the lower ratio readings (1:2 and lower) to the higher ratio readings (1:1) is caused by two factors: 1) greater dilution desorbs more Se; 2) measurements at higher ratios are at the HG-AAS method's detection limit, artificially raising the values. Due to problems with the HG-

AAS detection limit at lower ratios, 1:1 and 1:2 ratios were chosen, although the values measured for these two sediments are at the limit of quantification (most sediments and soils had higher concentrations of Dx-Se).

The Px extraction was tested at solid/solution ratios of 1:10, 1:5, and 1:1. Based on the results of extraction ratios, 1:5 was chosen for use because readings fell in a good range for the HG-AAS method, and extraction of Se by phosphate was optimized. With the two soils, MRP 3-1 and 3-2, the 1:5 and 1:10 ratios resulted in values of 2.90 and 1.90 ppb for MRP 3-1, and 2.60 and 1.81 ppb for MRP 3-2. The 1:10 ratio was not chosen because these readings are closer to the quantification limit of the HG-AAS method, and are therefore more sensitive to errors in measurement. 1:1 ratio extractions were also tested, but equilibrium concentrations were not significantly different from 1:5 ratio extractions (for MRP 3-2 1:1 = 2.62 versus 2.60 ppb for 1:5). The reason 1:1 and 1:5 extractions have similar Se concentrations is due to the dilution effect on the solutions and the increased desorption possible at greater dilutions. The implication is that desorption occurs up to some equilibrium concentration when solid/solution ratios are high. Once the solid/solution ratio decreases below a certain value (it appears to be close to 1:5 in this case), desorption has reached a maximum, and the additional desorption seen with lower ratios (1:10 and 1:20) releases less and less additional adsorbate (Sposito, 1989).

OHx extractions were run at a solid/solution ratio of 1:10, and solutions were then diluted five-fold for analysis to prevent OM interference (foaming in the hydride generation reactor). Foaming causes the baseline on the HG-AAS system to fluctuate too greatly, resulting in falsely high or low readings. Dilution reduces the concentration of OM in the solutions, diminishing the amount of foaming. Se concentrations in the dilutions were still high enough for accurate quantification. When solution concentrations fall to near or below the quantification limit, extraction solutions are diluted at lower ratios.

4.3.2 Distilled Water Extraction

Dx was done using distilled water from a Barnstead Fistreem II apparatus. Samples were shaken for 1 hr on a reciprocating shaker, and the supernatant was extracted for analysis with no further treatment.

4.3.3 Phosphate Extraction

Px extractions were done using 0.001 M Na_2PO_4 because previous studies have shown that higher concentrations of phosphate result in increased dissolution of silica, implying that occluded or precipitated Se is being released (Tokunaga et al., 1994) . To maximize desorption and achieve equilibrium, samples were shaken for 24 hr, and prepared for analysis with no further treatment.

4.3.4 Sodium Hydroxide Extraction

OHx extraction was tested to determine the best solution concentration for extraction. Solutions with molarities varying from 0.02 to 1.0 M were tested. Field samples were analyzed using a solution of 0.02 M NaOH, to prevent oxidation and dissolution problems associated with high ionic strength and pH (Hayes, 1994), and because OHx-Se concentrations did not increase significantly with stronger extracting solutions (MRP 3-2: 0.02 M = 86.3 versus 0.5 M = 87.0 and 1.0 M = 80 ppb; MRP 7-3: 0.02 M = 183.0 versus 0.5 M = 197.7 and 1.0 M = 200.1 ppb). Higher concentrations of OM in the more basic solutions result in more interference during analysis and increased variability in the measurements, making accurate quantification difficult.

The method calls for samples to be heated in an 85 °C bath for 2 hr with 5 min of shaking every 30 min. Extracts were diluted after filtration (1:5), to prevent OM interference in analysis.

The hypochlorite treatment, which is also used to remove OM from soils, requires addition of 4-5% NaOCl to soil in an 85°C bath. This method was used to determine the concentration of Se associated with OM, but elemental Se spikes showed that significant oxidation of other reduced Se fractions occurs, making the NaOCl extraction a less selective method than desired (see Section 4.4).

4.3.5 Acetate Extraction

The Ax extraction procedure was tested for efficiency of carbonate associated Se extraction. Ax extractions were done at a solid/solution ratio of 1:10 followed by a Px extraction of 1:20. These ratios were maintained for field sample analysis. Ax and AxPx extractions were done using 1.0 M sodium acetate (pH adjusted to pH 5.0 with glacial acetic acid) followed by a 0.001 M Na_2PO_4 solution. Each extraction solution was measured separately for total Se and selenite after filtration, with no additional preparation.

4.3.6 Sulfite Extraction

Sx extractions were done using 1.0 M Na_2SO_3 (pH adjusted to 7.0 using concentrated HCl), solid/solution ratios were maintained at 1:4, and extraction efficiency was tested by varying the sonication time of the slurry. Samples were initially sonicated for 1 min using an ultrasonic probe set to 2 kHz. Half of the samples were then sonicated for 60 min (method used on field samples), and the rest for 6 hr, in a sonicating bath. Sx was tested using both ground soil and residual soil from sequential extractions. After filtration, 5 mL of supernatant was placed in a 30 mL beaker. One mL of concentrated nitric acid was added to the beaker, and the solution was covered with a watch glass prior to heating on a 95°C hotplate. After refluxing for 1 hr, the beaker was uncovered and the solution evaporated to near dryness before 1 mL of distilled water was added, and the solution evaporated again. At near dryness, 1 mL of distilled water was added to dissolve any precipitates that may have formed, and the solution was diluted to 25 mL, after addition of 0.5 mL of urea, in a volumetric flask. The final solution was analyzed for total selenium.

4.3.7 Pyrite Extraction

The pyrite extraction is run sequentially after the sulfite extraction to prevent interference from elemental Se. Approximately 0.1 g of rinsed, dried, and ground sediment is added to the LN_2 trapping method stripper vessel with a magnetic stirring bar and 15 mL of distilled water (see Section 4.2). The stripper is assembled and purged for 2 min. After purging the trap is immersed in LN_2, and 5 mL of concentrated HCl and 10 mL of Cr (II) solution are added to the stripper. The Cr (II)

solution is made by adding elemental Zn pellets to a 1 M $CrCl_3$. After 30 min, the Cr(III) in the solution has been reduced to Cr(II) and the solution is ready for use. Solutions must be prepared daily.

After 25 min the reaction is complete, the trap is removed from the LN_2, and the trapped H_2Se and H_2S pass through the Porapak PS (50/80 mesh) column and into the AAS. The elution time for H_2Se should be 1.8 min, if the flow rate is set to 75 mL min^{-1} (Velinsky and Cutter, 1990).

4.4 Sequential Extraction Testing

4.4.1 Experimental Design

Given that many of the potential problems associated with different extraction techniques are unknown, it is essential that some of the suspected interferences be tested. Major interferences for Se analysis in anoxic sediments and soils stem from high OM concentrations. Determining which techniques allow for the analysis of Se associated with OM and do not remove other Se species such as pyrite-Se and elemental-Se is important to the success of this project. Additional problems of analysis exist, but OM interferences appear to be most significant at this point.

Experimentation on extraction efficiency and compatibility:

1. Phosphate extraction efficiency in high OM sediments and soils:
    a. Extracting selenite/selenate spikes from sediments.
    b. Extracting seleno-amino acids from sediments.

2. Testing NaOH extraction of O-Se.
    a. Extraction of O-Se from sediments with spikes of amino acid-, elemental- and pyrite-Se.
    b. Determining work-up procedure for extracts that eliminates Se and HG-AAS measurement interference by OM.
3. Testing acetate extraction efficiency and impact on achavalite solubility.

a. Measuring efficiency of the acetate extraction on the removal of carbonate-Se spiked sediments.

4. Testing sulfite extraction efficiency and impact on elemental- and O-Se removal.
   a. Measuring extraction efficiency on elemental-Se spiked soils and sediments.
   b. Measuring the effects of different pre-treatments (NaOCl and NaOH) on extractable elemental Se.
5. Testing Cr extraction efficiency and impact of pre-treatments on its efficacy.
   a. Testing effects of different OM extraction procedures on residual pyrite-Se concentrations (NaOCl and NaOH).
   b. Testing the effects of acetate extraction on pyrite-Se concentrations extracted by Cr stripping.

4.4.2 Selenate/Selenite and Seleno-Methionine Spikes

Sediments from the Martinez Regional Park were spiked with selenate, selenite, and seleno-methionine from standard, calibrated solutions. Spike solutions were added to a bulk mass of wet sediments, and shaken on a reciprocating shaker for 24 hr. The spiked sediments were then centrifuged at 10,000 rpm for 30 min, and the supernatant was filtered for analysis. Sequential extractions were then run on the sediments following the procedures outlined in Section 4.3 (pyrite extractions have not been completed on these sediments). 5.015 μg of selenium, as selenate and selenite, and seleno-methionine were added to three sediment layers with varying organic carbon (OC) concentrations and elemental compositions (Table 4.1). Total Se concentrations for these three sites ranged from 326 ppb for MRP 1-1 to 640 ppb for MRP 7-2.

Table 4.1 Elemental composition of soils used for selenate/selenite spike study.

Soil	% OC	Al ppm	Fe ppm	Ca ppm	Mg ppm	K ppm	Mn ppm
MRP 1-1	0.395	23810	24852	6194	6170	3140	597
MRP 6-2	0.514	23629	33945	8078	6297	3736	493
MRP 7-2	1.134	50764	37979	5090	11923	7930	279

These sediments are of varying texture ranging from a predominantly silt and fine sand layer (MRP 1-1) to a predominantly clay and fine silt layer (MRP 7-2). Based on initial visual assessment of texture, finer textured sediments tend to have higher Se concentrations. For example, the Martinez Regional Park, despite being in close proximity to the outfall of the Shell Refinery, had lower total Se concentrations than the finer-grained sediments of Southampton Bay.

To test the efficiency of the sequential extraction methods on different forms of Se, soils were spiked with various dissolved Se forms. Although most of the Se associated with OM is assumed to be adsorbed inorganic Se (Zawislanski and Zavarin, in press; Cutter, 1985) it is important to approximate the role of organic Se species. Seleno-methionine is assumed to be the most common form of organic Se, and was therefore chosen for analysis. Cutter (personal communication) claims that amino acid Se in the form of seleno-methionine, is quite labile, and susceptible to hydrolysis. Surface catalyzed hydrolysis could also be responsible for the dissociation. We were unable to verify this fact, but further analysis is ongoing.

4.4.3 Extraction Efficiency for Selenate/Selenite

Extraction efficiency for selenate and selenite varied with sediment properties, but no consistent trends were observed. As can be seen in Table 4.2, the percent recovery for each of the soils was very similar. MRP 6-2 had the highest extraction efficiency, but differences among the sediments were not statistically significant. In all three sediments the bulk of the spike was removed by the Dx and Px extractions, with both extractions accounting for approximately 70% of the total Se added. OHx accounted for the next largest pool of spike Se, having a value that ranged from 10.0 to 17.9%. Only in the sediment with higher OM concentrations was the Sx extracted Se contribution a large percentage of the spike Se pool (9.1% for MRP 7-2 versus 0.8 and 0.3% for MRP 1-1 and 6-2). Therefore the extraction sequence appears to remove selenite and selenate as desired, in the Dx, Px, and OHx extractions. Figure 4.1 shows the percent-recovery of the spike in each extraction as a fraction of the whole.

The fact that a high concentration of spiked Se is found in the OHx extraction supports the assertion that selenite adsorbs onto OM, forming strong linkages which phosphate appears to have difficulty breaking. The close association between OM and

Table 4.2 Selenium fraction recovery for selenate/selenite and selenomethionine spikes.

Sample Name:	MRP 1-1S	% Extr.	% Spike Extr.	Blank	% Blank Extr.	MRP 6-2S	% Extr.	% Spike Extr.	Blank	% Blank Extr.	MRP 7-2S	% Extr.	% Spike Extr.	Blank	% Blank Extr.
	ppb			ppb		ppb			ppb		ppb			ppb	
SeIV/VI spike															
Dx Se:	1099.7	29.7	32.2	11.0		1002.2	29.7	36.7	18.0	2.6	1385.6	32.0	36.9	21.5	3.4
Px Se:	1298.4	35.0	37.6	27.6	8.4	1021.9	30.2	35.8	62.7	8.9	1147.5	26.5	29.7	51.1	8.0
Nx Se	687.1	18.5	17.9	82.8	25.4	624.6	18.5	17.6	152.1	21.7	604.5	13.9	10.9	202.9	31.7
Ax + AxPx Se:	40.6	1.1	1.2	0.0	0.0	47.3	1.4	1.8	0.0	0.0	35.7	0.8	1.0	0.0	0.0
Sx Se:	152.6	4.1	0.8	123.9	38.0	191.1	5.7	0.3	184.2	26.3	407.3	9.4	9.1	71.7	11.2
Residual	427.9	11.5	10.3	81.0	24.8	492.7	14.6	7.8	284.7	40.6	756.0	17.4	12.5	293.4	45.8
Total Se:	3706.3	100.0	100.0	326.3	100.0	3379.8	100.0	100.0	701.7	100.0	4336.6	100.0	100.0	640.6	100.0
% Extracted			**89.7**		**75.2**			**92.2**		**59.5**			**87.5**		**54.3**
SeMeth spike															
Dx Se:	271.1	8.7	9.2	12.5	3.4	430.7	10.1	11.6	18.0	2.6	955.6	20.1	22.7	21.5	3.4
Px Se:	104.3	3.3	2.7	27.5	8.4	329.9	7.7	7.5	62.7	8.9	614.6	12.9	13.7	51.1	8.0
Nx Se	435.3	13.9	12.6	82.8	25.4	1424.7	33.4	35.7	152.1	21.7	699.7	14.7	12.1	202.9	31.7
Ax + AxPx Se:	17.7	0.6	0.6	0.0	0.0	136.1	3.2	3.8	0.0	0.0	313.3	6.6	7.6	0.0	0.0
Sx Se:	236.7	7.6	4.0	123.9	38.0	401.0	9.4	6.1	184.2	26.3	441.5	9.3	9.0	71.7	11.2
Residual Se:	2063.9	66.0	70.8	79.6	24.8	1543.3	36.2	35.3	284.7	40.6	1722.9	36.3	34.8	293.4	45.8
Total Se:	3129.0	100.0	100.0	326.3	100.0	4265.7	100.0	100.0	701.7	100.0	4747.6	100.0	100.0	640.6	100.0
% Extracted			**29.2**		**75.2**			**64.7**		**59.5**			**65.2**		**54.3**

Blank = unspiked sample

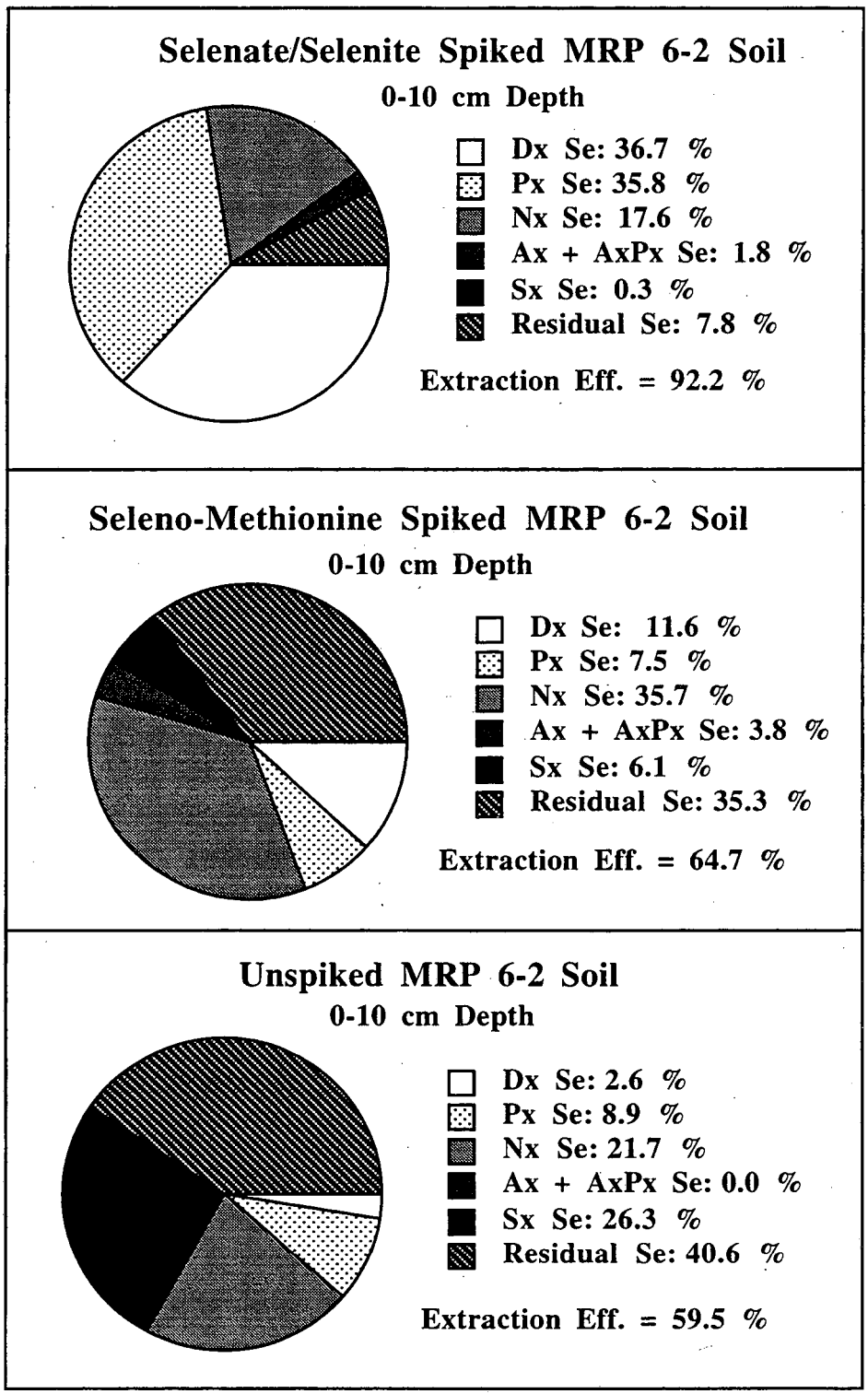

Figure 4.1 Extraction efficiency of selenite, selenate, and seleno-methionine in soil MRP 6-2.

Se may also account for a significant part of the unrecovered portion of the spike. The 7.8 to 12.5% of the Se that is not extracted can be accounted for by Se adsorption onto insoluble OM, and/or close association with OM that is not completely digested in sample preparation for Se analysis. Binding of Se to undigested OM in the Se analysis tubes prevents complete reduction of selenite to hydrogen selenide, diminishing the total amount of Se detected in a Px or OHx sample (Px removes significant concentrations of OM in OM-rich sediments). The operational difficulties of analyzing field samples that have a variety of interferences, makes precise distinction of Se fractions (selenate/selenite, OM-associated Se, adsorbed-Se, carbonate associated-Se, elemental Se, and pyrite-Se) difficult.

Due to OM interferences with analysis, exact measurement of selenite spike efficiency alone was very difficult. Digestion was always required to remove OM interferences, making selenate and selenite indistinguishable. In addition, Se associated with soluble OM in both Dx and Px was also impossible to determine because OM interfered with the measurement, and digestion was necessary. Acidification, which is part of the acid boil, causes the OM to precipitate, and at low pH up to 50 % of the selenite in solution will adsorb onto the OM (Lipton, 1991).

4.4.4 Extraction Efficiency for Seleno-Methionine

Extraction of seleno-methionine was not as efficient as selenate/selenite, due to irreversible adsorption and problems measuring OM-associated Se. MRP 1-1 efficiency was extremely low due to analysis error, but in MRP 6-2 and 7-2, where analysis problems were not a factor, spike recoveries were still well below our expectation of 90 % (64.7 and 65.2%, respectively). One reason for the low seleno-meth recovery is the difficulty of measuring OM-associated Se. Another is the tendency of seleno-meth to adhere to glassware and plastic. The poor extraction efficiency of seleno-meth indicates that perhaps OHx solutions need to be of higher concentration (0.5 and 1.0 M versus 0.02 M NaOH). One problem is that without using strong oxidizing agents, which will target all reduced Se not just OM associated-Se, extraction of compounds such as seleno-meth will be very difficult (Lipton, 1991; Geering et al., 1968). Repeated extractions may improve the extraction of seleno-meth, but dilution of extractions will make quantification difficult. Two OHx extractions may be sufficient to improve selemethionine removal without significantly affecting

quantification of the solution concentration. Addressing the problem of adsorption onto glass and plastic surfaces is more difficult. Both problems are currently being studied.

Only in sample 6-2 did the bulk of the spike Se come out in the OHx. In MRP 1-1 the spike Se appears not to be present, but this is most likely due to OM interference in the analysis of Se in the residue of the initial spike solution. In sample 7-2 the bulk of the Se was removed by all extractions in significant concentrations.

