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Benthic microalgae as bio-indicators of sediment quality in San Francisco Bay

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BENTHIC MICROALGAE AS BIO-INDICATORS OF SEDIMENT QUALITY IN SAN FRANCISCO BAY

A Thesis

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by

Andalusia I. Khechfe

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APPROVED FOR THE DEPARTMENT OF
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ABSTRACT

BENTHIC MICROALGAE AS BIO-INDICATORS OF SEDIMENT QUALITY IN SAN FRANCISCO BAY

by Andalusia I. Khechfe

Benthic microalgae are an important food source for many benthic organisms and comprise a large segment of the living community of sediments. Therefore, these algae could be significant vectors in the trophic transfer of pollutants. The focus of this study was to improve the collection method and analytical approach for measuring trace metals in benthic microalgal samples and to evaluate these algae as potential monitoring tools in assessing sediment quality.

Fine grain sediment particles can contaminate samples during collection. Correlating aluminum concentrations with trace metal concentrations measured in algal samples allows for the differentiation between trace metals associated with the algal component of the sample versus the sedimentary component of the sample. No clear indication of trophic transfer was observed between metal concentrations in the algae and metal concentrations in *M. balthica*. However, benthic microalgae may provide a good indicator of the *bioavailable* metals present in sediments and pore waters.

Key words: benthic microalgae, diatoms, bioavailability, sediment quality criteria, *Macoma balthica*, trace metals.

To my husband, Amine, with everlasting gratitude for his enduring love and constant encouragement and support throughout this endeavor.

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CHAPTER I

INTRODUCTION

Historical Impacts and Management of San Francisco Bay

During the past one hundred and fifty years, the San Francisco Estuary has been physically altered and transformed in ways that have resulted in negative impacts at all ecological levels. Economic growth and other benefits that have drawn people to the Bay area have taken a toll on the ecological health of the estuary. Growth in industry, agriculture, and an increasing human population have contributed to the reduction of wetlands, the pollution of water and sediments, and a general reduction in the quality of the estuary's natural resources. Additionally, the intentional and accidental introduction of non-native organisms to the Bay has resulted in uncertain ecological impacts and negative consequences for indigenous aquatic organisms.

Historically, threats to aquatic resources have been addressed using a crisis management approach usually focusing on a particular species in distress without sufficient understanding of interconnections between organisms and abiotic factors in the ecological system (San Francisco Estuary Project 1992). Scientists now recognize that this approach to aquatic resource management requires modification to include more unified studies addressing questions relating to food web processes and trophic connections (San Francisco Estuary Project 1992). In particular, scientists researching water quality issues and officials involved with developing water quality policies, such as

the Environmental Protection Agency and the Regional Water Quality Control Board, have come to recognize the importance of understanding the complex interactions between pollutants found in sediments, dissolved pollutants present in the overlying water column, and pollutants assimilated by benthic organisms exposed to these media.

Mudflats are large and important habitats in the San Francisco Estuary (California Regional Water Quality Control Board 1995). Since organisms such as snails, clams, and worms convert organic matter in the bottom mud to food for fish, crabs, and birds (California Regional Water Quality Control Board 1995), there is a clear need to re-evaluate the importance of sediments as a pollutant sink and source for organisms inhabiting the benthic environment. Sediment quality, like water quality, is important to the health of an aquatic ecosystem. Scientists recognize that although effluent from industrial sources may be in compliance with regulatory water quality criteria, over time, heavy metals and other contaminants can partition to the sediments resulting in very high sediment concentrations.

There exists only a limited understanding of the processes that govern bioavailability; the cellular uptake of contaminants by an organism from food or its environment (Luoma and Carter 1991). As a result, it is difficult to define how biota are affected by exposure to contaminants in nature. The total metal concentrations measured in the solute form and in sediments are not equally available to the biota (Sunda and Guillard 1976; Luoma 1983; Luoma 1989; as cited by Luoma and Carter 1991). Therefore, the best way of determining toxic exposure is to combine investigative

approaches that include sediment, water, and biological tissue measurements (Luoma and Carter 1991).

Sediment Quality Criteria and Current Approaches

The Regional Water Quality Control Board and the EPA are currently involved with the research and development of sediment quality criteria. Such criteria are important for the ecological health of the estuary and important for the development of policies that regulate activities that impact water and sediment quality in the Bay. Regulatory applications for such criteria are diverse. Criteria would assist in the designation of dump sites for industrial discharge or dredged materials as well as assist in the permitting process of dumping and disposal (Environmental Protection Agency 1987). Sediment quality criteria would also be useful for monitoring of waste disposal sites, for the identification or clean up of contaminated areas, and for the preparation of environmental impact statements (Environmental Protection Agency 1987).

Methods used for determining the toxicity of sediments have focused on sediment-water interface studies, the geochemical processes affecting metal speciation (the way in which metal ions are bonded to water molecules and organic or inorganic complexes present in their environment), and toxicity to specific organisms. Water quality regulators have difficulty agreeing on the best approaches to measure and manage sediment contamination and on developing a national, standardized sediment quality criteria plan (Adams, Kimerle, and Barnett 1992).

Adams, Kimerle, and Barnett (1992) list and describe the most common methods used for determining chemical specific sediment quality values. These methods include: the *apparent effects threshold* (AET) approach, the *equilibrium partitioning* approach (EP), and the *triad* approach. A common limitation to the three approaches is that they do not consider the living component of the sediment. That is, they do not consider the assimilation of pollutants that may occur in benthic organisms from major food sources such as benthic microalgae which comprise a large component of the living community of sediments.

There are several ways that contaminants can enter the food chain: through direct exposure, through ingestion of sediments or water, or through food sources. Luoma et al. (1992) examined the bioavailability of selenium to the benthic bivalve *Macoma balthica* and stressed the importance of understanding the contaminant vectors. In this study it was determined that the uptake of selenium was primarily from food sources rather than from the surrounding water. Given this, Luoma et al. (1992) emphasized that regulatory criteria (dependent upon solute toxicity assays) for selenium would fall short of protecting aquatic environments such as that of San Francisco Bay.

Metal uptake from food sources continues to be an area of needed research. Wang, Fisher, and Luoma (1995, 165) state that: "Metal uptake from food remains poorly understood. Quantifying the relative importance of dissolved and food sources is essential to the interpretation of data generated by environmental monitoring programs, but this also is poorly understood for most metals of environmental concern and most bio-

indicator species." Reinfelder and Fisher (1994) point out that understanding the assimilation of ingested elements by herbivorous marine organisms is important because trophic transfer of pollutants at the lowest levels of marine food chains can negatively impact organisms up through the entire food web.

The Significance of Benthic Microalgae

Unicellular algae consist of three major groups: *Euglenophyta*, *Pyrrophyta*, and *Chrysophyta* (Johnson 1983). Species of algae that reside in the sediments of aquatic bodies are referred to as benthic microalgae. These species include diatoms, blue-green algae, and flagellates, typically dominated by pennate diatoms; species possessing a raphe structure or two lateral halves (San Francisco Estuary Project 1992). The golden-brown algae often observed coating the surface of mudflats and estuarine marshes are part of the division *Chrysophyta* (Johnson 1983). This division includes closely related classes such as *Chrysophyceae*, *Prymnesiophyceae*, *Bacillariophyceae*, and *Phaeophyceae* (Lee 1980). The microalgae which are often dominant on estuarine mudflats are from the class *Bacillariophyceae* and are often pennate in form possessing a raphe system on both valves (Round 1971) in which silica is imbedded.

For decades, research has been conducted in an attempt to understand the biology and role that benthic microalgae play in their ecological niches. Some of the first recorded studies of diatoms were conducted during the late 1800s (Round 1971). Recent studies have observed that benthic microalgae are significant contributors to the primary

production of ecosystems and can provide a vital source of food to invertebrate grazers such as annelids, nematodes, flatworms, crustaceans, and mollusks (Gould and Gallagher 1990; Plante-Cuny and Plante 1986). These organisms are in turn consumed by birds and fish. Given this, benthic microalgae may act as a significant vector for contaminant bio-accumulation through coastal food chains (Absil 1993). In San Francisco Bay, benthic microalgae, along with settling phytoplankton (small floating plants in the water column), may be the most readily available source of food for bottom dwelling invertebrates (Nichols and Thompson 1985). Sullivan and Moncreiff (1988) determined that benthic microalgae could provide as much as one third of primary production in some ecosystems. This has been supported by research in south San Francisco Bay which estimates that benthic microalgae can contribute as much as 30% of primary production to the South Bay ecosystem --second to phytoplankton which may produce as much as 60% of primary production (San Francisco Estuary Project 1992).

Benthic Microalgae as a Tool in Aquatic Resource Management

In planning for the future of our aquatic resources, the San Francisco Estuary Project has called attention to the need for a comprehensive, holistic understanding of aquatic ecosystems. Estimating bioavailability is an important initial step in understanding the significance of chemicals sorbed to sediments (Adams, Kimerle, and Barnett 1992). Gaining a better understanding of food web processes and pollutant vectors can enable environmental policy and decision makers to improve ecological monitoring programs for

aquatic resources. Agencies such as the Regional Water Quality Control Board who are involved with developing numerical sediment quality objectives may find the quantification of trace metal concentrations in benthic microalgae useful in the development of sediment quality criteria.

The Bay Protection and Toxic Cleanup Legislation, established in 1989, is a factor that fueled the need to develop sediment quality criteria (California Regional Water Quality Control Board 1996). Some of the policies aimed at sediment contamination have focused on the assessment of the sediment quality of dredged material. Areas of concern for dredged sediments have included designating dump sites for dredged sediments; appropriate re-use of dredged material (wetland restoration and upland beneficial re-use), prediction and accumulation modeling of dissolved and particulate bound contaminants in dredged sediments, and disposal site investigations to monitor contaminants that could gradually exceed designated criteria limits over time (Environmental Protection Agency 1987; California Regional Water Quality Control Board 1996). In interviews with scientists and decision makers conducted by the EPA (Environmental Protection Agency 1987, A-13) most respondents felt that sediment criteria "..should consider the interrelationships between sediment contamination, bioaccumulation, and toxicity" and that "..both field and laboratory testing are necessary to support the sediment criteria." Considering benthic microalgae as contaminant vectors could be useful for establishing sediment criteria if applied to field investigations and in conjunction with comprehensive laboratory methods.

Focus

Researchers have discussed the importance of benthic microalgae as a food source to benthic macro invertebrates (Gould and Gallagher 1990; Plante-Cuny and Plante 1986). Other studies have investigated the role that benthic microalgae can play in the transfer of pollutants through the food chain and the methodological approaches to quantifying this phenomenon (Absil 1993; Stronkhorst, Vos, and Misdorp 1994).

The focus of this masters research was to build on the existing but limited knowledge pertaining to trophic transfer of trace metals from the sediments to sediment dwelling microalgae, and then to quantify trace metal concentrations affiliated with these algal organisms on a mudflat located in San Francisco Bay. This study was carried out to improve on a methodological and analytical approach for determining trace metal concentrations in benthic microalgae while identifying metal concentrations associated with potential sediment contamination that can occur with the collection technique. The final goal of this study was to evaluate the potential use of benthic microalgae as a monitoring tool that could be used with other scientific approaches to develop sediment quality objectives for San Francisco Bay.

General Procedures

This study was conducted in cooperation and with the support of the U. S. Geological Survey, Menlo Park, California. All sediment and clam data (*M. balthica*) were collected, analyzed, and interpreted by scientists at the U. S. Geological Survey as

part of an ongoing monitoring program at this site. I collected benthic microalgal samples for the analyses of chlorophyll and trace metals. This data was then compared with the sediment and clam data analyzed by the U. S. Geological Survey. Silver, copper, and nickel are of primary regulatory concern in the South Bay because their sources have been associated with point-source sewage treatment effluent which removes most but not all of the toxic pollutants from wastewater. Non-point source pollution originating from street and gutter runoff is also a significant problem contributing to trace metal concentrations found in San Francisco Bay .

Because benthic microalgae represents an important food source for many benthic organisms, seasonal trends in biomass (standing algal crop) and trace metal concentration were measured. Sediment core samples were collected on a monthly basis in 1995 (with the exception of the months of March and November due to weather and time constraints) for the analysis of chlorophyll (an indicator of biomass). Additional sediment core samples were taken in January, February, March, and April of 1996 for the measurement of chlorophyll. Benthic microalgal samples were also collected on ten occasions to measure trace metals associated with these algal cells.

Fluctuations in algal biomass were compared with *M. balthica's* condition index, an indication of the tissue weight and health of the clams, to determine the potential importance of benthic microalgae as a food source to the clams. There are a number of factors that influence the condition index of *M. balthica* and one of these factors is food availability. Another aim of the study was to determine whether or not metal tissue

concentrations of *M. balthica* fluctuated seasonally based upon the combined changes in benthic microalgal abundance, metal concentrations measured in benthic microalgal samples, and metal concentrations measured in sediments.

Trace metal analyses for silver, cadmium, chromium, copper, nickel, and zinc were conducted on benthic microalgae collected from Sand Point, Palo Alto Bay-lands Nature Preserve (Figure 1, Appendix B) to determine: a) whether or not a discernible correlation existed between trace metal concentrations in sediments and benthic microalgae inhabiting the sediment, and b) whether or not a trophic link could be observed between trace metal concentrations in benthic microalgae and trace metal concentrations in the clam species *Macoma balthica.*

The results of trace metal analyses on benthic microalgae were compared with trace metal values measured in ambient sediments and clams collected and analyzed from the same mudflat by S. Luoma, D. J. Cain, M. Hornberger, C. Brown, and R. Bouse at the U. S. Geological Survey to determine if a trophic link could be established and to evaluate the use of benthic microalgae as a potential indicator of bioavailable metal concentrations in intertidal estuarine sediments.

Objectives

Six research objectives are addressed in this thesis: a) To determine the temporal variability of benthic microalgal biomass at the selected study site in south San Francisco Bay; b) To determine whether or not benthic microalgae are an important food source for

benthic organisms like *M. balthica*, based on seasonal variability of biomass; c) To determine whether or not trace metal concentrations in benthic microalgae can be quantified with the use of a specific collection technique and analytical approach; d) To assess whether or not benthic microalgae reflect the variability of trace metal concentrations measured in the surrounding sediments; e) To determine whether or not a relationship can be observed between trace metal concentrations measured in benthic microalgae and trace metal concentrations measured in the clam species *M. balthica*; and f) Based upon the above five objectives, are benthic microalgae a useful tool to use in monitoring sediment quality?

