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Levels of metals from salt marsh plants from Southern California, USA

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Levels of Metals from Salt Marsh Plants from Southern California, USA

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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2009

The Thesis of Kimberly Ann Hoyt is approved and it is acceptable in quality and form for publication on micro film and electronically:

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ABSTRACT OF THE THESIS

Levels of Metals from Salt Marsh Plants from Southern California, USA

by

Kimberly Ann Hoyt

Master of Science in Biology

University of California, San Diego, 2009

Michael I. Latz, Chair

Elsa Cleland, Co-Chair

Salt marshes in Southern California are surrounded by urban development and therefore subjected to various levels of anthropogenic disturbances. In the San Diego area urban development increases in density from north to south, which would imply an impact on individual marshes. The objective of the field component of this study was to investigate the level of metal contamination in plants and associated with sediment surrounding plant roots in four salt marshes. Generally, metals associated with sediment showed a decreasing concentration gradient from northern to southern marsh. In contrast, plant metal concentration showed a reverse trend with an increasing gradient from northern to southern marsh. Increasing organic matter and decreasing grain size of sediment, which affect bioavailability of metals, appeared to be the main factors controlling such trend rather than the density of urbanization. Samples were collected in the summers of 2007-08, and winter of 2008. Winter season showed a 4.5x increase in metal content in plants compared to summer; however, metals associated with sediment increased 2.5x in winter. These results suggest that metal accumulation occurred mainly from dissolved metals in seawater in the winter. The laboratory objective was to test the rate of metal uptake by plants by a dose response of copper bioaccumulation experiment in aquaria using one of three local marsh plants. Metals were taken up by plant roots then subsequentially transported throughout the plant tissues. The linkage to marsh ecosystems for development of effective management strategies in biomonitoring programs for environmental quality assessment will be discussed.

Keywords: Contamination; Metal contamination; Salt marsh; marsh plants; metal; *Batis maritima* (saltwort); *Spartina foliosa* (cordgrass); *Sarcocornia pacifica* (pickleweed); pollution; San Diego

Introduction

Salt marshes are a complex environment situated in the transition zone between the land and sea (Levin et al., 2001). They therefore form a unique ecosystem with critical functions including nutrient cycling, production of organic material, physical protection from storms, and serving as nurseries for many marine fishes and birds (Faghorazzi et al., 2004). Salt marshes contain a variety of plants that are essential in sustaining these various functions. These plants, however, are vulnerable to various threats from habitat destruction to excessive chemical contamination.

In southern California, urban development has dramatically reduced and hydrologically altered salt marshes along the coastline. Estuaries and lagoons have been dredged to form shipping harbors and recreational water parks, or filled for commercial development. Watersheds associated with the marshes have been paved over and developed into dense residential and industrial areas. These changes to the watershed increase the delivery of contaminants into coastal waters. Contaminants, including metals, can adversely affect biological function (Lemly, 1997).

Potential non-point sources of metal contaminants include: 1) antifouling paints; 2) re-suspension of sediment through dredging activity; 3) atmospheric deposition; and 4) stormwater runoff. Metals can enter the seawater from fuel discharges from boats which contain elements like lead, and antifouling paints of their hull, which usually contain high concentrations of copper and tin (Schiff et al., 2004). These metals continuously leach in surrounding waters to protect the hull from settlement of fouling invertebrates. Another source is dredging of contaminated seafloor sediment, which re-suspends contaminants in the sediment, reintroducing historical metal contaminates that flow into the salt marshes through tidal flushing (Gosselink, J., 1980). Besides these potential sources, stormwater is considered the major source of metal contamination reaching ambient waters, increasing contaminant loads with the density of urbanization in the surrounding watershed (Davis et al., 2001; Sabin et al., 2005; Tiefenthaler et al., 2007). Many metals are attached to particulate matter in the water column (Reboreda et al., 2006) and carried by stormwater to ambient receiving waters where these particles tend to settle out in sediment (Tiefenthaler et al., 2007). Characterization of the contaminant source from stormwater has shown various land-use types have greater concentrations of metals (Sanger et al., 1999; Tiefenthaler et al., 2007; Davis et al., 2001).

Increased non-point source of metals has also been attributed to atmospheric deposition (Sabin et al., 2005; Sabin et al., 2006). Metal concentrations associated with sediment in tidal creeks have increased levels of anthropogenic contaminants with increased development in the watershed (Sanger et al., 1999). For metal loading in stormwater from 8 types of land use in the watersheds of the Los Angeles area, the greatest concentrations of metals in stormwater come from industrial land-use sites (Tiefenthaler et al., 2007). Contaminant loading depends on building and automobile density, with specific examples being building siding and roofs; automobiles brakes, tires, oil leakage; and wet and dry atmospheric deposition (Davis et al., 2001). Southern California has a semi-arid climate; contaminants tend to build up on the impervious surface of the watershed before being washed out in stormwater.

Because salt marsh vegetation slows the velocity of water, allowing deposition of suspended matter, retention and filtering of contaminants occur in salt marshes (Reboreda et al., 2006; Leonard et al., 1995; Gidley, 1993). Salt marches can act as a sink, sequestering metals in sediment, or a source, releasing metals back into the environment (Burke et al., 2000; Kraus et al., 1987; Kraus et al., 1988; Windham et al., 2002; Weis et al., 2003; Weis et al., 2004). Physical parameters that affect the mobility of metals in salt marsh sediment include anoxic sediment, pH, salinity, and inundation from tidal flows. Salt marsh sediment is an anoxic zone, metals can be bound to sulfides, immobilizing in a reduced state (Pardue et al., 1995; Reboreda et al., 2007). However, plants can oxygenate the sediment by transporting oxygen to the root zone, thereby remobilizing metal contaminants (Weis et al., 2004). A decrease in pH can acidify sediment surface and change metal speciation and solubility (Reddy et al., 1977; Wright et al., 1999). A salinity increase results in many metals precipitating out of solution, increasing deposition. Increased periods of inundation can affect physical factors of redox potential, pH, and salinity of salt marsh sediment, therefore affecting metal mobility with salt marsh sediment.

Salt marsh plant species generally have similar metal uptake patterns (Windham et al., 2002; Kraus et al., 1987). Some species have been shown to primarily retain metals in belowground biomass, while some species redistribute metals to aboveground biomass, especially the leaf (Kraus et al., 1987; Kraus et al., 1988; Burke et al., 2000). In *Spartina* sp., metals are excreted from leaf salt glands (Burke et al., 2000; Kraus et al., 1988). Plant litter, especially leaves, may become enriched in metals by cation absorption and particle incorporation, thereby entering the food web through detritus feeders and decomposers, releasing metals to re-enter sediment or be exported with tidal flushing (Windham et al., 2002; Kraus et al., 1986; Cabador et al., 2009).

Studies in San Francisco tidal salt marshes assessed anthropogenic contribution of contaminants by means of an enrichment factor, the metal concentration above natural inputs, in surface sediment samples, finding Pb to be the impacted metal of all marshes with an enrichment factor ranging from EF=8 to EF=49 (Hwang et al., 2006). Studies in San Diego Bay on the complexation of metals, most specifically Cu, have found that different forms, Cu associated with particulates in the water column and dissolved Cu^{2+} form in seawater, influence the bioavailability to invertebrates in the larval stage (Rosen et al., 2008). Under controlled experimental condition with seawater originating from San Diego Bay Rosen et al. (2008) assessed toxicity level to mussel and sea urchin larval stages by in seawater. This study showed that dissolved metals were present in ambient waters as a dissolved ionic form that is bioavailable. Though San Diego Bay has an increasing gradient of metals associated with sediment from the mouth to the back of the bay, concentration of dissolved metals in seawater showed an inverse trend throughout the bay (Deheyn and Latz, 2006). In the study by Deheyn and Latz (2006), the bioavailability of 15 metals was assessed using a benthic invertebrate (brittlestar) as a model. The arms of the brittlestar accumulated metals from the water column at greater concentrations at the mouth of the bay, showing increased bioavailability by means of absorption of dissolved metals in ambient water. However, ingested metals in the disk were at greater concentrations in the back of the bay. Deheyn and Latz (2006) showed that difference bioavailability of metals could be attributed to route of accumulation, uptake or absorbance, which was than reflected in the tissues. Interestingly the bioavailability of metals in ambient waters was contrary to the common thoughts. Indeed, the mouth of the bay appears clean, yet containing the most bioavailable metals, while the back of the bay, which is known for its brown water, showed the least amount of bioavailability. This emphasizes that particles and organic matter suspended in the water can greatly influence the metal uptake in organisms. Many studies on Cu speciation have been done in San Diego Bay. Blake et al. (2004) found free Cu ions at the mouth of the bay at 10^{-11} and 10^{-13} mg kg^{-1} and decline moving towards the back of the bay while total Cu increases in a gradient from the mouth to the back of the bay. There was also an observed increase in Cu concentration during the winter season two weeks after a rainfall (Blake et al., 2004). Cu ions, thus clearly appear to be bioavailable by absorption whereas, the bound Cu form appears to be ingested by organisms.

Non-point sources of Cu and other metal contaminants are partially due to harbor related activities. Vessel related contamination to coastal water from wastewater discharge and fuel leakages (Young et al., 1979). In particular, Cu has been attributed to antifouling paints on vessel hull. Schiff et al. (2004) measured dissolved Cu from passive leaching of antifouling paints on recreational boats to contribute 3.7 and 4.3 $\mu g/cm^2$/day. While Cu emission from antifouling paint during hull cleaning was attributed to Cu flux (Valkirs et al., 1994), measurement of 8.6 μg dissolved copper/cm^2/event contributes approximately 5% from hull cleaning as opposed to 95% from passive leaching from antifouling paints on vessel (Schiff et al., 2004). Urban wastewater and industrial effluent are a large factor in contributing metal contaminant to marine waters (Brown et al., 2001). Metals adhere to the suspended matter that is discharged into the coastal waters. Studies of metals associated in sediment and porewater included grain size evaluation and total organic carbon found an 85% toxicity level to amphipods in San Diego Bay (Fairey et al., 1998).

Metals associated with suspended matter can flow onto salt marshes where vegetations decreases water flow and increases deposition. Uptake of metals by plants to aboveground biomass can reintroduce these metals into the food web. The importance of salt marsh plants to such metal cycling was demonstrated with stable isotope analysis of the food web in coastal waters (Kwak et al., 1997). *Spartina* and macroaglae provide organic matter in the form of suspended matter that supports invertebrates and fish, which cycle up the food web to birds. The dynamics of the linkage between marine systems is partially due to the coastal water exchange and flow of water currents carrying suspended matter.

This study provides information about metal concentrations in salt marshes in southern California, USA. It compares of metals associated with sediment from four salt marshes representing a gradients of urban development in their watersheds. Comparison of plant species from different marsh zones to assessed environmental factors on tidal inundation that can influence exposure to various contaminant loads. Metal concentrations in plant tissues (root, stem, and leaf) to enhance information on metal fate and transport within the marsh ecosystem. Evaluation of seasonal influences in the semi-arid mild climate of southern California to provide information on stormwater and of the processes contaminant loads reaching local salt marshes.

San Diego County, Southern California, has an increasing gradient of urban development in the watersheds associated with local salt marshes from the north to the south. It is well known that increase development in the watershed increases metal contaminant loading in stormwater. Data presented in Chapter I discusses the field component were the examination of concentrations of metals associated with sediment from four salt marshes representing an increasing gradient of urban development in their watersheds. Along with sediment, salt marsh plants are essential for the function of this ecosystem. Salt marsh plants have shown a high tolerance for stressful physical and chemical conditions. The ability of vegetation to withstand extremely high salinity and contaminant levels that salt marshes in southern California endure, lead to the interest in understanding the fate and transport of metals in salt marsh plants and tissues. Within each individual salt marsh, a comparison of plants from the low marsh zone and the marsh plain was expected to show differences metal concentration. Variation in physical and chemical stresses from inundation resulting in greater exposure to contaminants was expected to show increase metal concentrations in leaf tissue. Increases in sediment salinity from evaporation was expected to show increases metal concentration in belowground biomass or associated with sediment. In order to provide comprehensive information on the annual cycling of metals in the salt marsh, a sampling period of one and half years was established. Winter season salt marshes were expected to have increase concentration of metals associated with sediment from contaminant stormwater inputs. Increases of metals in the sediment would then be transported to aboveground biomass with uptake of nutrients during the summer growing season. However, to understand the impact of metal contaminates in seawater and the mechanism of salt marsh plants in the process of filtering these contaminates from seawater, a laboratory component was needed.

Chapter II data will discuss the laboratory component were under controlled conditions with known metal contaminate concentration the rate plants uptake metals from the sediment could be calculated. Knowing the difficulty of laboratory experiment reflecting all the environmental parameters that are present in the field careful consideration was done to minimize these variables. Metal concentrations were used that reflected concentrations in the seawater of San Diego Bay. The objective of this component of the study was to provide information on the physiological rate of metal uptake and allocation of metals in plant tissue.

The discussion will provides a baseline and comprehensive information on the mechanism and processes of metal contamination in salt marsh ecosystem of southern California. A comparison of contaminant loading at four salt marshes with various levels of urban development in watershed will be considered. Environmental factors such as grain size and organic matter content that can influence how much of the metals present in the environment are biological availability to salt marsh plants will be discussed. This study examined seasonal variation and possible uptake of dissolved metals by salt marsh plant tissues. In the context of salt marsh plants important role in metal recycling within the ecosystem, discussion of using primarily plant leaf tissue as a biomonitoring tool for coastal managers information of the impact of metal contaminants in stormwater in the arid west climate.

Conclusions will be followed by a general discussion on the this list of objectives: (1) urban density is a factor affecting metal concentration associated with sediment of salt marshes in southern California; (2) assess the amount of anthropogenic contamination after categorization of salt marsh based on enrichment factor; (3) determine differences in bioavailability of metals is responsible for levels of metals found in salt marsh plants; (4) evaluate metal concentrations of plant species from different marsh zones; (5) differences in metal uptake in plants during winter season; (6) salt marsh plants are an important component of metal recycling; (7) salt marshes can act as a monitoring tool for metal bioavailability.

Chapter I

Bioavailability of metals to common plants from salt marshes in Southern California, USA

Abstract

Urban development in the watersheds has lead to increasing metal contaminants in stormwater draining into salt marshes. This study examined levels of metals content in plant tissues and associated with sediment from salt marshes along the Southern California coast. Four salt marshes were investigated, each exposed to various environmental constraints and inputs of contaminants from differences in the density of surrounding urban and industrial development. Three species of plants (*Batis maritima, Spartina foliosa,* and *Sarcocornia pacifica)* were analyzed for fifteen known anthropogenic metal contaminants (Ag, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, Sr, V, Ti, Zn). Overall, the level of metals found in plants did not necessarily reflect differences in surrounding urbanization or corresponding load of metals associated with sediment. Sediment showed enrichment of metal content with increasing organic matter, and decreasing grain size; these appeared to be the main factors controlling the bioavailability of metals accumulated in plants rather than the actual metal concentration associated with sediment. The results showed a positive correlation in the order of metals ranked by increasing concentration between sediment and plant leaf tissue. While no difference in metal concentrations was observed among leaf, stem and root tissues in the summer samples, winter samples showed a 4.5x increase of metal concentrations only in root tissue and a 2.5x increase in sediment in winter compared to summer. Greater increases in root tissue compared to sediment suggests metal accumulation occurred through dissolved metals in seawater in the winter, entering the

roots first before spreading to the rest of the plant. Accordingly, identifying the amount of metal contamination can be determined by using one tissue, the leaf, in the summer, for environmental quality assessment and monitoring level of metal contaminations in salt marshes.

I.1. Introduction

Southern California possesses numerous salt marshes along its coast, most of them being exposed to and influenced by major metropolitan and urban development. San Diego, in particular, is a major industrial port city surrounded by a high population density that poses a potential contamination source to surrounding coastal waters. As with other industrial ports, San Diego marine waters and coastal sediment are subjected to various hazardous contaminants (Alberts, et al., 1990; Chadwick, et al., 2004; Deheyn et al., 2006; Kraus, et al., 1986; SanudoWilhelmy, et al., 1996). Sources of contamination include watershed runoff, agricultural inputs, antifouling boat paints, fuel discharge in marinas, canneries, industry, shipyards, and sewage overflows (Burke, et al., 2000; Deheyn et al., 2006; Hwang, et al., 2006; Levin, et al., 2001; Pednekar et al., 2005). Inputs of contaminate from changes in rainfall and fires alter input of organic matter and sediment to estuaries and coastal waters. Contaminants entering ambient receiving waters contain high concentrations of metals that can be toxic to the environment.

The importance of metal contaminants in salt marsh and coastline habitat results from the cycling patterns of trace metals that occur there (Katz et al., 1981). Metals adsorb to particulate and suspended matter as they pass through the water column. Metals from different sources have different leachability that depends on the nature of the material (Katz et al., 1981) Metals from anthropogenic or biogenic sources can be released from particulates or suspended matter in the water column with a weak acid treatment. Metals occurring as natural sediment or rock formation are released with a complete dissolution of inorganic matter. Data has shown that transfer of metals to ocean and atmosphere by human activity exceeds natural inputs (Katz et al., 1981).