It is difficult to determine whether OM content had a significant effect on seleno-meth adsorption by the sediments. The concentration difference in OM between MRP 6-2 and 7-2, for example, is only 0.620 % of the total mass of the sample. However, 6.20 mg of OM can coat a significant portion of the surface of 1 g of sediment, making the impact of this small amount of OM significant in the adsorption of soluble species. The higher concentration of Al and Fe in sample 7-2 also could contribute to the adsorption of seleno-meth, but we would expect to see more seleno-meth desorbed by the Px.

Summarizing the spike recovery data of both selenite/selenate and seleno-meth, we find that the extraction techniques work for inorganic Se species, but are problematic for seleno-meth. Because most OM-associated Se has been shown to be in forms other than seleno-meth (Cutter, 1985), and because seleno-meth has a high affinity for mineral surfaces as do all amino acids (Geering et al., 1968), seleno-meth extractability may not predict the ease of removal of most OM-Se species. The problem of quantifying seleno-meth concentrations does emphasize the need for new methods to determine OM-associated Se, and we are currently exploring other methods of selenium analysis which are not as sensitive to OM interference, e.g. co-precipitation of Se in lanthanum hydroxide, Tao and Hansen (1994).

4.4.5 Extraction Efficiency for Elemental Se

Sample MRP 7-2 was spiked with black and red elemental Se to test the extractability of Se using Sx, and to determine whether the hypochlorite extraction would remove a significant portion of the elemental Se as well as OM Se and other reduced Se species. Red Se was used to optimize the efficiency of the Sx extract, and black to determine the efficiency of hypochlorite extraction. Lipton (1991) used a

hypochlorite extract to analyze for residual OM associated-Se assuming that elemental Se concentrations were low. Although this may be true for California's Central Valley sediments and soils, sediments and soils in San Francisco Bay wetlands and mudflats have significantly lower redox potentials and are therefore predicted to have much higher concentrations of elemental Se. This means that hypochlorite extraction could result in significant loss of the elemental Se pool, and convolute interpretation of sequential extraction data.

4.4.6 Sediment Spiking with Elemental Se

MRP 7-2 was spiked with black and MRP 7-3 was spiked with red elemental Se using serial dilutions. The sediment was ground into a fine powder on a ball mill after elemental Se addition. Grinding was continued for five 20 minute cycles during each serial dilution, to ensure that sample homogeneity was achieved.

4.4.7 Black Elemental Se Extractions

Black elemental Se spike data shows that the extraction efficiency using sulfite is only about 50-60% (Table 4.3). Velinsky and Cutter (1990) reported that the efficiency of the method using red Se is 91 ± 8.6 % using a spike that was over three orders of magnitude higher than that observed in the soils and sediments. Therefore, lower extraction efficiency is expected with a spike that is less than two orders of magnitude higher. The spike used in this study to test the extraction efficiency, is more realistic in testing extraction efficiency for low level samples. Nonetheless, extraction efficiencies below 70 %, even with a high level spike, represent extremely low recoveries. Even hypochlorite treatment did not appear to improve the extractability of elemental Se, despite the fact that hypochlorite did remove significant amounts of elemental Se which the OHx extraction did not. The high concentration of elemental Se removed by hypochlorite indicates that hypochlorite is not an appropriate agent for selective extraction of OM or elemental Se. Why Sx resulted in low elemental Se extraction efficiency was unclear and further improvements of the extraction method using longer sonication times and longer equilibration times are needed (see below).

Use of the Sx and OHx extractions appears to give better extraction efficiency for low-level Se than the hypochlorite extraction method. One reason why spike recoveries

are better than low level recoveries may be that the hypochlorite interferes with analysis on the AA, diminishing the value observed in the low level analysis, but not significantly affecting the spiked sample. There is greater variance in the OHx method due to interference from the OM extracted by the NaOH. In the hypochlorite extraction, the OM is removed because it is destroyed by the bleach, whereas in the OHx OM is removed intact, and is therefore in a more intractable form. As a result, Se analysis of OHx is problematic, and has a higher variance.

Table 4.3 Black elemental Se extraction efficiency.

Sample	Total [Se] ppm	NaOCl-Se ppm	OHx-Se ppm	Sx-Se ppm	% Extr.
MRP 7-2 spiked	36.23	20.13±3.7		1.93±0.27	60.9±9.7
MRP 7-2	0.781	0.21±0.01		0.19±0.06	50.7±8.1
MRP 7-2 spiked	36.23		0.20±0.03	20.1±0.37	56.0±1.1
MRP 7-2	0.781		0.15±0.04	0.46±0.04	78.1±10.3

4.4.8 Red Elemental Se Extraction

Elemental Se characterized in Kesterson marsh soils appear to bear a stronger structural resemblance to red Se than black or gray (T.K. Tokunaga, personal communication). Velinsky and Cutter (1990) tested the Sx method on red Se, and since different structural forms of elements can have drastically different solubilities in certain solvents, Sx was also tested in this study using red Se.

Sediment sample MRP 7-2 was ground on a ball mill and 25.00 g were spiked with 1.794 mg of red Se and ball-milled again. The mixture was then subjected to 1 hr and 6 hr of sonication in a sonicating bath (at 65 °C), after 1 min of sonic disruption at 2 kHz from an ultrasonicating probe. The rest of the reaction cleanup was identical to that done by Velinsky and Cutter (1990).

Results demonstrated that the extraction efficiency of red elemental Se extraction is comparable to that reported by Velinsky and Cutter (1990). The efficiency measured in the present study was between 72 and 75 %, whereas Velinsky and Cutter (1990) reported 91 ± 8.6 % using a higher concentration spike (over 10 times higher than the

spike used in this study). Higher concentration spikes improve recovery because adsorption and other interfering processes are a minor factor when compared to the high concentration of the spike. Sonication time appeared to have little effect on the extraction efficiency, as long as equilibration time was greater than 5 hr after sonication, and the soil was washed with one 1:1 aliquot of sulfite solution.

Using this method for analysis of the blank sample (MRP 7-2), demonstrated that as much as 95 % of the total Se in the sample can be extracted by the Sx method. This fact implies that Sx is efficient at removing residual OM-associated Se as well as elemental and other non-extractable forms. In addition, PYR-Se would be presumed to be quite low, accounting for no more than 0 to 5 % of the total Se. Another factor revealed by these two tests, is that this method is better at removing red Se than black Se.

4.4.9 Carbonate-Se Spikes

Extraction efficiency of carbonate-associated-Se was tested by addition of calcium carbonate laced with Se from a synthesis based on work by Doner and Zavarin (personal communication). Sediments from Martinez Regional Park were used to test the extraction efficiency using known additions of carbonate associated-Se. No other publications known to this researcher have reported testing the efficiency of the carbonate-Se extraction procedure using buffered sodium acetate. Lipton (1991) reports optimizing extraction conditions to remove adsorbed Se after the extraction, and increasing contact time for the acetate to dissolve the carbonate. In neither case did Lipton (1991) spike the sediments to test the extraction efficiency, making comparison of the present results to his or other studies impossible.

A 1.0 M calcium chloride solution was prepared with 100 ppm Se (as selenite and selenate) and pH adjusted to 9.0. CO_2 was then bubbled through the solution for 12 h, and the resulting precipitate was vacuum filtered and washed with saturated calcium carbonate prior to drying in a 40°C oven. Two sediments, MRP 2-2 and MRP 7-2, were spiked with 0.130 ppm carbonate-Se kg^{-1} sediment.

The sequential extraction removes a carbonate-Se spike at an efficiency of 95 to 105 %. A majority of the spike is removed by Dx, Px, OHx, and Sx. The Ax and AxPx

extractions only account for between 20 to 30 % of the total spike. Se extracted by the Dx, Px, and OHx extractions is soluble Se on the surface of the carbonate, or part of the synthetic carbonate that is easily dissolved (potentially residual sodium carbonate associated Se that was not removed by the washes). Table 4.4 summarizes the extraction efficiency of each method for the two sediments.

Table 4.4 Carbonate-Se extraction efficiency in two Martinez Regional Park sediments.

Treatment	MRP 2-2 Soil Se ppb	MRP 2-2 Spike Se ppb	MRP 2-2 % of Spike	MRP 7-2 Soil Se ppb	MRP 7-2 Spike Se ppb	MRP 7-2 % of Spike
Dx	20.24	4.2	3.1	38.9	25.0	20.1
Px	61.50	15.6	11.7	98.2	16.3	13.1
OHx	96.84	30.9	23.1	162.4	31.3	25.2
AxPx	53.87	39.0	29.2	44.8	24.5	19.6
Sx	161.49	39.5	29.5	206.2	33.4	26.9
Σ Extr.	394.0	129.2	96.6	550.6	130.5	104.9
Total Se	523.0	133.8	100.0	781.2	124.5	100.0

The spike-Se removed by the Sx extraction is surprisingly high, and implies that the acetate method does not completely extract carbonate-Se. Due to the fact that carbonate-Se in the sediments of the mudflats and the soils of the marshes accounts for between 0 and 5 % of the total Se, there was not much reason to pursue improving the efficiency of this method. In further testing, Ax and AxPx will not be conducted, and carbonate-Se will be assumed to come out predominantly in the Sx. Soils and sediments will be tested for effervescence upon addition of concentrated HCl to oven-dry soil. If samples effervesce perceptibly, then Ax and AxPx extractions will be run to estimate the carbonate-Se.

4.4.10 Metal Removal by Sequential Extraction

Metals in interstitial waters and extracted by the different sequential extractions were measured to determine the extent of mineral dissolution and the efficiency of individual extractions. Particular extractions are designed to remove individual mineral fractions or metals, such as the acetate extract which dissolves calcium and magnesium carbonates (Goldberg and Glaubig, 1988). In other cases, metal dissolution is an

undesired side reaction which disturbs the equilibrium in the soil and results in the release of Se species not targeted by the method. For example, phosphate extraction is meant to remove adsorbed species, but high phosphate concentrations can lead to the release of silica which is an indicator of mineral dissolution. Mineral dissolution is a problem with Px, because it means that structural Se species, or irreversibly bound Se will be extracted, resulting in an over-estimation of adsorbed Se.

OHx, Sx, and PYRx extractions result in the dissolution of a broad range of mineral species. OHx dissolves species that are associated with OM in soils such as amorphous oxides. Sx has the ability to dissolve a variety of metal oxides, although the high pH of the extract (pH 7) makes complete dissolution of metals such as Fe and Al unlikely. Pyrite extraction destroys the entire mineral fraction through acidification and reduction. After Cr (III) and concentrated HCl have dissolved the mineral soil, very few components remain. The problem with the pyrite extraction in sediments from the San Joaquin Valley, is that the mineral fraction may have significant amounts of Se in forms other than pyrite-Se. Therefore, there may be a potential source of error in this analysis that is unique to the San Francisco Bay sediments.

ICP analysis of the different extraction solutions reveals which metals are removed by each procedure (Table 4.5). Dx metals represent "free" metal ions in the soil/sediment solution. These metals are often coordinated with dissolved organic matter (DOM) and can therefore be in higher concentrations than their solubility would allow at the soil/sediment pH. Px metals represent metals displaced with anions which are replaced by phosphate on anion exchange sites. Px also displaces OM which contains coordinated metals. OHx removes many of the metals associated with OM such as humic acids, which do not dissolve at typical soil/sediment pH. Fe and Al tend to dominate the OM. Ax extracts remove predominantly salts, such as carbonates. The high concentration of Ca and Mg in Ax indicate that the carbonate extract is quite efficient. In samples other than MRP 7-2, the Ca removed by Ax was as high as 34 % of the total Ca (22 % in MRP 7-2). AxPx is principally a wash and therefore only removes the residual of the previous extraction. Sx is a strong extraction that removes many of the mono- and divalent cations. Based on the low concentrations of Fe and Al in the Sx it does not appear to remove oxyhydroxides or phyllosilicate minerals. The high proportion of Mn removed is due to the fact that the sample shown has low Mn concentration compared to other sediment samples.

Table 4.5 Metal concentrations in extract solutions for sample MRP 7-2.

Extract	Al ppm	Ca ppm	Fe ppm	K ppm	Mg ppm	Mn ppm
Dx	441 (1.0)	95 (2.2)	360 (1.1)	90 (1.3)	97 (1.0)	0
Px	1128 (2.6)	56 (1.3)	934 (2.9)	191 (2.8)	245 (2.4)	0
OHx	2712 (6.2)	182 (4.2)	2276 (7.0)	430 (6.3)	571 (5.6)	0
Ax	0	960 (22.0)	422 (1.3)	704 (10.4)	953 (9.3)	32 (13.2)
AxPx	7 (0.02)	0	64 (0.2)	69 (1.0)	0	0
Sx	0	467 (10.7)	91 (0.3)	1256 (18.5)	1234 (12.1)	127 (52.6)

() = the percentage of the element in the whole soil/sediment.

Silica concentrations show how extensive phyllosilicate dissolution is in each of the extraction solutions. Silica analysis is now in progress. Ax, Px, and Sx are the three extracting solutions which have the greatest potential for dissolving mineral surfaces, because of the anions being introduced into the system. OHx may also have great potential for dissolving minerals associated with OM, as mentioned above, but due to the extracting solution's low ionic strength, that problem is minimized (as is the case for Px as well). Desilication of soils is typically associated with acid extractions, and therefore, acetate (at pH 5) may prove to be the most destructive solution when silica concentrations are measured.

5 Analytical Procedures

The primary goal of the analytical work was to set up a reliable method for the analysis of parts-per-trillion-level selenium in an aqueous matrix. This method was to address two problems: achieve a low limit of detection for selenium and be able to cope with a sea water or brackish matrix. Both of these objectives were achieved and will be addressed in this section.

5.1 History

Low level selenium analysis has been performed by Cutter (1978). His system involved reacting selenium with sodium borohydride in a closed vessel and analyzing the evolved hydrogen selenide by flame atomic absorption spectrometry (FLAA). The hydrogen selenide is stripped from water using helium as a carrier. The helium then travels through two traps. The first trap, a U-Tube, is in isopropanol with dry ice. This trap will remove all of the water, hydrochloric acid, and unreacted sodium borohydride traveling with the helium stream. This trap is not cold enough to trap any of the hydrogen selenide. The second trap, a U-Tube with silanized glass wool, is in liquid nitrogen. This will trap everything that was not captured in the first trap, except for helium. Cutter's method has a detection limit of 5.0 ng/L for a 100 mL sample.

Steps were taken to duplicate Cutter's method. A 5 pptr detection limit should be low enough to detect selenium in all areas of the ecological system. U-Tube traps and stripping chamber were custom-made with an injection port for the sodium borohydride. The injection port was 4 cm above the stripper (where the helium is introduced to the water). 1/4" Teflon tubing connected the U-Tubes to each other using 1/4" Swagelok fittings. The stripping chamber was connected to the first trap with a glass joint that was attached to the trap with a 1/4" Swagelok fitting. A sketch is included in Figure 5.1.

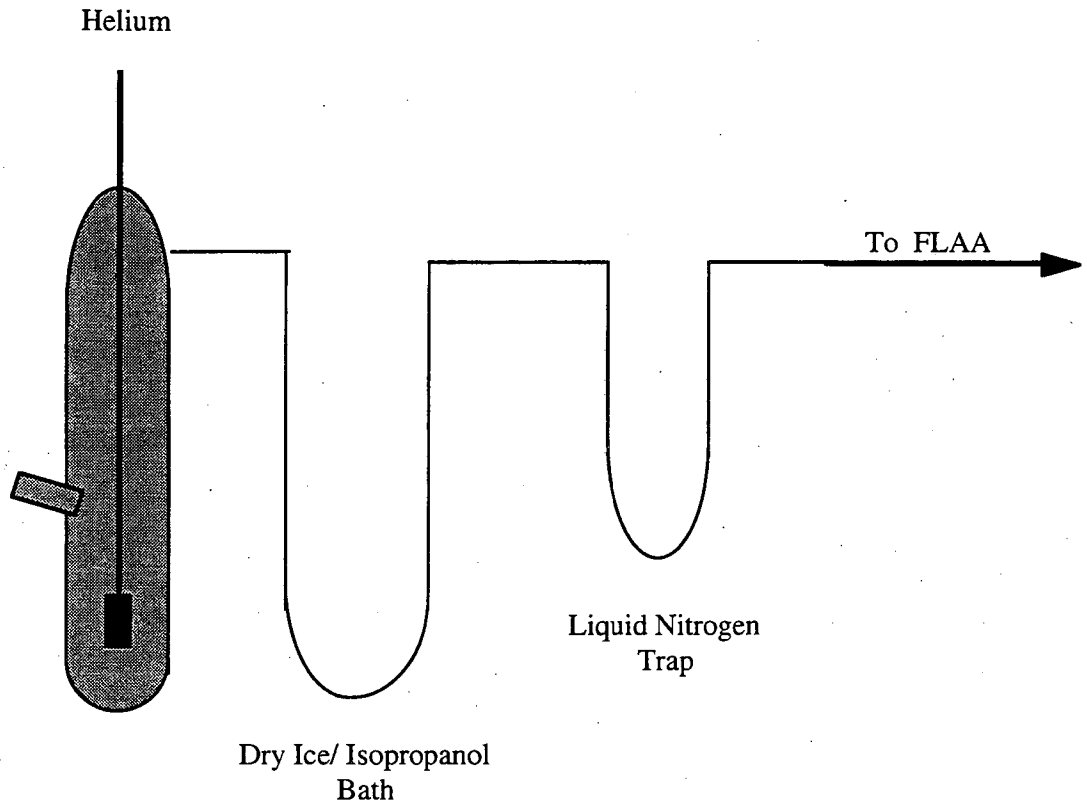

Figure 5.1 Schematic of apparatus for hydrogen selenide trapping (from Cutter, 1988)

5.2 Method Development

Because of several subtleties of this method, some difficulties were encountered, primarily affecting sensitivity. For instance, the silanization of glass wool for the liquid nitrogen trap is a work-intensive procedure, which on occasion produces inconsistent results (this procedure involves refluxing small amounts of glass wool in a 4% (v/v) dichlorodimethylsilane (DCDMS) solution in benzene at 110°C for 1 hr). This process is very tedious and exposes the analyst to chemicals that are toxic and known carcinogens. Commercially-produced silanized glass wool was found to save prep time for the wool and reduced the analyst's exposure to toxic chemicals.

Due to the large number of connections (up to twelve), there is a high potential for gas leaks. The entire system needs to be leak-tested prior to use. Of greatest significance is the need to replace the injection port septum at least every 15 samples.

The system needs to be tight to prevent the entry of carbon dioxide from the air. Carbon dioxide will overshadow the hydrogen selenide peak. To prevent this problem the sample is purged for 3 minutes with helium to ensure all the carbon dioxide is flushed from the system. The sodium borohydride solution must be purged constantly with helium to ensure that carbon dioxide does not react with sodium hydroxide in the borohydride solution. The sodium hydroxide is purchased as a prepared solution at a concentration of 30%. This prevents carbon dioxide from reacting with sodium hydroxide pellets before making the sodium borohydride solution.

There are three major causes for the loss of sensitivity. The first is the incomplete silanization of glassware, leading to potential adsorption of hydrogen selenide. Improved recovery is observed after complete silanization of the system. Unfortunately, silanized glassware is not available commercially. Glassware must be soaked in a 5% (v/v) DCDMS in toluene solution for a minimum of 4 hours prior to use. After treatment, glassware is rinsed with toluene and methanol and air dried. This treatment tends to last for a few hundred analyses. When water no longer beads up on the glassware, the treatment needs to be repeated. A standard operating procedure (SOP) has been written for this process and is included in Appendix B.

The second factor for decreased sensitivity is the wetting of the glass wool, which drastically reduces sensitivity. When this happens, the wool must be thrown away and the U-tube re-silanized. The most frequent cause is the condensation of water around the Swagelok fitting because of the temperature of the liquid nitrogen trap. Water does leak into the U-Tube over time. The tube needs to be wiped down after each use to prevent the build up of large water crystals at the Swagelok-U-Tube union.

The third cause for decreased sensitivity is the clogging of the water trap. The stripper can remove a lot of water and HCl. This can result in back pressure that causes the stripper and water chamber to pop loose. The usual remedy is changing the water trap every 4 - 5 samples.

Once all of the above mentioned factors were considered, the system was able to detect 1.00 ng of selenium, which is roughly twice the detection limit achieved by Cutter. Following discussions with Greg Cutter, a number of changes were made to the system.

First, the U-Tube in the liquid nitrogen trap was replaced with a V-Tube. This reduced the amount of void space in the tube, thereby reducing the amount of active sites for hydrogen selenide to absorb. The second change was the use of 1/8" tubing instead of 1/4" tubing. This reduces the amount of active sites and results in an increased signal. The third variation was the lowering of the injection port to increase mixing efficiency. The size of the injection port was also changed to prevent the formation of air pockets within the mixing chamber. The fourth change was to go to a higher purity of helium gas. While using 99.99% helium, quenching of the signal was observed whenever the tank reached half to one-quarter full. Scrubbers proved ineffective as they emitted other basic contaminants. Using higher purity helium (99.999%) eliminated the problem. The fifth, and probably most significant improvement was a re-design of the water trap from a U-Tube to a coil. A coil traps water and HCl more efficiently, resulting in a very stable baseline, and an increase in the signal to noise ratio. For a complete picture of the current setup, refer to Figure 5.2.