CHAPTER II

BACKGROUND AND RELATED RESEARCH

Sediment Characteristics, Bioavailability, and Biological Processes

Overall, there is a greater understanding of specific metal reactions in solution than in sediments (Luoma 1989). However, it is understood that pollutants will bind preferentially to different sediment types, sizes, and detrital particles (Baker 1980) which can result in varying toxic effects (Adams, Kimerle, and Barnett 1992). Sediments composed of carbonate minerals including calcium and magnesium can occur in shallow marine sediments and carbonates can play a role in the adsorption of trace metals and other ions (Sadiq 1992). Iron is another major component of sediments and is very reactive in adsorption processes in both sea water and marine sediments (Sadiq 1992). The binding strengths of pollutants to sediment surface sites is also dependent upon the concentration of the reacting species, the pH, the ionic strengths, and the presence of competing ions in solution (Baker 1980). Trace metal associations decrease with increasing quartz composition as sediments become more coarse grained (Salomons and Forstner 1984). In general, finer grained sediments consisting primarily of clay minerals, organic matter, fine grained quartz, carbonate and feldspar particles tend to contain higher metal content (Salomons and Forstner 1984).

Bioavailability of trace metals is highly dependent upon speciation. Different contaminant species will have different bioaccumulation trends, toxicity limits, adsorption

and desorption strengths on a given particulate, different rates of transfer to the sediment, and different transportation rates and vectors in a seawater system (Sadiq 1992). Also of key importance is the fluctuation in the oxidation-reduction conditions which can influence mineral transformation in an aquatic environment (Sadiq 1992).

Biological processes such as digestive mechanisms (gut retention of ingested material) and food sources can influence bioavailability. During digestion or as a result of extracellular secretions, plants and animals can alter forms of metal prior to assimilation (Davies 1976, Mcknight and Morel 1980; cited in Luoma 1989). Seasonal changes in reproductive states can also induce variability of trace metal concentrations in the tissues of some benthic organisms (Cain and Luoma 1986). Studies have also looked at the physiological mechanisms that govern bioavailability of trace elements in benthic organisms feeding on radio-labeled diatoms. Such processes as gut passage time, digestive partitioning, and pH conditions in the digestive tract can determine the extent to which trace metals are assimilated in benthic organisms (Wang, Fisher, and Luoma 1995; Reinfelder and Fisher 1994; pers. comm. Lee and Luoma, unpublished data).

Over the past decades researchers have examined the uptake of metals in phytoplankton, microalgal, and macroalgal species to determine the potential use of these organisms as tools which reflect aquatic pollution and to understand differences in the assimilation of contaminants among various algal species (Absil 1993; Haug, Melsom, and Ormang 1974; Trollope and Evans 1976; Wang, Fisher, and Luoma 1995). Hart (1982) cites a study by Hassett, Jennett, and Smith (1980) who studied a metal polluted stream in

Missouri where very high concentrations of lead, zinc, and copper were measured in freshwater algal samples. Hart (1982) also cites Salomons and Mook (1980) who found that algae accounted for 4-14% of the dissolved trace metal removal to the sediment at Lake IJssel (E. Netherlands). Another area of interest has been the measurement of biological and chemical cycling of elements in the aquatic environment from settling and decomposing diatoms, phytoplankton, and other algae (Ferguson and Bubela 1974; Lee and Fisher 1992; Lee and Fisher 1993; Lee and Fisher 1994). Such studies have been useful in understanding the fate and transport of pollutants from the water column to primary producers, to consumers (zooplankton), then back into the water column and sediments.

Field Studies of Benthic Microalgae

Very few published studies exist that specifically examine and quantify trace metals associated with benthic microalgal cells. More studies have explored the biological and environmental processes influencing contaminant assimilation and effects on cell development and survival of marine and freshwater phytoplankton and algal species (Gnassia-Barelli et al. 1983; Romeo and Gnassia-Barelli 1985; Gutknecht 1963; Ferguson and Bubela 1974; and Sunda and Guillard 1976). This dearth of data is probably due to the difficulty associated with obtaining a sediment-free sample of benthic microalgal biomass. This is a critical problem in determining the concentration of trace metals associated with benthic microalgal cells. Phytoplankton cells are much easier to work

with because the medium in which they exist (water column) facilitates the isolation and culturing of cells in the laboratory. The same is true for macroalgal species. Benthic microalgal species, on the other hand, are difficult to separate from sediments in the field.

A recent thesis study by Absil (1993) conducted in the Scheldt Estuary (Netherlands) explored a method to separate diatoms from sediment for the direct analysis of trace metals. The specific metals that were analyzed included cadmium, lead, copper, and zinc and the potential assimilation of these elements by the feeding clam *Macoma balthica*. The method utilized a combination of lens cleaning tissues and "planktonic gauze" to collect diatoms which were then concentrated by centrifugation and captured on filters. An almost linear relationship was found between lead and zinc in the sediment and lead and zinc in the diatoms at different locations suggesting that these organisms reflected the concentrations of the pollutants in the sediments. Stronkhorst, Vos, and Misdorp (1994) used a method similar to Absil's to collect diatoms for the analysis of trace metals and organic contaminants. This study compared concentrations of contaminants in surrounding sediments and bottom dwelling organisms in the Scheldt Estuary. The findings demonstrated that PCBs in diatoms were within the same range as PCBs found in the infaunal organisms. In the case of zinc, relatively high concentrations were found in *Macoma balitica*--suggesting a trophic connection.

The Absil and the Stronkhorst et al. studies emphasize the benefits of the planktonic gauze method as it allows for the collection of a clean, relatively sediment free benthic microalgal sample. However, neither study sufficiently addressed the issue of

contamination in the sample caused by fine grain sediment particles that can be incorporated into the sample even under the most careful handling conditions. Stronkhorst, Vos, and Misdorp (1994) do maintain that microscopic observations of the diatom samples showed "hardly any sediment particles." Nonetheless, visual observations may not be accurate when determining the effect that sediment contamination may have on the overall trace metal concentrations measured in the sample. In order to accurately assess the trace metal concentrations specifically associated with the algae, one must account for the interference caused by fine grain sediment particles.

CHAPTER III

METHODS

Study Site

The site for this study was located in the Palo Alto Baylands Nature Preserve at Sand Point (Figure 1, Appendix B). This site has been the focus of ongoing monitoring by the City of Palo Alto Waste Water Treatment Facility in collaboration with the U. S. Geological Survey since 1977 to evaluate the impact of improved waste water treatment on trace metal contamination in sediments and the indicator species, *M. balthica.* The sampling site is located approximately one kilometer south of the discharge point of the Palo Alto Sewage Treatment Facility. The major sources of copper and silver contamination are from local sewage outfalls (Thomson et al. 1984), and these metals, together with nickel, are of primary regulatory concern because of their significant toxicity to aquatic organisms. Luoma et al. (1995) compiled and compared trace metal data for clam tissues and sediments from 1977 to the present. The data suggest that over the past 17 years there has been a significant reduction in copper and silver concentrations in clam tissues since the implementation of improved wastewater treatment.

Major sources of trace metal input to the Palo Alto Waste Water Treatment Facility vary. According to the City of Palo Alto Clean Bay Plan (1996) the major point source of copper, up to 51%, originates from the corrosion of water supply piping in homes and businesses. Thirty-five percent of non-point source copper originates from

brake pads. Other point source metals, such as silver, originate from photo processing and x-ray developing, and zinc originates from vehicle service facilities and machine shops. Tissue analyses of *M. balthica* around the sewage outfall site have demonstrated a strong correlation between copper found in clam tissues and copper discharged from the sewage treatment facility (Luoma and Cloern 1982). Silver contamination in the South Bay is reflected in both sediments and in the clams (Luoma and Cloern 1982). Concentrations of metals such as cadmium and zinc found in the sediments are slightly higher than background and below the effects level (Luoma et al. 1995). Analyses of chromium in San Francisco Bay sediment core samples have revealed that high levels of this metal are of geologic origin making it difficult to differentiate between anthropogenic chromium inputs and natural background sources (M. Hornberger, U. S. Geological Survey, unpublished data; cited in Luoma et al. 1995). Nickel concentrations in *M. balthica* are high all over San Francisco Bay when compared to other aquatic environments, but it has also been found to comprise a significant portion of the estuary's natural sedimentary composition (Luoma et al. 1995). Cadmium, chromium, nickel, and zinc were included in this research in order to allow for a broader range of metals to compare between sediments and benthic microalgae and clams and benthic microalgae. Because trace metals bind preferentially and are assimilated preferentially under different geochemical and biochemical conditions, this study provides information on various metals that may or may not be assimilated in the food chain via vectors such as benthic microalgae.

Isolating diatoms

The success of isolating and collecting benthic microalgal samples is dependent upon present biomass, light intensity, water saturation in the sediment, and the tidal rhythm of the diatom population (Stronkhorst, Vos, and Misdorp 1994). The sampling procedure for this study involved the use of a nylon mesh called Nitex, a method adapted from a laboratory approach used by E. Canuel at the Virginia Institute of Marine Science modified for direct field sampling of benthic microalgae. The mesh opening size was 64 microns allowing the free-living mobile species of diatoms (epipelic) to move up through the Nitex by phototaxis and capillary action while screening out sediment particles larger than 64 microns. Prior to sampling, Nitex was acid washed in 10% HCL (cold soak overnight), rinsed thoroughly in de-ionized Milli-Q water, and allowed to air dry flat under a laminar flow hood equipped for ultra clean trace metal free procedures. The use of Nitex allowed for a relatively clean separation of benthic microalgae from the sediment for later processing and analysis.

A limitation of this method, also acknowledge by Absil (1993) and Stronkhorst, Vos, and Misdorp (1994), is that only the mobile epipelic species of microalgae are collected for trace metal quantification. Another limitation of the method is that smaller sediment and detrital particles may be incorporated into the sample caused by the movement of particles from surface water tension or transported with algal mucous secretions making a "pure" benthic microalgal sample difficult to achieve.

This limitation was considered and corrections were made for expected sediment

contamination. This correction was accomplished by analyzing aluminum in the samples. Aluminum is a good indicator of sediment contamination because it comprises approximately 4-7% of the fine grain sediment particles in San Francisco Bay; several orders of magnitude higher than what would be assimilated by benthic microalgae.

Pilot work was conducted to determine the best strategies for obtaining the cleanest sample of benthic microalgae of sufficient biomass for analysis. In February, collection began using: a) two Nitex layers and b) Nitex over clean-room lab tissues. At that point it was determined that a cleaner sample could be obtained using two layers of Nitex because it could be acid washed, unlike clean-room lab tissues.

During the sampling months of April, June, and July samples were collected by placing a sheet of Nitex in a plastic embroidery hoop (Figure 2, Appendix B) and laying another sheet of Nitex on top (cut to fit the hoop diameter). This was done to assist in laying the Nitex on the mudflat during times when wind made handling of the Nitex awkward and difficult. In many instances contact between the Nitex and the sediment surface was poor when using the embroidery hoops making it difficult to obtain a sufficient amount of biomass for trace metal analyses. In the remaining months of collection (August, September, November, and December) the samples were collected by laying Nitex directly onto the sediment with a smaller piece on top without using the embroidery hoop (Figure 3, Appendix B). Two Nitex sheets were carefully placed onto the sediment of the mudflat (one over the other) and left for approximately 30 minutes to one hour to allow microalgae to migrate up through the mesh openings (Figure 4,

Appendix B). In all schemes, the top Nitex layer was carefully removed by folding it over and inward upon itself. Top layers were placed into a trace metal free (10% HCL acid washed and thoroughly rinsed in de-ionized water) 250 mL volume, polypropylene, wide mouth container for transport back to the laboratory. The process of laying down the Nitex and collecting it required two people with clean, gloved hands to accomplish the task. Extreme care was maintained in the field to insure that general handling of the Nitex did not result in sediment contamination of the samples.

Benthic microalgal samples were also collected for species identification using the above described sampling method. Samples for cell identification were accomplished as described in Sherr and Sherr (1993) preserving and staining with 10% Lugol's solution and analyzed microscopically by R. Dufford, Phytoplankton Taxonomist, Fort Collins, Colorado.

Sampling for Standing Biomass

Chlorophyll, the green pigment in plants that absorbs light energy for photosynthesis, is an indicator of algal biomass--or standing crop. Samples for chlorophyll *a* measurements were collected monthly at low tide during the year 1995 except for the months of March (due to persistent rain) and November (due to time constraints) and for the first four months of 1996. Sampling for chlorophyll *a* and phaeopigment (product of degraded chlorophyll) involved a random sampling strategy from a grid area established at the site (Figure 5, Appendix B). The area was a 45' x 20' section which was subdivided

into 5' x 5' grids. These grids were further divided into 2.5' x 2.5' subsections. Four to six 5' x 5' grid areas were randomly selected prior to arrival onto the site. Four replicate cores were collected from each 2.5' x 2.5' subsection resulting in sixteen to twenty-four samples for the chlorophyll *a* analysis. The initial sampling strategy involved sampling six quadrants. However, a statistical power analysis indicated that four quadrants were sufficient in differentiating significant chlorophyll *a* values between samples (Table 1, Appendix A).

Samples were collected using 3/8" diameter acrylic tubes four to six inches in length. To collect the sediment core samples, each quadrant was approached with a 2.5' x 2.5' plastic board into which were drilled one hundred evenly spaced holes. Due to the patchy nature of benthic microalgal mats, the board was held over the sampling area as a non-biasing technique to prevent viewing of the quadrant during sampling (Figure 6, Appendix B). For each quadrant, four acrylic tubes were pushed at random through holes in the board. After insertion into the sediment, each tube was plugged at the top with a rubber stopper (to create a vacuum effect) to hold the sediment in the tube. After pulling the core up from the sediment, it was plugged from the bottom with a rubber stopper and placed in a cooler for transportation back to the laboratory. In the laboratory the samples were placed in an ice packed cooler (to prevent the degradation of chlorophyll) and prepared for analysis.

Laboratory Preparation and Analysis for Chlorophyll and Phaeopigment

During preparation, each core was separately removed from the ice cooler and the approximate top centimeter (measured with a metric ruler) was extruded from the tube using the plunger of a 3cc syringe. At selected times the second and third centimeters were individually extruded to compare chlorophyll *a* measurements at depth. Each extruded sample was smeared onto a pre-weighed 47 mm Gelman, glass-fiber filter and weighed. After recording the weight (in grams), the sample on the filter was placed on a clean, glass filtering assemblage and one milliliter of magnesium carbonate suspension (2 g $MgCO_3$ (heavy powder):200 mLs deionized water) was filtered through each sample using a hand-held vacuum pump. The magnesium carbonate was used to buffer the samples from potential acidification during the chlorophyll extraction technique. Acidification results in a degradation of chlorophyll *a* into phaeopigments. After buffering the samples, each filter was carefully folded in half and placed in a filter holder and frozen until analysis (one week to two months following collection).