Sediment quality criteria have developed interpretive tools to assess adverse biological effects for Ag, As, Cd, Cr, Cd, Pb, Ni, and Zn beginning at a concentration of 0.6 mg kg^{-1} (Long et al., 1995; MacDonald et al., 1996). Other anthropogenic metal contaminants from industrial and manufacturing uses include Al, Fe, Mn, Se, Sr, Ti, and V. The contamination of metals on invertebrates in the marine environment around San Diego is well studied (Middaugh, et al., 1993; Corbisier, et al., 1996; Tabak, et al., 2003; Chadwick, et al., 2004) In particular, Deheyn and Latz (2006) showed that metal accumulation in brittlestars occurred similarly throughout the bay even though the back of the bay had higher concentrations of metals associated with sediment (0.155mg g^{-1} Cu), indicating bioavailability was greater at the mouth of the bay where the levels of contamination were lower (0.0026mg g^{-1} Cu). However, the bioavailability of metals in the marine waters and sediment to marsh plants in southern California is poorly understood. These marshes are continuously and intensely exposed to hazardous contaminants. Today only a small portion of the original salt marshes in southern California remain due to excessive human population growth and urban development.

Salt marshes, by their location at the interface between land and the ocean, can be considered to act as sediment traps, thus filtering and retaining the contaminants that are carried by the sediment (Levin, et al., 2001). A majority of metals have a reactive behavior with solids, being adsorbed onto particles in the water column, transported, and eventually deposited in marsh sediment (Hwang, et al., 2005; Reboreda et al., 2007; SanudoWilhelmy, et al., 1996; Yamashita et al., 2008). Chemistry of the trapped sediment is then subject to change with variation in the physio-chemical properties of the water and/or sediment, possibly remobilizing the adsorbed metals into the surrounding environment (Reboreda, et al., 2008). However, the biological availability of the metal enriched sediment to the marsh fauna and flora remains relatively unexplored.

Physical processes including elevation, landscape location to tidal creeks and bayward edge, topography, and inundation control salt marsh habitat. Differences in frequencies of inundation support distinct vegetated zones with particular plant species. On the bayward edge plant species are frequently inundated for 9 hours a day and typically support *Spartina foliosa,* a cordgrass that has salt excreting glands on the leaves (Zedler, 1977). Mid and upper marsh habitat can be inundated from one to two hours daily or a few days a month on extremely high tides. In Southern California the mid and upper marsh vegetation support several species, in particular for this study, *Batis maritima,* saltwort, and *Sarcocornia pacifica,* pickleweed, which retain water in leaves. Salt marsh plants play an important role in nutrient cycling. Plants influence sediment by first removing nutrients from soil while alive, and then releasing them in the form of detritus (Weis, et al., 2002; Windham, et al., 2003). Metals can also be part of such recycling process, as plants can absorb and transport metals to aboveground tissues while alive, releasing them back to the environment in association with detritus. The mechanism of metal uptake depends on the mobility and availability of the metal in the sediment and grain size that is determined by a combination of factors including pH, concentration of organic matter (OM), salinity, and redox potential (Reboreda, et al., 2008). However, upon accumulation, some of the metals can induce toxicity and may possibly be lethal. Toxicity to marsh plants can modify the recycling process described above and possibly change the vegetation structure and thus impact the ecosystem biodiversity sustainability (Boyer, et al., 2001; Boyer, et al., 1998; Brinson, et al., 1996; Chapman, et al., 2006). This process of nutrient recycling sustains the biodiversity of fish nurseries, invertebrates, and avian communities in the marsh ecosystem. There are socio-economical and ecological reasons in preserving the biodiversity of salt marshes, from the ecosystem inherent ability to improve water quality to serving as a nursery for game fish and birds, and a habitat and breeding ground for threatened species (Prasad et al., 2003). Also, salt marshes situated in the midst of dense urbanization are used by migrating birds to rest and feed (Ambrose et al., 2002).

The bioavailability of metals to salt marsh flora in southern California is poorly known. In this study measurements of metals in sediment and common plants from four salt marshes surrounded by different levels of urban density and industrial development, used as a possible proxy for possible metals contamination source. We tested the following hypotheses (1) the metal concentration in salt marshes reflect the amount of surrounding urban development, (2) salt excreting plants have lower metal tissue concentration than non-salt excreting plants, and (3) there is a seasonal difference in sediment and plant metal content due to rainwater input.

I.2. Methods

This study was performed in August 2007 through August 2008 in urban salt marshes distributed along the Southern California coastline. The following four marshes were investigated (Fig. I.1): C_1. Los Peñasquitos Lagoon (LP), C_2. Kendall-Frost Reserve (KF), C_3. Sweetwater Marsh National Wildlife Refuge (SW), and C_4. Tijuana River Estuary (TJ).

I.2.1 Study Sites

Los Peñasquitos Lagoon (LP) is an estuary system located within Torrey Pines State Reserve between La Jolla and Del Mar, California (Fig. I.1). LP is the most northern site of this study, and though surrounded by housing development, this lagoon is isolated, closed to human access, and considered undisturbed. The lagoon consists of channel habitat that is fed by two creeks from Carmel Creek watershed on the east and Los Peñasquitos watershed on the southeast; the lagoon has been periodically closed to tidal flushing (Cole & Wahl, 2000; Greer & Stow, 2003; Nordby & Zedler, 1991). This watershed has an approximate population size of 400,000 and encompasses approximately 160 km^2 of drainage area that run off into the lagoon (Greer et al., 2003). LP is subjected to variable rainfall, stream flow, and some anthropogenic disturbances to sedimentation from dredging and wastewater inflows (Nordby et al., 1991).

Kendall-Frost Reserve (KF) is a salt marsh estuary located in the northeastern corner of Mission Bay that lies by the mouth of San Diego River and north of San Diego Bay (Fig. 1.1). KF is surrounded by suburban development yet has limited human access. Historically, Mission Bay consisted of a natural salt marsh estuary with intertidal flats, shallow water habitats, tidal channel and a small central bay (Fairey, et al., 1998; Zedler, 1996). KF watershed has an approximate population size of 100,000 and encompasses 58 km^2 along Rose Creek watershed (http://www.projectcleanwater.org). Intense urban development and canalization of the river has changed Mission Bay into a highly dredged lagoon that has reduced the salt marsh estuary to 15% of its traditional size (Zedler, 1996). Freshwater inputs are primarily limited to storm water runoffs from the streets, and Rose Creek Watershed stream discharges directly into the bay.

Sweetwater Marsh National Wildlife Refuge (SW) is a salt marsh in a metropolitan area located in the lower-mid southeastern portion of San Diego Bay (Fig.

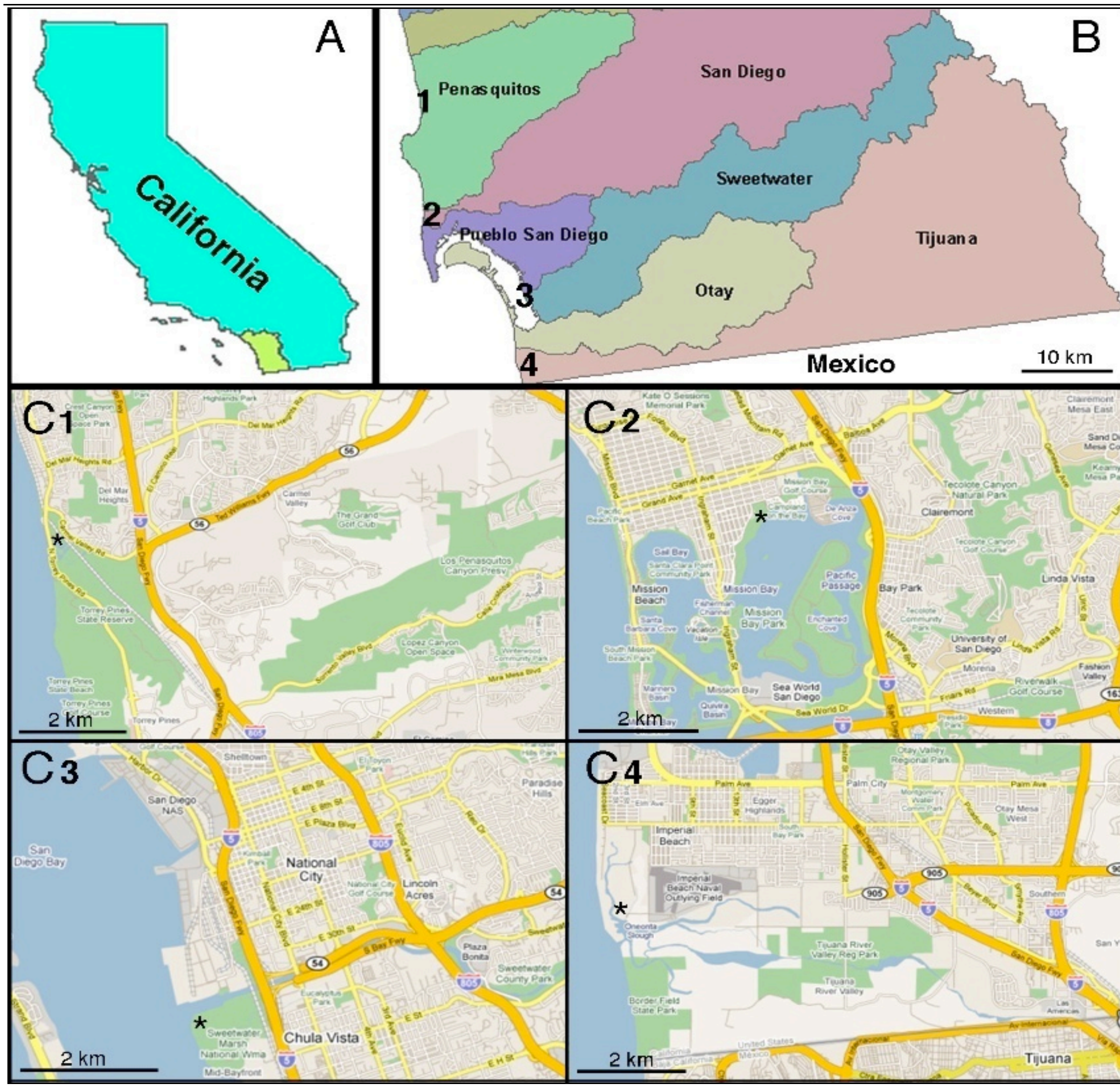

Fig. I.1. Location of the sampling sites in each of the salt marsh investigated in Southern California. A. Map of California showing in highlight location of the salt marshes. B. Close-up of the salt marshes location with description of watershed size feeding each marsh (1 thru 4). C1-4: Salt marsh from north to south are 1. Los Peñasquitos Lagoon (LP), 2. Kendall-Frost Reserve (KF), 3. San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW), and 4. Tijuana River Estuary (TJ). Asterisks represent sampling site.

I.1). San Diego Bay is a large urban and military harbor (Nichols, 1988) that receives large contamination loads from multiple human activities, past and present, such as sewage runoff, untreated industrial discharge from aircraft manufacturing plants, shipyards, recreational boating with antifouling contaminates in paint, transportation activities, fuel combustion, shipyards, and the spillage from many marinas (Burke, et al., 2000; Deheyn & Latz, 2006; Weis & Weis, 2004). Contaminants remain in the sediment from past contamination including from fish canneries, kelp processing facilities, and poor circulation in the marina and harbor areas (Deheyn & Latz, 2006; Schiff, et al., 2004). SW watershed has an approximate population size of 300,000 and consists of 370 km^2 from Sweetwater River Watershed, 97 km^2 from Pueblo San Diego Watershed with a population size of 500,000, and 267 km^2 from Otay River Watershed with a population size of 150,000 (http://www.projectcleanwater.org). San Diego Bay has been subjected to routine sampling and monitoring to assess the hazards of water quality to residents and wildlife. These find that dissolved copper ion concentrations of 1.7mg l^{-1} seawater often exceed the US EPA's water quality in marinas (Deheyn et al., 2006; Fairey, et al., 1998; Schiff, et al., 2004).

Tijuana River Estuary (TJ) is an intertidal estuary found along the California-Mexico border just south of San Diego Bay (Fig. I.1). TJ is the most southern site, surrounded by a dense urban development and industry located in the US side of the estuary, but also very extended in the Mexican side. The TJ Watershed encompasses approximately 2816 km^2 on either side of the California-Baja California border with an estimated population size of 1 million (http://www.projectcleanwater.org). TJ has been altered substantially by human activity resulting in poor water quality mainly influenced by wastewater flow from heavy urbanized and industrialized runoffs from the Tijuana area in Mexico (Fairey, et al., 1998; Ward, et al., 2003).

Overall, the four salt marshes encompass a range of exposure to urban and industrial development, which increase gradually from north to south implying an increasing gradient of contamination from north to south.

I.2.2 Sample collection and preparation

For each salt marsh, triplicate independent samples of sediment and plants were collected from three locations considered representative of the intertidal zone, with each species within a 2 m^2 plot, representative between the mean low-tide line (MLTL) and the mean high-tide line (MHTL). Sampling was completed on foot, at low tide based on NOAA tide prediction model (http://co-ops.nos.noaa.gov). Collection dates where in August 2007, 2008 and February 2008.

Salt marsh plants

The three most common plants were collected at each salt marsh and consisted of *Batis maritima* (saltwort), *Spartina foliosa* (cordgrass), and *Sarcocornia pacifica* (pickleweed). Triplicate samples of entire individual plants were collected close to each other (within a 2 m^2 area) and placed in a separate Ziplock® bag, placed in a cooler, and immediately transported to the laboratory to avoid any plant desiccation. In the laboratory, each plant was washed with filtered seawater and divided by tissue system (roots and rhizomes, stems, and leaves). Each put in separate containers and oven dried to a consistent weight. The dried plant samples were then ground to a powder by hand using a marble mortar and pestle. Each sample was then sub-sampled in triplicate for elemental analysis.

Salt marsh sediment

Sediment adjacent to each plant collected, was also collected using a 50 ml polypropylene Falcon® tube. The tube was used as a mini-corer, and the sediment collected included, on average, the top 5 cm. As done for the plants, the sediment samples were oven dried, homogenized with a mortar and pestle, and subsampled for further analysis.

All manipulations during collection and samples preparation were done under controlled conditions to avoid metal contamination, using metal free solutions, nitric acid washed containers, and disposable polypropylene or high-density polyethylene supplies, including forceps and tubes. Trace Metal Grade nitric acid (Fisher Scientific) was used in sample preparation with concentrations $<10^{-7}$ mg/g for each metal, thus they have a negligible effect on sample metal concentration (Deheyn, et al., 2005; Deheyn & Latz, 2006).

I.2.3 Metal analysis

A total of fifteen metals (Ag, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sr, Ti, V, Zn) were simultaneously analyzed using an Induced Coupled Plasma Atomic Emission Spectrum (ICP-AES) spectrometer (Optima 3000, Perkin Elmer), available at the Scripps Institution of Oceanography Analytical Facility. Calibration of the instrument was done before every run by dilution of a 100 ppm Multi-Element Instrument Calibration Standard solution (Fisher Scientific). Samples were run by marsh for plants, and separately for all marshes for sediment, in order to ensure running samples of similar matrix and range of metals concentration.

All methods and protocols were modifications from Deheyn and Latz (2006), which focused on sediment and invertebrates. For plant tissues each sample was fully digested in 0.5 mL of 70% nitric acid solution (Fisher Scientific) at 100°C for 40 min using an Ethos EZ Microwave Digestor (Milestone). The concentrated digest was then diluted (by weight) to 5% nitric acid using MilliQ water (Barnstead) and directly used for the ICP analysis.

Sediment samples were subject to a similar digestion and dilution process, yet using a mild 45% nitric acid digestion in order to assess the leachable and bioavailable fraction of metals associated with the sediment and not the metals constitutive of the geological matrix (Deheyn & Latz, 2006). The sediment in acid solution was microwave digested at 80°C for 20 min, and the resulting solution was then diluted (by weight) to 5% nitric acid using MilliQ water for ICP analysis.

I.2.4 Bioconcentration factor

The bioconcentration factor (BCF) was used to represent the concentration of metals present in plant tissues relative to that associated with the sediment, thus representing the bioavailable fraction of metals that plants have taken up from the sediment over time (Deheyn & Latz, 2006). BCF was calculated as a ratio of the mean concentration values of plant leaf to mean concentration values associated with sediment at each site, and for each plant species.

I.2.5 Sediment properties analysis

Sediment for samples collected in August 2007 was also processed for the mud to sand ratio of the particle size characteristics, following the protocol of Talley et al. (2001). In summary, sub-samples of the sediment (100 g) were treated with 25 mL of 35% H_2O_2 for 48 h for dissolution of the organic material, and sieved to separate the < 63 mm (mud) and ≥ 63 mm (sand) fractions.

Sediment samples were also processed for carbon and nitrogen content at the UC Davis analytical facility, using a PDZ Europa ANCA-GSI elemental analyzer with combustion set at 1020 °C.

I.2.6 Statistical analysis

Analysis of variance (ANOVA) with post-hoc pairwise comparison, non-parametric student t-test, and Spearman Rank Correlation analysis were used to test significance of the differences in metal concentrations observed among marshes for sediment and plants, for each marsh among plant species, and for each plant among plant tissues. Statistical analyses were done using JMP® 7.0 and Statview® 5.0 software (SAS Institute, Inc), with significance set at α=0.05. Unless indicated, each value represents the mean with one standard error.

I.3 Results

I.3.1 Sediment characteristics

Sediment characteristics in terms of percent carbon and mud to sand ratio were significantly different among the four marshes ($P<0.01$), while the nitrogen concentration showed no significant differences across marshes ($P>0.60$). The carbon concentration was the greatest at Kendall-Frost (KF), followed by Sweetwater (SW), Tijuana Estuary (TJ), and Los Peñasquitos (LP) (Fig. I.2). The mud to sand ratio followed the same trend with, however, KF having a much greater ratio value for mud then LP, SW, or TJ, indicative of a mud-dominant environment, LP had the lowest mud to sand ratio value, indicative of a sand-dominant environment (Fig. I.2).