Optimization of the He flow rate to remove baseline fluctuations resulted in an increase to 250 cc/min from Cutter's 200 cc/min. The increased flow rate caused the water trap to clog quicker than before. To compensate, the stripper was changed from a ground glass bubbler to a ball with four holes. This has reduced the clogging of the water trap, and the sensitivity has remained constant.

For analyzing seawater samples, sulfanilamide must be added to remove nitrite interference [Cutter, 1983]. The addition of 1.0 mL of a 2% (w/v) sulfanilamide solution is sufficient to remove this interference in Bay waters. This is discussed in more detail below. A complete SOP for the method is in Appendix C.

5.3 Results

Sulfanilamide was chosen over urea to remove nitrite interference. Table 5.1 shows a comparison between Se recoveries using the two agents. Clearly, sulfanilamide use results in more consistent recovery.

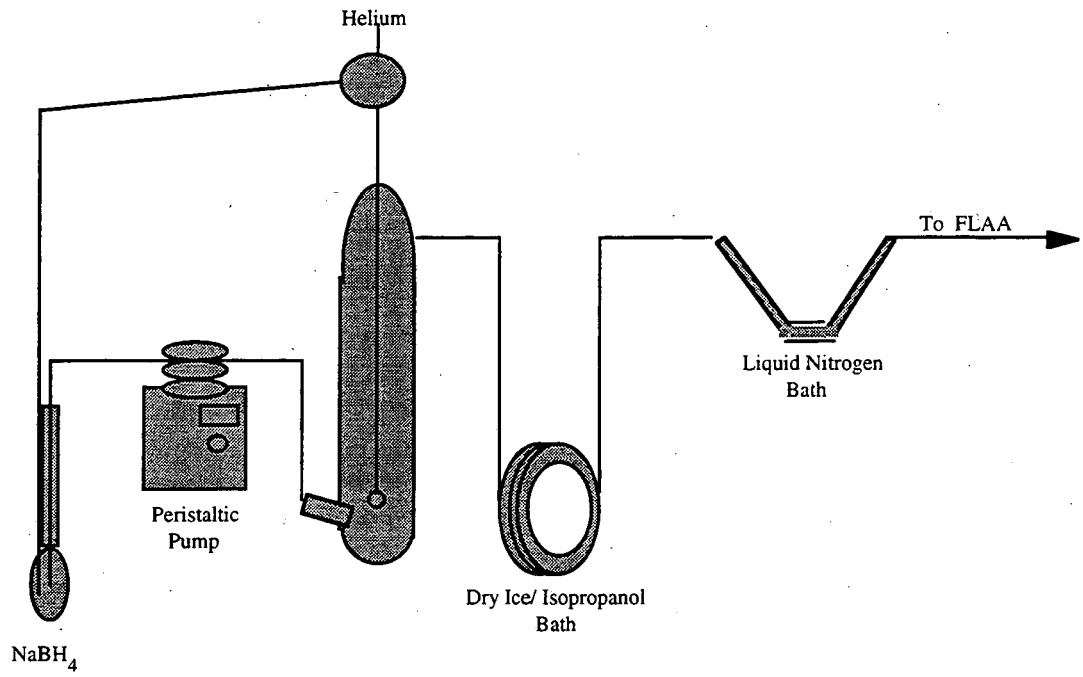

Figure 5.2 Current set-up for hydrogen selenide trapping.

The system was tested by analyzing blind check samples prepared by the QA/QC officer. The results are shown in Table 5.2. Recoveries are very good for these blind samples. The blank sample, B, was likely contaminated from an acid bath. The bath was analyzed and found to contain 2.17 ng of selenium. This could have produced the observed carry-over. The acid bath will be monitored on a regular basis to prevent future contamination.

For a series of samples that were analyzed, reproducibility has been very good. A sample collected from the Martinez Marina was analyzed five times over several days. The average Se mass for this sample was 1.06 ng with a standard deviation of 0.05 ng.

Table 5.1 Comparison of Se recovery using sulfanilamide and urea treatment.

	Urea Treated Sample		Sulfanilamide Treated Sample
	Peak area		*Peak area*
	1089500		1104200
	658400		1080000
	482700		1133400
			1197100
			1202200
Mean	743533	*Mean*	1143380
Std Dev	312230	*Std Dev*	54766
*RD**	42 %	*RD*	4.8 %

*RD = relative deviation.

Table 5.2 Blind check sample recovery.

Sample #	Sample Volume (mL)	Mass Se (ng)	Standard Deviation	[Se] (ng/L)	Actual Value	Percent Recovery
A	10	4.97	0.98	497	490	101 %
B	30	0.93	NA	31	0	NA
C	10	7.84	0.04	784	814	96 %
D	30	6.42	0.17	214	196	109 %

A method detection limit study was performed. Bay water from the Martinez Marina was spiked with 1.0 ng of selenite. The method detection limit calculations were performed following the procedure outlined by the California Department of Health Services (Glaser et. al., 1981). The method detection limit for seawater matrix using this method was determined to be 0.16 ng. This calculates to a concentration of 4.5 pptr for a 35 mL aqueous sample. This does not reflect the actual detection limit but the theoretical detection limit based on the calculation. The reporting detection limit is 10 pptr for 35 mL sample.

5.4 Summary

A reliable method to determine selenium at low levels in seawater has been established. An existing method has been applied and modified by introducing a coil

for the water trap, a V-tube for the liquid nitrogen trap, silanizing the glassware, relocating the injection port, and increasing the flow rate of the helium carrier gas. A series of blind samples was analyzed to validate the method. Selenium recoveries ranged from 96 - 109 %. Using seawater from the Martinez Marina, sulfanilamide was found to be more efficient at removing nitrite interference than urea. The method has a detection limit of 4.5 ng/L and a reporting detection limit of 10 ng/L.

6 Stable Isotope Methods

The goals of this study are to develop methods for measurement of Se isotope ratios in a variety of water samples and sediment extracts, and to apply these techniques toward a better understanding of the processes controlling Se mobility and the impact of refinery discharges in the Bay environment. Pioneering work has been required to accomplish the former goal. Existing experimental techniques were not sufficient, as is described below. The latter goal is now within reach, and measurement of Se isotope ratios in natural samples is under way.

6.1 Isotope Ratios as Environmental Tools

Isotope ratios have been used as environmental tools with increasing frequency in recent years, as measurement techniques have improved and environmental studies have become more complex. The most common use of isotope ratios is as a "fingerprinting" tool. For example, natural lead (Pb) dissolving from a soil usually has $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ ratios that are distinct from those of industrial Pb. Studies of Pb isotope ratios have succeeded in calculating the relative contributions of the two sources to Pb in stream water (Bullen and Shanley, 1994; Bacon and Bain, 1995). Similarly, nitrogen isotope ratios have been used to examine sources of nitrate in groundwater (Kendall et al., 1994).

6.2 Selenium Isotope Ratios

Se isotope ratio measurements have the potential to give key information about sources of selenium and chemical processes that control its fate and mobility. Chemical reactions alter the relative abundances of the six stable Se isotopes. For example, chemical reduction of Se is known to increase the abundance of the lighter isotopes relative to the heavier isotopes. Accordingly, the $^{80}Se/^{76}Se$ ratio reflects the chemical history of a Se sample.

There are two ways in which Se isotope data may be used. Because Se occurring in oil refinery wastewater has a very different chemical history from Se in river or sea water, it is likely that its $^{80}Se/^{76}Se$ ratio will be distinct from those of other sources. The isotope ratio should thus be useful as a tool for distinguishing refinery-source Se from Se of other origins. Perhaps more importantly, $^{80}Se/^{76}Se$ measurements provide a tool for studying the chemical processes that affect Se mobility. In field investigations, the relative values of $^{80}Se/^{76}Se$ in the various pools of selenium (dissolved, adsorbed, precipitated Se^0, pyrite Se) will give indications of the pathways through which those pools were formed. In general, the isotope ratios give information that complements the concentration measurements that are performed. Concentration data give the sizes of the various pools of Se, while the isotope ratios give information about their sources and/or the reactions that transfer Se between them.

6.3 Se Mass Spectrometry

Se isotope ratios are measured in this study using Thermal Ionization Mass Spectrometry (TIMS) (Wachsmann and Heumann, 1992). With this method, Se^- ions are formed by thermal effects on the surface of a hot (ca. 950°C) rhenium filament, accelerated by an electric field, and focused into a beam. The beam is deflected by a magnetic field, and splits into 6 separate beams -- one for each isotope. The intensities of the beams are measured and reflect the abundances in the sample.

The TIMS technique we are using has been available only in the last several years. It requires a mass spectrometer which can be operated in the negative polarity mode (most are designed to produce positive ion beams only), and accordingly, we are collaborating with Dr. Tom Bullen at the US Geological Survey in Menlo Park. Se isotope measurements made before 1985 used a very different method involving difficult procedures to synthesize SeF_6, a highly toxic gas. In contrast, the TIMS technique uses Se in the selenite form, which is the form most easily produced by our chemical separation techniques which are discussed below.

6.3.1 Machine Bias and Interferences

TIMS measurements usually differ slightly from the true ratios in the sample material, and this bias must be minimized or compensated for in order to obtain highly precise ratios. We have found that the machine bias for Se isotope ratio measurements is significant and variable, necessitating use of the "double spike" method to remove that bias. With this technique, two stable isotopes, selenium-74 and selenium-82, are added to the sample in known quantities. The 74/82 ratio of the added Se is known, and the measured 74/82 ratio can thus be used to evaluate the machine bias.

Purification of the Se is necessary before TIMS measurements can be made. Several elements, such as Cl, P, and S can interfere by either decreasing the ability of Se to ionize or by producing ions with the same mass as one of the Se isotopes (e.g., SeO_3^- at masses 80 and 82). The interfering species must be removed, or in some cases can be detected and corrected for during the Se isotope ratios measurements.

6.3.2 Previous Work

Se isotope ratios have been measured in a few previous studies. Early work (1962-1978) concentrated on laboratory and theoretical studies to determine the extent to which Se isotope ratios change during reduction reactions, and included a few measurements on ore materials (Rashid et al., 1978; Krouse and Thode, 1962). More recent work, enabled by the development of the TIMS method for Se (Tanzer and Heumann, 1991) has focused on concentration measurements at low levels (<30 parts per trillion), but has not been applied toward making isotope ratio measurements on natural samples.

6.4 Progress

At present, the analytical procedures for Se isotope ratios measurement have been developed and calibration of the measurement procedure is complete. The chemical procedures for separating Se from interfering elements are complete and measurements of isotope ratios in samples from the study area has begun.

6.4.1 Purification Chemistry

The separation of microgram quantities of Se from concentrated solutions such as Bay water and sediment extracts presents a significant challenge. Only small amounts of chloride and various other elements can be tolerated in the final purified selenium solution, and in saline waters, the molar ratio of chloride to selenium is over one million. Furthermore, the chemical process must minimize loss of Se and avoid oxidation or reduction steps, because these problems can change the isotope ratios.

The problem of efficiently separating Se from concentrated solutions was solved by a ferric iron hydroxide $Fe(OH)_3$ precipitation procedure. A literature search of the many techniques employed over the years to concentrate Se from seawater led to this one, which was used in the 60's (Chau and Riley, 1965). Hydride generation was also considered, but was rejected because the chemical reduction step would greatly alter the isotope ratios if small errors were made. The $Fe(OH)_3$ precipitation scrubs selenite from solution without a reduction step, has a yield between 95% and 100%, and can be carried out with very large volume samples. The procedure involves adding Fe^{3+} to the solution, adjusting the pH, filtering to recover the precipitate, dissolving it in acid, and removing the Fe^{3+} with cation exchange resin. This leaves selenite and a few other weak acid anions in the final solution.

Phosphate and organic acid anions brought with the selenite through the $Fe(OH)_3$ precipitation procedure can interfere with the measurements, so an anion chromatography procedure was developed. The solution is passed through a column of anion exchange resin, followed by a known amount of dilute acid. The selenite is separated from the other anions because it moves at a different rate through the column. This technique yields a sufficiently pure Se solution which can be used for mass spectrometry, and we have found that at least 90% of the selenium is recovered.

The complete chemical separation procedure appears to be working well. Analysis of the final product of an extraction of one microgram Se from one liter Bay water spiked with 30 μg P (as phosphate) yielded the results given in Table 6.1. The process is effective at removing Cl and P and retains a high percentage of the Se.

Table 6.1 Results of chemical purification of selenium

Element	Amount in final solution (μg)	Percent recovered
Se	0.91	91%
P	1	3%
Cl	10	0.00001%

6.4.2 Mass Spectrometry

Before the inception of this project, trial runs with Se on Tom Bullen's mass spectrometer at the US Geological Survey gave good results and suggested that measurements could be made with little additional development. Further investigation showed that the measurement bias was much larger than expected and varied with time, and this necessitated the use of the double spike technique described above. Calibration of the double spike was completed recently, and an initial series of repeat measurements of a working standard gave excellent results (Table 6.2).

Table 6.2. Results of Se isotope ratio measurements.

Sample	$^{80}Se/^{76}Se$ ratio
MH495-1 (working std.)	5.2982
MH495-2 (working std.)	5.2966
MH495-3 (working std.)	5.2958
Std. Dev for MH495	0.0008 (0.016%)
Shell Refinery effluent	5.3628

These are the first double-spike-calibrated Se isotope ratio measurements ever made. The precision indicated by the replicate analyses is better than with any earlier method, with a standard deviation of less than 0.02%. Thus, while the variation in $^{80}Se/^{76}Se$ is expected to be only a few percent, the uncertainty of the measurements is roughly one hundred times smaller than that variation. This provides ample opportunity to resolve subtle effects in the natural samples.

6.5 Future Work

Reconnaissance data for refinery effluents, Bay water, and sediments are being collected as of the writing of this report. It is expected that the Se in refinery effluent will have isotope ratios that are distinct from riverine Se, and that this difference will provide a means of "sourcing" of selenite at various field locations. Selenium in the water column and in shallow pore waters will be analyzed and the results compared to the refinery and background values.

Detailed study of Se isotope ratios within one or more field sites should provide information concerning the chemical processes at work. Measurements of $^{80}Se/^{76}Se$ on the various fractions of Se from sediment cores as a function of depth would give indications of Se cycling. For example, it is expected that elemental selenium in the sediment that has precipitated from pore waters has a lower isotope ratio because of the chemical reduction that produces it. In such a case, the isotope ratio of the adsorbed and dissolved selenite as a function of depth gives information about chemical processes. If selenite is reduced slowly and diffuses relatively rapidly from the sediment-water interface, the isotope ratios would be uniform and equal to the water above. If diffusion is slower relative to reduction, the selenite $^{80}Se/^{76}Se$ would increase with depth as elemental Se with a low ratio is removed. Finally, in a case where elemental selenium is being re-mobilized by oxidation, the values would equal those of the dissolving elemental Se at each depth.

The isotope ratio measurements may also be employed in tracer studies that track the evolution of labeled Se. In small-scale field experiments or laboratory models, stable isotope tracers can be introduced and used to track the evolution of Se as it interacts with solid matrix materials.

7 Selenium Levels and Fractionation in Intertidal Sediments

The purpose of site characterization is to provide baseline data for site selection, to compare Se concentrations in sites with varying selenium levels, and to evaluate the appropriateness of extraction methods at fractionating individual Se pools. The measurement of Se fractions and baseline concentrations in different sites facilitated evaluation of one of the main objectives in this study: to determine what factors control Se accumulation and mobility in the intertidal sediments, and develop a realistic model that can predict Se cycling in the intertidal zone.

Characterization of sediments and soils in the Martinez Regional Park and the Southampton Bay for total and fractionated selenium, organic carbon (OC), metals, and anions was done using a sequential extraction developed for this study (Chapter 4). Baseline data on the sites used for this study were determined from samples taken over a series of dates starting May 17,1995 through August 8, 1995. Samples were taken at low tide along transects beginning in the mudflats and ending deep into the marsh areas. Samples were spaced from 5 to 10 meters apart, and were collected over a depth series of 0-2, 2-10, and 10-20 cm, with the exception of the first sampling at Southampton Bay, where samples were taken from one depth interval of 0-12 cm. In areas where the 0-2 cm layer was difficult to separate without disturbing the sample, only 0-10 and 10-20 cm depths were collected. Samples were split after interstitial water removal and a subsample was dried and ground to a fine mass on a ball mill. Acid digests were run on the ground sediment/soils for total Se analysis.

The chosen depth delineation was based on observations of color changes at the surface (0-2 cm) and consultations with Dr. Sam Luoma, USGS Menlo Park. Lower horizons were divided simply to determine how Se speciation varies with further burial. The 0-2 cm layer represents what Dr. Luoma identified as the oxic zone in the mudflats. This layer is oxygenated by surface waters, and redox potential is assumed to be positive (to be verified using direct electrode measurement, see Section 7.3).

7.1 Total Selenium Levels

Total Se data for the Martinez Regional Park and Southampton Bay Park have distinctly different trends, which may be indicative of the manner in which Se enters each system. The MRP site shows a strong trend in which total Se concentrations in the mudflats are low and show small relative differences between 10 m intervals (Figure 7.1). The average mudflat total Se level is approximately 0.35 ppm, and the marsh values increase from 0.35 to as high as 0.75 ppm. At SHB, total Se concentrations are lowest in the mudflats and in the upper marsh (Figure 7.2). There appears to be a discontinuity in Se values between the mudflats, where total Se concentrations are approximately 0.65 ppm, and the border of the marsh and the mudflats, where total Se concentrations are as high as 0.850 to 0.990 ppm. Further sampling in the mudflats needs to be done in order to confirm this discontinuity.

7.1.1 Martinez Regional Park

At the MRP site, total Se concentrations are elevated in the mudflats (0.300 to 0.650 ppm), and in the marsh (Figure 7.1). Total Se concentrations of the sediments are relatively high for Bay sediments (mean value measured in 1990 and 1991 was 0.29 ppm (Lee, et. al., 1995)). Total Se concentrations were lower than those measured at Southampton Bay (Figure 7.2) which may be attributable to sediment texture. MRP sediments are coarser-textured and therefore do not contain high concentrations of fine colloids which adsorb dissolved Se species. Also, sediment deposition from the adjacent creek may dilute sediment Se concentrations (see Chapter 3)

There are no trends in the data within the mudflats with respect to location. The variability in the texture and in the concentration of OM of the sediments demonstrates that there is no deposition of contiguous layers that can be characterized as such (Figure 7.3). The heterogeneity of the sediment layering in the mudflats means that layers were disrupted by alternating deposition and erosion and the resulting layers do not represent sequential deposition. Changes in Se concentrations with depth also did not follow any trends, a likely result of the heterogeneity of deposition (Figure 7.4).

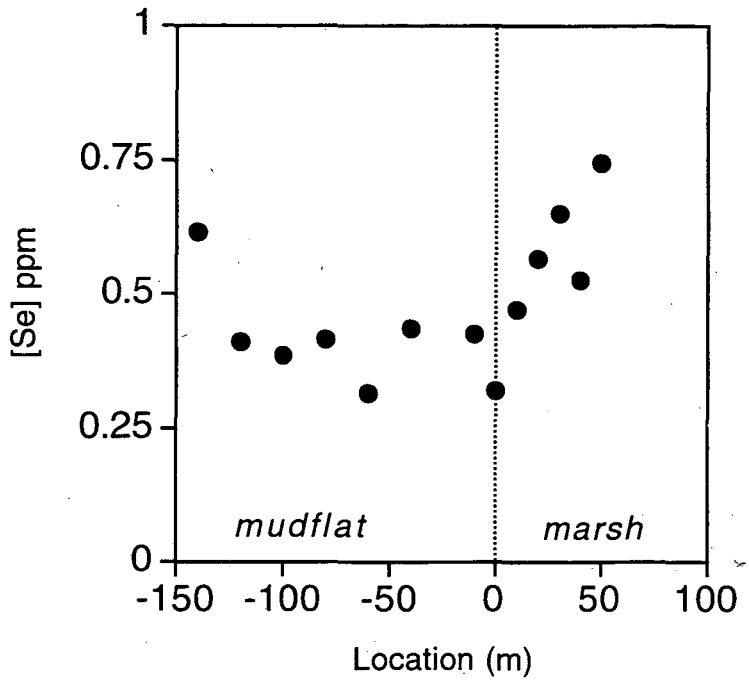

Figure 7.1 Total Se in MRP intertidal sediments, 0-20 cm depth, weight-averaged, along a transect perpendicular to shore.

Water sampled at the Martinez Marina dock, contained selenite concentrations between 30 to 250 pptr, and total Se concentrations between 197 and 328 pptr. These concentrations are higher than average values for the San Francisco Bay (100 pptr total Se, Ball and Arthur, 1986). The close proximity of the Shell refinery discharge may significantly influence Se concentrations.