Chlorophyll *a* and phaeopigment analyses were conducted by a chlorophyll extraction method as described by Thompson, Nichols, and Weinke (Feb. 1980-Feb. 1981) with variations to the procedure for intertidal benthic sampling and analyzed on an HP8452 Spectrophotometer. Using volumetric glassware, a solution of spectrophotometry grade acetone (diluted to 90% with distilled water) was made for each analysis. To prevent chlorophyll degradation, the samples, glassware, and acetone needed to remain as cool as possible during handling. The container holding the 90% acetone was

placed in an ice bath, the glass mortar and pestle tissue grinders used to grind the samples (15 mL volume) were placed in the freezer, and during the grinding process each mortar was held in an ice bath. After the mortars were cooled in the freezer, one mortar and one benthic chlorophyll sample were removed from the freezer at a time. The mortar was placed in the ice bath (ice in a Nalgene polyethylene 250 mL bottle). The benthic chlorophyll samples were carefully removed from the filter holder using forceps, broken up into small pieces, and dropped into the mortars. Grinding was accomplished by retro-fitting the pestle to a fixed position hand drill that was speed controlled using a foot pedal. Ten to fourteen milliliters of 90% acetone were added to the samples during the grinding process until the samples were thoroughly ground and rinsed into 15 mL test tubes. Immediately after grinding, the samples were plugged with a rubber stopper, bound at the top with electric tape (to prevent potential evaporation of the acetone), and placed in the freezer to extract over night. Prior to analysis by spectrophotometry, samples were centrifuged for half an hour to settle out the sediment particles. During analysis, 6 mLs of the supernatant were siphoned using volumetric glass pipettors and dispensed into the cuvette of the spectrophotometer.

The chlorophyl *a* and phaeopigment values were calculated using Lorenzen's equations (Lorenzen 1967) adapted for sediment samples as described in Thompson, Nichols, and Weinke (Feb. 1980-Feb. 1981):

$$\text{Chl } a \text{ (ug/g)} = \frac{A \times K \times (665o - 665a) \times v}{g \times l}$$

$$\text{Chl } a \text{ (g/cm}^2\text{)} = \frac{A \times K \times (665o - 665a) \times v}{cm \times l}$$

$$\text{phaeopigment (ug/g)} = \frac{A \times K\,(R[665a] - 665o) \times v}{g \times l}$$

$$\text{phaeopigment (ug/cm}^2\text{)} = \frac{A \times K\,(R[665a] - 665o) \times v}{cm \times l}$$

Where (Lorenzen 1967):

A absorption coefficient of chlorophyll a = 11.0

K factor to equate the reduction in absorbency to initial chlorophyll concentration, 1.7 : 0.7, or 2.43

665o absorbance at wavelength 665 before acidification

665a absorbance at wavelength 665 after acidification

v volume of acetone used for each extraction (mL)

l path length of the cuvette used for the spectrophotometer (5 cm in this thesis)

R maximum ratio of $665_o : 665_a$ in the absence of phaeopigments, 1.7

And (Thompson, Nichols, and Weinke Feb. 1980-Feb. 1981) :

g weight of sediment extruded (grams) for chlorophyll analysis

cm area of the core sample tube (.7126 cm^2 in this thesis)

*The absorbance values for *665o and 665a* are the highest value taken between the readings measured at wavelengths *664o and 666a*. The background value measured at wavelength *750* is subtracted from the highest values taken for *665o and 665a* before the number is inserted into the equation.

Carbon and Biomass Estimates

From the estimate of carbon and the measurement of pigments (chlorophyll *a* and phaeopigments), it is possible to estimate the biomass present at the time of sampling in a given area--in this case expressed as g dry weight/m^2. Carbon estimates were determined based upon chlorophyll *a* measurements and calculated by a method described in Jassby, Cloern, and Powell (1993). This is a point of interest because, among the numerous sources of carbon in San Francisco Bay, benthic microalgae is believed to be the second most important. The calculation estimates that the carbon:pigment ratio (pigment = chlorophyll *a* and phaeopigment values) is equal to forty (carbon=[chlorophyll *a* + phaeopigments] x 40). This allows for an "estimation" of the carbon specifically contributed by the benthic microalgae present at the study site during the time of sampling.

It must be noted that there may be significant error in this method due to the fact that different species of benthic microalgae possess various physiological differences that determine carbon content (De Jonge 1980 and Strathman 1967). Over a three year study period conducted in the estuary of the River Ems (Netherlands), De Jonge (1980) found that carbon:pigment ratios ranged from 10.2 to 153.9 depending on species composition. Temperature, daily irradiance, and nutrient-limitations are also factors that have been determined as key components for obtaining accurate estimations of population growth rates and biomass (Cloern, Grenz, and Vidergar-Lucas 1995). However, the scope of this study did not include an evaluation of these factors; therefore, a carbon:pigment ratio of forty was used because the major species composition in this study consisted of the class

Bacillariophyceae which possess silicate outer walls and thus contain a lower carbon content than phytoplankton species (pers. comm. J. Cloern, U. S. Geological Survey and J. Pinckney, University of North Carolina).

Because of the potential variability in benthic microalgal species composition, two estimates were made to measure biomass (pers. comm. J. Cloern, U.S. Geological Survey). One estimate assumed a sample composed primarily of phytoplankton species (which can settle out of the water column onto the mudflat). The cells of these species contain approximately 50% carbon dry weight. The second calculation assumed a sample composed primarily of diatom species that contain a lower (30%) carbon content. Calculations for biomass were accomplished by dividing the estimated carbon values (g C/m^2) by 0.50 and 0.30.

Procedures and Analyses for Determining Trace Metals in Algae

Once in the laboratory, the Nitex layers used to collect benthic microalgae in the field were carefully removed from the transportation containers (to avoid trace metal contamination) in a clean room designed with a laminar flow hood. Each Nitex sheet was rinsed in sea water to re-suspend the microalgae for filtration onto nuclepore filters. The sea water was obtained from U. C. Santa Cruz Marine Laboratory and diluted with ultra pure, de-ionized water to match the salinity found present on the date of sampling at the mudflat. These algae inhabit a saline environment and must be resuspended in saline water to prevent algal cells from losing water or rupturing. Ruptured cells can result in a loss of

trace metals from the microalgal cells during filtration. Filtered sea water from off the coast of Santa Cruz was used because it contains significantly lower trace metal concentrations compared to San Francisco Estuary waters. The Nitex was rinsed into an acid washed filtering funnel to remove the microalgae and filtered through acid washed 0.45 micron nuclepore filters (for trace metal analysis). Each sample was allowed to air dry under a positive pressure laminar flow hood to evaporate the sea water (approximately 2 days). An analysis was done to compare filter-blank trace metal values with values found in the sea water by filtering 200 mLs of sea water through nuclepore filters and analyzing them by graphite furnace atomic absorption spectroscopy (GFAAS). In all of the seven metals analyzed except for aluminum, the sea water filter-blanks were equivalent to the absorbance values measured for the regular filter-blanks (used to determine any background contribution from filter contamination (Table 2, Appendix A). The aluminum analysis demonstrated a slightly higher absorbance value in the sea water compared to the filter blanks.

Preparation for the analysis of trace metals involved a total decomposition (metals adsorbed as well as intercellular trace metal content of algal samples). Dry filters were carefully weighed (filter weight subtracted-based on the average filter weight for the packaged filters) to determine the dry weight of biomass accumulated on the filter. Filters were placed in a 10 mL acid washed teflon jar with an acid combination of 12N hydrochloric acid, 16N nitric acid, and concentrated hydrofluoric acid (a ratio of 3 mL:1 mL:200 *ul* respectively). These samples were allowed to digest overnight (about

19 hours) under a chemical hood while set on a hot plate designed to hold the teflon jars at a constant temperature of approximately 100 degrees Celsius. After the samples were digested and cooled, they were allowed to evaporate under a chemical hood while gently heated over a hot plate. After evaporation (approximately 12 to 24 hours) the samples were reconstituted in 5% hydrochloric acid to a volume of 3 mLs. Samples were transferred to acid washed 1.5 mL volume polypropylene centrifuge test tubes. The samples were centrifuged for ten minutes to allow particles to settle to the bottom. The supernatant was pipetted into another clean centrifuge test tube (carefully--not to include the settled particulates) and stored until analysis by graphite furnace atomic absorption spectroscopy with Zeeman background correction. For detailed information on GFAAS conditions refer to Table 3 (Appendix A).

Samples were diluted using a matrix modifier (Table 4, Appendix A) and analyzed by a standard additions method. The use of a matrix modifier during analysis allows a higher temperature to be used in the charring step of the GFAAS program enabling the removal of compounds that would otherwise interfere with the absorption reading by the instrument (Slavin 1984). With the volatization of these compounds that bind with the matrix modifier, the trace metal of concern is left behind to be measured and recorded by the instrument. With the standard additions method, a standard of a known concentration is added (in equal proportions 20 *u*l:20 *u*l) to the sample during analysis in the graphite tube. This is done to determine the slope of the calibration curve, allowing the concentration in the sample to be calculated.

A sodium (Na) analysis was also performed for each of the samples. This was done to subtract the "salt weight" contributed to the sample by the re-suspension in sea water. Five standards of known concentrations were analyzed (0 ppm, and 0.665 ppm-2.27 ppm) by flame spectroscopy. A regression analysis was done to obtain the equation of the line used to calculate the salt weight of each sample (Table 5, Appendix A). The calculations used to determine the trace metal concentrations in the benthic microalgal samples are as follows:

$A_s - A_m = A_{std}$

$A_m / A_{std} = A_{ratio}$

$A_{ratio} \times C_{std} = C_{sample}$ (ng/g)

$C_{sample} \times D \times V - F_{blnk} = S_{corr}$

$S_{corr} / S_{wt} - Na_{wt} = S_{conc}$ (ug/g)

Where:

A_m Sample + Matrix Modifier

A_s Sample + Standard

A_{std} Reflected Standard in Sample

A_{ratio} The Ratio of A_m/A_{std}

C_{std} Concentration of Standard Added (ppb)

C_{sample} Concentration of Sample in graphite Furnace

D Dilution Factor For Sample

V Sample Volume of Original Sample Following Final Preparation (mL)

F_{blnk} Trace Metal Associated With Filter Blank

S_{corr} Sample Concentration - Any Blank Correction

S_{wt} Original Sample Weight

Na_{wt} Salt Weight

S_{conc} Final Concentration of Sample (ug/g)

Correcting Trace Metal Concentrations in Samples Due to Sediment Contamination

Aluminum comprises a significant amount of the fine grain clay sediments of the San Francisco Estuary (pers. comm. S. Luoma, U. S. Geological Survey) and is more highly affiliated with sedimentary material than with algal cells. Algae contain aluminum concentrations that are several orders of magnitude lower that what is present in San Francisco Bay sediments. Aluminum concentrations were analyzed in each sample as an indicator of the presence of sediment, recorded as a percentage dry weight for each sample, and correlated with the concentration (ug/g) of each of the six trace metals analyzed. Through linear regression analysis (trace metal content as a function of aluminum content), trace metal content of the algal component of each sample was determined for each metal based on the Y intercept where aluminum concentration equaled zero.

CHAPTER IV

RESULTS AND DISCUSSION OF CHLOROPHYLL ANALYSES

Sampling and Collection for Chlorophyll

Table 6 summarizes the conditions for each sampling event and Figure 7 summarizes chlorophyll measured in the top first centimeter of samples collected in 1995 and 1996. The values for chlorophyll (mg/m^2) and phaeopigment (mg/m^2) presented in figure 7 and table 7 represent the mean values of all chlorophyll *a* and phaeopigment samples collected and analyzed for each sampling date accompanied by 95% confidence intervals. Of the ten months sampled in 1995, the month of February exhibited the highest peak in biomass on the mudflat as indicated by chlorophyll *a* (46.74 mg/m^2). Visually, the mudflat appeared heavily covered with a benthic microalgal mat--unlike the previous month of January. Chlorophyll *a* values steadily declined through the month of June when chlorophyll *a* was measured at 12.54 mg/m^2. Another slight peak in chlorophyll *a* was measured in July (23.1 mg/m^2) followed by a steady decline throughout the months of August, September, October, and December (5.67 mg/m^2).

Weather conditions such as periods of high wind speed can re-suspend benthic microalgae up into the water column incorporating them into the water column chlorophyll readings. Likewise, settling phytoplankton can be incorporated into the benthic region.

Table 6. Collection Dates and Sampling Conditions.

Sampling Date	Tide Out Golden Gate	Level of Low Tide	Time of Sampling	Salinity	Temperature Celsius	Wind Speed Miles Per Hour
1/23/95	10:27 am	1.4 ft	12:30 pm	*	12.80	7.99
2/23/95	12:24 pm	0.2 ft	2:30 pm	10 ppt	13.30	4.07
4/10/95	2:29 pm	0.5 ft	2:30 pm	11 ppt	13.92	5.82
5/17/95	8:02 am	-1.6 ft	10:00 am	15 ppt	14.36	5.75
6/19/95	11:09 am	0.6 ft	1:00 pm	15 ppt	15.20	8.38
7/18/95	10:30 am	1.2 ft	12:00 pm	15 ppt	18.60	7.38
8/29/95	7:44 am	1 ft	10:00 am	20 ppt	18.60	6.06
9/25/95	6:02 am	1 ft	8:15 am	21 ppt	17.82	6.04
10/24/95	6:16 pm	-0.6 ft	5:00 pm	22 ppt	14.83	4.35
12/5/95	4:31 pm	-0.4 ft	4:00 pm	20 ppt	15.30	3.29
1/17/96	2:57 pm	-0.7 ft	3:30 pm	19 ppt	11.06	6.86
2/13/96	12:41 pm	0.4 ft	1:20 pm	15 ppt	16.05	3.41
3/13/96	12:11 pm	0.1 ft	2:00 pm	8 ppt	11.70	9.17
4/10/96	11:32 pm	-0.1 ft	10:00 am	*	*	*

Unavailable data marked by *. (Wind speeds are daily mean values; pers. comm. L. Schemel, U.S. Geological Survey, Menlo Park, CA.).

January 1995 was marked by wind speeds ranging from 2.25 miles per hour up to 15.13 miles per hour in the days prior to the January 23rd sampling. February was marked by relatively lower wind speeds ranging from 1.95 miles per hour up to 6.14 miles per hour prior to sampling.