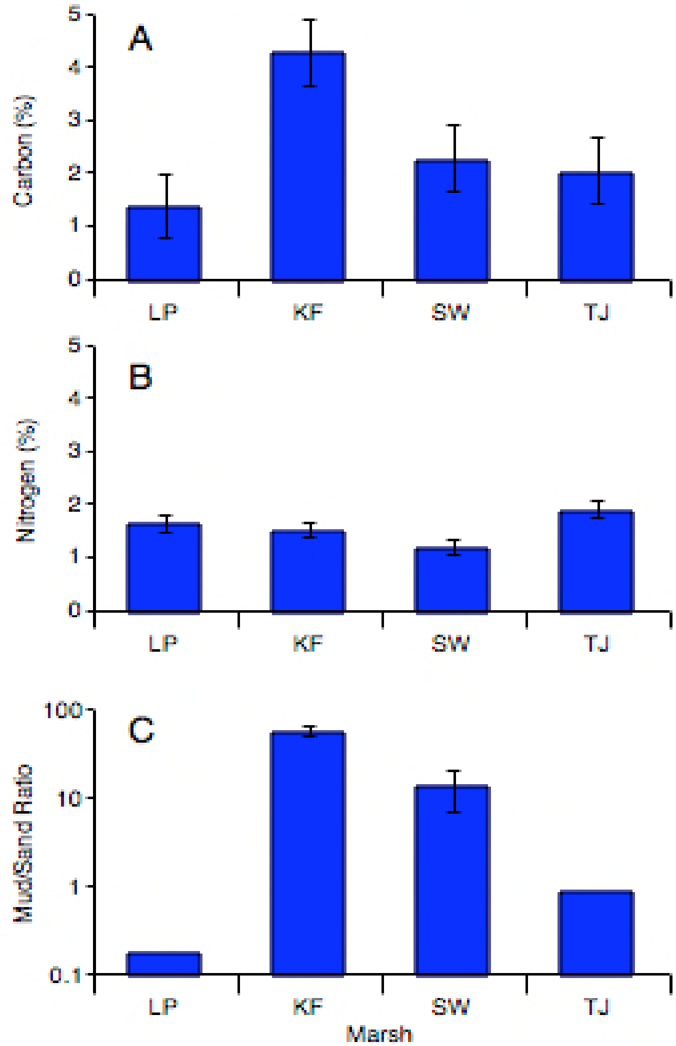

Fig. I.2. Sediment properties characteristics between four salt marshes: Los Peñasquitos Lagoon (LP), Kendall Frost Reserve (KF), San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW), and Tijuana River Estuary (TJ). (A) Percent carbon content, (B) percent nitrogen content, (C) mud to sand ratio.

I.3.2 Metal concentrations associated with sediment

Metal concentrations from samples collected in August 2008 were different among elements within the same marsh, and the order of metals ranked by decreasing concentration was as follow: Fe>Al>Mn>Sr>Ti>Pb>V>Cr>As>Ag>Se for LP; Al>Fe>Mn

>Sr>Ti>Pb>Zn>V>As>Cu>Cr>Ag>Se>Zn>Cd for KF; Fe>Al>Mn>Zn>Ti>Sr>Cu>V>Pb> Cr> Cd>As>Ag>Se for SW: and Fe>Al>Ti>Mn>Zn>Sr>Pb>V>Cu>C d>Cr>As>Ag>Se for TJ (Table I.1). In all cases, Al and Fe were the most concentrated metals while Ni was always below detection limits. No other metals were found below detection limits, except for Cu in sediment samples from Los Peñasquitos (Table I.1).

Table I.1. Metal concentration (Mean $\pm$ Standard Error (SE); in mg kg^{-1}) associated with sediment from four marshes: Los Peñasquitos Lagoon (LP), Kendall-Frost Reserve (KF), San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW), and Tijuana River Estuary (TJ). All samples were measured in triplicates. Collection date August 2007.

	LP		KF		SW		TJ	
Metal	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ag	1.98	0.24	0.62	0.32	2.71	0.40	2.58	0.07
Al	3,517.2	439.	5,674.20	699.	2,723.30	135.	2,212.70	193.
As	3.86	0.62	17.37	3.88	3.37	0.44	3.04	0.31
Cd	.99*	<0.0	0.37	0.12	5.72	0.48	3.78	0.35
Cr	0.99	0.62	9.65	1.05	5.75	0.34	3.06	0.34
Cu	BDL	–	16.29	0.39	23.95	0.97	5.36	0.55
Fe	5,658.0	676.	2,593.60	499.	5,479.00	170.	3,694.60	244.
Mn	95.20	6.26	186.70	59.6	98.43	10.3	50.24	3.65
Ni	BDL	–	BDL	–	BDL	–	BDL	–
Pb	20.03	2.41	70.73	6.34	13.41	0.69	14.98	0.78
Se	0.67	0.06	0.49	0.06	0.65	0.11	0.92	0.19
Sr	43.81	6.47	164.61	34.4	47.69	2.03	37.12	1.04
Ti	31.00	4.29	133.06	11.0	67.01	4.66	52.67	8.50
V	18.60	1.44	54.16	4.00	17.66	0.73	12.70	0.66
Zn	5.99*	<0.0	67.46	1.84	83.71	1.05	45.04	2.32

BDL: Below Detection Limit
–: Not Available
*: (x10^{-3})

Order of the ranking was highly preserved from one marsh to the other, as indicated by the significant correlation among the ranking order from the different marshes. Indeed, the correlation factor rho ranged from a min of ρ=0.67 (P<0.01) between LP and SW, to a max of ρ=0.97 (P<0.01) between SW and TJ.

In general, the concentration of metals associated with sediment was different from one marsh to another, and usually greater for KF and SW, and lower for TJ and LP (Table I.1). For example, KF showed the greatest concentrations for Al, As, Cr, Mn, Pb, Sr, Ti and V while these elements were among the least concentrated in TJ. Similarly, SW showed the greatest concentrations for most of the remaining elements, such as Ag, Cd, Cu, and Zn, most of these elements were among the least concentrated in LP (Table I.1).

I.3.3 Metal concentration in plant leaves across marshes

Metal concentration in plant leaves was significantly among marshes (P<0.05). In general, the highest concentrations for all metals were found in samples from SW for Ag, Al, As, Cr, Cu, Mn, Se, Ti, V, and Zn, the lowest in samples from LP for As, Cr, Fe, Pb, Sr and below detection limit BDL for As, Cr, Ti and Zn (Table I.2). Leaf metal concentrations also differed with plant species and showed the highest values were in *Batis maritima* > *Sarcocornia pacifica* at LP (P<0.05; F=3.25). This ranking could be different across marshes and at KF, *S. pacifica* had the greatest metal concentrations (P<0.04; F=2.34), with the exception of Sr, Se, Ni, which were higher in *B. maritima* and Mn, Ti, Cd which were greater in *Spartina foliosa* (P<0.01; F=8.91). At SW, *S. foliosa* had the highest leaf metal concentration (P<0.07; F=3.74), with the exception of As, Sr which were greater in *B. maritima* and Cr, Fe, Al, Ti, Ni which were

Table I.2. Metal concentration (Mean ± Standard Error (SE); in mg kg^{-1}) from leaf samples of three plant species (*Batis maritima, Spartina foliosa, Sarcocornia pacifica*) collected from four marshes: Los Peñasquitos Lagoon (LP), Kendall-Frost Reserve (KF), San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW) and Tijuana River Estuary (TJ). All samples in triplicates except for those indicated with asterisks (N=2). Date of collection August 2007.

		LP		KF		SW		TJ	
Metal	Species	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ag	*B. maritima*	3.46	0.15	5.59	0.19	8.91	0.65	5.86	0.38
($x10^0$)	*S. foliosa*	-	-	5.06	0.33	9.88	1.87	9.42	2.48
	S. pacifica	2.83	0.21	6.82	0.54	8.71	0.66	5.22	0.26
Al	*B. maritima*	30.56	4.76	7.57	2.87	13.09	0.15	34.97	1.47
($x10^0$)	*S. foliosa*	-	-	11.16	2.44	23.33	3.19	21.10	2.04
	S. pacifica	<0.01	<0.01	51.67	8.91	32.87	4.69	38.39	3.90
As	*B. maritima*	BDL	-	BDL	-	4,761.93	2,749.14	<0.01	<0.01
($x10^{-3}$)	*S. foliosa*	-	-	BDL	-	932.23	332.34	0.29	0.13
	S. pacifica	BDL	-	1,778.72	1,026.94	1,735.13	868.42	BDL	-
Cd	*B. maritima*	10,443.00	1,435.00	0.26	0.24	0.07	0.04	0.36	0.15
($x10^{-4}$)	*S. foliosa*	-	-	0.49	0.14*	432.94	249.90*	2,822.65	162.91
	S. pacifica	6,197.00	548.00	0.40	0.16	BDL	-	2.28	1.32
Cr	*B. maritima*	BDL	-	2.00	1.00	2.00	1.00	BDL	-
($x10^{-4}$)	*S. foliosa*	-	-	0.21	0.01	4,163.00	2,591.00	1.10	1.00
	S. pacifica	BDL	-	2.18	0.97	5,070.95	1,834.09	BDL	-
Cu	*B. maritima*	2.50	0.50	BDL	-	BDL	-	BDL	-
($x10^{-3}$)	*S. foliosa*	-	-	BDL	-	2.70	1.70	BDL	-
	S. pacifica	2.30	0.70	BDL	-	2.50	0.80	BDL	-
Fe	*B. maritima*	85.93	11.57	243.77	70.03	111.42	59.49	208.93	28.41
($x10^0$)	*S. foliosa*	-	-	389.62	75.94	422.83	49.22	116.99	60.03
	S. pacifica	24.98	3.41	401.40	238.33	497.09	113.71	158.83	45.80

BDL: Below Detection Limit - : Not Available

Table I.2. Metal concentration continued (Mean ± Standard Error (SE); in mg kg^{-1}) from leaf samples of three plant species (*Batis maritima, Spartina foliosa, Sarcocornia pacifica*) collected from four marshes: Los Peñasquitos Lagoon (LP), Kendall-Frost Reserve (KF), San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW) and Tijuana River Estuary (TJ). All samples in triplicates except for those indicated with asterisks (N=2). Date of collection August 2007.

		LP		KF		SW		TJ	
Metal	**Species**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Mn	*B. maritima*	11.58	0.99	8.81	0.96	32.94	17.83	4.95	0.77
($x10^0$)	*S. foliosa*	-	-	28.96	1.88	56.89	4.97	48.34	8.07
	S. pacifica	4.72	0.64	8.21	2.64	24.84	2.43	4.19	0.72
Ni	*B. maritima*	470.40	253.10	0.85	0.49*	0.60	0.10	0.41	0.24
($x10^{-3}$)	*S. foliosa*	-	-	BDL	-	0.60	0.20	0.20	0.11
	S. pacifica	407.00	250.50	BDL	-	0.70	0.30	BDL	-
Pb	*B. maritima*	65.15	19.55	69.58	36.39	BDL	-	0.20	0.12
($x10^{-2}$)	*S. foliosa*	-	-	786.41	454.04*	265.85	153.49*	BDL	-
	S. pacifica	BDL	-	787.24	454.52*	BDL	-	0.03	0.02
Se	*B. maritima*	12.30	3.98*	6.39	0.73	2.80	1.24	BDL	-
($x10^0$)	*S. foliosa*	-	-	2.69	1.67	104.86	3.52*	5.21	3.74*
	S. pacifica	0.65	0.12	3.31	1.15	BDL	-	10.57	3.74*
Sr	*B. maritima*	79.11	4.73*	216.06	22.45	108.02	52.49	199.90	13.26
($x10^0$)	*S. foliosa*	-	-	122.27	5.05	34.95	2.03	62.17	20.27
	S. pacifica	54.05	4.07	102.45	36.05	60.48	7.87	62.28	8.26
Ti	*B. maritima*	BDL	-	1.47	0.77	3.27	1.90	0.88	0.51*
($x10^0$)	*S. foliosa*	-	-	5.98	0.84	16.61	5.27	12.86	7.18*
	S. pacifica	BDL	-	5.96	3.42	22.07	4.56	2.05	0.72
V	*B. maritima*	82.82	54.37	71.05	38.83	266.15	152.86*	0.90	0.06
($x10^{-2}$)	*S. foliosa*	-	-	209.29	32.83	423.24	129.14	0.23	0.07
	S. pacifica	80.54	43.82	446.68	204.51*	153.57	25.96	0.70	0.08
Zn	*B. maritima*	BDL	-	BDL	-	0.02	0.02	BDL	-
($x10^{-2}$)	*S. foliosa*	-	-	BDL	-	2,574.80	138.39	BDL	-
	S. pacifica	BDL	-	BDL	-	1,809.50	140.65	BDL	-

BDL: Below Detection Limit - : Not Available greater in *S. pacifica* (P<0.03; F=5.58). At TJ, *S. foliosa* had the highest concentration (P<0.21; F ratio 1.74), with the exception of Fe, Sr, V, Ni, Pb, which were higher in *B. maritima*, and Al, Se that were higher in *S. pacifica* (P<0.07; F=3.59) (Table I.2).

I.3.4 Metal concentrations in plant tissues

Overall, for each metal during August 2007 collection, plant species and marsh, there was no significant difference in metal concentration between the different plant tissues considered, namely the root, stem and leaf (ANOVA: P>0.05). There were a few exceptions, which occurred 17x at KF, 11x at LP, 7x at SW, and 10x at TJ. Of these exceptions among the marshes, 19 were for *Batis,* 16 for *Sarcocornia,* and 10 for *Spartina,* that accounted for 45 out of the 540 tests completed. For these exceptions the trend was that metal concentration was greater in root > stem > leaf.

No plant species dominated with a consistently greater metal concentration among the marshes. In each marsh and plant species, different metals showed different concentrations within each plant tissue, and when ranked by decreasing value of metal concentration, the metals showed a ranking order that was directly correlated from one plant tissue to the other (0.77<ρ <0.91; P < 0.01).

I.3.5 Correlation between metal concentrations in plants and sediment

In order to optimize data presentation 6 metals (Cd, Cr, Cu, Mn, Pb, and Zn) that represent the major trends among metals are presented (Fig. I.3). For collection date August 2007, showing metal concentrations in plant tissues from the four salt marshes and metals associated with sediment.

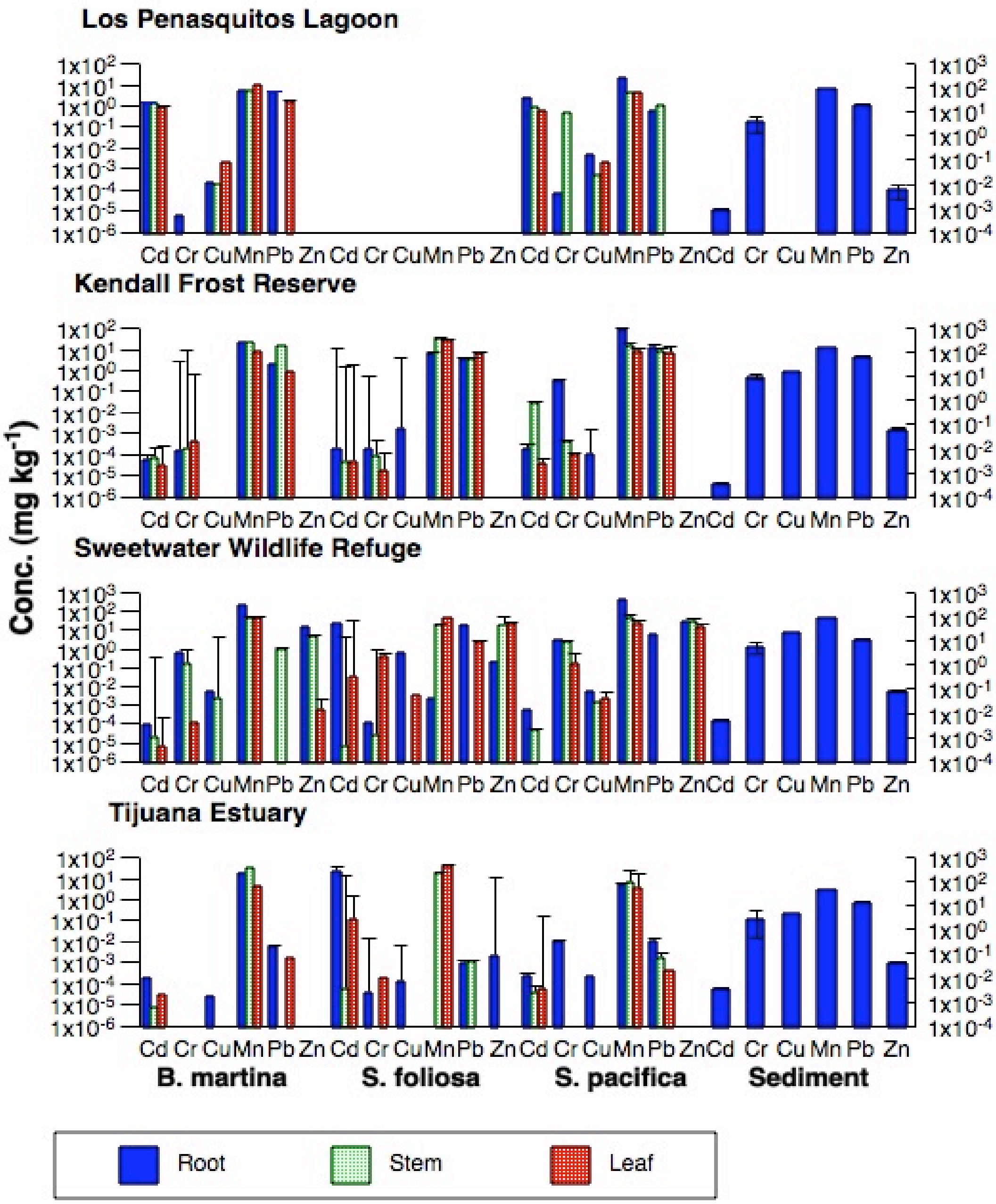

Fig. I.3. Metal concentration (Mean $\pm$ Standard Error (SE); in mg kg^{-1}) from sediment and plant tissue (root, stem, leaf) of three plant species (*Batis maritima, Spartina foliosa, Sarcocornia pacifica*) collected from four marshes: Los Peñasquitos Lagoon (LP), Kendall-Frost Reserve (KF), San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW) and Tijuana River Estuary (TJ). Sediment concentration on right, plant tissue concentration on left. Collection date was August 2007.