7.1.2 Southampton Bay

Southampton Bay total Se concentrations were higher than those in Martinez (Figure 7.2). This may be attributed to the fine particle size of the sediments and the low energy of the environment, which allows for greater rates of deposition and sediment accumulation. Se concentrations in the mudflats of the intertidal zone vary from spot to spot, but there is insufficient data to determine a trend at this point. Total Se concentrations along a transect from the mudflat into the marsh show a spike in concentration at the marsh edge, and a discontinuity in readings from the mudflats into the marsh (Figure 7.2). Why there is a sudden increase in total Se levels between the marsh and the mudflats is unclear, and further sampling is needed to determine whether

these values are typical of the area.

Due to the small number of mudflat samples and their proximity to the shore, no trends in the data could be found for this portion of the intertidal zone. Within the marsh, Se concentrations clearly decrease as the sample location becomes more positive (positive is defined as further into the marsh and away from the shore).

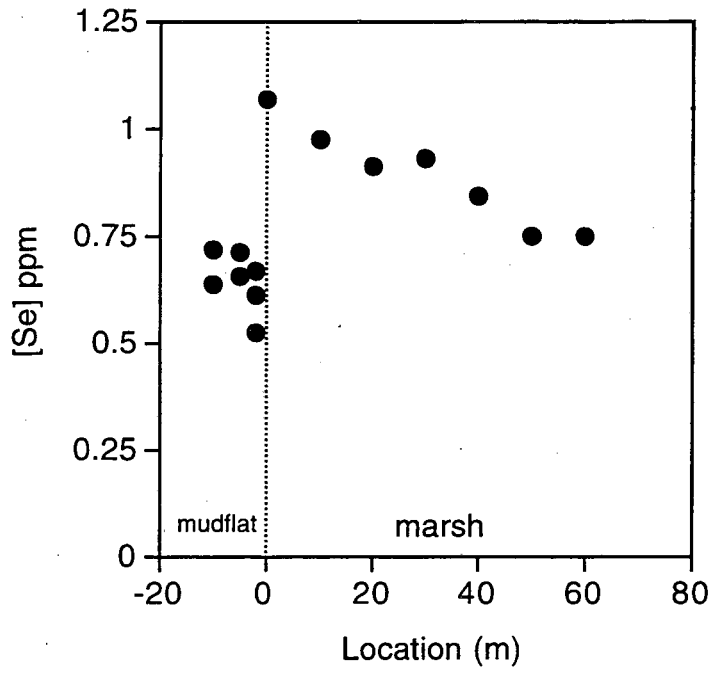

Figure 7.2 Total Se in SHB intertidal sediments, 0-12 cm depth interval, along a transect perpendicular to shore.

The decrease in total Se concentration follows an increase in total organic carbon (TOC), as the influence of plants on the soil composition increases deeper in the marsh. Plotting both %OC and total Se versus location demonstrates the inverse correlation between OM and Se concentration (Figure 7.5). This is likely not a cause-effect relationship, but simply a reflection of the fact that increased plant growth away from the shore coincides with decreasing influence of Se-containing tidal waters and sediments, and increasing influence of surface runoff.

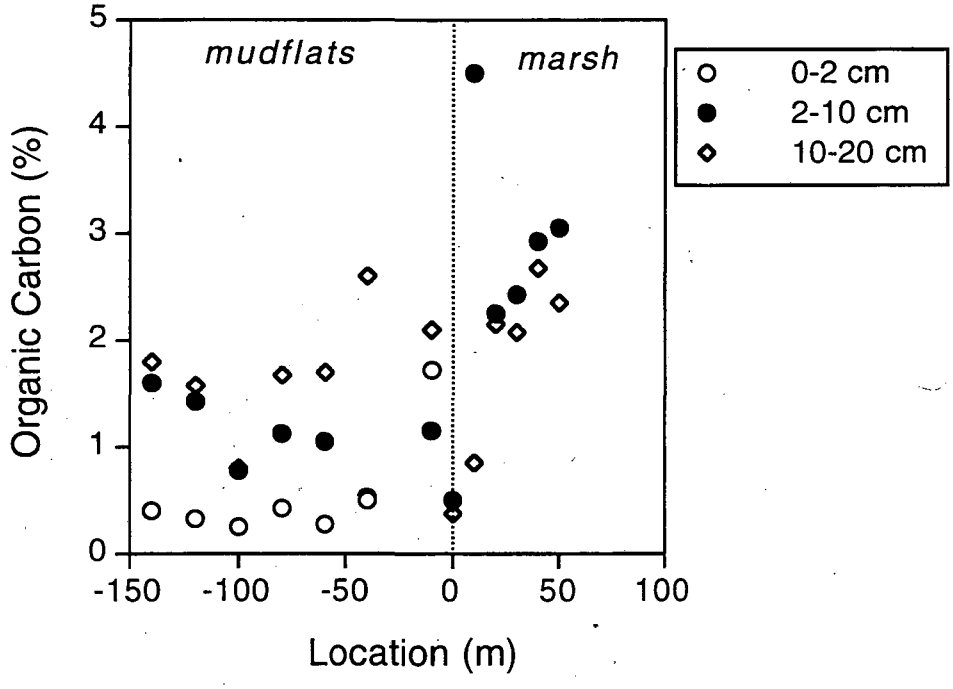

Figure 7.3 Organic carbon content in MRP intertidal sediments, at three depth intervals, along a transect perpendicular to shore. In the marsh, the "2-10 cm" interval is actually 0-10 cm (applies to all subsequent figures).

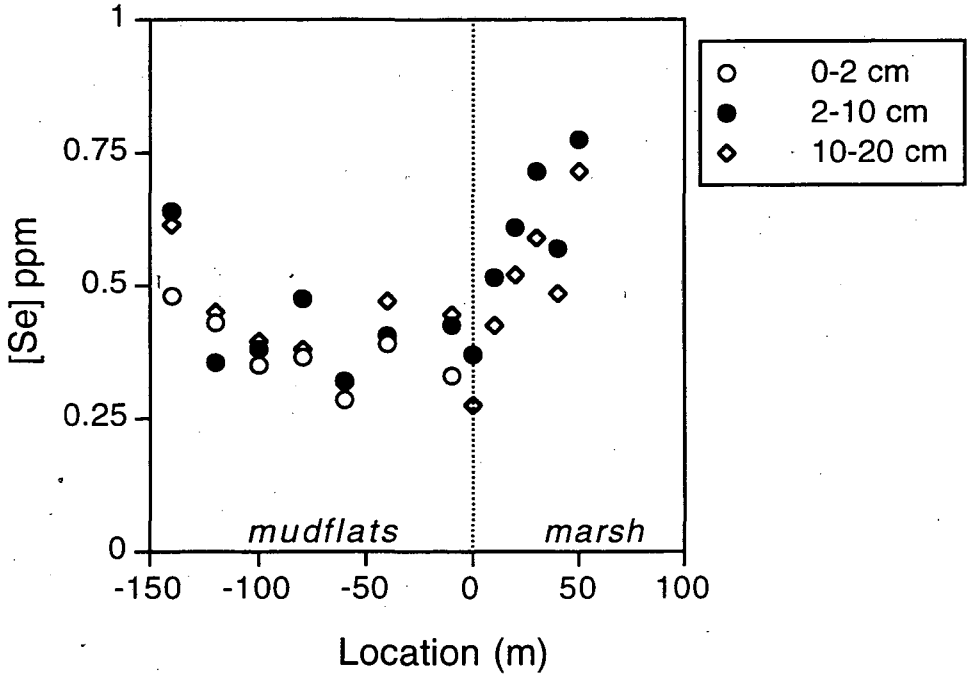

Figure 7.4 Total Se in MRP intertidal sediments, at three depth intervals, along a transect perpendicular to shore.

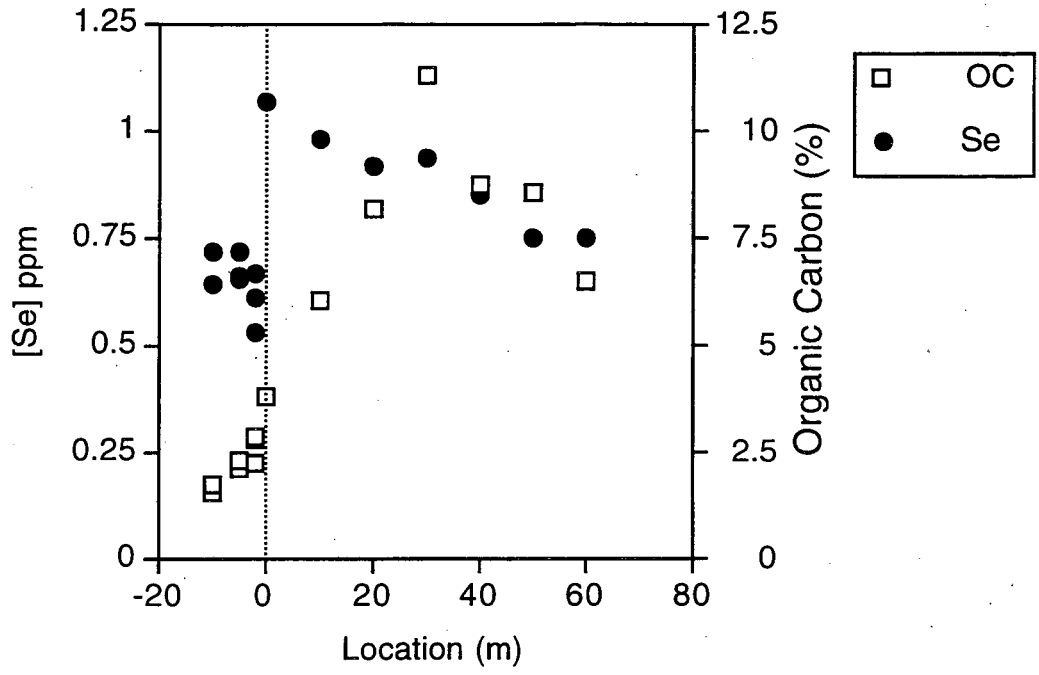

Figure 7.5 Total Se and organic carbon in SHB intertidal sediments, 0-12 cm depth interval, along a transect perpendicular to shore.

7.1.3 Total Se vs. Sediment Metal Content

Correlation of total Se to metals and other sediment/soil components did not reveal any discernible trends. Total acid digests of sediments/soils gave good indication of the concentration of mineral colloids in the samples, metals such as Al and Fe, for instance showed little correlation with Se concentrations. Digest data suggests that metal concentrations were fairly constant between samples, with the exception being Mn which gave a close correlation with the % OC.

7.2 Sequential Extractions

Sequential extractions for the two sites have a lower than desired efficiency when TAD Se measurements are compared to total extracted Se. Assuming TAD-Se represents 100% of the Se in a given sediment sample, which is quite well established (Weres et. al., 1989), sequential extraction efficiency is typically between 55 and 75%, before pyrite-Se extraction. Pyrite-Se extraction is ongoing given that the technique

developed by Velinsky and Cutter (1990) requires the use of the liquid nitrogen trapping apparatus, and requires run times of 20 min per sample (see Appendix A). In anoxic sediments, pyrite Se may prove to be a significant fraction of the overall Se. With the exception of Velinsky and Cutter (1990), no other study has quantified pyrite Se, and even their study did not extract organic Se, adsorbed Se, and carbonate Se and attempt to verify that the recoveries represent 100% of the Se in a given sample. Like most Se fractionation studies, they measured OM-Se by the difference between the total Se and the Se in the measured fractions. This results in assigning all cumulative error to the OM-Se value. In this study, every identified Se association is extracted using a technique that attempts to isolate that individual fraction. Sequential fractionation of each of these forms of Se, provides the most accurate assessment of the actual Se concentration in a given form.

One of the problems with extraction techniques is that they are often not as specific as would be desirable, making the extraction operationally defined. Therefore, Se released by a phosphate solution is referred to as phosphate-extractable Se, and likewise NaOH extracted Se is referred to as OH-extractable Se, even though these techniques have been shown to remove predominantly adsorbed Se and OM-Se, respectively. However, given the ongoing status of method development, results will be considered in terms of the operationally defined fractions.

7.2.1 Interstitial Water Selenium

Prior to chemical extraction, sediment samples are centrifuged to remove interstitial water (see Appendix A). Although some of the interstitial water will not be removed in this fashion, due to tension on silts and clays, the water which is removed can be analyzed for Se (Ix-Se), with results providing information on Se which is readily available for physical redistribution within the sediments and plant uptake in the marsh.

Results of Ix-Se measurements are shown in Figures 7.6 and 7.7, for the MRP and SHB sites, respectively. At the MRP site, Ix-Se in the surface layer (0-2 cm) increases to over 3.5 ppb at the marsh-mudflat interface. In the marsh, the 0-2 cm layer was not differentiated, and the 0-10 cm interval was sampled. Ix-Se in the marsh declines inland, down to near 0 at 50 m. A decline is also seen in the 10-20 cm interval. This is in contrast with total-Se concentrations, which increase in the marsh. Such a trend is

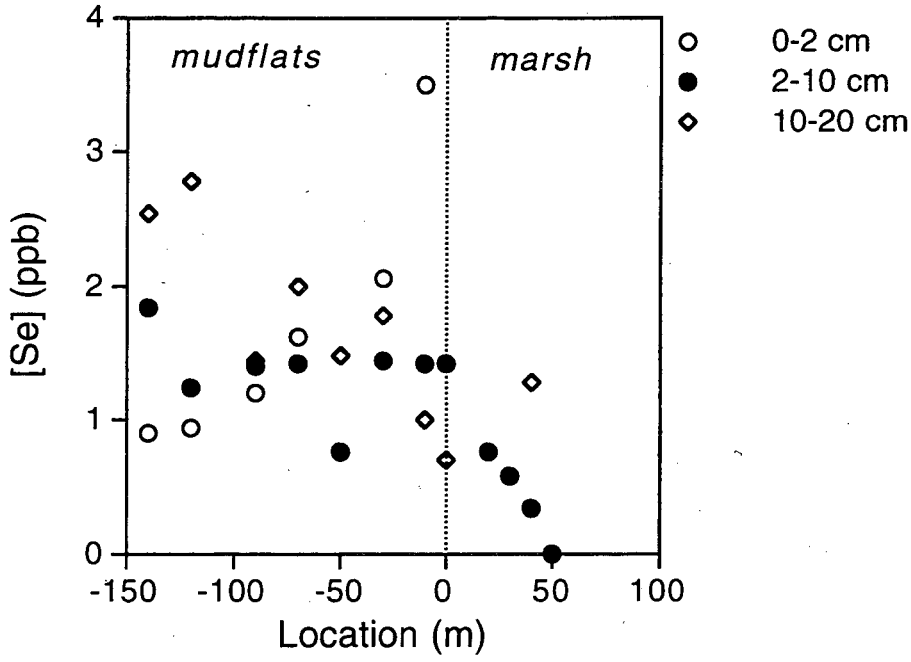

Figure 7.6 Interstitial-water-Se in MRP intertidal sediments, at three depth intervals, along a transect perpendicular to shore.

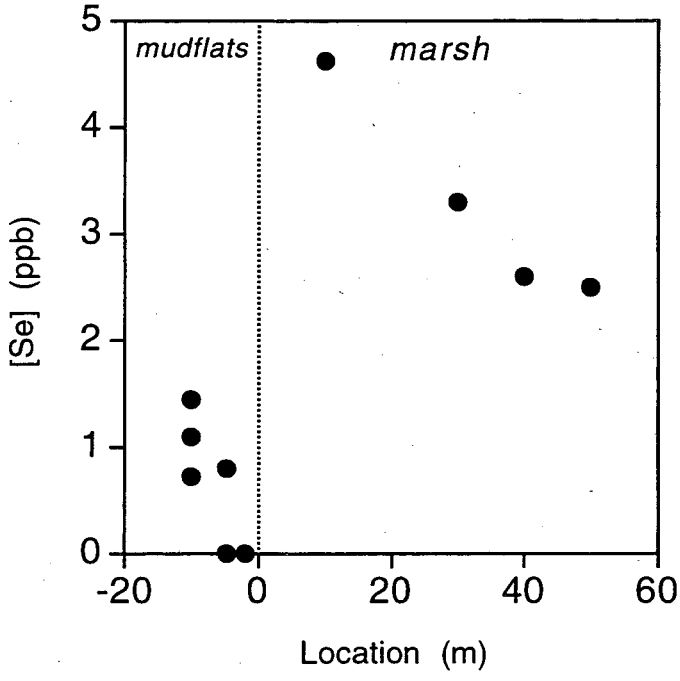

Figure 7.7 Interstitial-water-Se in SHB intertidal sediments, 0-12 cm depth interval, along a transect perpendicular to shore.

indicative of biologically-created reducing conditions in the marsh. It is not clear why there are higher concentrations of Ix-Se at depths below the 0-2 cm interval in the mudflats, although the same trend is observed in the total Se depth distribution (Fig. 7.4).

At SHB, Ix-Se concentrations (Fig. 7.7) show the same discontinuity in values as the total Se data (Fig. 7.2), where the mudflats have distinctly lower Se concentrations than the marsh samples. In fact, Ix-Se decreases sharply at the mudflat/marsh interface and increases to over 4.5 ppb at 10 m into the marsh. Because only the 0-12 cm interval was sampled at SHB, no depth profile of Ix-Se is available. Similarly to the MRP site, Ix-Se concentrations decrease further into the marsh.

Ix-Se was speciated and selenite was found to dominate. However, the results need further consideration and are not presented here.

7.2.2 Sequential Extraction Results

In the extracted sediments, Dx-Se and Px-Se are extremely variable in concentration. These extracts are often rich in dissolved organic matter, complicating analysis, and making the speciation of selenite, selenate, and OM-Se often impossible. OHx-Se and Sx-Se appear to be the dominant selenium fractions in the sediments. For the MRP site, OHx-Se is between 19 and 47 % of total Se (Figure 7.8). Sx-Se ranges from approximately 2 to 30 % of total Se (one sample had a value of 69 %, but this appears to be anomalous for this site, see Figure 7.9), with the lower concentrations generally being in the oxic 0-2 cm samples, and the higher concentrations being in the 2-10 and 10-20 cm samples. Based on the expected redox status for different depths in the soils and sediments, the observed values correlate well with theoretical predictions. The higher Sx-Se values in the marsh at site SHB also follow theoretical predictions based on the lower redox potential of marsh samples (Figure 7.13). There is a strong correlation between Sx-Se and % OC at the MRP site (Figure 7.10).

At the SHB site, OHx-Se concentrations were somewhat higher than Px-Se in the marsh, but significantly higher near the marsh mudflat interface and in the mudflats (Figure 7.11). There appears to be a slight decreasing trend towards shore in the OHx-Se data (Figure 7.11). Based on the location vs. OHx-Se plot, an inverse correlation

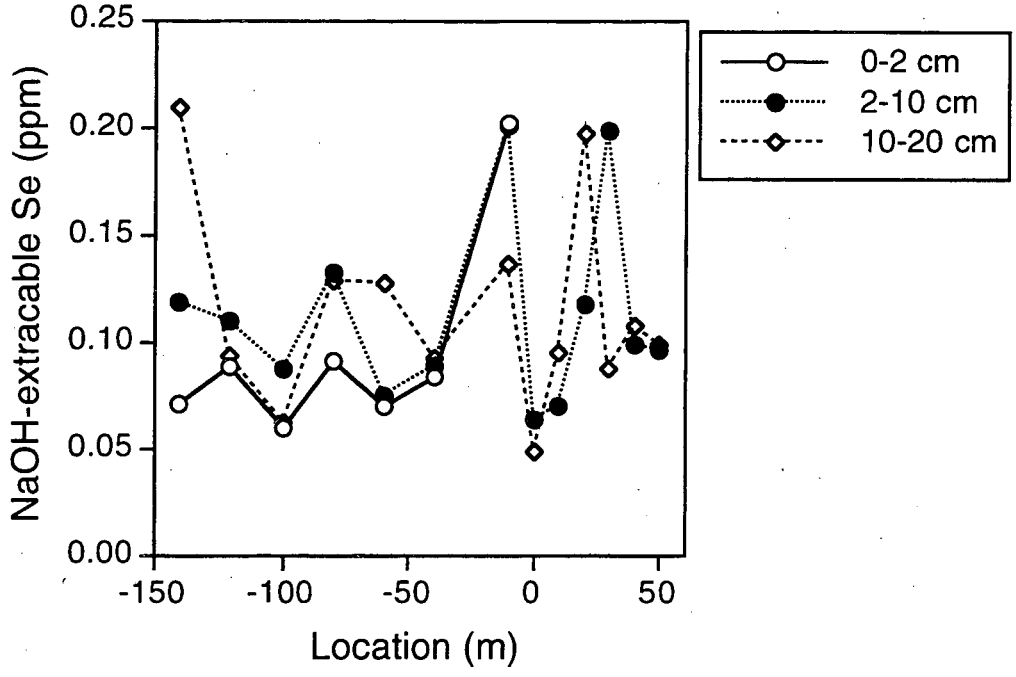

Figure 7.8 OH-extractable Se in MRP mudflat sediments, at three depth intervals, along a transect perpendicular to shore.