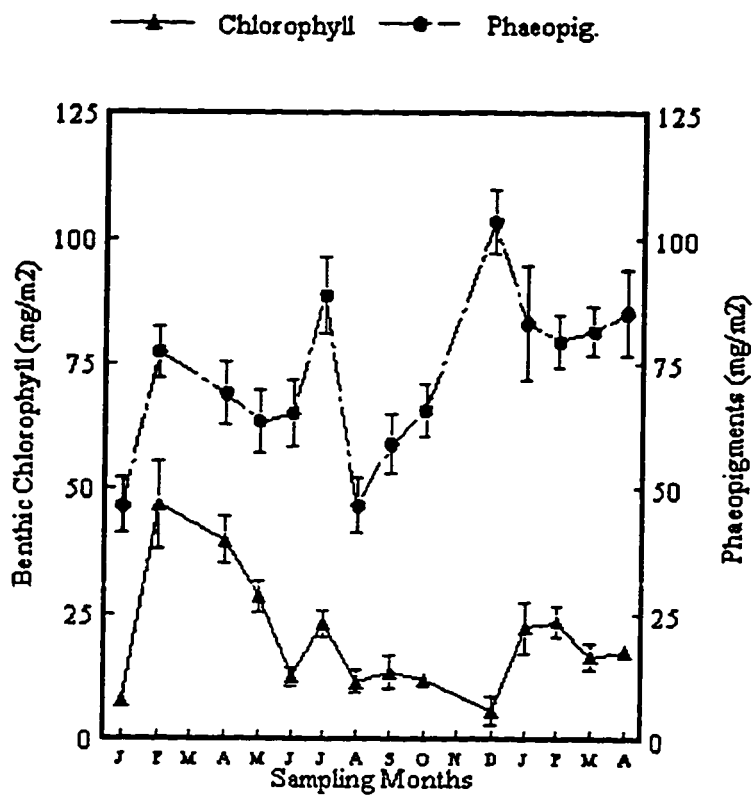

Figure 7. Chlorophyll and Phaeopigment in Top First Centimeter of Sediment (1995 and 1996).

Table 7. Summary of Chlorophyll and Phaeopigment Data for 1995 and 1996 from Sand Point, Palo Alto Mudflat.

Year	Date	n	mg/m² Chlor.	Chlor. Std.	95% Conf.	mg/m² Phaeo.	Phaeo. Std.	95% Conf.
1995	1/23	22	7.61	2.73	1.14	46.57	13.16	5.50
1995	2/23	24	46.74	21.83	8.73	77.45	12.99	5.20
1995	4/10	21	39.68	10.82	4.63	69.10	14.80	6.33
1995	5/17	15	28.52	6.03	3.05	63.41	12.66	6.41
1995	6/19	15	12.54	3.68	1.86	65.12	13.10	6.63
1995	7/18	15	23.10	4.69	2.73	88.83	15.10	7.64
1995	8/29	15	11.43	4.52	2.29	46.64	10.69	5.41
1995	9/25	13	13.37	6.25	3.40	58.88	10.92	5.94
1995	10/24	15	11.85	3.17	1.60	65.66	10.32	5.22
1995	12/5	14	5.67	5.61	2.94	103.50	12.35	6.47
1996	1/17	15	22.33	10.21	5.17	83.27	22.86	11.57
1996	2/13	16	23.42	5.92	2.90	79.59	11.05	5.41
1996	3/13	15	16.48	5.13	2.60	81.50	9.64	4.88
1996	4/10	15	17.65	3.36	1.70	85.34	17.25	8.73

***Includes standard deviation and 95 % confidence intervals.**

In 1996, only four months of data were collected for benthic microalgal chlorophyll analyses. Sampling for the first four months of 1996 indicated a rise in benthic chlorophyll concentrations occurring in January (22.33 mg/m^2) and February (23.42 mg/m^2). Data collected from the water column (pers. comm. J. Thompson U.S. Geological Survey, unpublished data) indicated an elevated chlorophyll reading occurring in April (13.21 ug/L) at station D11 (closest proximity to the benthic microalgal sampling site) and again in July (27.5 ug/L).

The sampling grid for chlorophyll *a* analyses in this study was angled approximately between 21 feet and 40 feet along the marsh shoreline (dominated by *Salicornia virginica* and *Spartina foliosa*). A study by Thompson and Nichols (1988) sampled benthic chlorophyll from the Palo Alto area, however, the sampling occurred much further out on the mudflat. Over a two year period (1983 to 1984) average measurements ranged between 0.5 mg/m^2 and 15 mg/m^2. The fact that the sampling site in the Thompson and Nichols study was further from the marsh than samples collected in this study may be a driving force behind the significantly higher values of chlorophyll *a* concentrations measured in this study. Different species dominate various ecological niches and there may be more biomass closer to shore than one would find further out on the mudflat due to variations in habitats, nutrients, and light availability.

Chlorophyll *a* Measured Below First Centimeter of Sediment Cores

Benthic microalgae primarily inhabit the upper 1 cm of sediments, however, they can also be found alive at greater depths (San Francisco Estuary Project 1992). Admiraal, Peletier, and Zomer (1982) estimated that 90% of the photosynthetically active diatom populations could be found in the upper 5 millimeters of sediment. Some mobile diatom species can move up and down through the sediment at a rate of 1 to 25 microns per second (Pinckney and Zingmark, 1991). Epipelic diatoms can be found to surprising depths as a result of weather conditions and bioturbation. Measurement of chlorophyll *a* was found to a depth of 12 cm by Joint (1978) on an intertidal mudflat in south western England during the month of June. Benthic core samples taken in the intertidal zones of San Francisco Bay have indicated that under extreme conditions (ie. intense summer heat and over drying sun exposure) benthic microalgae can be found (as indicated by chlorophyll *a* measurements) to a depth of 30 cms into the sediment (pers. comm. J. Thompson, U.S. Geological Survey). Benthic microalgae found below the top first centimeter of sediment, although not considered among the photosynthetically active population, may present a significant contribution to the carbon cycle. Therefore, at selected times in this study, samples were taken to measure chlorophyll *a* at two and three centimeters below the surface (further depths were not measured due to time and resource constraints). Table 8 summarizes the levels of chlorophyll *a* measured below the top centimeter (measurements below 1 cm were taken on five occasions). When this data is compared to chlorophyll *a* measured in the top centimeter of these samples very

comparable concentrations of chlorophyll *a* exist between the top and second or third layers of sediment (Table 9).

Other factors that can contribute to chlorophyll at lower depths in the sediment include natural diurnal rhythms combined with laboratory methodology. A number of studies cited by Pinckney and Zingmark (1991) (Palmer 1973; Palmer and Round 1967; Pearse 1977; Admiraal, Peletier, and Zomer 1982) observed the surface migration

Table 8. Chlorophyll Below First Centimeter of Sediment Layer.

Date	n	Depth	Mean chlor *a* mg/m²	95% Conf. Interval	Mean Chlor *a* ug/g	95% Conf. Interval
2/23/95	11	2 cm	16.05	1.51	1.42	0.14
6/19/95	11	2 cm	16.24	0.61	0.94	0.03
9/25/95	9	2 cm	13.32	1.04	0.80	0.05
2/13/96	10	2 cm	21.87	1.68	1.27	0.10
2/13/96	10	3 cm	19.33	1.51	1.53	0.09
3/13/96	7	2 cm	14.65	1.53	0.87	0.08

Table 9. Chlorophyll in First Centimeter of Sediment Layer.

Date	n	Depth	Mean Chlor *a* mg/m²	95% Conf. Interval	Mean Chlor *a* ug/g	95% Conf. Interval
2/23/95	24	1 cm	46.74	1.78	8.21	0.14
6/19/95	15	1 cm	12.54	0.48	1.17	0.18
9/25/95	15	1 cm	13.37	0.82	0.73	0.04
2/13/96	16	1 cm	23.42	0.73	1.46	0.06
3/13/96	15	1 cm	16.48	0.67	1.01	0.04

patterns of diatoms in sediments and their correlation to tidal and diurnal cycles. The fact that mobile species of diatoms have the ability to move rhythmically and cyclically through the sediment layer may affect the measurement of chlorophyll *a* found at different depths of the sediment because a significant amount of time passes (two to four hours total) before all samples are processed for chlorophyll analysis (ie. segmented between layers, placed on filters, etc). Also, the extrusion process of pushing the sediment up through the core can result in some mixing of biomass into the sub-layers as residual sediment is left behind from the top centimeter and met by the second and third layers as they are extruded up through the core.

Patchiness of Benthic Microalgal Mats

During sampling a great deal of patchiness was observed on the mudflat associated with benthic algal biomass. This patchiness is evident in comparing samples taken from each 5' x 5' section at the Palo Alto mudflat as summarized in table 10 (Appendix A) by the coefficients of variation for each quadrant.

A number of studies have addressed the phenomenon of benthic microalgal patchiness. Hopner and Wonneberger (1985, 282) note that nutrient limitations can cause patchiness and if the primary source of nutrients is from sediments, then "patchy detrital and biomass distribution on and within sediments as well as patchy distribution of re-mineralization activities" can result in diatom patchiness. The mucous layer created by benthic microalgal mats may also cause a diffusive boundary that limits chemical exchange

between the sediments and the water column (Gould and Gallagher 1990). Grazing pressures by benthic organisms can also affect benthic microalgal biomass (Pinckney and Sandulli 1990; citing Davis and Lee 1983; and Montagna 1984). Pinckney and Zingmark (1991) noted that productivity (the rate of algal production over a given time) in motile benthic diatoms varied with periodic migration up and down through the sediments as influenced by tidal stage and sun angles. Their study found that lowest production rates were on days when high tide occurred in the afternoon while highest production rates were observed on days when low tide occurred in the afternoon. This thesis study did not address productivity as did these other studies. However, the periods of highest variability are marked by the high chlorophyll concentrations measured in February and the lowest chlorophyll concentrations measured in December on the mudflat.

Ecological Niches and Species Identification

There are many different species of diatoms--both free living marine species and species living in and attached to the sediments of marshes and mudflats. In San Francisco Bay, Laws (1988) identified 273 different species of diatoms. Numerous species of benthic microalgae reside in the sediments at any given time and the composition of the community will vary with seasonal conditions. At the time of his study, Laws (1988) found that the most common species in the estuary included *Paralia sulcata*, *Thalassiosira decipiens*, *Nitzchia acuminata*, *Ditylum brightwellii*, and *Cyclotella striata*.

Diatoms are unique from other species of algae and phytoplankton in that their cell walls consist of two valves (referred to as frustules) that contain polymerized silica (Johnson 1983). Each species can inhabit a different ecological niche or zone. Round (1971) defines the different zonations as follows: epiphyton (growing attached to other plants), epipelon (growing on sediments), endopelon (growing within the sediment), epipsammon (growing attached to sand grains), epilithon (growing attached to rock surfaces), endolithon (growing within cavities of rocks), and epizoon (growing attached to animals). The free-living, mobile species of diatoms found in sediment and pennate in form are referred to as epipelic.

Species identification for this study at Sand Point was conducted by R. Dufford (Phytoplankton Taxonomist, Fort Collins, Colorado) to evaluate the dominant species of benthic microalgae present on the top film of sediments for the sampling dates of April 10, June 19, November 3, and December 5, 1995. Photomicrographs were taken by Sarah Spaulding of the U.S. Geological Survey, Boulder, Colorado. Table 11 (Appendix A) shows the classes and different species of benthic microalgae present at sampling, cell density, and cell volume. Figures 8-9 are photomicrographs of samples that were collected in April and December of 1995. April 10th was dominated by *Cylindrotheca gracilis* and December 5th was dominated by *Achnanthes hungarica* and *Nitzschia agnita.*

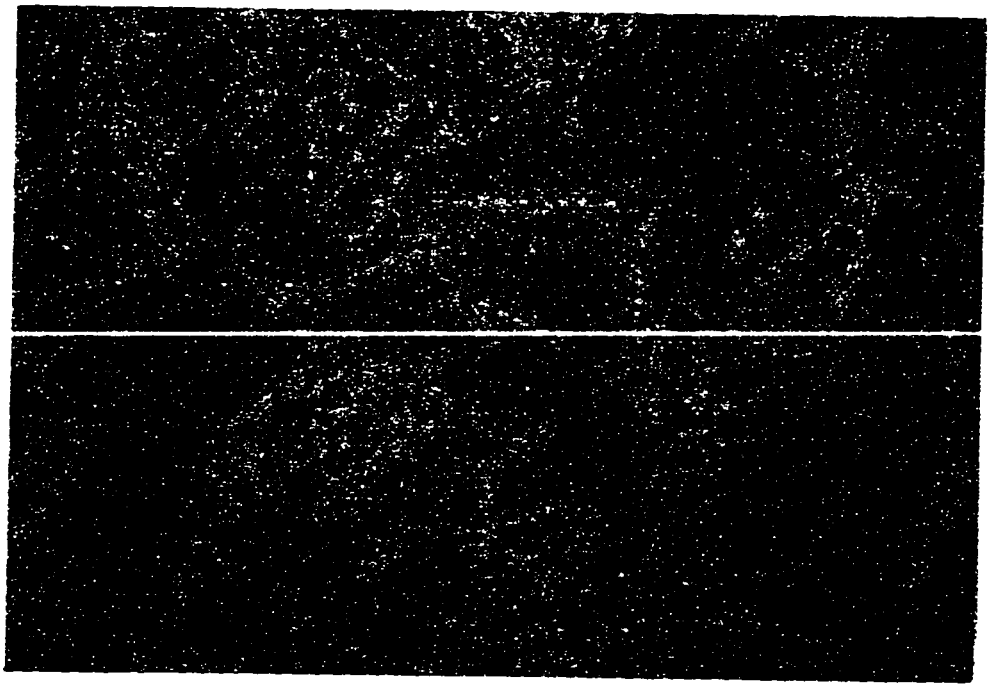

Figure 8. Sampling Date: April 10, 1995. ***Cylindrotheca gracilis.*** **(Species identification by R. Dufford, Phytoplankton Taxonomist, Fort Collins, Co. Photomicrograph by S. Spaulding, U. S. Geological Survey, Boulder, Co.).**