In general, the ranking of metals based on their concentrations in a given plant tissue (root, stem, or leaf) followed an order that was significantly correlated with the ranking of metal concentrations associated with sediment (ρ=0.54; $P<0.05$). Few exceptions were observed and included, for LP, no significant correlation in the metals ranking between the sediment, stem and root of *B. maritima* (ρ=0.33; $P>0.16$), and between the sediment, leaf and stem of *S. pacifica* (ρ=0.35; $P>0.18$). At KF no significant correlation of metals ranking was found between the sediment and *B. maritima* leaf (ρ=0.46; $P<0.09$), and between the sediment and *S. foliosa* stem (ρ=0.48; $P<0.07$). At SW the exception came from the lack of correlation between the metal ranking in the sediment and the one in leaves of *B. maritima* (ρ=0.40; $P>0.13$), while at TJ was with leaves of *B. maritima* and *S. foliosa* (ρ=0.49; $P>0.07$).

I.3.6 Metal bioconcentration factor

Metal bioconcentration factor was determined for 5 metals (Ag, Al, Fe, Mn, and V) that were representative of the major trends among metals and above detection limit at all marshes are presented (Fig. I.4). Overall, the bioconcentration factor (BCF) was <1 for most metals, at each marsh and for each plant species (Fig. I.4). The exception came from Ag which always had a BCF >1, then followed by Mn>Fe>V>Al (Fig. I.4). For *B. maritima*, the BCF decreased in KF with SW usually having the greatest BCF with the exception of Ag and Fe which had greater BCF at KF. In general, the BCF in *S. foliosa* increased from the northern marsh to the southern, with KF having the lowest BCF overall with the exception of Ag and Fe, which had greater BCF at KF. *S. pacifica* BCF increased from the northern marsh (LP) to the south, with SW having the greatest BCF with the exceptions of Ag and Fe, which had greater BCF at KF.

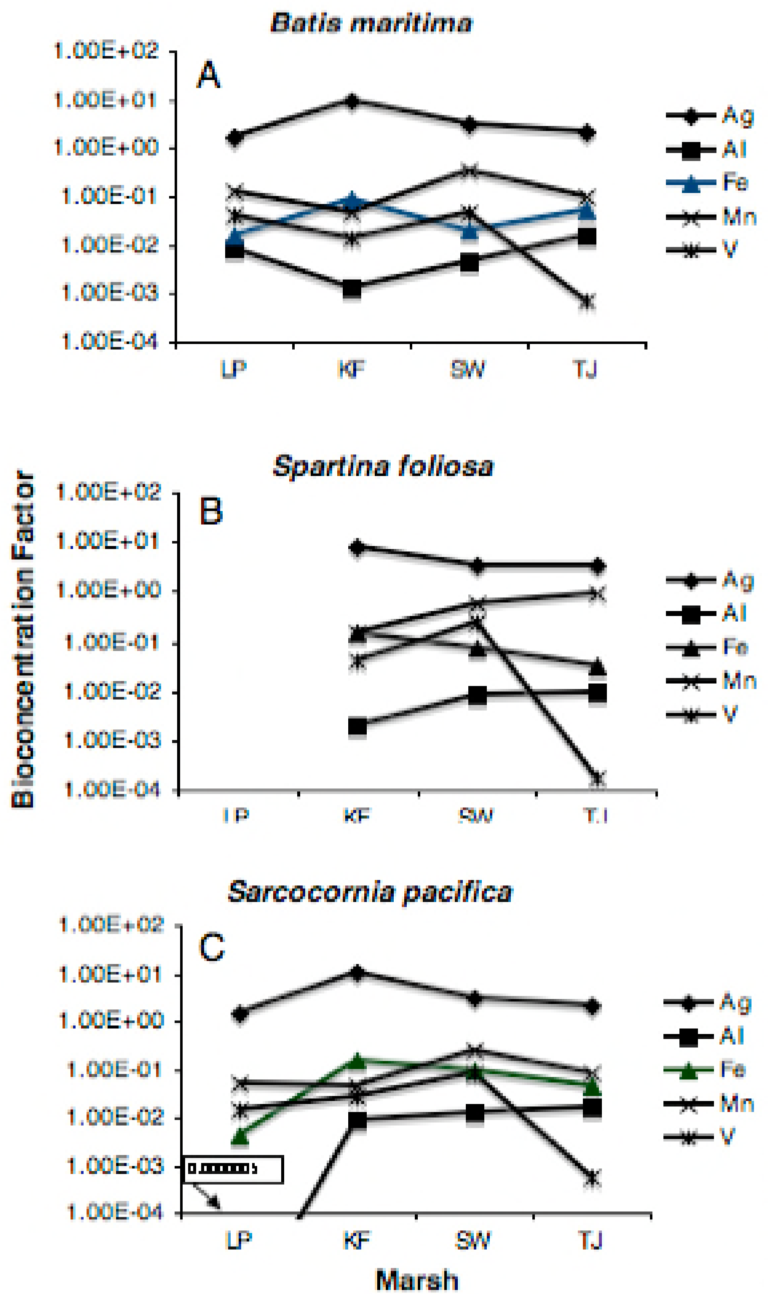

Fig. I.4. Bioconcentration factor (BCF) for five metals at each marsh and for each plant species. Marshes are Los Peñasquitos Lagoon (LP), Kendall-Frost Reserve (KF), San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW), and Tijuana River Estuary (TJ). Five representative metals (Ag, Al, Fe, Mn, V) were chosen to show the general trend of the BCF of each plant at each site. Collection date was August 2007. (A) *Batis maritima;* (B) *Spartina foliosa;* (C) *Sarcocornia pacifica.*

I.3.7 Rainfall and bioaccumulation of metals in plants

In general, metal concentration in plant species was significantly different between summer and winter ($P>0.01$) (Table I.3). KF had significantly different and greater concentration of metal content in plants during winter ($P<0.01$), with the exception of Ag, which was significantly different and greater in summer for all three species ($F=66.25$; $P<0.01$). SW had a significantly different and greater concentration of metal content in plant during winter ($P<0.01$), with the exception of As observed significantly different and greater in summer ($F=4.58$; $P<0.01$), Ni which was significantly different and greater in summer for all three species ($F=13.63$; $P<0.01$) and Mn showed no significant difference between seasons for all three species at SW ($F=2.02$; $P=0.09$).

In general, KF had a 4.09x increase and SW had a 2.62x increase in metal content in plants from summer to winter (Table I.3). With a few exceptions, KF had 90x greater Cr concentration in *S. foliosa* tissue in winter, 50x greater Ni concentration in *S. pacifica,* and a 20x increase in Zn for all plant species from summer to winter. SW, however, had increases in concentration from winter to summer of 2.7x for As for all plant species, and 4.0x for Ni for all species from winter to summer. KF had an average 4.9x increase from winter to summer for Ag content in all plant species.

Cu content in all plant species increased from summer to winter. KF had 3.8x increase for *B. maritima,* 3.4x increase for *S. pacifica,* and a 2.7x increase for *S. foliosa.* SW had 17.3x increase of Cu content from summer to winter for *B. maritima,* 1.9x increase for *S. pacifica,* and 2.7x increase for *S. foliosa.*

Table I.3. Metal concentration (Mean ± Standard Error (SE) in mg kg^{-1}) of three plant species (*Batis maritima*, *Sarcocornia pacifica*, and *Spartina foliosa*) collected from two salt marshes, Kendall Frost Reserve (KF) and San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW). Collection in August 2008 and February 2008.

		KF					SW				
		Summer		Winter		Ratio	Summer		Winter		Ratio
Metal	Species	Mean	SE	Mean	SE	W/S	Mean	SE	Mean	SE	W/S
Ag	*B. maritima*	8.94	1.90	1.78	0.80	0.20	7.92	1.95	17.24	3.40	2.18
	S. pacifica	9.44	2.09	1.96	0.99	0.21	9.24	1.97	19.68	6.39	2.13
	S. foliosa	8.80	1.54	1.82	0.95	0.21	9.21	1.63	18.15	5.51	1.97
Al	*B. maritima*	34.15	31.29	190.61	130.40	5.58	317.96	290.43	375.96	353.79	1.18
	S. pacifica	152.00	134.54	754.64	489.95	4.96	381.42	408.19	1070.58	995.94	2.81
	S. foliosa	89.66	71.07	201.61	197.80	2.25	207.37	190.31	179.67	204.42	0.87
As	*B. maritima*	6.42	2.21	0.87	0.25	0.14	10.39	3.70	6.05	2.95	0.58
	S. pacifica	1.44	1.17	1.54	0.49	1.07	7.94	5.72	4.63	1.79	0.58
	S. foliosa	4.40	1.95	9.65	5.27	2.19	6.57	4.19	1.42	0.39	0.22
Cd	*B. maritima*	0.51	0.42	2.19	0.90	4.29	0.93	0.49	1.53	0.99	1.65
	S. pacifica	0.53	0.23	2.58	1.00	4.87	0.80	0.59	1.72	0.69	2.15
	S. foliosa	0.69	0.41	3.40	0.72	4.93	0.67	0.54	1.54	0.53	2.30
Cr	*B. maritima*	0.66	0.21	3.04	2.71	4.61	0.64	0.45	4.44	1.29	6.94
	S. pacifica	0.95	0.36	3.10	22.99	4.03	1.07	1.13	5.03	1.59	4.70
	S. foliosa	0.77	0.28	85.57	2.79	90.07	2.11	1.27	4.26	0.95	2.02
Cu	*B. maritima*	1.50	1.24	5.63	4.13	3.75	3.97	2.70	68.51	24.29	17.26
	S. pacifica	2.46	1.35	8.45	39.90	3.43	8.50	6.30	16.81	9.99	1.98
	S. foliosa	3.97	3.91	10.77	9.20	2.71	5.81	2.13	15.58	14.92	2.68
Fe	*B. maritima*	5,255.30	1,977.90	490.48	390.91	0.09	4,567.30	2,299.39	12,299.42	4,171.36	2.69
	S. pacifica	2,078.72	1,299.20	2,739.18	1,699.01	1.32	2,747.62	1,800.97	6,621.37	3,959.60	2.41
	S. foliosa	1,578.94	732.97	4,183.86	2,625.92	2.65	1,773.34	767.59	1,293.00	907.02	0.73

Table I.3. Metal concentration continued (Mean ± Standard Error (SE) in mg kg^{-1}) of three plant species (*Batis maritima*, *Sarcocornia pacifica*, and *Spartina foliosa*) collected from two salt marshes, Kendall Frost Reserve (KF) and San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW). Collection in August 2008 and February 2008.

		KF					SW				
		Summer		Winter		Ratio	Summer		Winter		Ratio
Metal	Species	Mean	SE	Mean	SE	W/S	Mean	SE	Mean	SE	W/S
Mn	*B. maritima*	13.96	9.08	74.15	58.94	5.31	374.89	74.89	622.67	62.67	1.66
	S. pacifica	17.61	16.59	91.86	65.92	5.22	148.48	122.75	316.03	100.39	2.13
	S. foliosa	13.19	9.36	34.76	19.70	2.64	92.11	90.89	88.88	78.92	0.96
Ni	*B. maritima*	1.27	0.49	9.06	6.10	7.13	11.26	4.89	8.02	4.50	0.71
	S. pacifica	1.84	0.79	92.24	30.75	50.13	10.46	7.02	4.01	1.69	0.38
	S. foliosa	1.32	0.95	7.51	7.29	5.69	11.46	6.62	1.43	3.36	0.12
Pb	*B. maritima*	46.43	12.01	4.87	4.08	0.10	6.37	1.89	57.19	21.90	8.98
	S. pacifica	4.38	2.98	7.58	6.75	1.73	4.55	2.76	10.11	9.97	2.22
	S. foliosa	13.74	7.90	19.23	14.79	1.40	5.47	2.97	6.05	6.01	1.11
Se	*B. maritima*	1.15	0.70	5.17	3.96	4.50	1.33	0.37	6.72	4.07	5.05
	S. pacifica	1.24	0.99	5.42	3.22	4.37	7.45	7.32	8.24	4.42	1.11
	S. foliosa	1.08	0.94	5.16	3.12	4.78	16.92	10.69	8.93	4.06	0.53
Sr	*B. maritima*	144.98	59.77	246.79	91.34	1.70	145.37	90.30	373.69	171.07	2.57
	S. pacifica	33.89	10.21	97.39	21.89	2.87	95.10	31.97	126.46	54.72	1.33
	S. foliosa	23.01	15.00	53.59	24.94	2.33	42.00	17.49	56.70	36.69	1.35
Ti	*B. maritima*	2.39	1.70	7.10	3.82	2.97	10.53	10.49	22.07	19.86	2.10
	S. pacifica	6.22	5.92	12.27	15.36	1.97	18.21	15.21	46.13	39.86	2.53
	S. foliosa	4.40	3.96	11.02	9.42	2.50	10.43	10.35	14.16	11.00	1.36
V	*B. maritima*	11.63	10.94	17.78	6.65	1.53	21.15	14.12	67.03	46.72	3.17
	S. pacifica	7.32	3.59	22.69	9.00	3.10	21.47	7.90	35.42	21.79	1.65
	S. foliosa	5.40	3.95	15.32	12.41	2.84	8.28	7.10	22.58	5.96	2.73
Zn	*B. maritima*	0.04	0.005	2.80	1.10	70.00	20.79	16.17	110.46	32.99	5.31
	S. pacifica	1.33	0.51	14.55	12.14	10.94	17.44	11.54	26.09	14.90	1.50
	S. foliosa	0.23	0.04	15.41	10.41	67.00	16.05	10.43	32.74	29.90	2.04

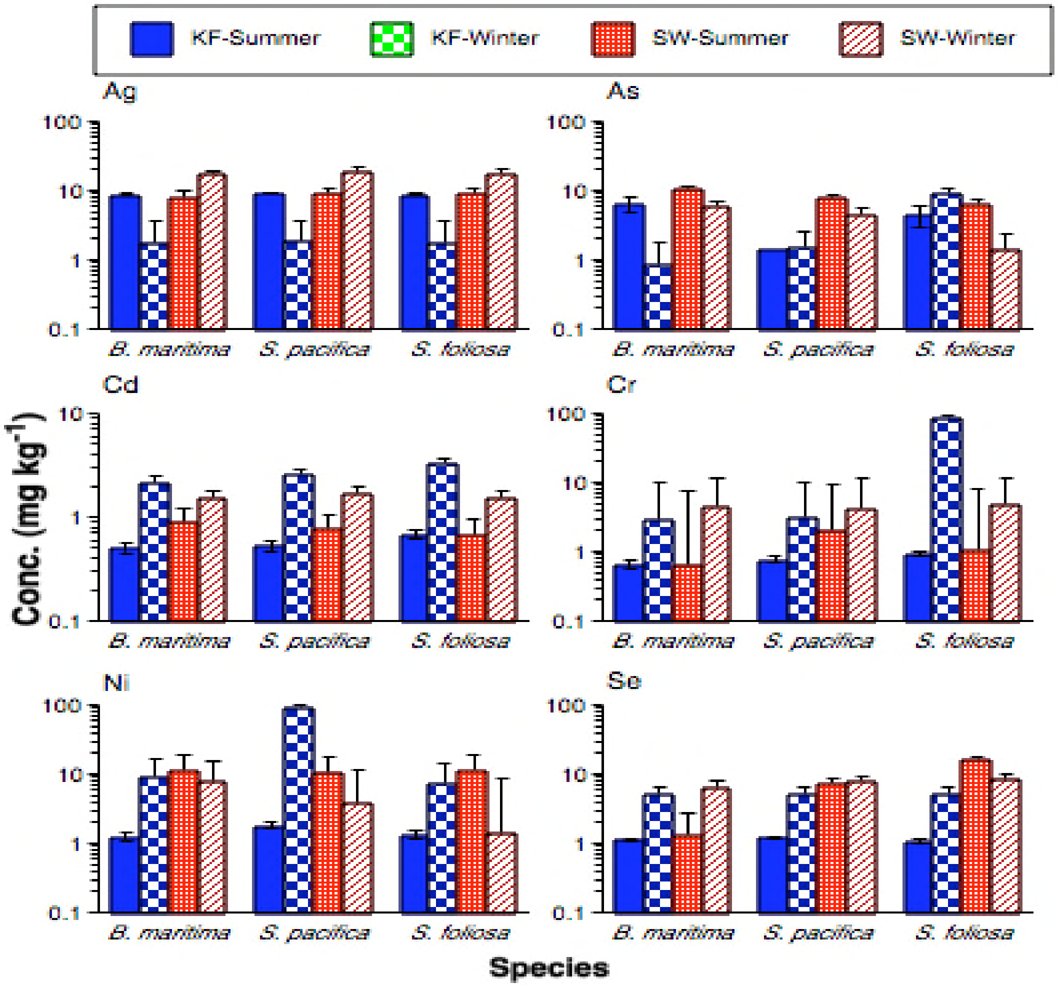

Fig. I.5. Metal concentration (Mean ± Standard Error (SE) in mg kg^{-1}) from three plant species *Batis maritima, Spartina foliosa, Sarcocornia pacifica*) collected from two marshes: Kendall-Frost Reserve (KF) and San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW). Samples collected August 2008 and February 2008.