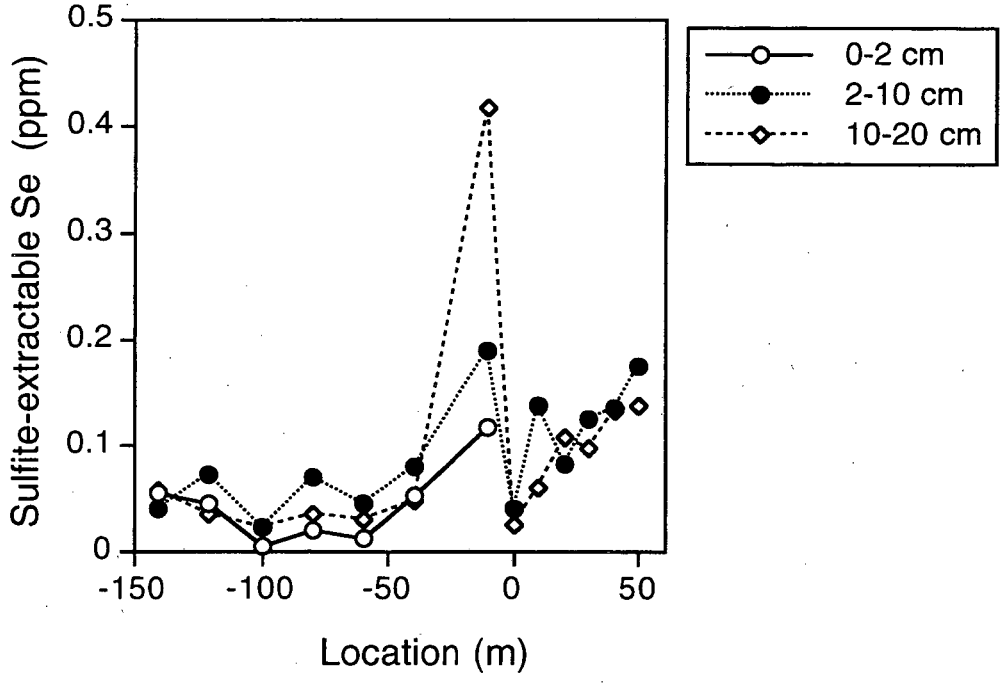

Figure 7.9 Sulfite-extractable Se in MRP mudflat sediments, at three depth intervals, along a transect perpendicular to shore.

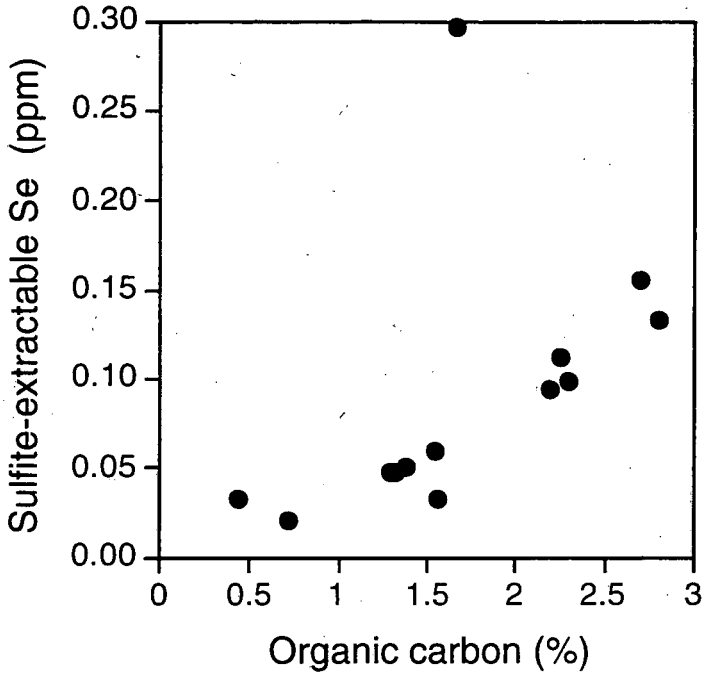

Figure 7.10 Sulfite-extractable Se in MRP mudflat sediments, 0-20 cm, weight-averaged, as a function of sediment organic carbon content.

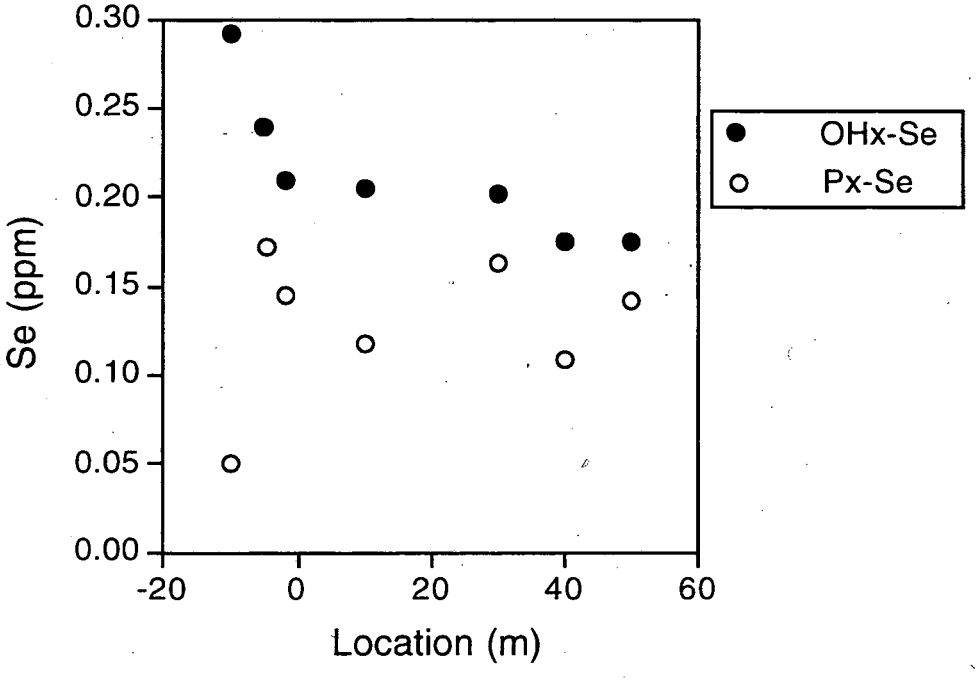

Figure 7.11 Phosphate- and OH-extractable Se in SHB intertidal sediments, 0-12 cm, along a transect perpendicular to shore.

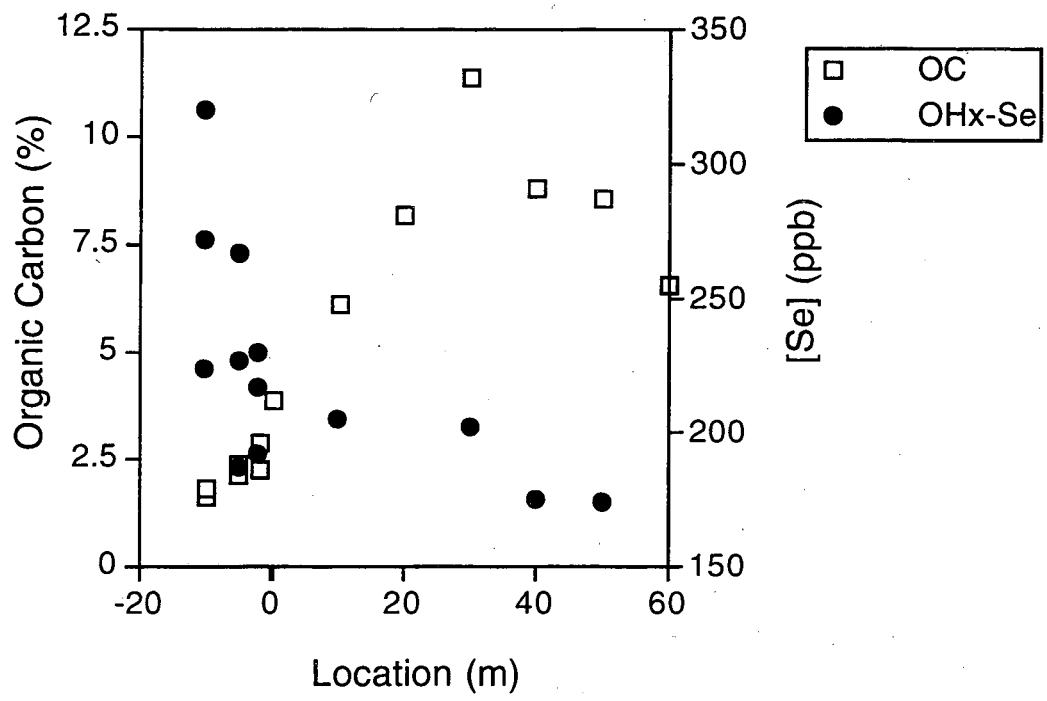

Figure 7.12 OH-extractable Se and organic carbon in SHB intertidal sediments, 0-12 cm depth interval, along a transect perpendicular to shore.

was found between OHx-Se and OM, where, in the marsh soils of Southampton Bay, the weighted average OHx-Se concentrations decrease or remain constant as %OC increases (Figure 7.12). Similar correlations cannot be made at the MRP site because sediment texture varies too greatly, significantly altering the %OC of the sediments, and their adsorptive capacity.

Sx-Se concentrations increased in a transect from the edge of the marsh inland, and the percentage of elemental Se in the samples was consistently the highest of all fractions in the marsh, ranging from 9.8 to 35 % (Figure 7.13). More extensive sampling is necessary to verify the trend measured in the SHB marsh, but the present numbers support the basic understanding that Se farther in the marsh is more likely to be reduced to elemental Se due to greater biological activity. Also, the high degree of saturation throughout the portions of the marsh sampled, even in the dry season, demonstrate that drying and oxidation are not likely. Plotting Sx-Se versus % OC, yields a strong trend that has an r^2 value of 0.72 (Figure 7.14). Thus the Sx-Se follows a similar trend as that seen in the MRP marsh, despite the fact that total Se concentrations decrease at SHB farther into the marsh, and the total Se concentration

increases in MRP.

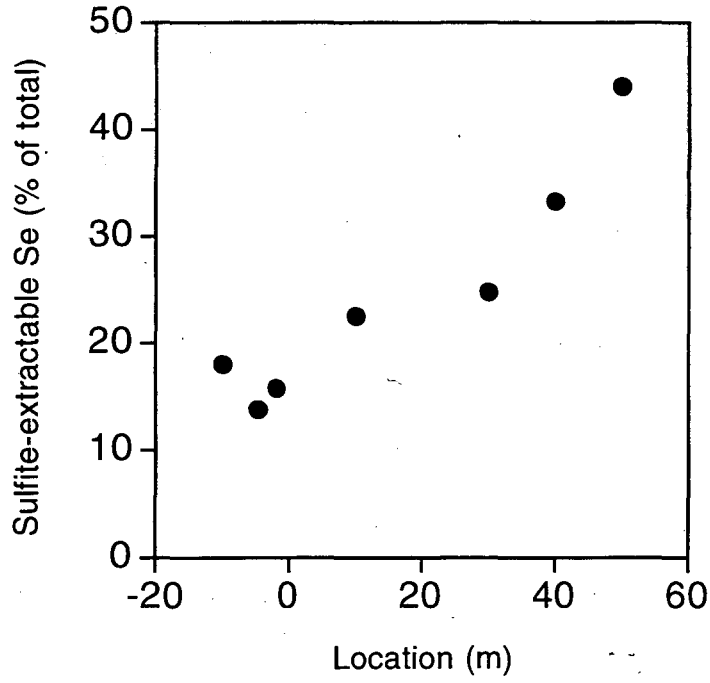

Figure 7.13 Sulfite-extractable Se in SHB intertidal sediments, 0-12 cm, along a transect perpendicular to shore.

The relatively high extraction efficiency in the SHB samples (67 to 95 %) implies that pyrite-Se is not a significant fraction of the overall Se, and that fraction which Lipton (1991) refers to as residual or structural Se (defined as non-extractable mineral Se) is also not present. Most of what Lipton (1991) defined as structural residual Se, is pyrite-Se that survives NaOCl extraction (the efficiency of NaOCl at removing pyrite-Se should be relatively high, meaning pyrite-Se is a small fraction of the residual Se Lipton refers to). In the coarser-textured sediments of the MRP site, more mineral Se may be present, which remains in the residual fraction because of its resistance to dissolution.

7.2.3 Summary of Sequential Extraction Results

The sequential extraction data for the sites can be summarized in a series of pie charts which show the Se concentration of the individual fractions in the sediments (Figure 7.15 and 7.16). Although only selected samples are presented, they show typical breakdowns of the extractable Se in the sediments. Given the heterogeneity

of the two sites, an average speciation value is meaningless, because it does not account

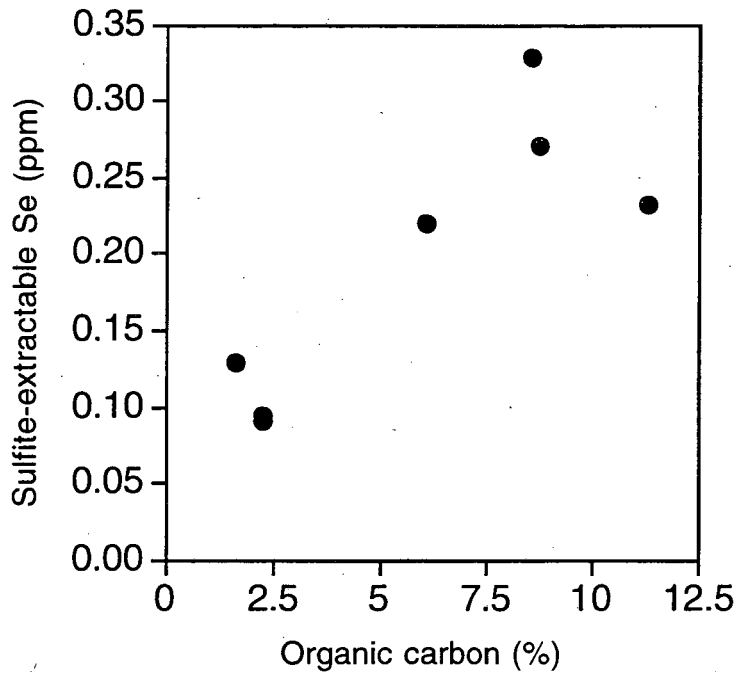

Figure 7.14 Sulfite-extractable Se in SHB intertidal sediments, 0-12 cm, as a function of sediment organic carbon content.

for the changes in Se levels with depth and location of the individual cores. The pie charts do emphasize changes in Se speciation with depth for particular samples when analyzed on an individual basis. Therefore, the profile described by the pie charts in Figure 7.16, shows how burial (i.e. age) affects the speciation of particular fractions and emphasizes what processes may be important in the transformation of Se. In both of these cases, the reduction of oxidized Se to elemental Se appears to be the most important transformation observed. These conclusions are supported by the Sx-Se vs. location plot shown in Figures 7.9 and 7.13.

Profiles of both locations are presented in Figures 7.17, 7.18, 7.19, 7.20, where stack charts show clearly how total-Se and individual Se fractions vary with location and depth (no depth is shown for Southampton Bay because marsh samples were taken over one depth interval).

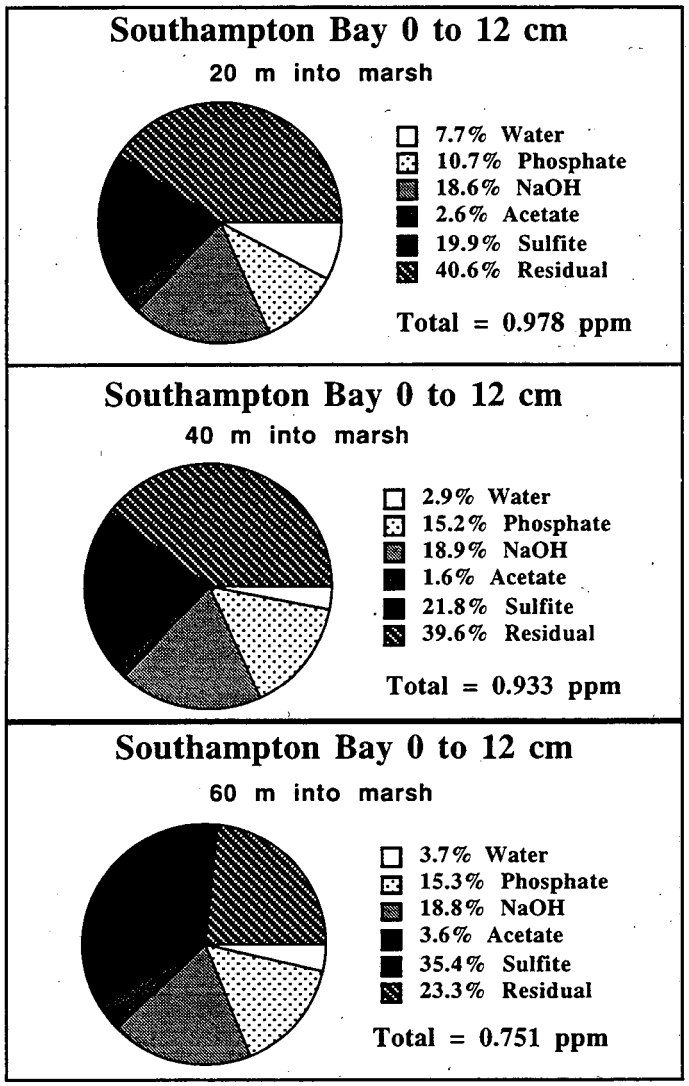

Figure 7.15 Results of sequential extractions of SHB marsh sediments, 0-12 cm, taken at three points upgradient from the mudflat/marsh interface.

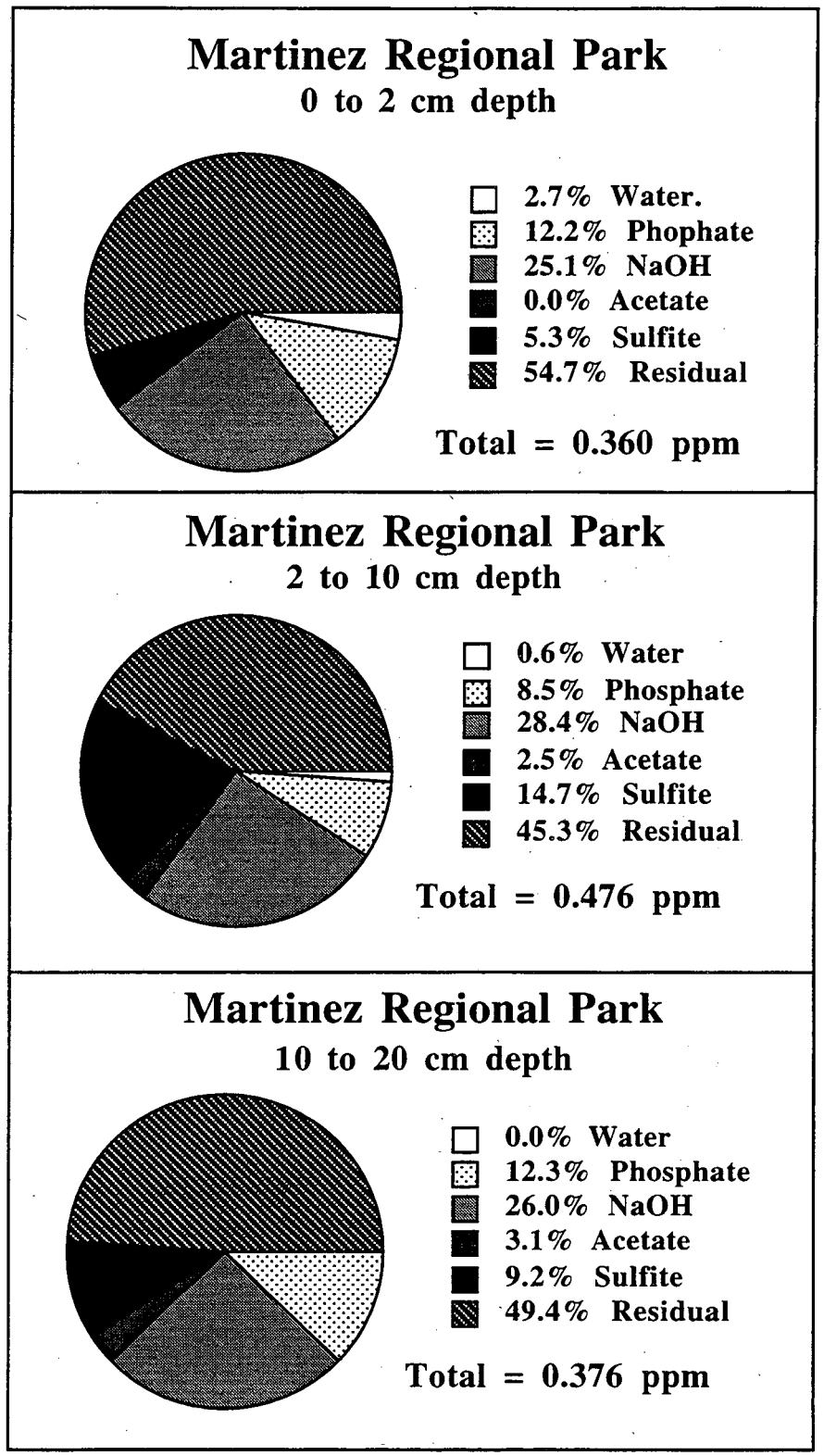

Figure 7.16 Results of sequential extractions of MRP mudflat sediments, three depth intervals, 80 m from marsh/mudflat interface.