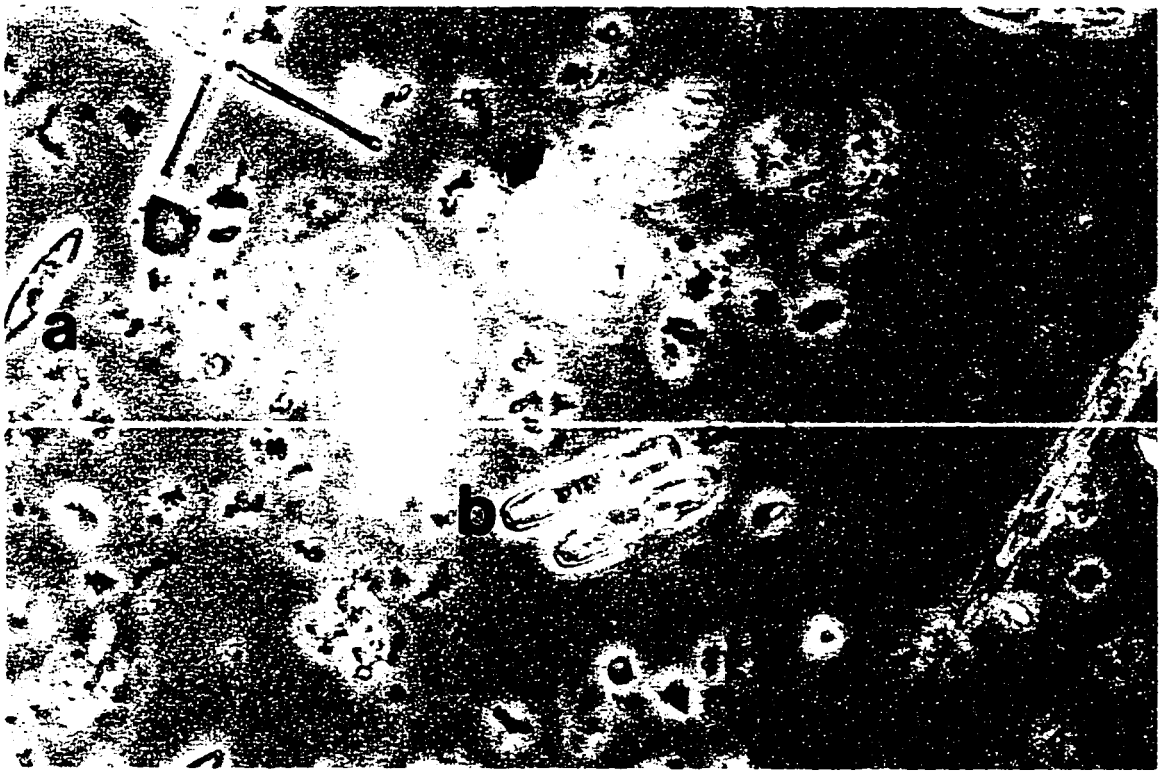

Figure 9. December 5, 1995. a) *Nitzschia agnita* and, b) *Achnanthes hungarica*. Species identification by R. Dufford, Phytoplankton Taxonomist, Fort Collins, Co. Photomicrograph by S. Spaulding, U. S. Geological Survey, Boulder, Co.

Carbon Estimation Based Upon Chlorophyll Measurements

Because of the important and significant contribution that benthic microalgae may play in the carbon cycle, carbon estimates were made based upon chlorophyll *a* and phaeopigment measured in the samples assuming a carbon:pigment ratio of 40. Tables 12 and 13 summarize the monthly carbon estimate associated with the algae for 1995 and 1996. Carbon estimates for this study range from 2167 mg C m^{-2} to 4477 mg C m^{-2} in 1995. Carbon estimates ranged from 3919 mg C m^{-2} to 4224 mg C m^{-2} in 1996.

Table 12. Carbon Estimates for 1995 in Top First Centimeter of Sediment.

Date	1/23	2/23	4/10	5/17	6/19	7/18	8/29	9/25	10/24	12/5
n	21	24	21	15	15	15	15	13	15	14
mg C m^{-2}	2167	4145	4351	3677	3106	4477	2323	2890	3100	4367
95%	239	544	367	294	317	314	290	213	245	216

Table 13. Carbon Estimates for 1996 in Top First Centimeter of Sediment.

Date	1/17	2/13	3/13	4/10
n	15	16	15	15
mg C m^{-2}	4224	4121	3919	4119
95%	435	209	263	336

This is a "general" estimate and the limitations to this approach have been discussed in Chapter 3. Studies of annual production values for other estuaries (Knox 1986; Colijn and De Jonge 1984; cited in San Francisco Estuary Project 1992) found that most of the

annual production values fall within 50 to 200 g C m^{-2} y^{-1} (5 x 10^4 to 2 x 10^5 mg C $m^{-2}y^{-1}$). Because no long-term data of annual production has been collected for San Francisco Bay, carbon estimates have been made from these values. A midpoint of 120 g C m^{-2} y^{-1} is considered to be the best estimate for average benthic primary production for various parts of the Bay (San Francisco Estuary Project 1992). Carbon estimates for this study are not based on "annual productivity" but do provide a snap-shot of carbon biomass present during the time of sampling. So, in comparison to carbon estimates for benthic primary production, carbon biomass in this study provides insight into the potential contribution to the carbon cycle from this small section of the estuary.

Biomass and Clam Condition Index

There are many factors that control and influence the health and condition index of a clam, one of them being food sources. Condition index is a measure of the tissue mass of a clam and is indicative of a clam's well being as well as its reproductive cycle (Luoma et al. 1996). Condition index is determined by measuring the tissue weight of a clam of a given length. As described in Luoma et al. (1996), a clam of a given shell length will add glycogen and weight seasonally as it prepares for reproduction. During and after reproduction, glycogen is metabolized and weight is lost during and after the reproductive cycle. Condition index can be reduced as a result of stress from pollutant exposure, changes in salinity, or lack of food--all of which can lead to increased energy consumption affecting net glycogen accumulation (Luoma et al. 1996).

Figure 10 shows chlorophyll *a* (an indication of biomass) plotted together with the condition index of an average 25 mm clam over the year 1995. Peak chlorophyll *a* measurements occurred during the sampling date of February 23, 1995. Following the peak in benthic chlorophyll there was an increase in clam condition index suggesting a link between benthic microalgal food sources and the well being of the clams. The phytoplankton bloom in the water column (61.6 ug/L) coincided with the benthic microalgal bloom on the mudflat in February 1995 and significantly declined steadily through the end of the year (with a minor peak in water column chlorophyll during April of 36.9 ug/L). Benthic chlorophyll declined consistently following the February bloom with a slight elevation (23.1 mg/m^2) on the sampling date July 18, 1995.

When comparing chlorophyll measured in the water column with benthic chlorophyll measurements in 1995, benthic microalgae appeared to present a consistent although variable food source for benthic organisms over the entire year. Thompson and Nichols (1988) addressed the issue of growth rates and food availability in the clam *M. balthica* in a study conducted in 1983 and 1984 in San Francisco Bay. This study determined that growth rates and tissue weights of clams were highly influenced by food availability when comparing various chlorophyll *a* values measured at several sites around the Bay. The highest tissue weights were found at the sites with the highest chlorophyll measurements and *M. balthica* appeared to rely on both water column phytoplankton and benthic microalgal food sources. This thesis work also demonstrates a link between clam tissue weight and benthic microalgal food availability.

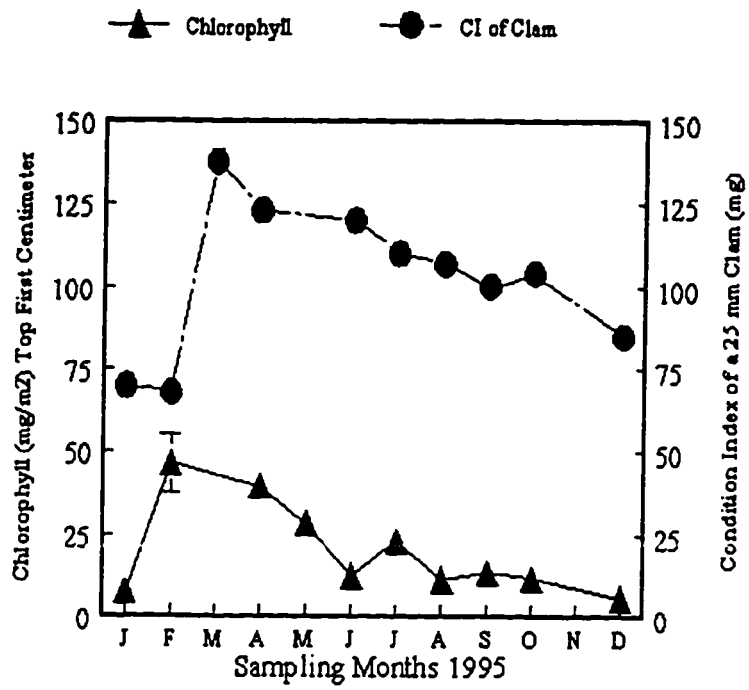

Figure 10. Chlorophyll Data Compared With Condition Index of a 25 mm Clam (1995).

CHAPTER V

RESULTS AND DISCUSSION OF TRACE METAL ANALYSES

Quality Assurance in Trace Metal Analyses by Graphite Furnace AAS

During the analysis of benthic microalgal samples, certified reference standards (National Bureau of Standards "NBS") of a known concentration were analyzed as a means of ensuring that: a) instrument conditions were optimal, and b) that the digestion process of the samples was sufficient to extract trace elements from particulates and bring them into solution.

Table 14 (Appendix A) illustrates the NBS sample data for each of the analyses. There is evidence of analytical variability as indicated by differences in NBS recovery of the same digestion analyzed on different dates. This variability can be attributed to instrument background variations or sampling cup contamination. Contamination can be particularly problematic in the analysis of zinc. Variability also occurs among digestions. Although hydrofluoric acid was used in an attempt to obtain total metal recovery by total decomposition, some metals, such as aluminum and chromium, are not easily extracted from particulates and one hundred percent recovery is not always achieved (pers. comm. S. Luoma, U. S. Geological Survey).

As expected, aluminum recovery was less than certified NBS values with a recovery range of 59% to 74% over the three digestions. As expected, chromium recovery was also lower than certified NBS values and recovery varied among digestions.

Variability also occurred in the recovery of the cadmium NBS samples; although two out of three of the recoveries fell within the range of expected concentrations for NBS values. For further details on National Bureau of Standard (NBS) recovery data refer to Table 14 (Appendix A).

Aluminum as an Indicator of Sediment Present in Algal Samples

Although extreme care was maintained during the collection of benthic microalgae for trace metal analyses, there was the inevitable problem of fine grained sediment particles moving up through the Nitex mesh when it was placed onto the mudflat surface. Aluminum is a significant component associated with the geological composition of clay sediments in San Francisco Bay. Because of this, aluminum was measured in each sample as an indicator of the presence of sediment and recorded as a percentage dry weight of the sample. Aluminum is a good indicator of sediment contamination because it is more highly associated with the sediment component (approximately 4 to 7 % by weight in San Francisco Bay sediments) and is less associated with assimilation by algal cells.

Results and observations from ongoing research on trace metals in sediments at the Palo Alto Mudflat (Luoma et al. 1996) has shown that sediments were more sandy in the fall of 1995 (July through September) because seasonal winds tend to re-suspend fine sediments through the summer months (Thompson-Becker and Luoma 1985). Table 15 illustrates the aluminum content in the concentrate of algal samples collected during the year 1995. From this collection of data, a correlation between seasonal changes in surface

sediment composition and variability of aluminum content observed in the algal samples cannot be made.

Table 15. Aluminum Content in Algal Samples.

Sample Date	n	Mean Concentration (% by Weight)	Standard Deviation
2/23/95	2	1.80	0.15
4/10/95	4	0.66	0.28
6/22/95	3	2.50	0.04
7/18/95	1	0.35	N/A
7/20/95	1	3.80	N/A
7/31/95	3	1.50	1.60
8/29/95	4	5.30	0.59
9/25/95	2	5.90	0.03
11/3/95	4	4.30	0.64
12/5/95	5	6.90	0.32

Samples collected from February through July (with the exception of July 20, due to sampling difficulties) appeared to contain significantly lower aluminum concentrations when compared with samples collected from August through December. Environmental conditions such as rainfall prior to sampling and wind speeds during sampling can make conditions for collection on the mudflat difficult. Excess water on the mudflat can draw fine grained sediments up through the Nitex layers and wind conditions can make handling of the mesh precarious or result in wind-born contamination from mud surface waters

carrying sediment particles. Prior sampling days for June and July were marked by breezy conditions and intermittent, light rain (except July 31st, which was a sunny warm day). Rain also occurred on the sampling date of December 5th, 1995.

Differentiating Between Algal vs. Sedimentary Associated Metals in Samples

In measuring trace metals collected by the method described in this thesis, it is important to differentiate between the metals associated with the algal component of the sample and the sedimentary component of the sample. Of the metals analyzed in this study, one would expect the following characteristics to be displayed in terms of most readily assimilated metal to least readily assimilated metal (bioavailable to the algae): Cd>Zn>Ni>Cu>Cr; with cadmium and zinc having a higher propensity for assimilation into the cells of algae and chromium being more of a surface reactive metal more highly affiliated with the sedimentary component of the sample.

In the case of silver, the processes that are important in sorbing silver to sediments also favor enhanced bioavailability (Luoma, Ho, and Bryan 1995). In the presence of chloride, (such as Bay waters) silver precipitates stoichiometrically as $AgCl_{(s)}$. As Luoma, Ho, and Bryan (1995, 52) point out "The abundant chloro-complex is available to biota and the amorphous aggregated coatings enhance Ag accumulation in sediments as well as Ag uptake from sediments by deposit feeders." Luoma, Ho, and Bryan (1995) cite Bryan (1985) who found that among deposit feeders from two estuaries in the UK (clams: *Scrobicularia plana* and *M. balthica*, and the polychaete *Nereis diversicolor*), silver was

the most strongly bioaccumulated of all the elements studied in all three species from the study sites.