I.3.8 Change of metal bioaccumulation with seasons

In general, all marsh plant species had higher metal concentrations during the winter season then during the summer season ($P<0.05$) (Fig. I.5). *S. foliosa,* which excretes salt on it's leaf, had similar accumulation levels of metals as *B. maritima* and *S. pacifica,* which retain water in their leaves. With a few exceptions, KF had significantly greater concentrations of Ag in the summer for all three species ($F=66.25$; P,0.01). In contrast

SW had significantly greater concentrations of Ag for all three species during winter season (F=13.63; P<0.01) SW had significantly greater concentrations during summer for NI in all three species (F=13.63; P<0.01) and As (F=4.58; P0.01). KF had significantly greater concentration of Cr in *Sarcocornia* in the winter season (F=3.5; P<0.04). At SW there was no significant difference between seasons and plants observed for Mn (F=2.02; P>0.09).

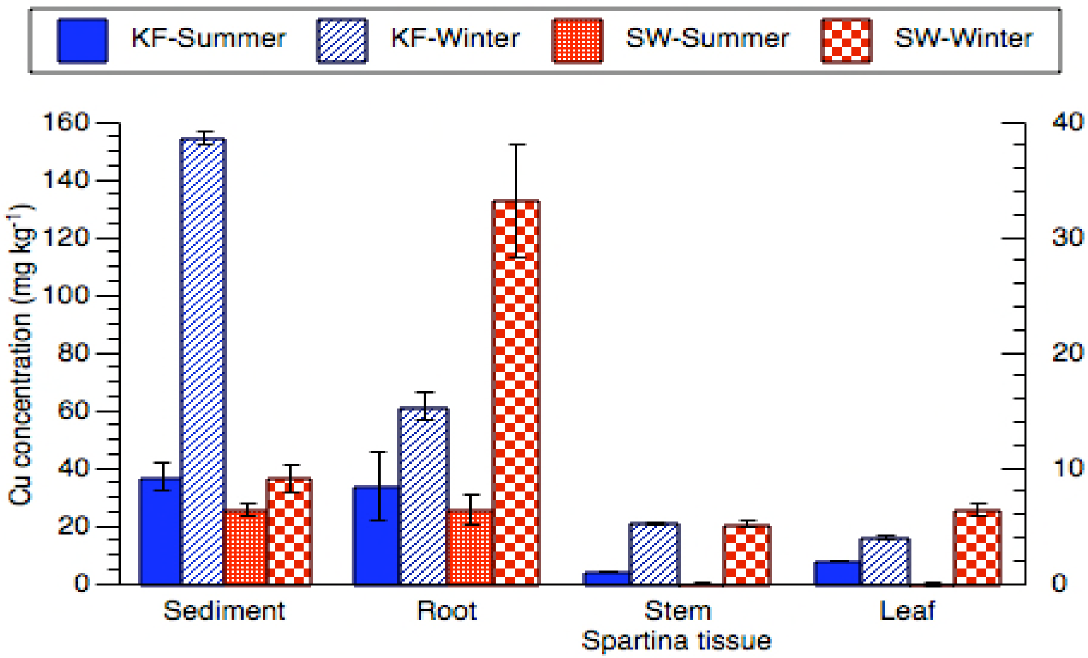

Fig. I.6. Seasonal variation of copper concentration in *Spartina foliosa* plant tissues (root, stem, leaf) and associated with sediment. Samples collected August 2008 and February 2008.

I.3.9 Seasonal bioavailability of metal to plants

In order to optimize presentation of data, Cu in *S. foliosa* that was representative of the major trends for Cu and Zn in all three plant species is presented (Fig. I.6). Metal concentration content observed at KF and SW in the plant was greatest in the root tissue

Table I.4. Metal concentration (Mean ± Standard Error (SE) in mg kg^{-1}) in sediment from two marshes, Kendall Frost Reserve (KF) and San Diego Bay Sweetwater Marsh National Wildlife Refuge (SW). Sample collection in summer (August 2008) and winter (February 2008).

	KF					SW				
	Summer		Winter		Ratio	Summer		Winter		Ratio
Metal	Mean	SE	Mean	SE	W/S	Mean	SE	Mean	SE	W/S
Ag	1.64	0.62	3.59	0.89	2.19	1.98	0.61	3.76	1.06	1.90
Al	9,856.64	3,089.53	10,822.63	3,694.94	1.10	9,069.79	5,885.29	15,463.46	6,070.63	1.70
As	12.55	4.37	21.71	8.29	1.73	4.77	2.66	6.12	4.30	1.28
Cd	1.18	0.36	2.63	0.74	2.23	1.01	0.78	3.16	1.89	3.13
Cr	20.88	5.14	25.22	7.03	1.21	19.51	12.41	34.71	16.07	1.78
Cu	36.90	9.27	154.70	89.55	4.19	25.63	8.80	36.46	31.02	1.42
Fe	24,137.15	11,549.92	30,499.01	11,472.45	1.26	14,824.33	8,125.06	21,967.43	8,347.14	1.48
Mn	295.03	117.20	295.52	178.30	1.00	372.57	351.74	686.13	606.10	1.84
Ni	11.26	2.20	17.09	2.83	1.52	8.55	5.70	17.34	5.33	2.03
Pb	79.71	24.77	115.32	32.29	1.45	39.98	26.50	107.49	23.46	2.69
Se	>0.01	>0.01	0.95	0.37	-	0.36	0.08	0.65	0.21	1.81
Sr	141.63	83.02	208.80	46.54	1.47	130.82	101.07	422.24	315.03	3.23
Ti	198.90	51.95	244.06	54.79	1.23	242.83	141.63	396.98	166.31	1.63
V	84.79	24.17	128.36	17.57	1.51	65.93	34.69	118.79	29.53	1.80
Zn	26.87	13.09	104.76	48.19	3.90	13.76	7.03	169.65	77.92	12.33

for Cu during the winter season ($F=4.96$; $P<0.01$; $F=27.64$; $P<0.01$) respectively. SW had a significantly greater Cu concentration in root then KF during winter ($F=12.07$; $P<0.01$). In contrast, KF had greater concentration of Cu associated with sediment during winter ($F=30.71$; $P<0.01$). There was no significant difference between KF and SW Cu concentrations observed during the summer season ($F=2.10$; $P>0.15$).

Metal concentration associated with sediment was significantly greater in winter for Ag ($F=6.39$; $P<0.01$), Cd ($F=71.74$; $P<0.01$), Cu ($F=179.86$; $P<0.01$) for KF. SW had significant increase for Ag, Cd, Ni, Pb, Sr, and Zn associated with sediment from summer to winter ($F=8.38$; $P<0.01$) (Table 1.4). All metals associated with sediment showed an increase from summer to winter. However, there was no significant difference observed between the seasons and marsh for all other metals ($P>0.05$) (Table I.4). The average increase in metals associated with sediment was 3.0x for KF and 2.0x for SW from summer to winter season.

I.4. Discussion

Marshes in southern California have been subjected to major anthropogenic changes associated with urban development. Over 90% of the salt marshes in the San Diego area have been lost due to urban development. Marshes are sediment traps, filtering metal contaminants that are considered non-point sources because of their emergence from multiple individual sources (e.g., boats, marinas, shipyards, watershed and storm drain runoff) being deposited from tidal flushing and land runoff (Burke, et al., 2000; Deheyn & Latz, 2006). Contaminants from urban runoff and industrial pollutants have resulted the local marshes, Los Peñasquitos (LP), Kendall-Frost Reserve (KF), Sweetwater Marsh National Wildlife Refuge (SW), and Tijuana River Estuary (TJ), being listed as impacted waterbodies (Schiff, 2007). Urban development surrounding these marshes and their respective watersheds has an implied perspective of how contaminated the marsh environment may be. There is a social and economic gradient where the north is surrounded by suburban housing development, with an increasing urban density gradient with industrial activity as one moves to the south, which borders Mexico, surrounded by dense urban development and heavy industrial activity.

I.4.1 Biogeochemical characteristics affect concentrations of metal associated with sediment

Most metals in the water column are associated with suspended particulate matter. Understanding the different binding affinities between metals and organic matter (OM) is important in determining the bioavailability of metals to plants (Yamashita et al., 2008). Some metals (Al, Cu, Fe, and Mn) have an affinity to form complexes with particulates at low salinity (SanudoWilhelmy, et al., 1996) and OM. This would suggest that these metals are less bioavailable to plants. Vascular plants are known to pump O_2 to their root system changing the microenvironment in surrounding soil. This biogeochemical process can form-chelating complexes that mobilize the metals allowing uptake by the plants (Weis, et al., 2002). Other metals (Ag, Cd, and Sr) with a low affinity to particulates (SanudoWilhelmy, et al., 1996) have high accumulation levels in leaves when compared to concentration content associated with sediment.

Metals associated with course-grained particulates settle out close to the source of contamination. Finer particulate sizes stay suspended longer and are dispersed by water circulation (Katz et al., 1981). Vegetation in salt marshes slow the velocity of the water flow increasing deposition of fine particles. However, sediment concentrations of metals associated with sediment did not reflect the amount of surrounding development. One site, KF, had different biogeochemical properties, where the metal contaminant levels associated with sediment were proportionately greater for half of the metals. Therefore, particulate size and OM have a major impact on the sorption of metal contaminants in sediment to plants. Sites at LP and TJ had medium/course/sand grain size sediment, and lower organic content and had lower levels of enriched metals content. OM and small grain size in sediment increases the salt marshes ability for phytostabilization by sequestering metals belowground, where they are not bioavailable to the ecosystem.

I.4.2 Metals cycle through leaf tissue

Accumulation of metals in leaves leads to exporting metal contaminants to environment due to excretion through salt glands (Kraus, et al., 1986) and plant detritus breaking down releasing metals into the marsh habitats. Results of this study support the transport of metals to leaf tissue. *B. maritima and S. pacifica,* a C_3 plant, uptake water and store the moisture inside its leaves. *S. foliosa,* a C_4 plant, has salt glands on its leaves for excreting salt from water uptake. Distinctive structural features contribute to the specificity of the mechanism for plant uptake and transport of salt in the leaves (Horie et al., 2004). Although the physiology of these plants differ, the patterns of metal uptake and concentration in aboveground tissues were similar. *B. maritima, S. foliosa,* and *S. pacifica* from the four sites accumulated metal contaminants in leaf tissues. Overall, the level of metal contaminants associated with the sediment matched bioavailability to marsh plants. However, KF had higher concentrations of metals associated with sediment that was not reflected in level of metal content in leaf matter.

I.4.3 Variation of metal concentration in plant tissues

Plants accumulate greater levels of contaminants in the roots, carrying the metal burden in belowground tissues (Weis, et al., 2002). In this study the winter season showed greater levels of metal content in roots; however, by summer the metals were transported to the aboveground biomass with all plant tissues having relatively similar levels of metal content. The evaluation of metals distributed among the tissues indicates that metal contaminants were transported and redistributed to aboveground biomass after a 6 month period, furthermore, accumulating in the leaves of these plants. Vascular plants and their detritus are intrinsic parts of the marsh nutrient cycle, therefore metal accumulation in aboveground tissues is considered bioavailable to the marsh ecosystem (Weis, 2002).

Sites located at LP are surrounded by suburban development with the vegetation lying above the high tide mark and not subjected to tidal flushing; therefore, the source of contaminants is considered to be from the watershed, storm drain runoffs, and atmospheric deposition. The sites at TJ, which lies by the Mexican border, are surrounded by urban and industrial development. *S. foliosa,* which was not found at LP, grows along the mean to low tide line and is exposed to tidal flushing, but *B. maritima* and *S. pacifica* grows above the mean high tide line (MHTL) with no tidal flushing. Growth above the MHTL suggests that *B. maritima* and *S. pacifica* at these two marshes would reflect contaminants from their watersheds, atmospheric deposition, and storm drain runoffs. Contrary to the first hypothesis, the salt marshes did not show a gradient of source contaminants due to surrounding urban industrial development, but sediment characteristics such as grain size and OM influenced the bioavailability of metals to marsh plants. LP and TJ have channel type salt marsh topography, with an inlet directly into the ocean leading to sand deposition. Whereas KF and SW are marshes lying in a semi-enclosed bay. They consist of mudflats, with all three plants exposed to tidal flushing from the bay and stormwater, ground run off, from surrounding land and watershed. SW lies in San Diego Bay, a large port with heavy industrial use surrounded by a metropolis development. SW had a metal signature for seasonal variation consisting of As, Cr, and Ni. A metal signature for seasonal variation at KF consisted of Al, Ni, and V. KF lies in Mission Bay and is surrounded by a suburban urban development and a bay primarily used for recreational boat use. KF, with the greatest mud to sand ratio, also exhibited the greatest concentration of metals in sediment. Metal speciation associated with particulate matter in the water column influences the fate and bioavailability of metals in salt marsh habitat.

I.4.4 Bioaccumulation of metal contaminants among non-salt excreting and salt excreting leaf tissue

Assuming that metal accumulation among plant species is similar, hypothesis #2 was refuted because non-salt excreting plants did not accumulate greater concentrations of metals then salt excreting plants. There were similar levels of metal content among plant species within a particular marsh. Identifying non-point source contaminants, however, can be determined by topographical location of specific species. *B. maritima* and *S. pacifica* at LP and TJ lie above the mean high tide line (MHTL) at these marshes, therefore are not exposed to tidal flushing. Contaminant loads for these marshes are from their watersheds, storm drain runoffs, and atmospheric deposition. Though LP is perceived as less impacted and TJ as highly contaminated, in this study LP had higher levels of metal contaminants in leaves for several metals that are considered to have adverse biological effects in high concentrations (e.g., Cd, Mn, Ni, Pb), while the other metals had a striking similarity to the level of contaminants in leaves from the TJ sites. This suggests that atmospheric deposition has a impact on contaminants in the salt marsh ecosystem.

I.4.5 Metals levels showed changes in bioavailability with season

Metal contaminants found in plant leaves from KF and SW marshes reflected the different bioavailability of contaminants between suburban and dense urban-industrial developments, respectively. Metal concentration in plant tissue revealed differences due to marsh location, sediment characteristics, and seasonality. Metals are introduced to salt marshes through tidal flushing of stormwater, watershed run off, industrial discharge, urban sewage spills, and atmospheric deposition. SW lies in San Diego Bay, a highly industrial and military harbor. SW's watershed is densely, with over 7,000 populated per square mile, with 45% developed for industrial use. Whereas KF is surrounded by suburban development whose bay is primarily used for recreational boat use. KF watershed has approximately 2,700 population per square mile, with inputs of urban runoff and sewage spills. It was expected that SW would have the greater contaminant load. There was an observed increase in metal concentrations in winter than summer season. These results support hypothesis #3 that there were greater concentrations of metal in the winter time then the summer time. However, SW showed 4.5x greater increase in root tissue metal content in winter than in summer season, with a small increase in metals associated with sediment. The significant increase in root tissue but small increase in metals associated with sediment suggest that metals are absorbed directly from the water column. KF, however, showed 2.5x greater increase in root tissue metal content and 4.0x greater metals associated with sediment in winter then summer season. Earlier studies had observed greater metal content associated with sediment at KF, with a lower metal content in plants. Sediment at KF showed the greatest increased enrichment of Cu content during the winter, in contrast to SW, which showed the greatest increase in Cu content in root tissue during winter season. Bioavailability of metals, thus clearly appears to be greater at SW were absorption of dissolved metals from water column was observed, than KF marsh, were the greatest metal load was retained in the sediment.

This shows two process of phytoremediation, the process of phytostabilization and sequestering metals in sediment vs. phytoextration, requiring efficient metal accumulation in plants. Phytoremediation is an effective technique for cleaning up wastewater contaminants (Kraus, et al., 1989; Mingorance et al., 2007; Windham et al., 2003). Metal analysis from salt marsh plants have been done in the frame of studies on phyto-extraction, for plants ability to hyper-accumulate metal in aboveground tissues (Weis et al., 2004). However, harvesting of the contaminated plant biomass and proper disposal (Prasad et al., 2003) is harsh on the salt marsh habitat. Marsh habitat is not conducive to plant harvesting, thus making use of marsh plants challenging.

Summary

It was hypothesized that the type of metal contaminant and the level of contamination found at different marsh sites is due to different non-point sources of urban and industrial runoffs creating a gradient of contaminants to these marshes. However, the results suggested that the organic matter (OM), grain size, and water circulation patterns had a significant impact on the bioavailability of metals to plants. Sediments with greater OM and smaller grain size characteristics reflected enriched metal content. Seasonality had little influence on sediment metal content levels; however, plants showed a significant increase in metal concentration during winter, showing that metals originate from runoff.