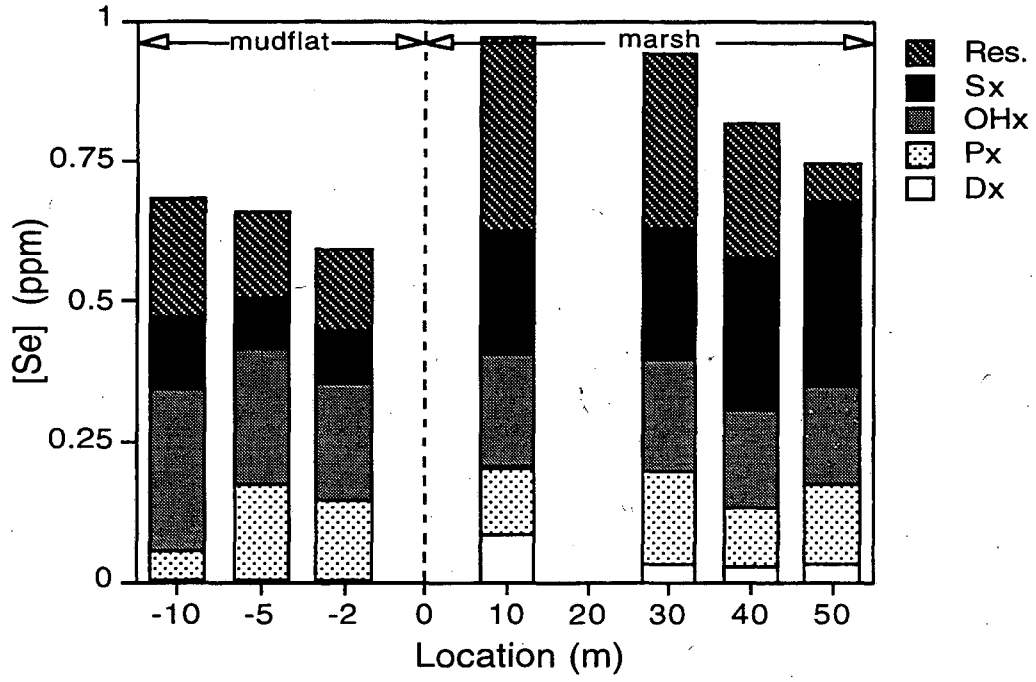

Figure 7.17 Selenium fractionation in SHB sediments, 0-12 cm depth interval, sampled along a transect perpendicular to shore.

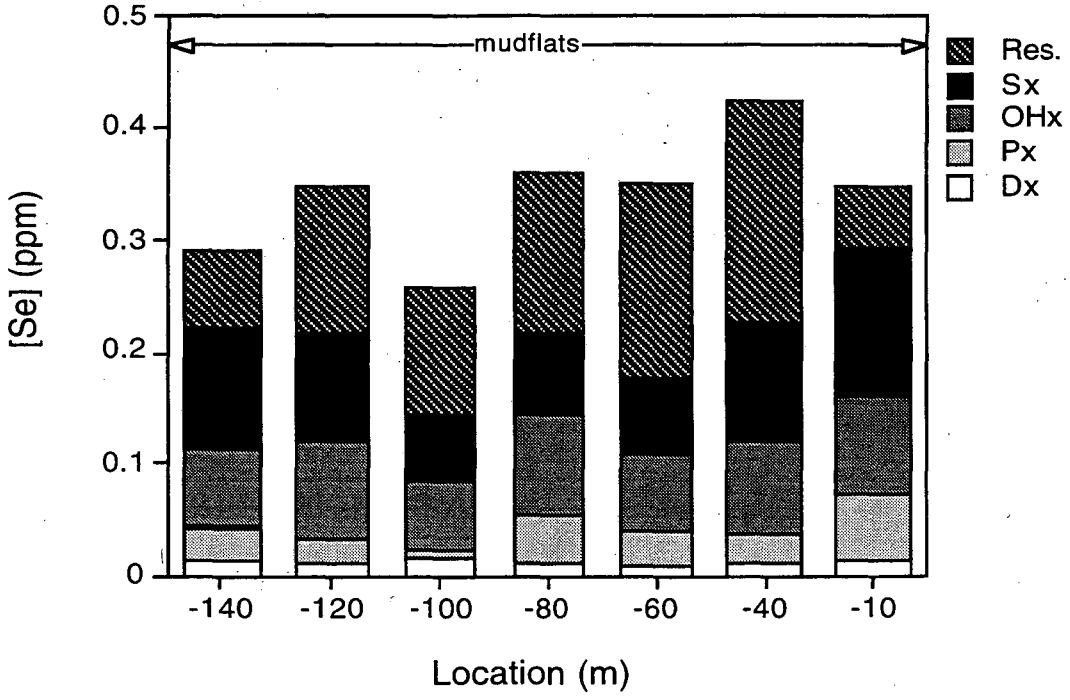

Figure 7.18 Selenium fractionation in MRP mudflat sediments, 0-2 cm depth interval, sampled along a transect perpendicular to shore.

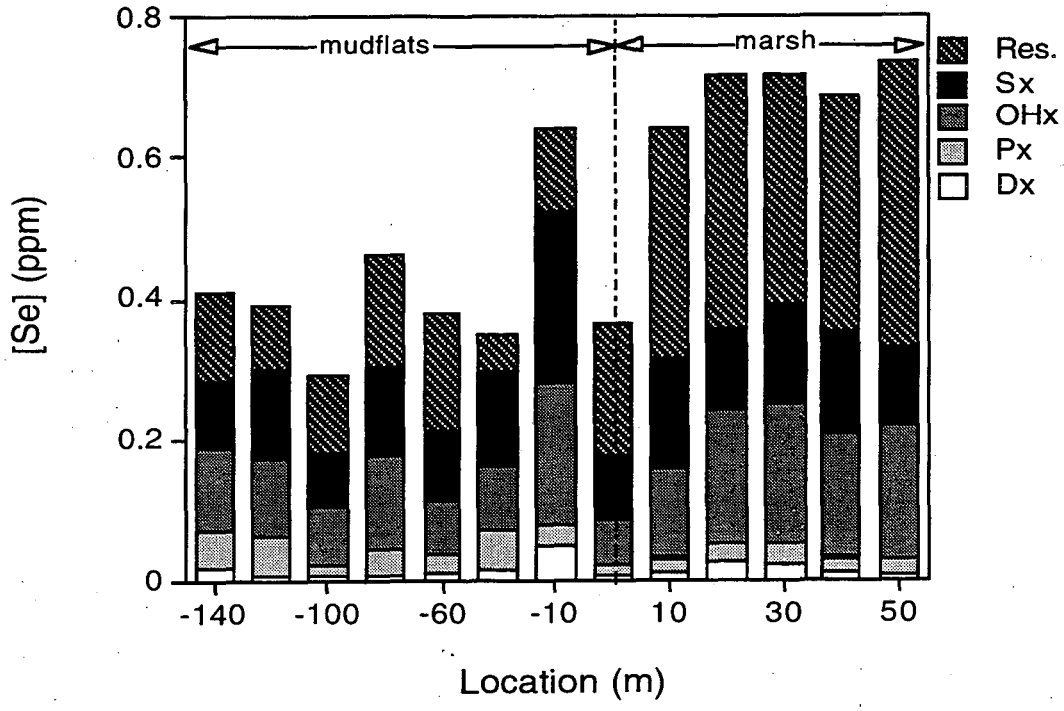

Figure 7.19 Selenium fractionation in MRP intertidal sediments, 2-10 cm depth in the mudflats, 0-10 cm in the marsh.

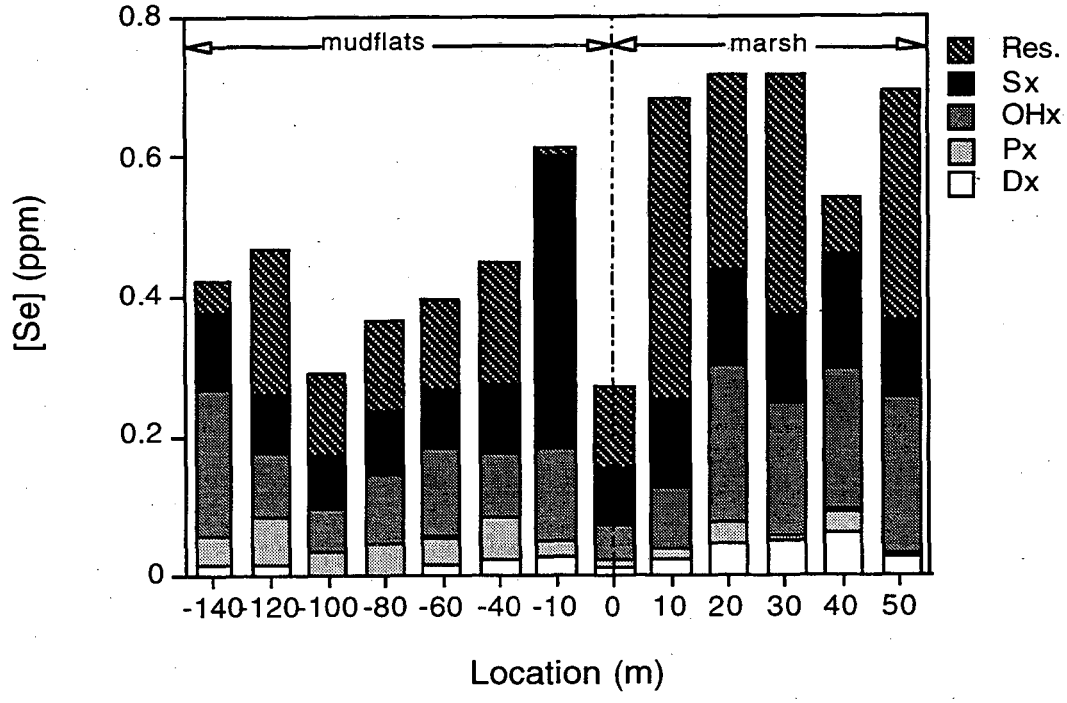

Figure 7.20 Selenium fractionation in MRP intertidal sediments, 10-20 cm depth interval.

7.3 Redox Potential Measurements

Preliminary measurements of redox potential have been made in soils from both sites. The ultimate purpose of Eh measurements is to gain an understanding of Se reduction rates as influenced by anoxic conditions. The initial measurements were performed on sediments collected on 8/8/95, along a transect at site MRP, and at two selected locations near the mudflat/marsh interface at site SHB. Soils at MRP were collected at points (-20 m), (0 m), and (20 m), covering a 40 m transect straddling the edge of the marsh. Given the results presented in the previous two sections, far more extensive sampling is necessary to distinguish meaningful trends, and such sampling will occur in the near future.

Samples were collected using a prototype piston sampler, which allows for accurate sectioning of the sample. Core at point (-20) was subdivided into the following intervals: 0-2.5 cm, 2.5-5.0 cm, 5-10 cm, 10-15 cm, and 15-17.5 cm. Core at point (0) was subdivided into the same intervals, except the deepest interval was 15-20 cm. The core collected in the lower marsh, at (20 m), was only 10 cm deep, and was subdivided into intervals: 0-2.5 cm, 2.5-5.0 cm, and 5-10 cm. The samples were homogenized in a closed freezer bag to reduce contact with air. This was possible because they were moist enough to be almost a slurry. The Eh measurements were made by inserting a combination redox electrode (Orion, model #96-78) into a saturation paste of each sample, made by adding just enough distilled water to make mixing in a small beaker possible. It was found that varying the amount of distilled water did not have a significant effect on Eh values, suggesting that the soil is strongly poised. The clear disadvantage of this method is that the sediment is at least somewhat exposed to air (i.e. oxygen) and the possibility of oxidation exists. While this is not likely to affect Eh measurements made very shortly after sample collection, it is not a preferred approach. In the future, measurements will be made using a platinum wire electrode, inserted into an intact core, still in a plastic sleeve, thereby even further limiting sediment contact with air. Field measurements are also possible.

The results of the measurements, along with Se fractionation, are presented in Fig. 7.21. Se fractionation is not complete, in that the residual fraction is not shown, but based on other results from this site (Fig. 7.19), it comprises between 25% and 50% of

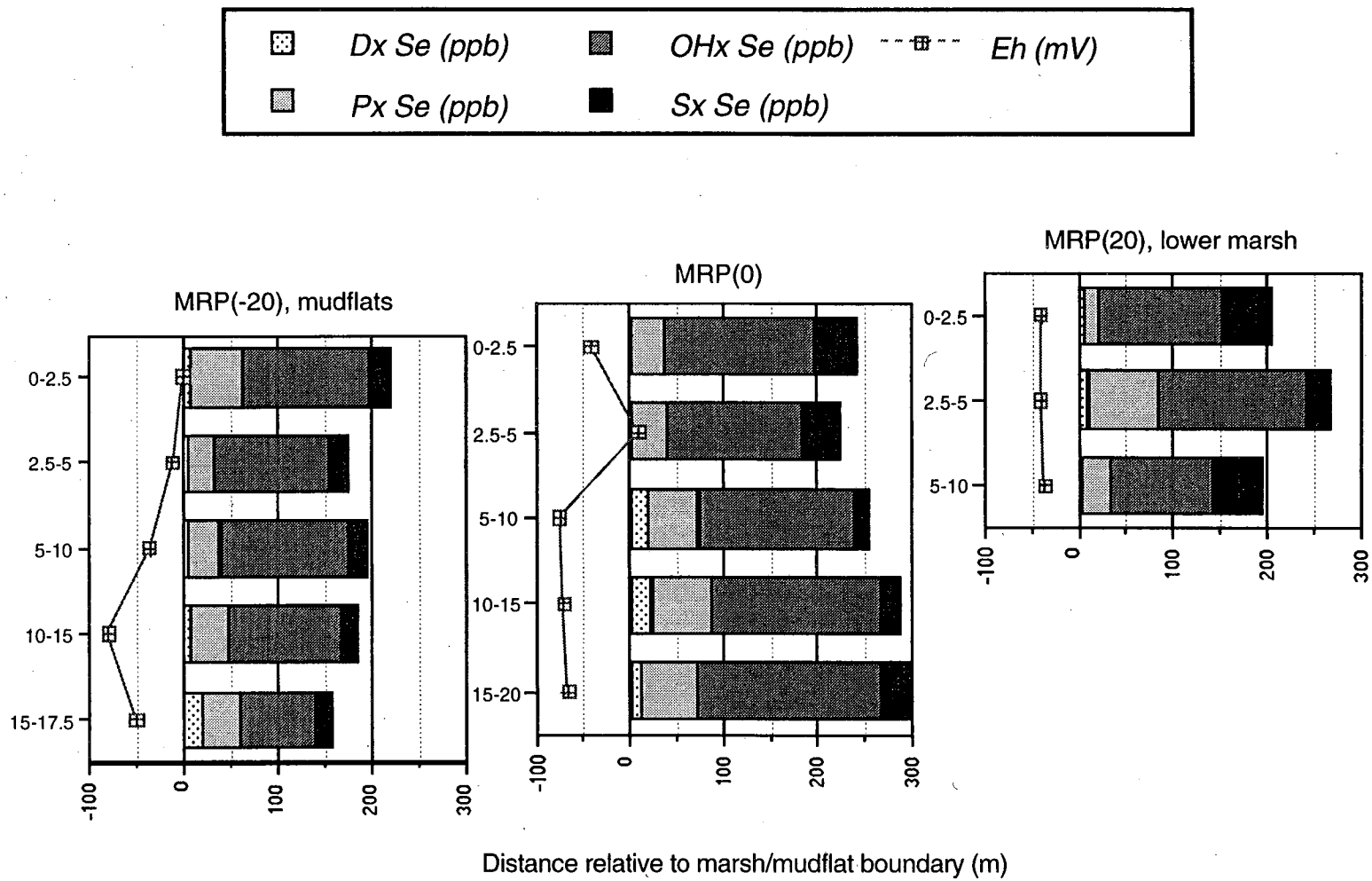

Figure 7.21 Selenium fractionation and oxidation potential of MRP sediments at three sample points along a transect perpendicular to shore.

the sample. There is very little variation in redox potential in these cores. The total range is from -90 mV to +10 mV. This may be one reason why trends are hard to discern. The only trend appears in core (-20), where Eh gradually decreases downward, except at 15-17.5 cm, where an increase is observed. Such a trend is reasonable, assuming that the sediments become more anoxic with depth. However, this trend is not easily discerned in core (0), and no trend is seen in core (20). Also, there are not significant differences between the absolute Eh values in the three cores. One interesting aspect of the Se fractionation is the increase in selenate and selenite with depth. This agrees with other results from the MRP marsh, but not the MRP mudflats (cf. Fig. 7.18-20). There appears to be no correlation between Eh and Se fractionation. Overall, differences in total Se (minus pyrite-Se), with depth or distance are not significant, although the highest concentrations are observed at point (0), or the mudflat/marsh interface.

A far greater number of cores farther inland and in the mudflats needs to be collected. Measurements need to be made both deeper in the profile, and at very fine intervals (sub-cm) near the core surface, in order to define the "oxic" zone. Clearly, in these samples, the oxic zone is not the top 2 cm, as the Eh in all 0-2.5 cm intervals was negative.

8 Summary and Future Work

Although method development was the predominant task over the last year of research, analysis of field samples has revealed interesting information which will lead to further field and laboratory work. The successful adaptation of analytical procedures and the development of novel stable isotope methods will be further utilized over the next twelve months. The variations observed in Se fractionation will need to be confirmed with more extensive sampling and more detail analysis.

8.1 The Selenium Cycle -- A Hypothesis Revisited

Results obtained over the first year of this project suggest that Se cycling in the sediment-water system is controlled to some degree by location relative to the marsh/mudflat interface and depth. The available results make it possible to revisit the preliminary selenium cycling hypothesis posted in Section 2.2.

- Total Se concentrations are higher in the marsh than in the mudflats of both sites, suggesting that in-situ reduction and immobilization of Se is a significant process relative to SPM-Se deposition.

- Adsorbed Se (Px-Se) levels are higher in the mudflats, possibly because of higher pH in the marsh, which would lead to selenite desorption. Strong selenate and selenite reduction is observed in the marsh, with decreasing interstitial Se concentrations (Ix-Se) and increasing organic-Se (OHx-Se) and elemental Se (Sx-Se) farther inland. This agrees with the assumption that Se reduction in the marsh is more rapid than in the mudflats. Why interstitial Se increases so sharply at the mudflat/marsh interface at site SHB is not clear.

- Interstitial Se levels are one order of magnitude higher than Bay water, likely because of the solubilization of adsorbed selenite under higher pH conditions in the marsh. This being the case, there is an upward dissolved Se gradient. Therefore, upward

diffusion of Se should be expected, although the relative significance of this process may be minor.

- It appears that residual Se is a very important fraction in the marsh. This may be because of a more intimate association of Se with organic matter, one which needs to be further investigated. On the other hand, pyrite-Se may indeed be significant in this environment.

- Contrary to the postulated hypothesis, elemental Se does not dominate mudflat sediments. However, most of the Se is reduced and between 25% and 50% is not extractable using the sequential extraction series prior to pyrite-Se extraction, suggesting that pyrite-Se may be an important fraction. Further work is needed to define the Eh-pH regime and deduce its influence on Se speciation. Research on pyrite-Se extractability is ongoing.

- Given the relatively low concentrations of interstitial water Se, it appears that plants in these areas will not accumulate excessively high Se in their tissue. An estimate of 0.5 ppm Se in plants is reasonable, suggesting an average Se accumulation rate of 0.5 mg Se m^{-2} yr^{-1}.

- There appear to be no significant trends in total Se concentrations with depth, although finer intervals near the sediment surface need to be analyzed to accurately measure this gradient. Work in this area is ongoing. Surprisingly, on the limited scale over which measurements were made, Se fractionation does not vary significantly with depth. Again, a more detail look both near the surface and with greater depths may reveal different trends. Finer subdivision of sediment cores may help define the extent of the oxic layer, which may in fact not exist in some of the mudflat sediments, especially those with high clay content, and therefore high moisture retention.

8.2 Future Work

Work over the next four to six months will focus on more comprehensive field surveys, which will include core sampling along several transects, both normal to and parallel to the marsh/mudflat interface. Suspended sediment analysis will be performed, along with the speciation of Se on SPM. Plant sampling and analysis will be performed. A sediment budget for each field site will be established, which will allow an estimation of SPM-Se movement. The results of these measurements will help further shape our notion of the Se cycle. When the Se, Eh, pH, T, and salinity distributions and inter-relationships at each site are well understood, laboratory microcosms will be set up to study in greater detail the dynamics of Se cycling in intertidal sediments.