Tables 16 and 17 (Appendix A) list the mean concentrations of each metal measured for each sampling date with the standard deviations (except 7/18/95 and 7/19/95 where only one sample was collected) and the regression analyses of each metal with aluminum. As previously discussed, aluminum is an indicator of the presence of sediment. In other words, aluminum content in the sample provides an indication of the proportion of sample mass that is composed of sediment versus algae. Regression analysis with aluminum as the independent variable and each metal as the dependent variable (dependent upon the presence of sediment) shows that copper, nickel, and chromium appear to be more associated with the sedimentary components of the sample with R^2 values of 0.94, 0.98, and 0.75 respectively. Silver and cadmium, on the other hand, demonstrate a significantly lower association with the presence of aluminum with an R^2 of 0.02 and 0.42, respectively. These two metals are more closely associated with the algal component of the samples. By graphing the trace metal as a dependent of the aluminum present in the sample, one can determine the algal metal content based on the Y intercept if zero aluminum is in the sample. In the case of copper (Figure 11), there is a strong correlation with sedimentary affiliation. However, based upon the regression of the curve, the Y intercept demonstrates that the copper associated with the algal component of the sample is 7 ug/g when aluminum is equal to zero (ie. no sediment is present). Chromium (Figure 12, Appendix B) at zero aluminum, (the Y intercept), is negative which indicates that

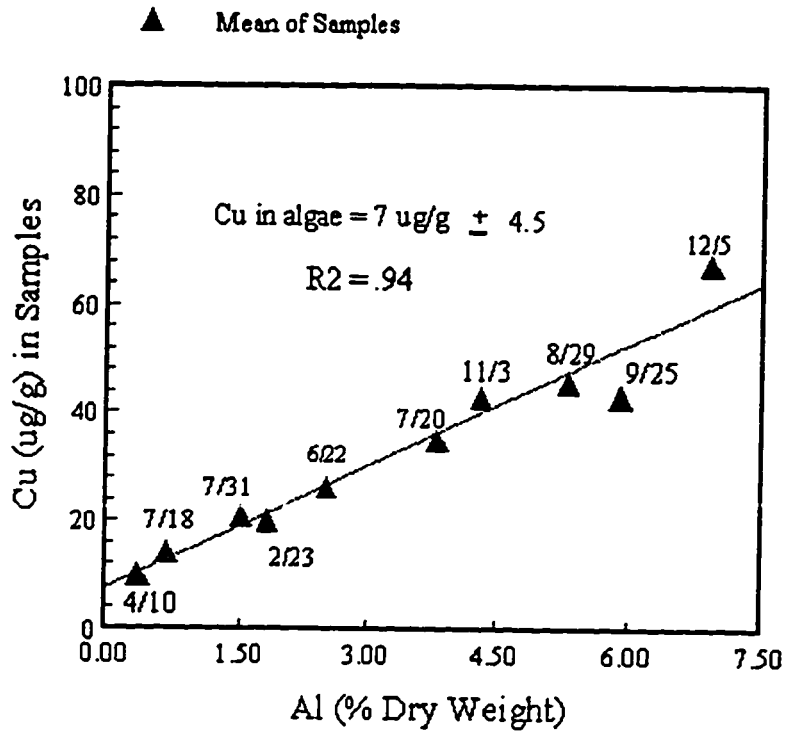

Figure 11. Algal Sample Aluminum and Copper Correlation.

chromium is so highly associated with the sedimentary component of the sample that it is not possible to distinguish chromium associated with the algal component of the sample. Nickel is also highly associated with the sedimentary component of the sample. However, through regression analysis, the algal component is determined to be 6 ug/g (figure 13, Appendix B).

Silver and cadmium are demonstrably more affiliated with the algal components of the sample and in these cases a range of numbers is reported (0.26 ug/g-1.35 ug/g for silver and 0.06 ug/g - 0.17 ug/g for cadmium). In neither case do silver or cadmium correlate with aluminum content. As aluminum content increases, cadmium (Figure 14) and silver (Figure 15, Appendix B) content do not progressively increase. In the case of cadmium, there appears to be a dilution process connected with the increase in sediment contamination. This is particularly evident in the August through December samples where increased aluminum concentrations associated with the sediments coincide with a lower cadmium concentration.

The correlation of aluminum with zinc demonstrates an interesting relationship. Figure 16 (Appendix B) demonstrates a zinc correlation with aluminum with the exception of sampling dates July 31st, August 29th, and September 25th which are outlier points from the regression curve. In figure 17 (Appendix B), these outlier points are removed from the regression analysis and all data points (not the mean of each sampling date) are regressed to demonstrate an R^2 of 0.91.

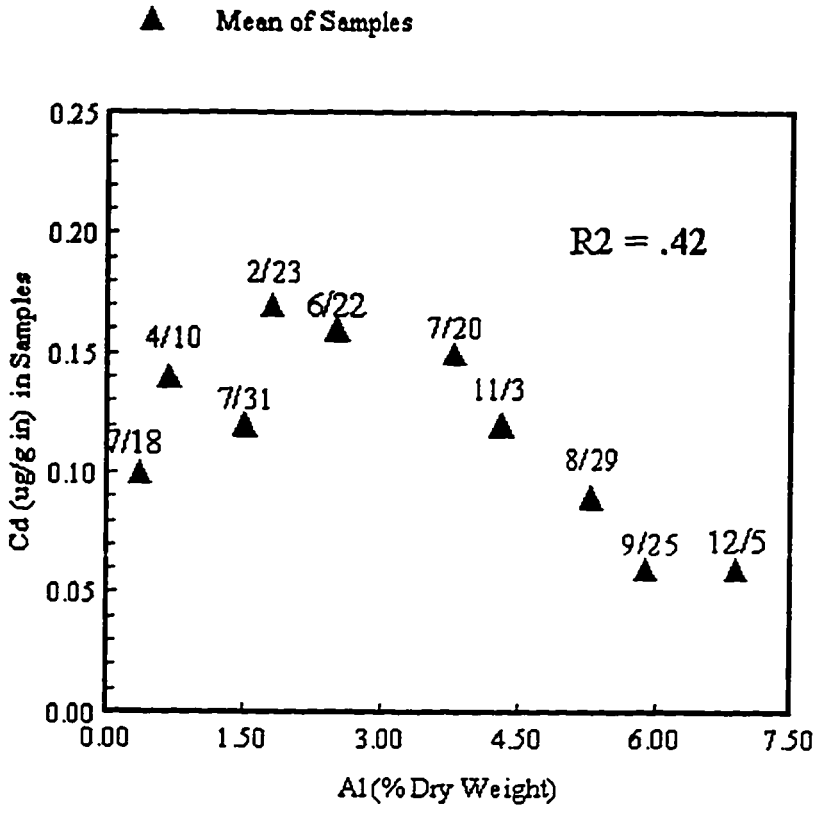

Figure 14. Algal Sample Aluminum and Cadmium Correlation.

Figure 18 (Appendix B) adds these outlier points to the regression curve to depict what may be a significant trend in seasonal variability for zinc assimilation in these algal samples.

Tables 18 and 19 demonstrate the importance of differentiating between the trace metal values obtained from the concentrate of the sample (before correcting for sediment contamination) and the values after correcting for sediment contamination. Before correcting for sediment contamination, the algal trace metal concentrations are very similar to concentrations measured in the sediments. After correcting for sediment contamination, the metals associated with the algal component of the samples are demonstrably lower. By using aluminum as an indicator of sediment contamination, it is possible to obtain an understanding of the metal associated with the algal component of the sample.

Variability of Trace Metal Concentrations in Concentrate of Algal Samples

Table 16 (Appendix A) summarizes the mean concentration of each metal and the standard deviations for each sampling date. There are two types of variability: variability among samples collected on the same date, and temporal variability. Figures 19 through 25 (Appendix B) graphically depict measurements taken on each sampling date accompanied by error bars (standard deviations). Sampling for the month of July occurred on three occasions: July 18th, July 20th, and July 31st. The initial two sampling experiences only resulted in enough biomass for one filtered sample due to sampling

difficulty caused by light rain. Also the presence of ripples on the mudflat resulted in poor contact of the Nitex with the mudflat surface. Significant variability in trace metal concentrations can be seen among these July dates particularly for aluminum, chromium, copper, nickel, and zinc. Much of this variability can be attributed to sediment contamination among samples with the exception of zinc which demonstrated what may be a significant incidence of trace metal assimilation in samples collected on July 31st.

Determining Seasonal Variability of Trace Metal Concentrations in Algae

For copper, nickel and chromium it is not possible to determine any seasonal variability from the data because these metals are very closely associated with the sediment content of the sample. Cadmium is more highly associated with the organic component of the samples; however, the differences observed over the months are not significant enough to draw any conclusions on seasonal trends. Silver and zinc may demonstrate some seasonal variability. In the case of silver (Figure 15, Appendix B) a pattern of increased silver appears in the July samples with a peak of 1.35 ug/g in August. A decrease in concentrations is seen from September through December. As previously discussed, these silver values are actual concentrate values without accounting for the sediment correction, because, based upon the aluminum analysis, almost no correlation exists (R^2=0.02) between the silver and aluminum regression.

In the case of zinc, there is an apparent differentiation between what is algal versus what is associated with the sediments in the sample. However, a deviation is noted

Table 18. Total Metal (ug/g) in Concentrate of Algal Samples Compared With Sediments Trace Metal Concentrations (ug/g) in 1995.

Trace Metal	Algal Mean n=30	95%	Sediment Mean n=20	95%
Ag	0.68	0.13	0.56	0.06
Cd	0.11	0.02	0.22	0.03
Zn	294	98	136	6.82
Cu	37	6.8	44	3.15
Cr	116	36	116	7.01
Ni	62	13	95	4.28

Annual means of total metal (ug/g) in concentrate of algal sample (before sediment correction) with 95% confidence intervals compared with annual means of total metal in fine grain sediments (ug/g) at the Palo Alto mudflat (sediment data obtained from Luoma et al. 1996).

Table 19. Corrected Algal Metal Content Compared With Trace Metal Concentrations in Sediments.

Trace Metal	Algal Mean n=30	Sediment Mean n=20
Ag	0.26-1.35	0.56
Cd	0.06-0.17	0.22
Zn*	44	136
Cu	7	44
Cr	<1	116
Ni	6	95

Annual means of corrected algal trace metal concentrations (ug/g, based on aluminum content) compared with annual means of total metal concentrations in fine grain sediments (ug/g) at Palo Alto mudflat. *Zn algal mean does not include 7/31, 8/29, and 9/25 data where n=20.

during the sampling dates of July 31st, August 29th, and September 25th as depicted in figure 16 (Appendix B). Because these sampling dates were significant and consistent outlier points from the regression, an additional calculation was conducted to assess a differentiation between the sedimentary versus the algal zinc in these samples. Based upon the regression analysis of all samples excluding July 31st, August 29th, and September 25th samples (Figure 17, Appendix B), 44 ug/g of zinc was determined to be associated with the algal component of the samples. To predict the inorganic versus the organic concentration of zinc associated with these outlier points three steps were taken:

1) $Y' = a + bx$

2) $Y' - a$ = the inorganic zinc

3) $Zn_{algal} = [(Zn_{conc} - Y') + a]$

where: Y' = the sum of the benthic microalgal zinc (44 ug/g) plus the sedimentary zinc associated with the samples.

a = the Y intercept of 44 ug/g of benthic microalgal zinc (from the regression in figure 17, Appendix B)

b = the X coefficient from the regression curve in figure 17 (Appendix B) (37)

x = the aluminum concentration in the sample

Zn_{algal} = Zn associated with the algal component of the outlier points

Zn_{conc} = Zn associated with the concentrate of the sample (without subtracting for sediment interference)

Table 20 (Appendix A) summarizes the data for this calculation. When this data is graphically presented over time (Figure 26, Appendix B) these outlier points are

significantly higher during these sampling periods than during the rest of the year. This implies that there may have existed ecological conditions that made zinc more bioavailable to benthic microalgae during this period.

Algal Metal Estimates Per Square Area

Because the metal content of the algae can be determined using the linear regression method with aluminum described in this thesis, it is of considerable value to estimate the algal metal per unit area at a given site. By knowing the algal metal content and the biomass present at the time of sampling (estimated from chlorophyll *a* and carbon estimates), an estimate of algal metal per square meter can be determined. Table 21 (Appendix A) summarizes biomass values used in the calculations of this data. Because these populations can mix depending upon environmental conditions, concentrations per square meter were calculated for both the lower carbon content that would be associated with a benthic microalgal species composition consisting of primarily diatoms (30%) and a population consisting of primarily phytoplankton species (50%). Figures 27 through 31 (Appendix B) graphically depict the seasonal algal metal (ug)/m^2 for Ag, Cd, Cu, Ni, and Zn that was analyzed with the metal concentration (ug/g) of an average 25 mm clam.

The amount of algal metal (ug/m^2) is of interest because it tests the hypothesis that an increase in biomass and hence of algal metal (ug/m^2) would result in an increase in trace metals found in the tissues of *M. balthica*. A laboratory study by Lee and Luoma (unpublished data) examined the uptake of metals in the clam species *Macoma balthica*

and *Potamocorbula amurensis* feeding on radioisotope labeled (^{109}Cd, ^{51}Cr, and ^{65}Zn) benthic microalgae and sediments collected from the creek beds of the upper marsh of the Palo Alto mudflat near the sampling site for this study. Three feeding mediums were used to ascertain assimilation efficiency of trace metals: a suspension of radio-labeled sediments, a suspension of radio-labeled benthic microalgae, and a suspension combining radio-labeled sediments and benthic microalgae. Lee and Luoma's study found that both clam species assimilated more cadmium, chromium, and zinc from the medium dominated by benthic microalgal particles than from sediment sources (pers. comm. Drs. S. Luoma and B. G. Lee , U. S. Geological Survey, Menlo Park, CA). This finding is consistent with investigations and results of other studies (Absil 1993; Stronkhorst, Vos, and Misdorp 1994; and Wang et al. 1995) which determined that pollutant uptake from primary food sources is an important, significant, and overlooked area of research necessary for understanding and describing the distribution of contaminants through the food chain.

In this thesis study at Sand Point on the Palo Alto mudflat, silver (Figure 27, Appendix B), may demonstrate what could be a delayed reflection of trace metal uptake in clams following increased levels of trace metals from increased biomass on the mudflat. This same trend would be expected in the case of zinc (Figure 31, Appendix B) where algal zinc concentrations /m^2 increased significantly during the months of July, August, and September. However, no increase in zinc concentrations was noted in the clams following the algal zinc increase. Because it can take up to 200 days for organisms to

reflect pollutant concentrations from their environments, it would be necessary to examine zinc concentrations in clam tissues beyond the sampling dates used in this study.

It is important to note that in this study it is difficult to draw definitive conclusions relating to the trophic transfer of pollutants with one year of data because there are a large number of variables that can affect and influence bioaccumulation. However, this study provides a foundation of data on this subject matter for San Francisco Bay.

Trace Metal Transfer Through the Food Chain

Two further objectives of this study were to determine whether or not there is a detectable correlation between trace metals found in sediments and trace metals found in benthic microalgae, and, whether or not there is any correlation between trace metal concentrations measured in *M. balthica* and trace metal concentrations measured in the algal food sources over time. Figures 32 through 34 (Appendix B) compare silver, cadmium, and zinc measured in the algal samples with trace metals measured in the sediments (chromium, nickel, and copper showed no significant seasonal variability). None of these metals showed a significant correlation between sediment trace metal concentrations and benthic microalgal trace metal concentrations. For cadmium, in particular, there are very low levels associated with the algal samples suggesting that some factor may make cadmium less bioavailable to the algae in this environment. Some studies (Kuwabara et al. 1996; Kuwabara and Luther 1993) have investigated the importance of dissolved sulfides in affecting chemical speciation of trace metals like

cadmium, copper, and zinc in San Francisco Bay. Sulfides are generated from bacterial action on organic matter under anaerobic conditions (California Regional Water Quality Control Board 1995). Trace metal interaction with sulfides can present very thermodynamically stable complexes in an aquatic environment which can result in reduced bioavailability of some trace metals to planktonic and benthic organisms (Kuwabara and Luther 1993).