Although the results of this study show that metals are bioavailable to salt marsh plants in southern California, sediment characteristics and circulation patterns impact the level of contaminants in this ecosystem. Identifying source of contaminants as sediments become enriched with metal contaminants is of paramount concern in environmental assessment. Plant transport patterns that redistribute metal contaminants to aboveground biomass lead to the possibility of biomonitoring to determining metal contamination levels and bioavailability to marsh plants, by evaluating metal level content in leaf matter. Only bioavailable metals have the potential to be toxic with adverse effects on organisms and ecosystems. Assessments of metal concentrations and bioavailability, as considered with this study, would provide useful information if integrated into environmental quality assessment assessing contaminant loads to evaluate the ecological impact.

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Chapter II

Kinetics of copper bioaccumulation in tissue of the cordgrass, *Spartina foliosa,* under experimental conditions of contamination

Abstract

Salt marshes located in the proximity of urban areas are exposed to contaminants due to anthropogenic discharge. Once in the salt marsh, contaminants can be bioaccumulated in flora tissues with potential toxic effects and cycling through the ecosystem. This study evaluated the fate of copper and assessed accumulation kinetics under experimental conditions within sediment and tissue (root, stem, and leaf)of the cordgrass, *Spartina foliosa.*. Sediment and *S. foliosa* were collected from a salt marsh and transplanted in two enclosed circuit aquaria under natural light conditions. Cu^{2+} exposure concentrations were similar to those found in local marine waters. All samples exhibited elevated levels of Copper after exposure to Cu^{2+}, sediment starting on day 1, root on day 3, stem on day 7, and leaf on day 14. Copper concentrations of leaf tissue were 15% greater than for the stem. However, concentrations in sediment and root samples were at 10 times greater than stem and leaf. There was a 3.5x greater accumulation in plant tissue metal content than metal associated with sediment increase. Thus it clearly appears that metal accumulation occurred from dissolved metals in seawater. These results demonstrate that sediment and *S. foliosa* roots initially immobilize metals, storing a greater percentage of the copper in below ground biomass before transporting to above ground biomass.

Key words: Phytostabilization, bioavailability, salt marsh

II.1. Introduction

Southern California coastline and salt marsh habitat have been heavily impacted and nearly eliminated by urban and industrial development. Salt marshes, in particular, are exposed to dynamic sources of pollution through stormwater runoff and tidal flushing. Located at the interface of land and the ocean (Levin, et al., 2001) the proximity of densely urbanized areas expose salt marshes to urban and industrial discharges that contain metal contaminants (Carvalho, et al., 2006; Sanders et al., 1985). Dense urban development has encroached on watershed habitats that drain into the local salt marshes as well (Pednekar, et al., 2005). The inherent nature of salt marshes results in the absorption and retention of pollutants from the water column acting as a sink for contaminants (Carvalho, et al., 2006). Vegetation reduces the velocity of water circulation allowing particulates to settle out. Bioavailability and toxicity of metal contaminants depend on the physio-chemical speciation between amount of organic and inorganic particulates that are suspended in the water column (Ignacio, et al., 2005; Rosen, et al., 2008; Shafer, et al., 2004; Zirino, et al., 1998).

Southern California salt marshes are inundated with pulse source contamination from stormwater runoff and tidal flushing, re-suspension particles following dredging activity. Seasonal variability of rainfall in the local area is attributed to pulse flushes of stormwater runoff from agricultural and urban development that surround salt marshes and their watersheds (Carvalho, et al., 2006; Pednekar, et al., 2005). Dredging of marinas and harbors for shipping and recreational boat use re-suspend metal contaminants in sediment (Schiff, Diehl, & Valkirs, 2004) resulting in a pulse tidal mixing that flushes onto salt marshes. Stormwater runoff and dredging activity simulate pulse flushing of contaminants onto salt marshes, however, pollutants from military, recreational vessels, waterfront infrastructure (fluid leakage, fuel spills, antifouling paints), and discharge from industrial facilities are a continuous source of pollutants.

Copper is an important element of concern contaminating coastal water, specifically marinas, harbors, and bays. In San Diego Bay copper concentrations in sediment and seawater often exceed US EPA water quality criteria, with measured levels of 0.2 mg l^{-1} to 3.0 mg l^{-1} marinas (Blake, et al., 2004; Fairey, et al., 1998; Valkirs, et al., 1987). Contaminants in stormwater contain copper originating from fertilizers, herbicides, automobiles (tires, brakes, fluids), buildings (weathering paints and metal parts), and atmospheric deposition (Blake, et al., 2004; Davis, et al., 2003; Gallagher, et al., 2005; Giblin, et al., 1980; Malhi et al., 2006; Pednekar, et al., 2005; Rehm, 2008). Copper is used as a biocide in antifouling paints on vessel hulls of recreational boats, cargo ships, and naval vessels (Blake, et al., 2004; Comber, et al., 2002; Nichols, 1988; Schiff, et al., 2004; Valkirs, et al., 2003; Zirino, et al., 1998). Many studies in San Diego Bay have been done to assess copper speciation, in particular the bioavailability of metals to marine fauna and ecological risks related to toxicity of contaminants (Blake, et al., 2004; Deheyn and Latz, 2006; Fairey, et al., 1998; Rosen, et al., 2008; Schiff, et al., 2007). The Fairey et al. (1998) showed the levels of copper in porewater had toxic affect on the survival of amphipods. Rosen et al., (2007) showed through experimentally spiking seawater that excessive metal concentrations inhibits the development of sea urchin larval. Metal concentrations found in harbors and bays clearly appear to adversely affect the developmental stages of invertebrates in the plankton stage.

However, it is unknown if short exposure has a significant impact on flora and fauna in Southern California salt marshes.

Salt marshes are sources and sinks for metal contaminants (Burke, et al., 2000; Kraus, et al., 1986; Weis, et al., 2003; Windham, et al., 2004). Salt marsh plants influence the sediment by removing carbon and nutrients from the soil when alive, and returning them in the form of dead material biomass (Weis, et al., 2002; Windham, et al., 2003). Metals can be a part of this recycling process, as plants absorb metals and transport them to aboveground biomass as phytoextraction, then releasing them back to the environment in association with detritus. Salt marshes are also used in phytoremediation to increase water quality around waste treatment plants. Giblin et al. (1980) found that salt marshes surrounded by metropolitan areas showed elevated levels of metal contaminants in living *Spartina,* however salt marsh sediment retains 55-80% of the copper load, thereby acting as a sink. Salt marsh plants have the ability to retain metal contaminants in sediment, along with retaining metals in belowground biomass by storage of metals in roots (Windham, et al., 2003). The intrinsic ability of salt marsh plants to preferentially absorbed and accumulate contaminants in belowground biomass, therefore, results in phytostabilization where the bioavailability of metals to the environment is decreased. This study focused on the impact of dissolved Cu^{2+} seawater on the local cordgrass, *Spartina foliosa,* in aquaria exposed to natural light. The objective was to evaluate the fate of copper and assess the rate of copper accumulation under experimental conditions within the sediment and plant tissue. Our hypothesis was that metals dissolved in seawater are first taken up by root tissue then subsequently transported through the rest of the plant tissue.

II.2. Methods

This study was performed from August 7 to November 14, 2007 under controlled laboratory conditions exposing salt marsh plants to a known copper concentration. Full intact specimens of *Spartina foliosa* (n=20) along with sediment were collected from Kendal-Frost Reserve (KF) in San Diego, California, and transported to the Scripps Institution of Oceanography. Plant tissues (root, stem, and leaf) along with sediment were sampled.

To allow for water exchange, partitioning one corner of the aquaria to restrain sediment, allowing space for exchanging seawater. Seawater was filled to the level of 1 cm standing water. Seawater was changed twice a week. To maintain temperature at below 30˚C, aquaria were placed in a water bath that had a flow-through system that delivered warm (28˚C) seawater as part of the Scripps Experimental Aquarium Facility. Ambient water temperature at collection site was 25˚C. Aquaria were exposed to natural sunlight outdoors.

II.2.1 Experimental Design

A copper concentration of 1.7 mg l^{-1} $CuCl_2$ in seawater reflected levels found in the water column of the mid portion of San Diego Bay, California (Blake, et al., 2004; Fairey, et al., 1998; Rosen, et al., 2008; Schiff, et al., 2004; Valkirs, et al., 2003). For quality assurance a control condition used a non-contaminated aquarium with plants and sediment collected at the same site. Treatments began August 14, 2007, with water changes (either contaminated or control) twice a week for the first month. Then beginning on 4 days later, weekly contamination was performed for four consecutive weeks. San Diego County suffered major fires affecting air quality and the aquaria were exposed to reduced air quality and ash conditions for the week of October 21-27, 2007. One final contamination was performed on October 29, 2007. Samples were collected on days 0 (before contamination), 1, 3, 7, 14, 21, 28, 60, and 90.

II.2.2 Sample preparation

Plant tissues were collected in triplicates, for each organ system (roots and rhizomes, stems, and leaves), thoroughly washed with filtered seawater, and placed in separate containers to be oven dried to a consistent weight for approximately 48 h. The dried plant samples were then ground and homogenized by hand using a marble mortar and pestle. Each stock of ground material was then sub-sampled in triplicate for elemental analysis.

As done for the plants, the sediment samples were oven dried, ground and homogenized with a mortar and pestle, and subsampled for further analysis. All manipulations during collection and sample preparation were done under controlled conditions to avoid metal contamination, using metal free solutions, nitric acid washed containers, disposable polypropylene or high-density polyethylene supplies, including forceps and tubes. Trace Metal Grade nitric acid (Fisher Scientific) was used in sample preparation with concentrations $<10^{-7}$ mg/g for each metal, thus having a negligible effect on sample metal concentration (Deheyn, et al., 2005; Deheyn et al., 2006).

II.2.3 Metal Analysis

Copper was analyzed using an Induced Coupled Plasma Atomic Emission Spectrum (ICP-AES) spectrometer (Optima 3000, Perkin Elmer) at the Scripps Institution of Oceanography Analytical Facility. The instrument was calibrated before every run by dilution of a 100 ppm Multi-Element Instrument Calibration Standard solution (Fisher Scientific). Samples of plant and sediment were analyzed separately to ensure running samples of similar matrix and range of metals concentration.

All methods and protocols were modified from Deheyn and Latz (2006), which focused on sediment and invertebrates. For plant tissues each sample was fully digested in 0.5 mL of 70% nitric acid solution (Fisher Scientific) at 100°C for 100 min using an Ethos EZ Microwave Digestor (Milestone). No sediment particles were seen in plant tissue samples after digestion. This highly metal and acid concentrated digest was then diluted (by weight) to 5% nitric acid using MilliQ water (Barnstead) and then directly used to run the ICP analysis.

Sediment samples were subject to a similar digestion and dilution process, yet using a mild 45% nitric acid digestion to assess the leachable and bioavailable fraction of metal associated with the sediment, thus not including the metal constitutive of the geological matrix (Deheyn et al., 2006). The sediment in acid solution was microwave digested at 80°C for 20 min, and the resulting solution was then diluted by weight in MilliQ water for ICP analysis.

II.2.4 Statistical Analysis

Analysis of variance (ANOVA) and Student's t-test was used to test significance of the differences in metal concentration observed for each concentration change with the exposure for sediment and plant, for plant tissues. Statistical analysis were done using JMP® 7.0 (SAS Institute Inc ©2007). Unless indicated, each value represents the median with one standard deviation. Uptake kinetics were determined from second-order kinetics exponential model; $y = ue^{-be^{-kt}}$, where *b* is the concentration at time *t* (day^{-1}) for the lag phase, u is the difference between concentration at time t (day) and concentration maximum, respectively and k is the rate constant (day^{-1}) (Deheyn et al., 2004).

II.3. Results

Copper addition to the aquaria resulted in contamination of sediment and *Spartina foliosa* tissue (Table II.1). In general, sediment showed immediate increase in copper levels on day 1 followed by root on day 3, stem on day 7 and leaf on day 14. Sediment and root showed a significant increase in copper content on day 14 for sediment (ANOVA; $F>4.15$; $DF=8$; $P<0.01$) and day 3 for root (ANOVA; $F>7.40$; $DF=8$; $P<0.04$) (Table II.1).

II.3.1 Kinetics of bioaccumulation in plant tissue

Overall, sediment and root had the greatest copper concentration values, reaching maximum concentration of 49 $\mu g\ g^{-1}$ sediment and 36 $\mu g\ g^{-1}$ root, observed at 21 days and 28 days after exposure, respectively. Cu^{2+} concentration in sediment before contamination was 17 $\mu g\ g^{-1}$, after contamination increased 2.9x from the beginning concentration to the concentration maximum (Table II.1) Plant tissue increased 10.9x for root, 3.8x for stem, and 14.0x for leaf tissue from the beginning concentration to concentration max. The average was a 9,6x increase in concentration over the 90 day period. Stem had an observed significant difference in tissue copper concentration on day 14 ($F>3.13$; $DF=5$; $P<0.05$). Leaf had an observed significant difference in tissue copper concentration on day 14 ($F>9.05$; $DF=8$; $P<0.01$). However, stem and leaf reached a maximum concentration of 2.4 $\mu g\ g^{-1}$ and 3.9 $\mu g\ g^{-1}$ at 90 days, respectively. The effect of copper accumulation after exposure showed the leaf concentration was

TABLE II.1. Metal concentration (Median ± Standard Error (SE); in mg kg^{-1}) from sediment and root, stem , and leaf of *Spartina foliosa* exposed in aquaria to a known copper concentration for 90 days. Uptake factor (UF) comparison between concentration at day 0 and day 60.

Time	Sediment		Root		Stem		Leaf	
day	Conc.	SE	Conc.	SE	Conc.	SE	Conc.	SE
0	16.56	1.19	3.30	0.05	<0.01	<0.01	0.28	0.19
1	21.81	3.19	1.85	1.45	NA	NA	0.15	0.06
3	24.54	2.90	12.79	6.71	NA	NA	0.26	0.35
7	33.50	5.91	18.95	10.95	0.64	0.64	0.31	0.12
14	33.02	5.98	28.08	6.34	1.24	<0.01	1.95	1.03
21	40.86	5.99	30.62	4.00	NA	NA	2.42	0.52
28	36.24	3.46	36.02	6.15	2.20	0.64	2.68	0.36
60	48.69	9.82	34.58	14.17	2.37	1.75	2.83	1.00
90	26.85	0.68	22.99	1.96	2.40	1.10	3.93	1.04
UF	2.47		10.92		3.75		14.04	

NA : Not Analyzed

observed at a greater level significantly different then the stem on day 28 ($F>5.95$; $DF=44$; $P<0.01$). There was no qualitative difference in the growth of *S. foliosa* between the control and the contaminated aquaria.

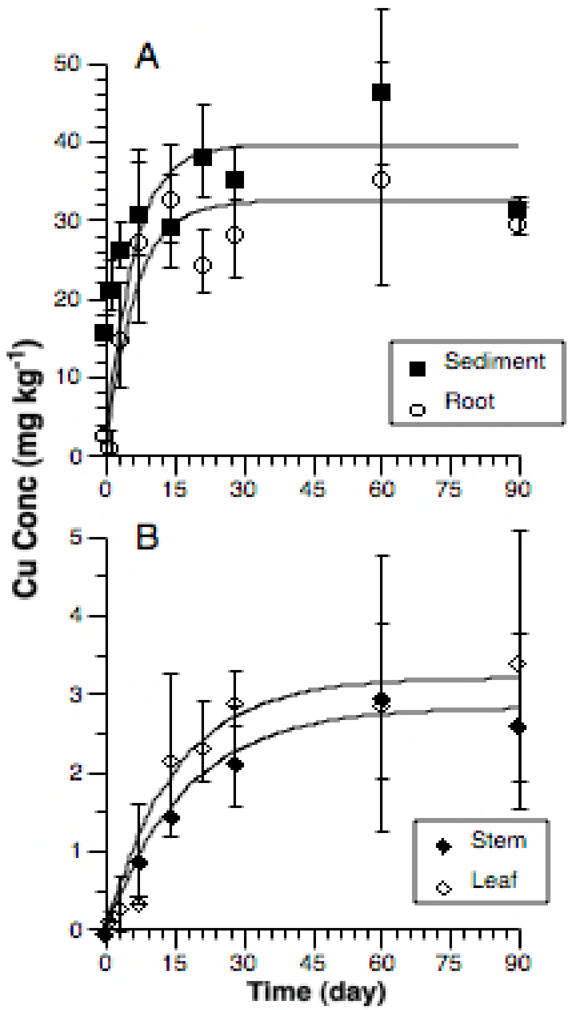

Fig.. II.1. Copper concentration in sediment and tissue of *Spartina foliosa*. Values are mean with standard error. Lines represent a double-component second-order kinetics exponential model for the change of copper concentration as a function of time. (A) Sediment and *S. foliosa* roots. (B) *S. foliosa* stems and leaves. Time zero is before Cu^{2+} contamination.