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Appendix A.-- SOPs for Sediment Extraction

A.1 Interstitial Water Extraction and Sample Homogenization

Wetland soils and intertidal sediments have a high water content in the field, and therefore require de-watering before moisture content can be determined. The following method is used to extract interstitial water and leave sediments and soils at a constant water content.

OBJECTIVE

To remove excess interstitial water, and homogenize the soil sample.

APPARATUS

250 mL centrifuge bottles
High speed centrifuge
Milipore 0.45 µm filter cartridges
60 mL syringes
Metal bowl
Soil chopper

PROCEDURE

Mix sample to homogenize material. Weigh out 300 g of wet soil. Centrifuge at 10,000 rpm for 30 min and remove all of the supernatant solution. Filter the interstitial water using the syringe and 0.45 µm filter apparatus. If suspended sediments persist after initial centrifugation, samples should be centrifuged for additional time. Sediments should be rehomogenized, and pebbles and plant material greater in size than 0.5 cm should be removed.

A subsample of the homogenized soil (10.0 g) should be weighed into a tared soil can and dried at 104.5°C for 24 hr. Moisture content (θ) values are calculated from the oven-dry mass (OD) using the formula:

$$\theta = \frac{M_W}{M_{OD}},$$

where M_W is the mass of water removed from the soil by dessication (M_i - M_{OD}) and M_{OD} is the OD mass of the soil after dessication. θ can be used to determine the mass of residual water in the original homogenized sample, and determine the amount of OD

soil in a specific mass of homogenized soil. To obtain the mass of OD, use the following formula:

$$M_{OD}=\frac{M_i}{(1+\theta)}$$

where M_i is the mass of homogenized moist soil, θ is the water content or theta value, and M_{OD} is the oven-dry mass of soil.

The supernatant solution is saved for Se analysis (selenite, selenite + selenate, and total selenium). Soils are frozen for use in sequential extractions, the final mass of the soil + residual water are recorded in order to correct subsequent sequential extractions (Distilled Water Extraction) for residual water extracted Se in the soils.

A.2 Distilled Water Extraction

OBJECTIVE

To extract free or unadsorbed Se from soils and sediments in wetland and intertidal areas.

APPARATUS

250 mL centrifuge bottles
High speed centrifuge
Milipore 0.45 µm filter cartridges
60 mL syringes
Distilled water
Reciprocating shaker

PROCEDURE

Weigh out 10.00 g (2.0 g) of dewatered-homogenized soil . Add distilled water to the soil at a soil:water ratio of 1:2 (if 2.0 g are used the ratio should be 4:1), accounting for the residual water calculted from the θ measurement. Shake samples on a reciprocating shaker for 1 hr, and centrifuge at 10,000 rpm for 30 min. Filter the supernatant solution. using the syringe and 0.45 µm filter apparatus. If suspended sediments persist after initial centrifugation, samples should be centrifuged for additional time to minimize filtering time and loss of supernatant.

The supernatant solution is saved for Se analysis (selenite, selenite+ selenate, and total selenium). If Se concentrations are too low for efficient quantification, the method of standard additions should be used to improve quantification. Soils are also saved for additional sequential extractions, the final mass of the soil + residual water are recorded in order to correct subsequent sequential extractions (Phosphate Extraction) for residual water extracted Se in the soils.

A.3 Phosphate Extraction

APPARATUS

250 mL centrifuge bottles
Reciprocating shaker
High speed centrifuge
Milipore 0.45 μm filter cartridges
60 mL syringes

REAGENTS

0.001 M Na_2PO_4

PROCEDURE

Usually this procedure is done sequentially after distilled water extraction of the soil. In the event that the procedure is done independently, weigh out the equivalent of 10.00 g of oven dried (OD) soil accounting for moisture contents measured from the OD weight of the soil (see procedure for obtaining θ values). Phosphate solution is added at a 5:1 ratio of solution to OD soil mass. Corrections for soil water are made using the θ values. After phosphate addition, the sample is shaken on the reciprocating shaker for 24 hr. The time the sample is placed on the shaker, and removed should be recorded. Samples are then centrifuged at 10,000 rpm for 30 min. and the supernatant solution filtered using a syringe and 0.45 μm filter apparatus. If suspended sediments persist after initial filtration, samples should be centrifuged for additional time.

The supernatant liquid is saved for Se analysis (selenite, selenite+ selenate, and total selenium). Soils are also saved for additional sequential extractions, final weight of the soil + residual phosphate solution are recorded in order to correct sequential extractions (Sodium Hydroxide Extraction) for residual phosphate extracted Se in the soils.

A.4 Sodium Hydroxide Extraction

APPARATUS

250 mL centrifuge bottles
Reciprocating shaker
High speed centrifuge
Milipore 0.45 μm filter cartridges
60 mL syringes

REAGENTS

0.02 M NaOH (or 0.50 or 1.0 M NaOH)

PROCEDURE

Usually this procedure is done sequentially after distilled water and phosphate extraction of the soil. In the event that the procedure is done independently, weigh out the equivalent of 10.00 g of oven dried (OD) soil accounting for moisture content (see procedure for obtaining θ values). Sodium hydroxide solution (0.02, 0.50, or 1.0 M) is added at a 10:1 ratio of solution to OD soil mass. Corrections for soil water are made using the θ values. The sample is heated in an 85°C bath for 2 h and shaken for 5 min every 30 min. Samples are then centrifuged at 10,000 rpm for up to 30 min or until all sediments have been removed from solution, and the supernatant solution is filtered using a syringe and 0.45 μm filter apparatus.

The supernatant liquid is saved for Se analysis (selenite+ selenate and total selenium). Soils are also saved for additional sequential extractions, final weight of the soil + residual sodium hydroxide solution are recorded in order to correct sequential extractions (Acetate extraction) for residual sodium hydroxide extracted Se in the soils.

A.5 Sodium Hypochlorite Extraction

APPARATUS

250 mL centrifuge bottles
Reciprocating shaker
High speed centrifuge
Milipore 0.45 μm filter cartridges
60 mL syringes

REAGENTS

4-5 % NaOCl (pH adjusted to 9.5)

PROCEDURE

Usually this procedure is done sequentially after distilled water and phosphate extraction of the soil. In the event that the procedure is done independently, weigh out the equivalent of 10.00 g of oven dried (OD) soil accounting for moisture content (see procedure for obtaining θ values). Sodium hypochlorite solution (4-5 %) is added a 1:1 ratio of solution to OD soil mass. Corrections for soil water are made using the theta values. The sample is heated in a boiling water bath for 15 min. during each addition of hypochlorite. Samples are then centrifuged at 10,000 rpm for up to 30 min. or until all sediments have been removed from solution, and the supernatant solution is filtered using a syringe and 0.45 μm filter apparatus. Additional sodium hypochlorite is added and the soil is treated until no visible reaction occurs (up to 5 times). The supernatant is removed and collected after each treatment.

The supernatant liquid is saved for Se analysis (selenite + selenate and total selenium). Soils are also saved for additional sequential extractions, final weight of the soil + residual sodium hydroxide solution are recorded in order to correct sequential extractions (Acetate extraction) for residual sodium hypochlorite extracted Se in the soils.

A.6 Sodium Acetate Extraction

APPARATUS

250 mL centrifuges bottles or 50 mL centrifuge tubes
Reciprocating shaker
High speed centrifuge
Milipore 0.45 μm filter cartridges
60 mL syringes

REAGENTS

1.0 M CH_3COONa (pH adjusted to 5.0 using glacial acetic acid, approx. 60 mL/2L)

PROCEDURE

Usually this procedure is done sequentially after the sodium hydroxide extraction of the soil. In the event that the procedure is done independently, weigh out the equivalent of 10.00 g (2.0 g can be used in 50 mL centrifuge tubes) of oven dried (OD) soil accounting for moisture content (see procedure for obtaining θ values). Acetate solution (1.0 M) is added at a 10:1 ratio of solution to OD soil mass. Corrections for soil water are made using the theta values. After acetate addition the sample is shaken for 5 hr on the reciprocating shaker. Samples are then centrifuged at 10,000 rpm for 30 min and the supernatant solution filtered using a syringe and 0.45 μm filter apparatus.

Samples are then run through a phosphate extraction to remove selenite that may have been readsorbed after release from carbonate minerals. (See section A.3.)

The individual supernatant solutions are analyzed separately for Se (selenite, selenite + selenate, and total selenium). Soils are also saved for additional sequential extractions, final weight of the soil + residual phosphate solution are recorded in order to correct sequential extractions (sulfite extraction) for residual phosphate extracted Se in the soils.

A.7 Sodium Sulfite Extraction

APPARATUS

250 mL centrifuges bottles or 50 mL centrifuge tubes
Sonicating bath
Ultrasonic Probe (with microprobe attachment)
Reciprocating shaker
High speed centrifuge
Milipore 0.45 μm filter cartridges
60 mL syringes

REAGENTS

1.0 M Na_2SO_3 (pH adjusted to 7.0 using concentrated HCl)
Concentrated nitric acid
8 M Urea

4 M HCl

PROCEDURE

Usually this procedure is done sequentially after the acetate or sodium hydroxide extraction of the soil. In the event that the procedure is done independently, weigh out the equivalent of 10.00 g (2.0 g can be used in 50 mL centrifuge tubes) of oven dried (OD) soil accounting for moisture content (see procedure for obtaining θ values). Sulfite solution is added at a 4:1 ratio of solution to OD soil mass. Corrections for soil water are made using the θ values. After sulfite addition the sample is sonicated for one minutes with the Ultrasonic probe (at a setting of 1.5) and then sonicated in the sonicating bath for 1 hr. After sonication the sample is shaken for 12-24 hr on the reciprocating shaker. Samples are then centrifuged at 10,000 rpm for 30 min. and the supernatant solution filtered using a syringe and 0.45 μm filter apparatus. Two washes of sulfite solution are used to rinse out the remaining sulfite-extracted-Se (e.g. 5 mL for 2.0 g samples) with a final rinse of distilled water to remove the residual sulfite solution from the soils prior to drying. The rinses and the extract are combined and digested using nitric acid.

5 mL of extract is added to a 30 mL beaker and 1 mL of concentrated nitric acid is added to the beaker. The beaker is covered with a watch-glass and the solution is heated on a hot plate to reflux for 1 hr (set hot plate temperature to ~95° C). Uncover beakers after 1 hr and allow the sample to evaporate to near-dry. Add 0.5 mL and again allow the sample to reach near dryness before adding an additional 0.5 mL of water. If sample is to be stored for a significant period of time (> 2 days) add 10 mL of 4 M HCl to dissolve the sample, 0.5 mL 8 M urea (to remove residual nitric acid interference in analysis) and bring the volume up to 25 mL in a volumetric flask. If samples will be analyzed immediately (< 2 days), omit the 4 M HCl.

The supernatant solution is saved for Se analysis (total selenium) using an acid boil. Soils are also saved for additional sequential extractions, final weight of the soil + residual phosphate solution are recorded in order to correct sequential extractions (pyrite extraction) for residual phosphate extracted Se in the soils.

A.8 Pyrite-Se Extraction

APPARATUS

LN_2 HGAAS

REAGENTS

1 M $CrCl_3$
Conc. HCl
Zn shot

Procedure:

Samples from the sulfite extract are washed three times, dried, and ball milled in preparation for Pyr-Se analysis. The stripper vessel in the LN_2-HGAAS apparatus is loaded with approximately 0.080 g of soil/sediment with a magnetic stir bar. 15 mL of

distilled water is added to the stripper and the apparatus is reassembled for low-level Se determination. The system is purged for 3 min with He (flow rate of 75 mL min^{-1}) and the trap is then dropped into liquid nitrogen. 10 mL of acidic Cr (II) solution is added (Cr (II) solution is made from 1 M $CrCl_3$ acidified with conc. HCl to which is added Zn shot to reduce the Cr (III) to Cr (II)) to the stripper, and allowed to react for 25 min. After 25 min the trap is removed from the liquid nitrogen and the sample is analyzed on the AAS. The LN_2 HGAAS system is calibrated using a selenite standard and sodium borohydride as the stripping reagent (see Appendix C).

A.9 Total Acid Digestion (TAD) Procedure

Materials:

balance--0.000g
mortar and pestle
Teflon FEP tubes (50ml)
hot plate
aluminum digestion block
sieve (425 micrometer, 35 mesh)
25 ml volumetric flasks
filter, 45 micrometer
Ultrasonic cleaner with rack and tube clips
centrifuge

Chemicals:

conc. HNO_3
30% H_2O_2
6M HCL
8M Urea

Soil Preparation:

1. Air dry for 2 days or oven dry at least 10 grams of soil from a pre-homogenized bulk sample.
2. Crush with a mortar and pestle. Sift through a 425 micrometer sieve.
3. Place 1 gram of soil into a teflon FEP tube.

Digestion Procedure:

1. Add 2.0 ml of concentrated HNO_3 to each tube. Swirl gently.
2. Add 1 ml of H_2O_2 to each tube. If vigorous effervescence occurs, mix and tap gently on the tube to prevent overflow. When the reaction has ceased, add additional hydrogen peroxide to those tubes in which the reaction has occurred(add enough so that the reaction subsides, not to exceed 10 mls).
3. Insert tubes into the preheated digestion block (approximately 90 °C) for a peroid of 24 hours. Ensure that the tube caps are somewhat loosened to avoid excessive pressure build-up.
4. Approximately 3 hours before the 24 hour period ends, i.e., at hour 21, remove the caps from the tubes and evaporate the HNO_3/H_2O_2 mixture from the tubes. AVOID

complete evaporation. When the liquid level has diminished so that the soil is damp, this process is complete. Do not let the soil go completely dry.

Dissolution Method:

1. To each of the tubes, add 15 ml of 6M HCL. Sonicate samples for 3 minutes in a heated bath. Maximum temperature for the bath is 60°C. Return tubes to the digestion block for 24 hours.
2. At the end of the 24 hour period, remove the tubes from the block and centrifuge them at 7000 rpm for 3-5 minutes. Be sure that the caps are tightened before centrifuging.
3. Remove the tubes from the centrifuge and decant the supernatent liquid into a 25 ml volumetric flask.
4. Add 10 ml of 6M HCL to each tube. Sonicate the samples for 3 minutes in a 60°C bath. Put samples back on the digestion block and let them digest for 30 minutes at 90°C.
5. Centrifuge the tubes at 7000 rpm and decant the supernatant solution into 25 ml volumetric flasks.
6. When warm, add 0.5 ml of 8M urea to each volumetric flask.
7. Top-off with distilled water.

Appendix B.-- SOP for Silanizing Glassware or Other Glass Materials

---

1.0 Safety

1.1 Each chemical used in this method must be regarded as potential health hazard and exposure to these compounds should be minimized.
1.2 Make sure that labcoat and safety glasses are worn at all times during this procedure.
1.3 This must be performed in a fume hood.

2.0 Scope and Application

2.1 This method is used to silanize glassware to prevent absorption of arsenic, mercury or selenium onto the glass surface.

3.0 Summary of Method

3.1 A 5% dichlorodimethylsilane in toluene solution is prepared. The glassware is allowed to soak in this solution overnight. The glassware is then washed with toluene and methanol and air dried.

4.0 Sampling and Storage

4.1 Toluene must be stored in a flammables cabinet.
4.2 Dichlorodimethylsilane is stored in a refrigerator.

5.0 Apparatus

5.1 Large photographic tray
5.2 Large beaker

6.0 Reagents

6.1 Dichlorodimethylsilane
6.2 Toluene, reagent grade
6.3 Methanol, reagent grade

7.0 Procedure

7.1 Make 1 liter of 5% dichlorodimethylsilane. (50 mL of dichlorodimethylsilane is added to 950 mL of toluene).
7.2 Place glassware in bath. Make sure all the glass is under the solution. NO AIR BUBBLES!!
7.3 Allow glassware to soak for a minimum of 4 hours prior to use.

7.4 Remove 5% dichlorodimethylsilane solution to a container for later. Rinse glassware with toluene a minimum of two times. Rinse again with methanol two times and air dry.

7.5 Glassware is ready to use once it is completely dry.

8.0 Calculations

8.1 none

9.0 Quality Control

9.1 none

Appendix C. -- SOP for Low Level Selenium Analysis using Gaseous Borohydride by Flame Atomic Absorption

---

1.0 Safety

1.1 Each chemical used in this method must be regarded as potential health hazard and exposure to these compounds should be minimized.
1.2 Make sure that labcoat and safety glasses are worn at all times during this procedure.

2.0 Scope and Application

2.1 This method is used to determine selenium levels (10 ng/L - 400 ng/L) in extracts, fresh water, and seawater.

3.0 Summary of Method

3.1 Samples are prepared following digestion with hydrochloric acid and ammonium persulfate.
3.2 The volatile hydride is transported into an air-acetylene flame heated quartz cell located in the optical path of the atomic absorption spectrometer. The reading of the lamp radiation is directly proportional to the concentration of the selenium.

4.0 Sampling and Storage

4.1 Digestate should be covered at all times to prevent contamination.

5.0 Apparatus

5.1 Peristaltic pump
5.2 Peristaltic pump tubing
5.3 Stripping chamber
5.4 Dewar Flasks
5.5 Cooling coil
5.6 Alltech silanized glasswool
5.7 V-trapping tube
5.8 Syringe needle
5.9 Gas flow regulators
5.10 Atomic absorption spectrometer with Flame burner assembly
5.11 Electrodeless discharge lamp (EDL) Box
5.12 EDL Selenium Lamp
5.13 Integrator

5.14 Printer
5.15 Screw cap test tubes
5.16 Volumetric flasks

6.0 Reagents

6.1 Trace metal grade concentrated hydrochloric acid: 25.0 mL is added to 50.0 mL aliquot of sample.
6.2 ASTM type II grade water
6.3 Reagent grade ammonium persulfate, 2.0% w/w: 2.0 g of ammonium persulfate is dissolved in 100 mL of ASTM type II water.
6.4 Sodium hydroxide, 50% w/w solution
6.5 Sodium borohydride, 0.1% w/w: 0.25 g of $NaBH_4$ plus 1.00 mL of NaOH are dissolved into 250 mL ASTM type II water.
6.6 Certified selenium stock solution, 1000 mg/L
6.7 Acetylene gas
6.8 Helium gas
6.9 2-propanol
6.10 Dry ice
6.11 Liquid nitrogen
6.12 Sulfanilamide, 2% w/v: 2 g of sulfanilamide plus 4 mL of concentrated HCl are dissolved and diluted to 100 mL with ASTM type II water

7.0 Procedure

7.1 Turn on FLAA and use selenium program with wavelength set at 196.0 nm.
7.2 Turn on EDL system and set current to 280 mV and allow 45 min. for warm up.
7.3 Prepare first Dewar, see figure 1, with 2-propanol/Dry Ice bath for water/HCl trap. Add enough 2-propanol to completely cover cooling coils. Make sure temp is at least -20.0 C at all times.
7.4 Prepare second Dewar, see figure 1, with liquid nitrogen (LN2). Do not place the V-Tube in LN2
7.5 Prepare sodium borohydride solution and degas throughout experiment with helium. (Flow rate of 1.5 mL/min.)
7.6 Digest sample using the appropriate method either 3010 for aqueous or 3050 for solids.
7.7 Prepare a working curve using the selenium stock solution. Recommended range of 10 ng/L to 300 ng/L.
7.8 Set integrator using: ATT 2 = 7.0, CHT SP = 3.0, PK WD = 0.04, THRSH = 7, and AR REJ = 0. Use peak area for quanitation.
7.9 Place a 50 mL aliquot of digestate into stripping chamber.
7.10 For seawater samples add 1.0 mL of 2% sulfanilamide solution to remove nitrite interferences.

7.0 Procedure

7.11 Mix sample thoroughly and allow to stand for 4 minutes minimum.
7.12 Add 25.0 mL of hydrochloric acid to sample and purge sample until all carbon dioxide has been removed
7.13 Place V-Tube in LN2 and top off Dewar with LN2.
7.14 Place syringe in stripper inlet and add sodium borohydride at a rate of 2.0 mL/min.

7.15 Continue adding sodium borohydride for 4 minutes then stop peristaltic pump and remove syringe.
7.16 Allow system to continue stripping for 3 min. and then start integration.
7.17 After 30 seconds, remove V-Tube, and look for selenium peak at 0.80 min. (± 0.1 min.) It is crucial that the V-Tube not come into contact with anything outside of the LN2 trap. If it touches anything a split peak will occur.
7.18 Dispose of waste and wash stripper and bubbler with ASTM type II water.
7.19 Repeat steps 7.9 through 7.17 for additional samples.