Bioconcentration Factors: A Comparison Between Studies

Both Absil (1993) and Stronkhorst, Vos, and Misdorp (1994) focused on bioconcentration factors "BCF" (a ratio indicative of trophic transfer of pollutants) of metals from sediments to algae and algae to benthic feeding organisms. Although bioconcentration factors do not provide an accurate assessment of trace metal transfer through the food chain from contaminant vectors, they do provide a useful numerical ratio that can be used to compare data between studies and study sites. Table 22 compares Palo Alto data with these two studies (cadmium, copper, and zinc concentrations in benthic microalgae, sediments, and benthic organisms) and bioconcentration factors associated with them. When comparing cadmium associated with algae between this study and the Absil study, Absil reports cadmium concentrations similar to the Palo Alto cadmium data up to an order of magnitude higher--depending upon the sampling site. Bioconcentration factors of cadmium in the algae from sediments range from an order of magnitude lower to an order of magnitude higher than the range of BCF for cadmium in

Palo Alto samples. The mean algal cadmium concentration in the Stronkhorst et al. study was 2.5 times greater than the highest seasonal range value measured in the Palo Alto study and in both studies bioconcentration factors were <1.

Copper values for Palo Alto algal samples fell within the range of values for Absil's study but were an order of magnitude below the mean value measured in the Stronkhorst et al. study. Bioconcentration factors (algae/sediments) for Palo Alto copper data were <1. Absil's data indicated bioconcentration factors ranging from <<1 to 15.0 at one site whereas Stronkhorst et al. demonstrated bioconcentration factors of <1 to 1.50.

In the case of zinc, Palo Alto algal concentrations fell within the site range values listed in Absil's study (with the exception of Palo Alto sampling dates 7/31, 8/29, and 9/25 which were significantly higher), and both studies demonstrated similar bioconcentration factors for zinc. When compared to the Stronkhorst et al. study, the Palo Alto algal zinc concentrations were slightly lower (with the exception of the above mentioned dates) and overall bioconcentration factors in the Stronkhorst study were similar or lower compared to the Palo Alto data.

In comparing bioconcentration factors of benthic organisms, metal concentrations/algal metal concentrations, the Stronkhorst study demonstrated lower bioconcentration factors for both cadmium and copper. Zinc bioconcentration factors in the Stronkhorst study fell within the range of seasonal values for *M. balthica* found at the Palo Alto site. Again, bioconcentration factors provide a useful way in which to compare

results and data between studies and study sites.

Table 22. Comparison of Bioconcentration Factors (BCF) Between Studies for Algae and Sediments and Clams and Algae.

Description	Cadmium	Copper	Zinc
P. A. Seasonal Range of Algal Mental Conc. (ug/g)	0.06-0.17	7	44, 235 (7/31), 520 (8/29), 439 (9/25)
Absil's Algal Metal Conc. Range of All Sites (ug/g)	0.1027-1.403	3-26	30-182
Stronkhorst et al. Algal Metal Conc.	0.43± 0.26	32±15	89±37
P.A. Sediment Seasonal Metal Conc. Range (ug/g)	0.16-0.32	33-56	112-158
Absil's Sediment Metal Conc. Range All Sites (ug/g)	0.06-7.9	1.2-195	10-430
Stronkhorst et al. Sediment Metal Conc.	5.24± 3.21	61±32	319± 109
P.A. BCF Algae/Seds.	0.50-0.87	0.14-0.21	0.30-4.5
Absil's BCF Algae/Seds.	0.013-21.7	0.08-15	0.24-4.4
Stronkhorst et al. BCF Range Algae/Seds.	0.01-0.30	0.19-1.5	0.10-0.54
P.A. Clam Metal Conc. (ug/g)	0.29-0.9	24-109	291-584
Stronkhorst et al. M. balitica Mean Metal Conc. (ug/g)	1.46±0.69	43±6	647±20
P.A. BCF Clam/Algae	2.07-8.33	3-9	0.72-13
Stronkhorst et al. BCF Clams/Algae	1.62	0.86	4

*(Palo Alto Mudflat "P. A.", Absil 1993, and Stronkhorst, Vos, and Misdorp 1994)

CHAPTER VI

CONCLUSIONS

Although many trends and cause and effect relationships are linked to specific sources of sediment pollution in San Francisco Bay, many of the underlying mechanisms of food chain transfer require further research. As several studies have pointed out, benthic microalgae are important contributors to estuarine carbon cycles and have been overlooked and insufficiently understood as a potential pollutant vector for benthic organisms in estuarine environments (Absil 1993; Stronkhorst, Vos, and Misdorp 1994; Sullivan and Moncreiff 1988). This study examined the potential use of benthic microalgae as an additional tool in monitoring sediment quality and defining sediment quality criteria by: a) quantifying trace metals associated with the algae, b) determining whether or not benthic microalgae reflects surrounding sediment quality, and c) determining whether or not increases in benthic microalgal abundance and trace metal concentrations are reflected in the deposit-feeding clam, *M. balthica*.

The first two objectives of this study were to determine the seasonal variability in benthic microalgal biomass on the mudflat and to determine the significance of benthic microalgae as a food source for benthic organisms such as *M. balthica*. The chlorophyll data in this study provides insight into the food availability seen at the Palo Alto mudflat for the benthic organism *M. balthica*. Benthic microalgae seemed to provide a continuous although variable food source over the entire year of 1995. An increase in clam condition

index followed the rise in chlorophyll *a* measured in both benthic and water column sources suggesting the importance of these food sources to benthic organisms. When determining contaminant assimilation mechanisms, there is a special need for considering food chain processes. It is also important to improve upon and add to the methods used in determining sediment quality criteria including studies of more complex processes-such as trophic transfer and bioaccumulation. In recent years, the need for development of sediment quality criteria has arisen out of a recognition that sediment contamination plays a role in the distribution of pollutants in the aquatic environment. As several studies have pointed out (Luoma et al. 1992; Wang, Fisher, and Luoma 1995; Reinfelder and Fisher 1994) metal uptake from food vectors is an area of needed research and is essential for understanding data that is generated by environmental monitoring programs researching trophic transfer of pollutants through the food chain.

The third objective of this study was to quantify trace metal concentrations associated with benthic microalgae. The use of Nitex allows for a relatively clean separation of epipelic species of diatoms from the sediment surface for trace metal analyses. However, significant contamination can still occur even under the most careful handling conditions and this sediment contamination must be quantified. The concurrent analysis of aluminum, a metal highly affiliated with the sediments of San Francisco Bay provides a good indicator of sediment contamination in the algal samples. Through linear regression (trace metal concentration versus Al % by weight in samples) a correction for the sediment contamination can be assessed by determining the value of the Y intercept.

Trace metal concentrations in benthic microalgae can be quantified using the collection technique presented in this study. However, care must be taken to account for fine grain sediment contamination in the samples. The technique of correlating aluminum concentrations with the trace metal concentrations of concern is an effective approach used to differentiate between the metal associated with the algal component of the sample versus the sedimentary component of the sample. This study demonstrated that chromium, copper, and nickel were more highly associated with the sedimentary component of the samples when correcting for sediment contamination using the linear regression approach. Cadmium and silver were found to be more highly associated with the algal component of the samples.

The fourth objective of this study was to determine whether or not benthic microalgae directly reflected the seasonal variability of trace metals measured in the sediments. Chromium is highly affiliated with the sediment component of the sample and is indistinguishable from that associated with the algal component of the sample using this correlation technique. Copper and nickel are also highly correlated to the sediments but demonstrate a value associated with the algal component (7 ug/g and 6 ug/g respectively). With these three metals it is not possible to discern any seasonal variability in trace metal concentrations because none of the values vary significantly enough from the regression curve (R^2 = 0.75, 0.94, and 0.98 respectively).

Cadmium and silver, on the other hand, demonstrate a greater affiliation with the algal component of the sample and display no linear relationship with the regression

against aluminum ($R^2 = 0.42$ and 0.02 respectively). For both cadmium and silver, a range of numbers is reported (0.06-0.17 ug/g and 0.26-1.35 ug/g respectively). The cadmium concentrations are very low in the algae and any variability between sampling dates is virtually insignificant. After correcting for sediment contamination in the samples, the concentrations of trace metals (Cu, Cr, Ni, and Zn) are significantly lower than the concentrations measured in the sediments. Benthic microalgae did not reflect the seasonal variability of trace metal concentrations observed in the sediments in a one-to-one fashion.

The fifth objective of this study was to determine whether or not trace metal concentrations in *M. balthica* reflected trace metal concentrations measured in the benthic microalgal samples. In the case of silver, an interesting relationship is seen between benthic microalgal silver concentrations on July 31st, August 29th, and September 25th, and the silver concentrations measured in *M. balthica* on September 25th, October 24th, and December 5th. A peak in silver concentrations was measured in the algae on August 29th (1.35 ug/g). The clam data indicates a peak in silver concentrations on October 24th (8.4 ug/g). This peak in silver measured in the clams occurred in a delayed fashion that one might expect for trace metal uptake from natural food sources.

In the case of zinc, there exists a linear relationship with aluminum concentrations with the exception of samples collected on July 31st, August 29th, and September 25th. A linear regression of the data against aluminum concentrations (excluding the afore mentioned dates) indicates algal zinc to be 44 ug/g with an R^2 of 0.92. The data for July 31st, August 29th, and September 25th indicate a significant rise in algal zinc

concentrations (235 ug/g, 520 ug/g, and 439 ug/g respectively). When comparing this data to the concentrations of zinc found in *M. balthica*, there is no significant increase in clam tissue zinc concentrations as was observed in the case of silver. Because it can take approximately 200 days for organisms to reflect the pollutant concentrations in their environments, further information on clam tissue zinc concentrations (beyond the December 1995 data) would have been valuable because it may have demonstrated an assimilation from algal sources. Overall, no simple or clear relationship exists between concentrations of metals in the algae and concentrations of metals in *M. balthica*. More long-term field data would be required to establish trends of this nature.

The similar methods used in this study and in the studies by Absil (1993) and Stronkhorst, Vos, and Misdorp (1994) have demonstrated an effective approach to collecting benthic microalgae in the field for the investigation of trace metal and organic pollutants. The approach used in this study to identify algal metal versus sediment metal in the samples is very useful because of the difficulty associated with obtaining a "pure" benthic microalgal sample. Visual inspection of algal samples to identify sediment contamination is not the best approach as it does not quantify the level of contamination. This study has demonstrated the significant error that can occur in determining trace metal concentrations in benthic microalgae if the sediment contamination is not quantified.

The final objective of this study was to determine whether or not benthic microalgae could provide an additional tool for sediment quality monitoring and/or establishing sediment quality criteria. The results of this study have provided preliminary

insight into the uptake of trace metals in benthic microalgae at the study site in south San Francisco Bay. This study has also demonstrated that by correcting for sediment contamination in the samples, trace metal concentrations in the algae are not within the same range (Cr, Cu, Ni, Zn) as concentrations of metals measured in the sediments. This is important because it may demonstrate that these algae could provide an indication of the *bioavailable* metals in the sediments and the pore waters.

The numerical trace metal data derived from this study may be useful for incorporating into ecological models of San Francisco Bay. Wang, Fisher and Luoma (1996, 92) state that " Models are necessary to supplement monitoring and predict bioaccumulation across [a] variety of conditions....Models can help identify pathways and the most important processes responsible for contaminant bioaccumulation." More research would be necessary to interpret the usefulness of benthic microalgae as an indicator organism. It is hoped that studies such as this one will lead to a better understanding of contaminant vectors in the food chain and that such data will assist in developing new tools and methods that can assist in protecting aquatic environments such as San Francisco Bay.

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TABLES

APPENDIX A

Table 1. Power Analysis for Chlorophyll Sampling.

Date	s^2	n	Mean Chlor. mg/m^2	X_{power}
1/23/95	2.73	22	7.61	1.34
2/23/95	21.83	24	46.74	3.61
4/10/95	10.82	21	39.68	2.74
5/17/95	6.03	15	28.52	2.48
6/19/95	3.68	16	12.54	1.86
7/18/95	4.69	15	23.1	2.18

Power Analysis Calculation:

n = sample size

s^2 = standard deviation

u = actual population mean

u_0 = the mean specified in the null hypothesis

X_{power} = the difference between u and u_0

So: X_{power} = the square root of (s^2/n) {t 0.05, (2), n-1 + t 0.10, (1), n-1}

Where: t 0.05, (2), n-1 = 5% level of significance and t 0.10, (1), n-1 = 90% chance of detecting a difference between means (using critical values of student's t-distribution tables).

Table 2. Trace Metals in Filter Blanks vs. Trace Metals in Sea Water Filter Blanks.

Trace Metal	Mean Blank Absorbance	Mean Sea Water Blank Absorbance	Filt. Blank *ng* of Metal in Digestion Bomb	Sea Water Blank *ng* of Metal in Digestion Bomb
Ag	0	0	0	0
Cd	9.5	0	.31	0
Cr	230	228	402	83
Cr	325	276	53	65
Cu	3	6	6	12
Ni	0	0	0	0
Zn	66	150	8	17
Zn	168	140	22	18
Zn	125	191	18	25
Al	17	122	95	445

***All samples from digestion date 2/7/95. Multiple analyses were run for Cr and Zn.**

Table 3. General Program for Graphite Furnace AAS.

Step	Temp.	Ramp Time	Hold Time	Argon Gas flow	Read	Record
1	100	1	1	300	—	—
2	200	7	45	300	—	—
3	see table 4	10	10	300	—	—
4	see table 4	1	1	10	—	*
5	see table 4	0	4	10	*	*
6	2600	1	4	300	—	—
7	20	1	1	300	—	—

Table 4. Conditions for Trace Metals Analyzed by Graphite Furnace AAS.

Metal	Detection Limit (*pg*)	Pyrolysis Temp. (Steps 3-4)	Atomization Temp. (Step 5)	HGA Rinse Water	Matrix Modifier
Ag	0.5	1000	1800	2% HCL	340 ul H_3PO_4
Al	4.0	1700	2500	D. I. Water	"
Cd	0.3	900	1600	"	"
Cr	1	1650	2500	"	"
Cu	1	1300	2500	"	"
Ni	10	1400	2500	"	"
Zn	0.1	700	1800	1% HNO_3	30 ul $Mg(NO_3)_2$

***All matrix modifiers diluted in 200 mLs of de-ionized water and 4 mLs HNO_3 except for zinc which used 2 mLs HNO_3. Zinc and cadmium were analyzed with platform graphite tubes, all other metals were analyzed with non-platform tubes.**

Table 5. Salt Weight Analysis of Benthic Microalgal Samples By Flame Spectroscopy.