II.3.2 Differences of bioaccumulation among tissues indicated in model

The Cu^{2+} accumulation rate was modeled using a second-order exponential model (Fig. II.1). In general, the copper concentrations of sediment and root were 10x greater than stem and leaf. Sediment concentrations increased exponentially at 0.172 d^{-1} to the highest values by 21 d (Fig. II.1; Table II.2). Root tissue concentrations increased at the slower rate of 0.167 d^{-1} but had a similar profile to the highest values by 30 d (Fig. II.1; Table II.2). There was a lag phase in copper accumulation shown in parameter *b* of the exponential model for root, stem, and leaf (Table II.2). Leaf tissue was observed to have a longer lag phase than stem, followed by a greater exponential increase in uptake rate then stem. Leaf tissue had a greater saturation level than stem by the end of exposure. Overall, sediment did not exhibit a lag phase with the time resolution of the experiment (Table II.2).

Table II.2. Kinetic accumulation values observed over time from exposure and parameter (*k, u, b,* and R^2) of a double-component second-order kinetics exponential model describing change in mean copper level between salt marsh sediment and *Spartina foliosa* tissues (root, stem, and leaf).

Sample	k (d^{-1})	u (mg kg^{-1})	b	R^2
Sediment	0.172	38,19	0.57	0.66
Root	0.167	35.41	2.18	0.87
Stem	0.101	2.45	2.74	0.99
Leaf	0.112	3.36	3.53	0.94

II.3.3 Plateau concentration differences

There was a significant difference in the level of plateau for max concentration between root and stem-leaf samples ($P<0.05$). Root tissue reached the plateau of max concentration of 35.41 mg kg^{-1} by day 28. Stem reached the plateau of max concentration of 2.45 mg kg^{-1} by day 90. Leaf reached the plateau of max concentration of 3.36 mg kg^{-1} by day 90. Root tissue began to plateau at day 14 of exposure to Cu contamination, whereas stem and leaf tissue began to plateau at 21 days.

II.4. Discussion

Although there have been many studies on the toxicity of metals in the marine environment around San Diego (Chadwick, et al., 2004; Corbisier, et al., 1996; Middaugh, et al., 1993; Tabak, et al., 2003) little is known about the fate of metals in the salt marsh ecosystem in Southern California. This study focused on *Spartina foliosa,* due to its ability to tolerate and absorb nutrients in a saline environment, excreting salt on its leaves. Though salt marsh plants, exhibiting resistance to harsh environmental conditions, they have not been known to be accumulators of metals. However, several freshwater algae species, bacteria, pteridophytes and mycorrhizae are known to be hyperaccumulators, having the potential to clean up metal contaminants (Prasad 2003). Salt marsh sediment is an anoxic reducing environment that acts as sinks for metals. Plants oxidize the sediment surrounding roots, making metals more mobile, thus bioavailable to fauna (Weis et al., 2004). Nevertheless, salt marsh sediment generally acts as a sink despite plant effects on metal biogeochemistry and mobility. While several studies have shown that plant litter absorbs greater metal concentrations (Windham et al., 2004, Giblin et al., 1089), the bioavailability of metals to detritus feeders has not been assessed. Isotope analysis has observed that marsh infauna does not fed on living or decomposing *Spartina,* but feed on microalgae (Currin,1990). Isotope analysis on the west coast have shown benthic infauna to feed on *S. pacifica* detritus (Whitcraft et al., 2008) and *S. foliosa* provide organic matter that support invertebrate fish (Kwak et al., 1997). Further studies are needed to assess the impact of *Spartina* detritus on food webs.

II.4.1 Contribution of this study to current knowledge

In this study, the sediment and root tissue retained copper in below ground biomass at the same levels as observed at SW during winter seasons of the field studies. However, over the duration of 90 days exposure to copper, the elevated levels of copper observed in the stem and leaf where 1.5x greater in the field studies during winter. Cu accumulation was 3.5x greater in plant tissue then sediment in the laboratory and 5.2x greater in plant tissue then sediment during winter in the field. This suggests the metal accumulation is absorbed from water column. In general, a major trend in plant tissues (root, stem, and leaf) observed a similar profile of kinetic rate in accumulation uptake. However, copper concentrations were an order of magnitude greater for root, than either stem or leaf. This largely resulted in the phytostabilization of copper being stored below ground. The mechanism of phytostabilization of metals reduces the bioavailability and toxicity of metals in the marine waters and sediment to biota.

II.4.2 Contribution to coastal managers

The implications of this study support the need for management strategies to determine non-point source contaminants in urban areas to identify anthropogenic sources that impact water quality. Policies on monitoring contaminate levels by assessing levels in marsh plants can contribute towards understanding the levels of metals that are bioavailable to organisms from metals dissolved in seawater and uptake from sediment, in determining the ecological impact of contaminants. Further studies to assess the cycling of metals in estuary habitat would include assessing metal accumulation in detritus material, impact on benthic infauna community structure, grain size evaluation across individual marshes, and laboratory experimentation of Cu spiked sediment to determine kinetic absorption rates from sediment verses dissolved in seawater.

Summary

Identifying environmental factors that mobilize metals, thereby, increasing the bioavailability to biota in marine waters is a key component in developing biomonitoring techniques for environmental quality assessment. This study showed the importance of laboratory experimentation that determined contaminants dissolved in seawater are first taken up by root tissue then subsequently transported through the rest of the plant tissue. Implications of metal being absorbed directly from the water column into the roots was observed with a 10.92x increase in root tissue as opposed to a 2.47x increase in metals associated with sediment. The laboratory experimentation was supported by field observations of increased metal content in plant root tissue during winter season ensuing the transport through all plant tissues by summer, 6 months later. Interestingly, the plateau Cu concentration levels where similar to levels observed in SW during the winter. However, in the field study, by summer the level of metal content in plant tissue not only decreased but the plant tissues (root, stem, leaf) had similar levels of metal content. Metals can be remobilized in the salt marsh habitat through detritus and dissolved organic matter. Identifying the cycling of metals within the salt marsh ecosystem can lead to the linkage between the bioavailability of metals to larva and

invertebrates in coastal waters. Developing biomonitoring techniques for environmental quality assessment that include the mobilization of metals through salt marsh plants and ultimately the impact of contaminants in coastal waters.

Chapter III

General Discussion

III.1 Urban density is not the main factor affecting metal concentration associated with sediment of salt marshes in southern California

Urban stormwater is considered a major non-point source of contamination of metals to coastal receiving water. Therefore, increasing development in watersheds usually leads to greater flow of stormwater containing a greater load of contaminants due to the imperious surface area, the later then based on land-use whether urban (developed) or non-urban (undeveloped) watersheds (Tiefenthaler et al., 2007). Usually metal contamination is found the greatest in stormwater from industrial sites, followed by residential, then commercial, and finally open space. In some cases the annual metal loading of Cu and Pb can be 2x greater in industrial compared to residential stormwater, and Zn can be up to 5x greater (Tiefenthaler et al., 2007). It is clear therefore that metal input to coastal areas increases with urbanization/industrialization, at least in terms of dissolved metals.

When dissolved in water, metal inherently reacts and adsorbs to suspended solids and organic matter; thus industrial stormwater can wash away both dissolved metals and perhaps more importantly metals associated with particles. Analysis of stormwater runoff following specific storm events show that the peak of suspended solids concentration coincides with the peak of metal concentrations in stormwater runoff (Stein et al., 2007; Ackerman et al., 2003; ACWA, 1997; Pitt et al., 2003). Along Southern California, San Diego county has a north to south increasing gradient of land-use of the watershed, with the northern land-use being essentially residential increasing southward to commercial/residential then being heavy industrial land-use further south with exposure to raw sewage inputs along the southern border to Mexico. These watersheds usually drain into salt marshes and lagoons that have the inherent ability to retain suspended matter and improve water quality along the coastline. Vegetation in tidal marshes decrease flow velocity increasing deposition of suspended matter and dissolved organic matter (Leonard et al., 1995). This leads to the assumption that salt marsh metal content increases with increased urban development along the coastline.

This study observed the greatest concentration of metals associated with sediment at a northern marsh, Kendall Frost Reserve (KF), with watershed land-use primarily residential and recreational, with only limited commercial development. Such elevated metal load in salt marshes therefore originates from other factors than just stormwater runoff. Other factors include the transport of metal loaded particles by tidal flushing. Dredging of the bays for commercial shipping and recreational boating re-suspends historical pooled contaminants associated with sediment that are then redistributed by tidal flushing (Rohatgi et al., 1975). KF, which lies on Mission Bay was historically a salt marsh shallow lagoon habitat which was transformed through extensive dredging into a heavily used aquatic park surrounded by some residential and commercial development. In addition to metal inputs from stormwater runoff and re-suspended particles another source is the passive leaching of metals, Cu in particular, from antifouling paints used for water vessels, an important source of contamination in marinas, bays, and harbors (Schiff et al., 2004).

However, suspended matter and metal contaminant loads in residential development of San Diego County is equal to or greater than industrial development

(Schiff et al., 1997). This could originate from increasing atmospheric deposition of contaminated particle as a major contributor of metal contaminant in stormwater runoff. In semi-arid urbanized area like San Diego, atmospheric deposition can account for 57-100% of metal contaminant in stormwater runoff (Schiff et al., 2005; Sabin et al., 2007). Dry atmospheric deposition onto land surface area within the watershed has a large influence on quality of stormwater runoff from urban areas (Sabin et al., 2006). These metal loaded particles eventually reach coastal areas where they can precipitate and accumulate, thus forming a continuously growing end reservoir of metal concentrations. The influence of atmospheric deposition on the watersheds in San Diego County could therefore to be one of the major factors contributing to the lack of north to south gradient of metal concentrations in salt marsh sediment.

III.2 Lack of a north to south gradient of anthropogenic contamination

Environmental managers are continuously challenged in determining the criteria for monitoring the input of anthropogenic contaminants. Characterizing contaminant source can aid in policy-making management that reduce contaminant loads that reach coastal waters. Criteria or tools used to assess the impact include monitoring the concentration of contaminant in seawater, monitoring stormwater inputs, assessing concentration of total metals in sediment, and determining an enrichment factor. These assessments are limited in that they do not account for characterizing the contaminant source, environmental effects of contaminants, and environmental endpoints (Stein et al., 2009).

One tool that can be used for policy-making management is the enrichment factor (EF), usually used to assess the extent of anthropogenic contaminant in coastal areas, including salt marshes (Chen, et al., 2007). The foundation of EF is that metals originate from both anthropogenic and natural sources. A natural source of metals is by weathering of soil and rock. Anthropogenic metals associated with sediment, however, are usually adsorbed to the surface area of particle and can therefore be leached with a weak acid (Katz et al., 1981). Natural occurring metals in the environment are usually bound within the particles in a lattice formation and can only be released with complete dissolution of sample sediment (Katz et al., 1981). EF is a technique used to determine the difference between naturally occurring metals and anthropogenic metals.

In calculating EF a baseline is determined from naturally occurring metals in the earth's crust. This technique normalizes sediment characteristics with Al as aluminum silicate is usually found in coastal sediment. EF values were interpreted as suggested by Birth (2003) for the metals studied with respect to the earth crust average (Taylor, 1964). EF of this study is defined as:

$$EF = (X/Al)_{sed} / (X/Al)_{crust}$$

Where X/Al is the ratio of metal (X) to Al. The scale is: EF<1 no enrichment, EF<3 minor enrichment, EF=3-5 moderate enrichment. EF=5-10 moderate to severe enrichment, EF=10-25 severe enrichment, EF=25-50 very severe enrichment, and EF>50 extremely severe enrichment.

EF values were not previously known for the four salt marshes considered in this study. Overall, Ag, As, Pb, and Se were severely enriched (Table II.3). Ag was the most severely enriched metal among all marshes, followed by Pb. Pb contamination in San Francisco Bay area is attributed to atmospheric deposition in tidal salt marshes, with EF values of 8-49,(Hwang et al., 2006). This study observed EF values of Pb between 20-50 among all salt marshes. Sr and V were moderately enrichment at all marshes. Kendall

Frost Reserve (KF), Sweetwater Wildlife Refuge (SW), and Tijuana Estuary (TJ) had severe enrichment of Cd, and Zn, and moderate enrichment of Mn and Cu.

Table III.1. Enrichment factors (EF) of metals in sediments from four salt marshes, Los Peñasquitos Lagoon (LP), Kendall Frost Reserve (KF), San Diego Bay's Sweetwater Marsh National Wildlife Refuge (SW), and Tijuana Estuary (TJ). Sediment samples collected August 2007.

Metal	LP	KF	SW	TJ
Ag	**643.37**	**124.88**	**1,137.28**	**1,332.57**
Al	0.04	1.00	1.00	1.00
As	**6.75**	**18.84**	**7.62**	**8.45**
Cd	0.08	**17.39**	**560.10**	**455.55**
Cr	1.02	1.51	1.88	1.23
Cu	>0.01	**5.10**	**15.63**	**4.31**
Fe	2.73	0.77	**3.41**	2.83
Mn	2.55	**3.10**	**3.40**	2.14
Ni	-	-	-	-
Pb	**22.78**	**49.86**	**19.70**	**27.08**
Se	**25.40**	**11.51**	**31.82**	**55.44**
Sr	**3.32**	**7.74**	**4.67**	**4.47**
Ti	0.15	0.41	0.43	0.41
V	**3.25**	**5.87**	**3.99**	**3.53**
Zn	0.00	**10.01**	**25.89**	**17.14**

Contrary to the assumption that Los Peñasquitos Lagoon (LP) was a relatively undisturbed salt marsh, it had EF values indicating moderately severe to extremely severe enrichment by metals. KF was observed to have the greatest concentration of metals associated with sediment; however, after considering EF values KF no longer had the greatest values, SW had the greatest EF values indicative of the heavy industrial and military use of the harbor. SW lies in San Diego Bay, which has a historical contaminant reservoir attributed to industrial and sewage discharge directly into the bay. TJ had similar EF values as SW. TJ is subjected to sewage spills and industrial waste in stormwater from a watershed that extends across the border into Mexico. Thus EF values provide evidence that metals are retained within salt marsh sediment.

A large part of the retention ability is the capacity of the vegetation to decrease water velocity, thereby increasing the deposition of suspended matter and promoting more anoxic sediment within the salt marsh. Salt marsh are known to improve water quality by retention of contaminants with marsh ecosystem. The ability of salt marshes to act as a sink, retaining metal contaminants, makes this habitat a central site for developing an understanding of the mechanism and processes increase the biological availability of metals to biota.

III.3 Bioavailability of metals is responsible for levels of metals found in salt marsh plants

Not all metals present in the environment are biologically available to organisms. Those that are biologically available are considered bioavailable. Some of these metals are essential for life in small quantity (Cu, Cr, Fe, Mn, Zn) and some are non-essential metals (Pb, As, Hg). Though metals are neither created nor destroyed, they undergo transformation between chemical speciation (Reiley, 2007). In solution metals are associated with ligands of suspended matter and dissolved organic matter (Luoma, 1989; Reiley, 2007). The affinity of metals to a particle increases with decreasing size of the particle. Metals associated with sediment partition into three defined fractions: weakly bound to sediment that is exchangeable and bioavailable to biota, bound to oxides such as Fe and Mn, and bound to organic matter (Almeida et al., 2005). Salt marshes thus clearly appear to act as sinks with increased deposition and adsorption of metal to organic matter and storing metals in sediment bound oxides.

However, there are several physical and chemical parameters that drive the bioavailability of metals in marine organisms: variations in sediment geochemistry including grain size, pH, salinity, alkalinity, and redox potential (Luoma, 1989). These factors can change the speciation of metals, adding to the complexity of explaining or predicting the effect of metal bioavailability.

Criteria developed to assess the biological effects of metals need to address the complexity of different levels of bioavailability between various organisms. Each metal species has a unique fate and transport for the bioavailability and bioaccumulation characteristic (Reiley, 2007). Environmental factors including grain size, salinity, and organic matter influence bioavailability of metals to biota. For example, San Diego Bay has an increasing gradient of metals associated with sediment from the mouth of the bay to the back of the bay, with a similar concentration of dissolved metals in seawater throughout the bay. Deheyn and Latz (2006) found that the arms of the brittlestar accumulated dissolved metals from the water column at greater concentrations at the mouth of the bay and ingested metals in the disk at greater concentrations in the back of the bay. Particle size was a key factor affecting the absorption of dissolved metals (Deheyn et al., 2006). However, bioavailability of metals can change with varied uptake and transport patterns. One of the main factors affecting bioaccumulation of metals was the route of ingestion, with deposit feeders having the greatest accumulation of metals and carnivores the least (Dauvin, 2008). Accumulation patterns can change with salinity; therefore salinity can be a factor affecting metal bioavailability to benthic organism in the salt marsh (Dauvin, 2008).

In this study Kendall Frost Reserve (KF) had the greatest concentration of metals associated with sediment. Despite the increased level of metal contamination, salt marsh plants accumulated metal concentrations inversely to sediment concentrations. This is indicative of different levels of bioavailability of metals among the salt marshes. Sediment characteristics such as small grain size and increased organic matter concentration are known to increase retention of metals in sediment (Laing et al., 2008). This study supports these findings, where enrichment of metal content in sediment increased with decreasing grain size and increasing organic matter, therefore affecting the bioavailability of metal to plants. Grain size and organic matter appeared to be the main factors controlling bioavailability of metals to salt marsh plants. Salinity is another environmental factor affecting the mobility of metal in sediment (Laing et al., 2008); however, in this study salinity did not vary among all four salt marshes.

III.4 Plant species from different marsh zones have different metal concentrations

Tidal inundation and duration influence the structure of salt marshes dictating vegetation zones and sediment deposition (Boyer et al., 1998; Silvestri et al., 2005). The lower marsh zone is inundated for periods up to 9 hours a day whereas marsh plain and high marsh is inundated periodically for less then an hour on average. During tidal inundation, the salt marsh vegetation is exposed to contaminants in the ambient waters. Many of these contaminants are metals associated with suspended matter and dissolved organic matter. As the ambient waters are flushed across the salt marsh, the vegetation slows the velocity of the water increasing deposition of suspended mater. Thus vegetation in the low marsh zone is exposed to increased levels of metal contamination, therefore, have greater concentrations of metals in sediment and plant tissue.