8.0 Calculations

8.1 Concentrations are based on the results when interpolated from the calibration curve.

9.0 Quality Control

9.1 One method blank per 20%
9.2 One duplicate sample per 20%
9.3 One matrix spike per 20%
9.4 One matrix spike duplicate per 20%
9.5 CCB and CCV every 10 samples
9.6 CCV must be within 15% of calculated value.
9.7 Spike recovery must be ± 20% for drinking waters
9.8 Spike recovery must be ± 25% for seawater and waste waters
9.9 Duplicates must be ± 20% RPD for values > 2X CRDL
9.10 One laboratory control sample must be run per batch
9.11 Laboratory control sample must.be within manufacturer's limits of acceptance.

Appendix D. -- Quality Assurance and Control

The staff of the LBNL Earth Science Division (ESD) has been performing quality-controlled selenium analyses since 1987. The quality assurance program was established as a contract requirement with the U.S. Bureau of Reclamation and it has proven convenient to apply this program to all subsequent studies. Selenium analyses are performed by the staff of the Environmental Measurements Laboratory (EML). Previous work was aimed at quantification of Se at ppb to ppm levels. Such levels are also found in extracts of sediments collected for this study. Parts-per-trillion levels found in Bay waters are analyzed for using a cold-trap method described in Chapter V, Scetion 2. Quality control procedures described below apply generically to all Se analyses.

D.1 Analytic Technique

Se analyses are performed on a Perkin-Elmer 3030 Atomic Absorption Spectrophotometer with a Varian Hydride Generator (AAS-HG). Selenite is analyzed by introducing the sample directly into the hydride generator. Total selenium is prepared for analysis by mixing 5.0 ml of a sample with an equal volume of concentrated (~37%) hydrochloric acid and between 0.2 and 0.5 ml of a 2% ammonium persulfate solution to oxidize any organic selenium compounds and other potentially interfering organic compounds. The mixture is heated at 98° C for 10 minutes to reduce all selenate to selenite, then allowed to cool and is introduced into the hydride generator for reading. The values reported to investigators are selenite and total selenium concentrations. Selenate concentration may be calculated from these values but is itself not directly subject to quality control because it is a derived quantity.

D.2 The Quality Control Process

Investigators submit sets of samples typically numbering from 40 to 80 samples, with empty containers dispersed throughout for blind quality control samples. The Quality Control Manager prepares the various types of QC samples and inserts them in the set, then passes the set on to the analyst. After analysis is complete the QC manager evaluates the QC results and advises the investigator, the analyst and the EML Manager of the control status of the set and designates the reanalysis brackets, if needed. Reanalyses are performed at the option of the investigator but analyses not in control are not to be used in any report or publication released outside of LBNL.

D.2.1 Types and Frequencies of Quality Control Samples

Two levels of quality control exist. The first is operational quality control, performed by the analyst in real time, to monitor the performance of the AAS-HG. This consists of the analyst running a continuing calibration check after each ten research samples and, in addition, a blank, a duplicate and, when meaningful, a post-spiked duplicate after each twenty research samples. When an operational QC check fails, the analyst makes any necessary adjustments to the instruments and repeats the previous 10 (or 20) analyses.

The second level of QC involves running blind QC samples to monitor the performance of both instrument and analyst. These amount to approximately 14% of the total research sample load and consist of blanks, reference standards, duplicates and spiked duplicates, refered to after this as spikes. The minimum number of blind QC samples for a set of samples submitted is one of each of these four classes for a total of four. When the number of research samples in a set is larger, the additional blind QC samples consist of standards, duplicates and spikes in as nearly equal numbers as possible. On the rare occassions when a set requires seventeen or more blind QC samples, two blanks are included. When unequal numbers of these samples are required, preference as to the nature of the most frequent is given to spikes followed by standards and then duplicates. This policy is a reflection of our experience that matrix interference is the most common cause of out-of-control results, followed by the need to recalibrate, erratic hydride generator performance and finally contamination.

D.2.2 Preparation of Quality Control Samples

Blanks

Blanks are prepared from quartz distilled water from a Barnstead Nanopure still by simply putting it in a sample container.

Reference Standards

The oxidation state (speciation) of Se in a water sample is of interest. Therefore, both selenite and total selenium analyses are performed. To meet this need, reference standards containing both selenite and selenate have been established. A liter each of two stock solutions are prepared with total selenium concentrations approximately 600 ppb and 2700 ppb, respectively. The ratio of selenite to total selenium has ranged from 34% to 51%. The actual concentrations of these solutions are established by analyses over a period of months. The low level stock is used at 4 different dilutions and the high level stock at 2 dilutions. Dilutions are chosen to check detection and quantification limits for both selenite and total selenium as well as gage error over a wide range of concentrations. Newly formulated stock is used to prepare only a third of all reference standards until a sufficient number of meaurements have been made, typically 5 to 7 at each dilution, to provide a preliminary estimate of the stock concentration. At this point, the newly formulated stock is used to prepare half to two thirds of all reference standards until 10 to 12 measurements have been made at each dilution. Thereafter, the new stock is used exclusively for reference standards but the old stock continues to be used for spiking.

Duplicates

Duplicates are prepared by simply decanting a portion of a research sample into a QC sample bottle. Samples are chosen for duplicate analysis from samples of adequate volume near the center of the bracket between the QC duplicate and the next QC sample.

Spiked Duplicates

Samples for spiked duplicate analysis are chosen according to the same criteria as duplicates. In addition, the investigator requesting the analysis is asked to give an estimate of the likely selenium concentration so that the spike may be as close to the

initial concentration as possible. If no estimate is available, samples are spiked at a variety of concentrations. Depending on the volume of sample available a portion of the sample is decanted into a 10 ml or 25 ml volumetric flask so as not to fill the flask. A known volume of a well-characterized reference standard stock solution is pipetted into the volumetric flask and the solution is made up to volume with more of the sample.

D.2.3 Calculation of Quality Control Measures

Blanks

The instrument limit of detection (IDL) has been determined by analyzing a series of very dilute selenite solutions. This limit is 0.15 ppb selenium. Blank values for selenite have been accumulated and the geometric mean determined, with the conversion of all values less than 0.15 ppb to 0.15 for this purpose. The selenite limit of detection is the same as the IDL. The total selenium limit of detection is 0.30 ppb because the solutions have been diluted by a factor of 2. The mathematically determined total selenium limit of detection is somewhat less. The limits of quantification are calculated by multiplying the mean by two times the anti-log of the standard deviation of the average log values. This is the equivalent of adding two standard deviations to the mean.

Standards

Measured values for various reference standards are accumulated and mean and standard deviation are calculated. Values for reference standards which are more than two standard deviations from the mean are outside the warning limit. A reference standard analysis outside the warning limit in a set is not out of control but may result in a discussion of the the analytic process. Values for reference standards which are more than three standard deviations from the mean are outside the control limit and mandate reanalysis.

Duplicates

Duplicate quality for selenium concentrations above the limit of quantification is determined by the relative difference which is calculated according to the following formula as percent:

$$RD = 200\left(\frac{(C_1 - C_2)}{(C_1 + C_2)}\right)$$

where C_1 and C_2 are the duplicate measures of selenium concentrations. Relative differences between 80% and 120% are in control. Below the limit of quantification, both values must be either between the limit of quantification and the limit of detection, or below the limit of detection.

Spiked Duplicates

Spike recoveries are calculated by the following formula as percent:

$$R = 100(C_s)\left(C_f - C_i\left(\frac{V_f - V_s}{V_f}\right)\right)$$

where C_f is the concentration of the spiked solution
C_i is the concentration of the unspiked solution
V_f is the total spiked sample volume
V_s is the spike volume
C_s is the concentration of the spiking solution

Recoveries between 80% and 120% are in control. When the spike concentration amounts to less than 25% of the initial selenium concentration the result is considered to be not statistically meaningful and is treated as if the result were in control, regardless of the calculated recovery.

D.3 Current Measures of Quality

Data for blanks and reference standards shown in the following section includes all samples run, including those from programs other than the Bay Selenium Project. Data for spiked duplicate, duplicates and completion come only from Bay samples.

Blanks

The limit of quantification is also the control limit. For total selenium this is currently 0.48 ppb and for selenite it is 0.40 ppb. The limit of detection is also the warning limit. For total selenium this is currently 0.30 ppb and for selenite it is 0.15 ppb. Our experience has been that out-of-control total selenium blanks are caused by cross-contamination due to errors in sample preparation rather than contaminated reagents. An example of this is point 72 on Figure D.1. The total selenium reported was actually twenty times greater than shown on the chart but was compressed to keep the chart within a reasonable scale. This extremely high value was probably due to a slip in the sequence of samples during preparation. It was a very good duplicate of the next sample in sequence.

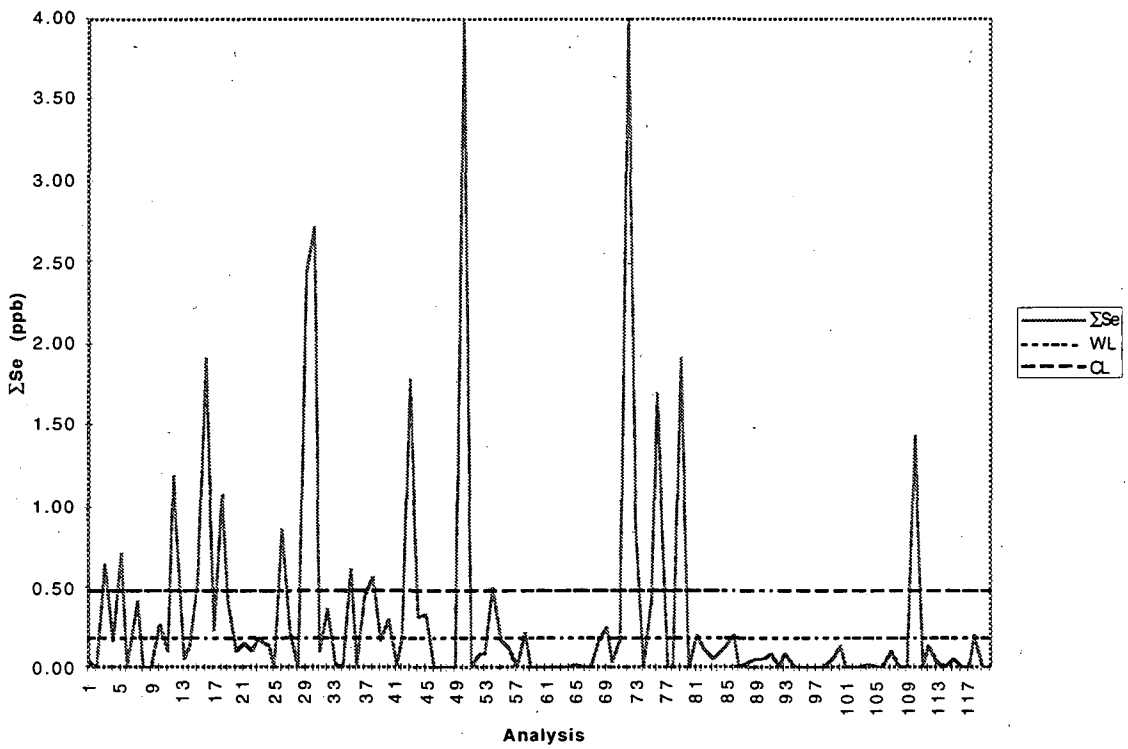

Figure D.1. Total selenium concentrations in QC blanks. Includes data from other projects.

Selenite blanks have been considerably less of a problem with only two above the warning limit as shown in Figure D.2. This is probably due to the fact that for selenite analysis the solution is fed directly into the instrument from the sample container.

Reference Standard Measurements

For reference standard analyses, two standard deviations from the mean is considered the warning limit and three standard deviations is the control limit. Relative deviation as a function of selenium concentration is derived from statistics on reference standard analyses and provides a means of gaging the error of any measurement, as shown in Figure D.3 and Figure D.4.

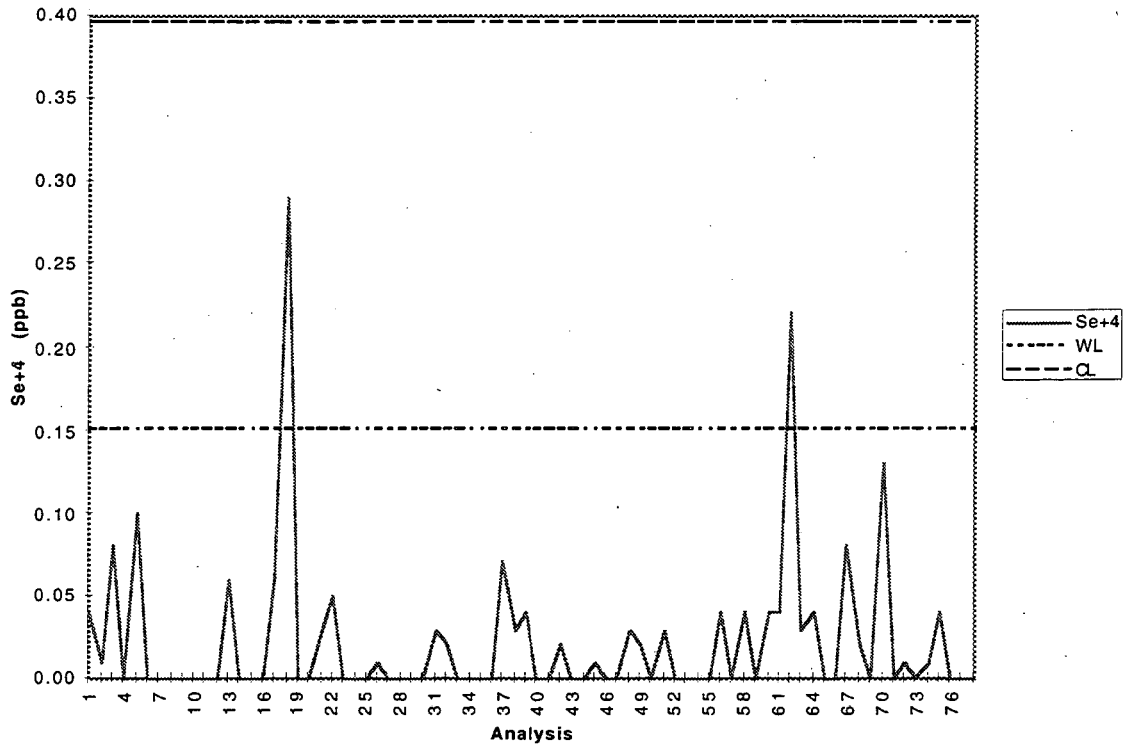

Figure D.2. Selenite concentrations in QC blanks. Includes data from other projects.

Spike Recoveries

A total of 117 spiked sample analyses, both for selenite and total selenium were performed, with 17 done as reanalyses. Of all 117 analyses, 60 were in control, 52 were out of control and 5 were not statistically significant because the spike amounted to less than 25% of the initial concentration. Of those in control, 6 were reanalyses. Of those out of control 11 were reanalyses which remained out of control, probably due to intractable matrix problems.

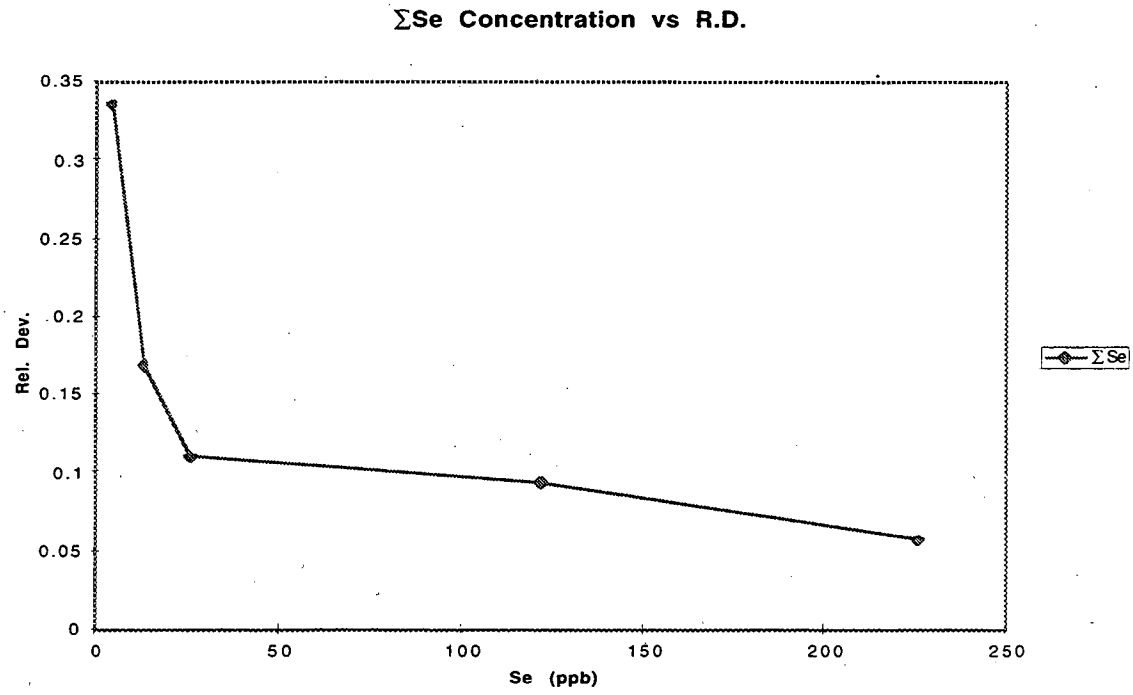

Figure D.3. Relative deviation vs. total selenium concentrations in QC standards. Includes data from other projects.

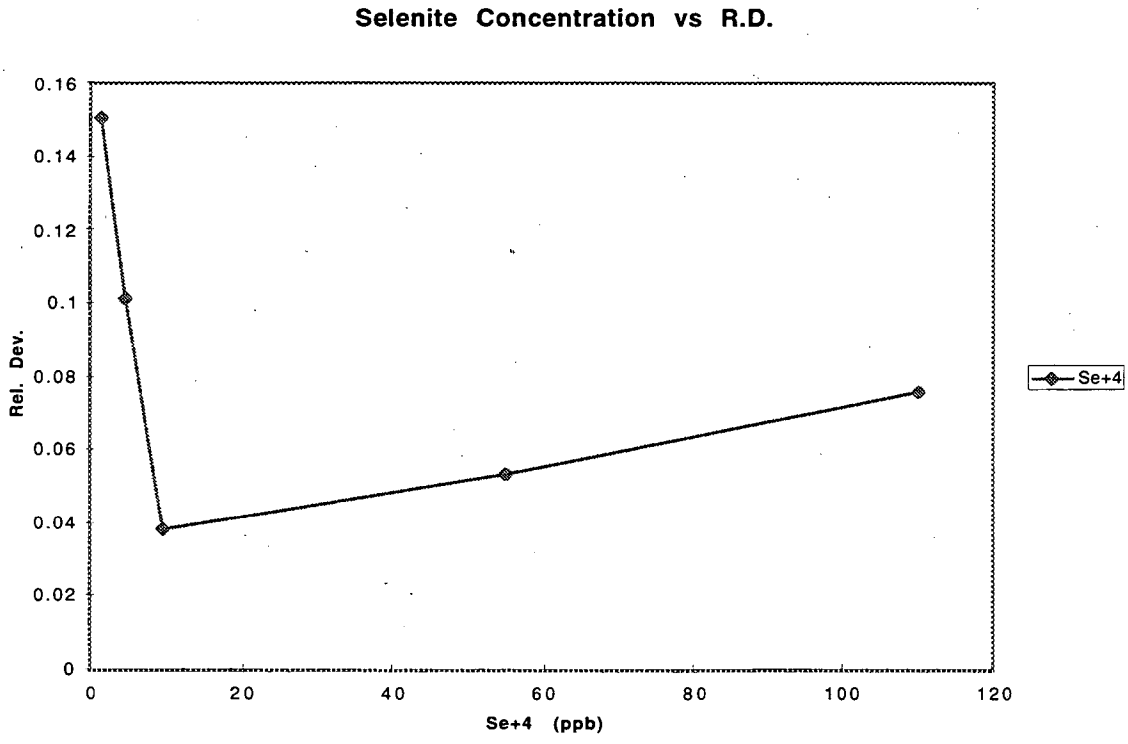

Figure D.4. Relative deviation vs. selenite concentrations in QC standards. Includes data from other projects.

Duplicates

A total of 78 duplicate analyses, both for selenite and total selenium were performed, with 11 done as reanalyses. Of all 79 analyses, 48 were in control, 21 were out of control and 10 had relative differences which were not statistically meaningful because both measurements were between the limit of quantification and the limit of detection or both were below the limit of detection. Of those in control, 8 were reanalyses. Of those out of control, 2 were reanalyses that remained out of control. One of the reanalyses was not statistically meaningful. This arose because, of the initial duplicates one was below the detection limit while the other was between detection and quantification limits. On reanalysis both concentration were between detection and quantification limits.

Completion

To date the Bay Selenium Project requested total selenium analysis for 2106 samples and EML completed 2085 for a completion rate of 99.0%. Selenite analysis requests numbered 993 and EML completed 843 of these for a completion rate of 84.9%. This low figure for selenite analysis is due entirely to matrix problems. Completed analyses include those both in and out of control.

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