However, in this study salt marsh plants from different vegetation zones had similar metal concentrations. This could result from differences in biology of these three plants. *Spartina* sp., the low marsh plant, is known for its ability to release metals through salt glands on leaves (Burke et al., 2000; Kraus et al., 1986; Windham et al., 2003). The release of metals through salt glands may explain the lack of higher metal concentration in leaf tissue from this plant. In addition, differences in plant carbon cycling may influence the variability in the ability for plants to exclude metals (Windham et al., 2003). *S. foliosa*, uses C_4 carbon cycle and the marsh plain plants *S. pacifica* and *B. maritima* use C_3 carbon cycle.

Also considered for possible metal accumulation differences were two plants from the marsh plain, *S. pacifica* and *B. maritima* with minor differences in physiology. *S. pacifica* has an upright morphology, high canopy and differs in biomass and nitrogen accumulation from *B. maritima. B. maritima* has a trailing morphology and growth is low to the ground. However, the differences in physiology did not overcome bioavailability of metals. It clearly appears that plant zones and tidal inundation have a limited affect on metal bioavailability in the framework of this study.

III.4.1 Comparison to Mediterranean salt marshes

Much is known about metal cycling and bioavailability including gradient of urbanization, difference marsh zones, different plant species, different seasons, in the Mediterranean, which has a climate similar to that in southern California.

- *Gradient of urbanization*

Most studies were either on heavily impacted marshes and lagoons or relatively undisturbed sites; however, a few observed increasing gradient of metal contamination in plants and seawater between their sites. Campanella et al. (2001) showed an increase in metal (Cd, Cr, Cu, Pb, and Zn) concentration in seagrass from a site in which was the local harbor and impacted by human activity. However, Campanella et al. (2001) focus in this study was to establish a baseline for natural metal concentrations in seagrass. Favero et al. (2002) observed increases in metal contaminant (Al, Mn, Fe, Cu, Zn, Cr, Co, Ni, and Cd) in the leaf tissue of macroalgae across Venice Lagoon, Italy; however, only one site showed increased concentration of metals associated with sediment. This site was known as a reservoir for historical metal contamination.

- *Difference marsh vegetation zones*

There were studies on vegetation zones in salt marsh habitat (Silvestri et al., 2005), however, none reflected the impact of inundation and marsh vegetation zones on metal accumulation in salt marshes or lagoons.

- *Different plant species*

Portugal studies determined how the type of salt marsh vegetation affects retention capacity of metals (Reboreda et al., 2006). *Spartina* sp. was more effective as a sink, in a phytostabilization process that immobilizes the metals (Cu, Zn, and Pg) in sediment than *Halimione* sp., which was a source of metal contaminant, re-introducing metals back into the marsh ecosystem. In another study the affect that different plants can have on sediment physical and chemical properties showed plant roots could change the surrounding sediment redox potential (Reboreda et al., 2007). Typically, salt marsh sediment is reductive, causing metals to be bound to sulfides and retained in sediment.

Halimione sp. was observed to transport oxygen to roots thereby oxidizing sediment, thus increasing mobility of metals for plant uptake (Reboreda et al., 2007). Different environmental parameters thus clearly appear to impact the bioavailability of metals to marsh plants.

- *Different plant tissues*

Three plant species and plant tissues (root, stem, leaf) were observed to uptake metals (Zn, Cu, Cd, and Co) during the growing season (Cacabor et al., 2009). The greatest increase in leaf concentration was in the autumn (Cacabor et al., 2009) as seen in San Diego salt marshes. As above ground biomass increased, the uptake of metals with nutrients was reflected with increase metal concentration in leaf tissue (Cacabor et al., 2000). Increases in metal concentration in root tissue were followed by an increase in root biomass. Clearly it appears that metals are being uptaken by the roots from the sediment during the growing season. Favero et al. (2002) observed metal concentrations in leaf tissue in macroalgae which do not have roots, were equal to metal concentrations in seawater at same sites. The increased metals in leaf tissue appear to be adsorbed from dissolved metals in seawater. What is unique about this study was the integration of environmental factors, including grain size and organic matter, along with seasons, to assess route of metal uptake by salt marsh plants.

- *Different seasons*

Metal concentration decreased in root tissue during the winter season (Cacabor et al. (2000). Contrary to this study in San Diego marshes, were metal concentration increased in root tissue during winter season. The increase in root tissue of appeared to be adsorption of dissolved metal from seawater. Similar to Favero et al. (2002) findings on macroalgae in Venice Lagoon. Understanding contaminant sources and uptake patterns may explain some of these differences. The possibility that dissolved metal contaminants in stormwater is uptaken by plant roots may be attributed to less suspended solids for metals to bound in ambient water.

- *Bioavailability*

Campanella (1999) determined an increase in metal concentration (Cd, Cr, Cu, Pb, and Zn) in leaf tip compared to leaf basal. Some metals suggested different uptake routes, the metal profile of Cd and Zn exhibiting uptake from water through photosynthetic tissue whereas Cr and Pb where shown in decreasing concentrations of old leaf > root > new leaf (Campanella et al., 1999). Increased concentration in older leaf tissue is indicative that leaf tissue continuously accumulating metal, possibly exporting metal through leaf.

III.4.2 Comparison to East Coast USA salt marshes

Many studies come from the East Coast (>70), with just a subset that pertain to this study reflected here. These studies have demonstrated that different metals have various profiles that influence absorption and translocation of metals from sediment to above ground biomass (Windham et al., 2003). Windham et al. (2003) reported greater concentrations in root tissue than leaf tissue of the two species *Spartina alterniflora* and *Phragmites austrilia.* In contrast, this study found comparable levels of Cr, Pb, and Zn in aboveground biomass of three species *Spartina foliosa, Sarcocornia pacifica* and *Batis maritima*. Industrial areas showed increased concentrations of metals in the sediment with plant accumulation of Cu, Hg, and Mn in leaf tissue (Albert et al., 1990). Comparatively, Kraus et al. (1986) found *Spartina* accumulated Hg and Cu in leaf tissue.

Kraus et al. (1986) reported *Spartina* excreted metal through salt glands in the leaf tissue. This demonstration of release of metals through salt glands is supported by other studies (Burke et al., 2000, Kraus, 1988; Kraus et al., 1986). The release of metals through salt glands may explain why low marsh plant *S. foliosa* had lower metal concentrations then expected.

III.5 Metal uptake is increased in plants during winter season

Southern California has a semi-arid climate, with seasonal rainfall, implying that stormwater runoff from surrounding watershed is a primary source of winter contamination. Biomonitoring of contaminant load during stormwater runoff has observed increases in metal contaminant from stormwater (Stein et al., 2007). Seasonal measurements in San Diego Bay observed different speciation of metals for winter and summer (Blake et al., 2004). Winter season showed an increase in Cu^{2+} concentrations and a decrease in total suspended solids (TSS), although dissolved Cu remained the same (Blake et al., 2004). Decrease in TSS can be explained by the decrease of phytoplankton and zooplankton during the winter season, resulting in lower levels of TSS. The free form of metal in the ambient waters may be readily available for plant uptake; however, plants uptake in nutrient usually coincides with summer growing season.

Plants show an increase in biomass during the summer season. Quarterly sampling of *Sarcocornia pacifica* in California salt marshes revealed a strong trend of 2x greater biomass during summer season (Boyer et al., 2001). Increase in above ground biomass in salt marsh plants during the summer season also occurs on the East Coast, USA (Weis et al., 2003). Increased levels of nitrogen following spraying of a nitrogen mixture during the winter were followed by increased levels of nitrogen in the biomass of *S. pacifica* during the summer (Boyer et al., 2001). An assumption is that the increase in biomass would reflect increased concentrations of metals in the plant tissue uptaken from the sediment with the nutrients during the summer season.

In this study, metal concentrations in root tissue in plants from San Diego Bay's Sweetwater Marsh (SW) were 4x greater in winter than summer, with only minor increases in metals associated with sediment. At Kendall Frost Reserve (KF), root metal concentration was 1.5x greater in winter season than summer; however, metals associated with sediment were 4x greater in winter season than summer at this site. Differences in metal increase may be due to differences in the sediment characteristics. KF sediment characteristic showed the greatest content of organic matter of all four sites. It is well know that organic matter in marsh sediment increases the salt marshes ability to act as a sink and retain metals in sediment (Weis et al., 2003; Reboreda et al., 2006). Another factor is grain size; KF site consisted mainly of silt and clay. Smaller particle size has a stronger affinity for metals, therefore retains metals in sediment, whereas the other sites consisted of a larger percentage of sand. Other environmental factors can cause differences in the content of contaminants in stormwater. Contaminants from different type of urban development within watershed may have an influence on the variation of metal contaminants in the stormwater. There are possible other environmental parameters influencing the bioavailability of metals to marsh plants at these different salt marshes. However, there was a significant increase of metal concentration in root tissue from all marshes, thus it clearly appears that dissolved metals are bioavailable for uptake in plant root tissue during winter season. In the Mediterranean, studies on macroalgae and sea lettuce have shown metal concentrations in leaf tissue are similar to those found in ambient seawater (Campenella et al., 1999; Favero et al., 2002).

III.6 Salt marsh plants are an important component of metal recycling

To understand the cycling of metals in estuarine ecosystems, it is imperative to assess how the metal is accumulated, thereby, determining the bioavailability to flora and fauna. Metal speciation is associated to particulates, organic and inorganic, suspended in the water column (Fig. II.2). A salt marsh's ability to protect the coastline is due to vegetation capacity to attenuate the flow of water and induces sedimentation of suspended matter. Input of land run off is filtered through the transition zone of the salt marsh vegetation,

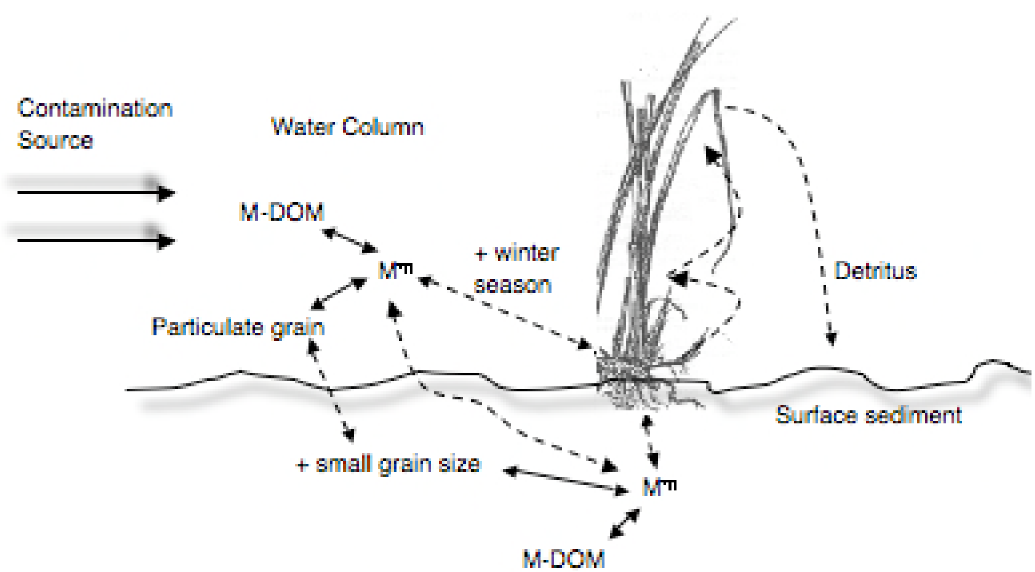

Fig. III.1. A schematic diagram of metal interaction that affect bioavailability to marsh plants. Simplified interaction with suspended matter in the water column, sedimentation, along with an individual plant. Dashed lines indicate possible exchanges between biogeochemical states. M^N refers to metal and M-DOM refers to metal bounded dissolved organic matter.

which retains and removes metals from the water before entering the ocean. Understanding the mechanisms that affect bioavailability of contaminants within the salt marsh ecosystem can lead to improvement in environmental water quality.

Essential ecological functions of the salt marsh include nutrient cycling, nutrient production and decomposition (Levin et al., 2001). *Spartina* plays an important role in metal cycling in salt marshes (Kraus et al., 1986). Metals taken up from sediment by plant root tissue can then be transported through the stem to leaf tissue. *Spartina* excretes Hg, Cd, and Zn on leaf salt glands (Weis et al., 2003). Excretion of metals on salt glands of leaf can enter into ambient water during tidal flushing. Weis et al. (2003) reported lower leaves, which are older, have greater concentrations of metals then younger leaves that are higher on the plant. This implies that leaf tissues continue to accumulate metals, or possibly increases leaf tissue accumulation. *Spartina* and *Phragmites* export metals in senesced leaf tissue as a detoxification mechanism (Weis et al., 2003). Metal enrichment in detritus exceeds levels in surrounding sediment (Windham et al., 2003), thus supporting the idea that leaf loss contributes to metal recycling. Increased accumulation of metals in detritus has been reported in association with fungal litter colonizer (Laing et al., 2005; Windham et al., 2003) Fungi, bacteria, and benthic invertebrates regulate nutrient flux and particles by decomposition of plant litter. As plant litter is broken down the metals are released and may return to sediment or be exported with tidal flushing.

This study demonstrates the importance of assessing the fate of metals in the salt marsh ecosystem because of salt marsh's inherent ability to retain a majority of the metal contaminants. However, the increasing loads of contamination can impair the ability of salt marshes to immobilize metals in below ground sediment and biomass. Salt marsh plants ability to retain metals in belowground biomass during winter season, transports to aboveground biomass is imperative for possible uses for biomonitoring contaminant metal levels in environmental quality assessment. Because Southern California salt marshes have been reduced to less then 15% of their historical size due to urbanization, more monitoring is needed.

III.7 Leaf from salt marshes as monitoring tool for metals bioavailability

Southern California currently has multiple assessment monitoring programs that assist environmental managers in evaluating and reducing contaminant loads through management programs and policies. Monitoring of effluent, sediment, benthos, and fish has lead to major infrastructure improvements for reducing endpoint source discharge (Stein et al., 2009). The effluent from waste treatment plants is monitored for constituents including heavy metals and suspended solids. The reduction in mass emission is reflected in sediment cores, with a reduction in nutrients and refractory (metals and pollutants). Evaluation of the reduction in effluent on the benthic community was assessed by annual and biannual transects measuring the species diversity and abundance. Including the top consumer by evaluating the health of bottom fish for fin erosion and tumors (Stein et al., 2009). Improvements in reducing contaminant loads from mass emission discharge has allowed environmental managers to move resources to non-point source contaminants, such as stormwater. Biomonitoring of stormwater can provide information about non-source contaminant concentrations that are useful to environmental management. However, this study has shown that biomonitoring by sampling of leaves is a tool for environmental managers to assess bioavailable metals from winter stormwater.

This study shows the importance of assessing dissolved metal contamination from winter stormwater by monitoring metal concentrations in salt marsh leaf tissue. Advantages of using salt marsh plant leaf tissue include leaf metal concentration with contaminate from past winter; collected leaf from dry plant equal contamination of metal input as dissolved metals; easy, fast, non-invasive and non-destructive since whole plant not necessary. Possibility of variation between leaves, even on the same plant, therefore a large sampling of leaves should be collected to get accurate data (Weis et al., 2003; Windham et al., 2003).

Moving beyond biomonitoring, the inherent ability for salt marsh vegetation to improve water quality makes them optimal in the use of wetlands restoration. An approach that encompasses wetland mitigation, not only for replacing habitat that will be lost due to development, but including sites where heavy contaminant loads are known to reach ambient water can improve water quality. Incorporation of salt marsh vegetation can filter out contaminants before entering into ambient receiving waters. Biomonitoring assesses past contaminant loads leading to improvement for environmental management plans. However, future solutions for wetland mitigation to improve water quality can lead to biological infrastructure improvement for reducing endpoint source discharge at stormwater drains.

IV.8 Future Work and Recommendations

Few studies have been done on the level of metal contamination in salt marshes of southern California, and hopefully this thesis will open new avenues for further work in this field. In particular, additional studies would be needed to assess the excretion of metals in salt glands on the leaf of *S. foliosa*, which might be key for understanding possible cycling of metals in salt marshes. Increased accumulation of metals in plant litter may be a factor in cycling of metals, and further studies would help with evaluating contribution to transfer up the food web. Such studies would benefit from the large amount of work already done on salt marshes on the East Coast USA, which might serve as a template. However, southern California's semi-arid climate will probably affect some of these mechanisms and processes, which deserves quantification.

Effective monitoring should provide environmental managers with useful information for any decision and policy-making processes. A successful monitoring strategy to assess salt marshes health and their ability to retain and recycle contaminants would develop into three parts. 1. Monitoring contaminant loads (dissolved and particulate) being washed out of urbanized areas, especially during storm events, which would evaluate the contaminants exposure through runoff. 2. Monitoring contaminant loads (associated with sediment) across salt marshes to evaluate contaminant loads that are retained or not within this ecosystem. 3. Monitoring the levels of bioavailable metals using as model salt marsh plants, in particular by analyzing metals content of leaf tissue from *S. foliosa* in the summer which would evaluate metal concentrations made bioavailable during winter in stormwater runoff.

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