Digest Date	Sample Absor-bance	Conc. in Diluted Sample PPM	Dilution	Sample Conc. PPM	Volume (mLs)	Na Weight (mg)	NaCl Weight (mg)
5/4/95	45	0.086	470	40.19	3	0.12	0.39
"	58	0.11	470	51.79	3	0.16	0.51
"	94	0.18	470	83.94	3	0.25	0.82
"	80	0.15	470	71.44	3	0.21	0.70
"	100	0.19	470	89.30	3	0.27	0.88
"	75	0.14	470	66.98	3	0.20	0.66
8/2/95	171	0.32	470	152.70	3	0.46	1.50
"	169	0.32	470	150.92	3	0.45	1.48
"	138	0.26	470	123.23	3	0.37	1.21
"	451	0.86	470	402.74	3	1.21	3.95
"	180	0.34	470	160.74	3	0.48	1.58
"	403	0.77	470	359.88	3	1.08	3.53
"	40	0.08	470	35.72	3	0.11	0.35
"	339	0.64	470	302.73	3	0.91	2.97
"	144	0.27	470	128.59	3	0.39	1.26
"	195	0.37	470	174.14	3	0.52	1.71
"	98	0.19	470	87.51	3	0.26	0.86
2/7/95	105	0.20	470	93.77	3	0.28	0.92
"	166	0.32	470	148.24	3	0.45	1.45
"	189	0.36	470	168.78	3	0.51	1.65
"	115	0.22	470	102.70	3	0.31	1.01

Table 5 Continued...

Digest Date	Sample Absor-bance	Conc. in Diluted Sample PPM	Dilution	Sample Conc. PPM	Volume (mLs)	Na Weight (*mg*)	NaCl Weight (*mg*)
2/7/95	186	0.35	470	166.10	3	0.50	1.63
"	248	0.47	470	221.46	3	0.66	2.17
"	1146	2.18	470	1023.40	3	3.07	10.03
"	710	1.35	470	634.03	3	1.90	6.21
"	211	0.40	470	188042	3	0.57	1.85
"	230	0.44	470	205.39	3	0.62	2.01
"	224	0.43	470	200.03	3	0.60	1.96
"	257	0.49	470	229.50	3	0.69	2.25
"	235	0.45	470	209.86	3	0.63	2.06
"	134	0.25	470	119.66	3	0.36	1.17
"	277	0.53	470	247.36	3	0.74	2.42
"	270	0.51	470	241.11	3	0.72	2.36
"	238	0.45	470	212.53	3	0.64	2.08
"	283	0.54	470	252.72	3	0.76	2.48
"	351	0.67	470	313.44	3	0.94	3.07

***Salt weight analysis based upon the regression analysis of standards (0 - 6 PPM) run by flame spectroscopy with benthic microalgal samples.**

$$\frac{\{(Abs * x + b) * D\} * v}{1000} = \text{Na Wt. (mg)} \qquad \frac{\text{Na Wt. (mg)}}{0.3061} = \text{NaCl Wt. (mg)}$$

Where: Abs = absorbance of sample by flame spectroscopy
x = X coefficient of regression = .0019
b = constant (Y intercept) = 0
v = total volume of sample
D = dilution from original sample

*** Na accounts for approximately 30% of the total salt weight of NaCl**

Table 10. Coefficients of Variation Per Quadrant for Chlorophyll Measured in the Top Sediment Layer: 1995

Date	Q1	Q2	Q3	Q4	Q5	Q6
	n=4	n=4	n=4	n=4	n=4	n=4
2/23/95	cv=50%	cv=30%	cv=58%	cv=50%	cv=17%	cv=60%
	n=3	n=4	n=4	n=4	n=3	n=4
4/10/95	cv=7%	cv=34%	cv=17%	cv=25%	cv=10%	cv=39%
	n=4	n=4	n=4	n=3		
5/17/95	cv=19%	cv=13%	cv=11%	cv=13%		
	n=3	n=4	n=4	n=4		
6/19/95	cv=31%	cv=34%	cv=12%	cv=48%		
	n=4	n=4	n=4	n=3		
7/18/95	cv=11%	cv=15%	cv=26%	cv=33%		
	n=3	n=4	n=4	n=4		
8/29/95	cv=45%	cv=35%	cv=25%	cv=28%		
	n=3	n=3	n=4	n=3		
9/25/95	cv=72%	cv=37%	cv=16%	cv=28%		
	n=4	n=4	n=3	n=4		
10/24/95	cv=35%	cv=12%	cv=12%	cv=19%		
	n=3	n=4	n=3	n=4		
12/5/95	cv=85%	cv=57%	cv=87%	cv=62%		

Q = Quadrant. n = the number of samples for each quadrant. January data is not included because samples were not separated to identify with quadrants during processing.

Table 11. Species Identification of Samples Collected from Palo Alto Baylands Nature Preserve in 1995.

Species	4/10/95 Density	4/10/95 Volume	6/19/95 Density	6/19/95 Volume
BACILLARIO-PHYTA				
Achnanthes hungarica?	1625	532610	169796.3	42007604.6
Cylindrotheca gracilis	140437.5	128247525	48375	56734200
Cymatopleura elliptica	0	0	387	356040
Gyrosigma fasciola	12.5	161603.8	1451.3	18762841.8
Navicula cryptocephala	0	0	2902.5	1281744
Navicula halophila	250	1335595.5	387	294661.8
Navicula pygmaea	0	0	387	437422.2
Nitzschia fonticola	0	0	22252.5	6907176
EUGLENO-PHYTA				
Euglena sp.	15.6	262992.6	0	0
TOTAL	142340.6	130540327	245938.6	126781690

Table 11 continued...

Table 11 Continued..

Species	11/3/95 Density	11/3/95 Volume	12/5/95 Density	12/5/95 Volume
BACILLARIO-PHYTA				
Achnanthes hungarica	101935	33414293	8742.5	2865791.5
Cyclotella meneghiniana	0	0	2017.5	268096.4
Cyclotella striata	0	0	3362.5	272178.2
Cylindrotheca gracilis	23147.5	14977.8	672.5	430131
Nitzschia agnita	557887.5	154423260	321455	88014379
Nitzschia longissima	1377.5	4620685	0	0
Stauophora amphioxys	4132.5	6317766	0	0
TOTAL	688480	198790981.8	336250	91850576.1

Density = cells/mLs in 100 mL of sample

Volume = cubic micrometers/mL in 100 mLs of sample

Table 14. NBS (National Bureau of Standards) Recovery of Certified Standards.

Metal/ NBS range	Digestion Date	n	Mean (ug/g)	Standard Deviation	% Recovery of Mean NBS Value
Ag (No NBS Certification)	5/4/95	5	0.10	0.03	N/A
	8/2/95	5	0.08	0.01	N/A
	2/7/95	4	0.12	0.01	N/A
Al 6.25 ± 0.20	5/4/95	5	3.69	1.23	59%
(% Dry Weight)	8/2/95	5	4.36	0.22	70%
	2/7/95	5	4.65	1.37	74%
Cd 0.36 ± 0.07	5/4/95	5	0.29	0.01	81%
	8/2/95	5	0.32	0.02	89%
	2/7/95	4	0.27	0.01	75%
Cu 18 ± 3	5/4/95	5	20.27	6.67	113%
	8/2/95	5	16.29	1.19	91%
	2/7/95	4	16.40	0.91	91%
Cr 76 ± 3	5/4/95	5	37.73	10.59	50%
	8/2/95	5	67.8	2.98	89%
	2/7/95	3	58.09	4.71	76%
	2/7/95	4	64.45	3.85	85%
Ni 32 ± 3	5/4/95	5	33.60	4.79	105%
	8/2/95	5	34.41	4.79	108%
	2/7/95	5	29.15	2.22	91%
Zn 138 ± 6	5/4/95	5	132.14	7.23	96%
	8/2/95	5	131.70	14.05	95%
	2/7/95	5	135.84	8.57	98%
	2/7/95	3	160.57	35.61	116%
	2/7/95	5	124.65	20.40	90%

Table 16. Mean Concentrations and Standard Deviations of Trace Metals in All Benthic Microalgal Samples.

Date	Metal	Mean Concentration (ug/g)	Standard Deviation	n
2/23/95	Ag	0.59	0.06	2
4/10/95	Ag	0.51	0.15	4
6/22/95	Ag	0.45	0.07	3
7/18/95	Ag	0.70	N/A	1
7/20/95	Ag	1.16	N/A	1
7/31/95	Ag	0.80	0.09	3
8/29/95	Ag	1.35	0.18	4
9/25/95	Ag	0.86	0.54	2
11/3/95	Ag	0.66	0.25	4
12/5/95	Ag	0.26	0.06	5
2/23/95	Cd	0.17	0.01	2
4/10/95	Cd	0.14	0.14	4
6/22/95	Cd	0.16	0.02	3
7/18/95	Cd	0.10	N/A	1
7/20/95	Cd	0.15	N/A	1
7/31/95	Cd	0.12	0.05	3
8/29/95	Cd	0.09	0.02	4
9/25/95	Cd	0.06	0.01	2
11/3/95	Cd	0.12	0.00	4
12/5/95	Cd	0.14	0.01	5

Table 16 Continued...

Table 16 Continued...

Date	Metal	Mean Concentration (ug/g)	Standard Deviation	n
2/23/95	Cr	37.00	4.50	2
4/10/95	Cr	11.24	5.00	4
6/22/95	Cr	79.32	7.94	3
7/18/95	Cr	13.48	N/A	1
7/20/95	Cr	112.71	N/A	1
7/31/95	Cr	33.35	40.07	3
8/29/95	Cr	81.12	8.86	4
9/25/95	Cr	140.72	5.64	2
11/3/95	Cr	152.88	11.23	4
12/5/95	Cr	309.90	12.88	5
2/23/95	Cu	19.90	2.53	2
4/10/95	Cu	14.04	2.05	4
6/22/95	Cu	26.21	4.06	3
7/18/95	Cu	9.88	N/A	1
7/20/95	Cu	34.73	N/A	1
7/31/95	Cu	20.75	8.78	3
8/29/95	Cu	45.70	5.33	4
9/25/95	Cu	43.02	1.48	2
11/3/95	Cu	42.86	2.94	4
12/5/95	Cu	67.42	9.87	5
2/23/95	Ni	34.14	1.18	2

Table 16 Continued...

Table 16 Continued...

Date	Metal	Mean Concentration (ug/g)	Standard Deviation	n
4/10/95	Ni	10.93	5.06	4
6/22/95	Ni	47.99	9.05	3
7/18/95	Ni	6.30	N/A	1
7/20/95	Ni	69.71	N/A	1
7/31/95	Ni	31.77	35.69	3
8/29/95	Ni	91.04	2.67	4
9/25/95	Ni	91.05	12.44	2
11/3/95	Ni	78.39	7.40	4
12/5/95	Ni	102.95	5.51	5
2/23/95	Zn	111.97	38.72	2
4/10/95	Zn	90.37	15.85	4
6/22/95	Zn	108.50	11.57	3
7/18/95	Zn	58	N/A	1
7/20/95	Zn	221	N/A	1
7/31/95	Zn	290.20	139.75	3
8/29/95	Zn	718.10	449.30	4
9/25/95	Zn	655.43	119.48	2
11/3/95	Zn	178.35	12.12	4
12/5/95	Zn	314.57	32.40	5

Table 16 Continued...

Table 16 Continued...

Date	Metal	Mean Concentration (% Dry Weight)	Standard Deviation	n
2/23/95	Al	1.80	0.15	2
4/10/95	Al	0.66	0.28	4
6/22/95	Al	2.50	0.38	3
7/18/95	Al	0.35	N/A	1
7/20/95	Al	3.80	N/A	1
7/31/95	Al	1.50	1.60	3
8/29/95	Al	5.30	0.59	4
9/25/95	Al	5.90	0.03	2
11/3/95	Al	4.30	0.64	4
12/5/95	Al	6.90	0.32	5

Table 17. Regression Analyses of Trace Metals vs. Aluminum Content in Benthic Microalgal Samples.

Trace Metal	Y Intercept	Std. Error of Y Est.	R^2	No. Of Observ.	Degrees of Freedom	X Coeff.
Ag	0.66	0.34	0.02	10	8	0.02
Cd	0.15	0.03	0.42	10	8	-0.01
Cr	-16.02	47.66	0.75	10	8	34.29
Cu	7.64	4.52	0.94	10	8	7.51
Ni	6.46	5.76	0.98	10	8	15.13
Zn	36.66	175.88	0.50	10	8	72.10

***Zn = 44 ug/g if outlier points are excluded.**

Table 20. Summary of Calculations for Zinc Concentrations in Outlier Points (July 31st, August 29th, and September 25th 1995).

Date	Zn in Concentrate of Sample (ug/g)	Al (% By Weight)	Individual Sample Sediment Zn (ug/g)	Individual Sample Algal Zn (ug/g)	Sampling Date Mean Algal Zn (ug/g)	Sampling Date Standard Deviation
7/31	448	0.76	28	419	235	162
7/31	238	3.3	122	116		
7/31	184	0.38	14	170		
8/29	1384	5.8	214	1170	520	436
8/29	430	4.6	170	260		
8/29	588	5.8	214	373		
8/29	470	5.2	192	278		
9/25	740	5.9	218.18	521.82	439.17	116.88
9/25	571	5.8	214.48	356.52		

Table 21. Carbon and Biomass Estimates.

Date	n	Depth	mgC/m²	Biomass mg dry wt./m² 50%	Biomass mg dry wt./m² 30%
1/23/95	22	1cm	2167	4334	7223
2/23/95	24	1cm	4967	9934	16557
2/23/95	11	2cm	3476	6952	11587
2/23/95	4	3cm	2875	5750	9583
4/10/95	21	1cm	4351	8702	14503
5/17/95	15	1cm	3677	7354	12257
6/19/95	15	1cm	3106	6212	10353
6/19/95	11	2cm	3415	6830	11383
7/18/95	15	1cm	4477	8954	14923
8/29/95	15	1cm	2322	4644	7740
9/25/95	14	1cm	2890	5780	9633
9/25/95	9	2cm	2631	5262	8770
10/24/95	15	1cm	3100	6200	10333
12/5/95	14	1cm	4367	8734	14557
1/17/96	15	1cm	4224	8448	14080
2/13/96	16	1cm	4119	8238	13730
2/13/96	10	2cm	3790	7580	12633
2/13/96	10	3cm	3232	6464	10773
3/13/96	15	1cm	3919	7838	13063
4/10/96	15	1cm	4119	8238	13730

***Based upon chlorophyll and phaeopigment measurements. Biomass estimates calculated for both 50% and 30% carbon content based upon the variability that may exist due to species diversity in algal mats.**

FIGURES

APPENDIX B

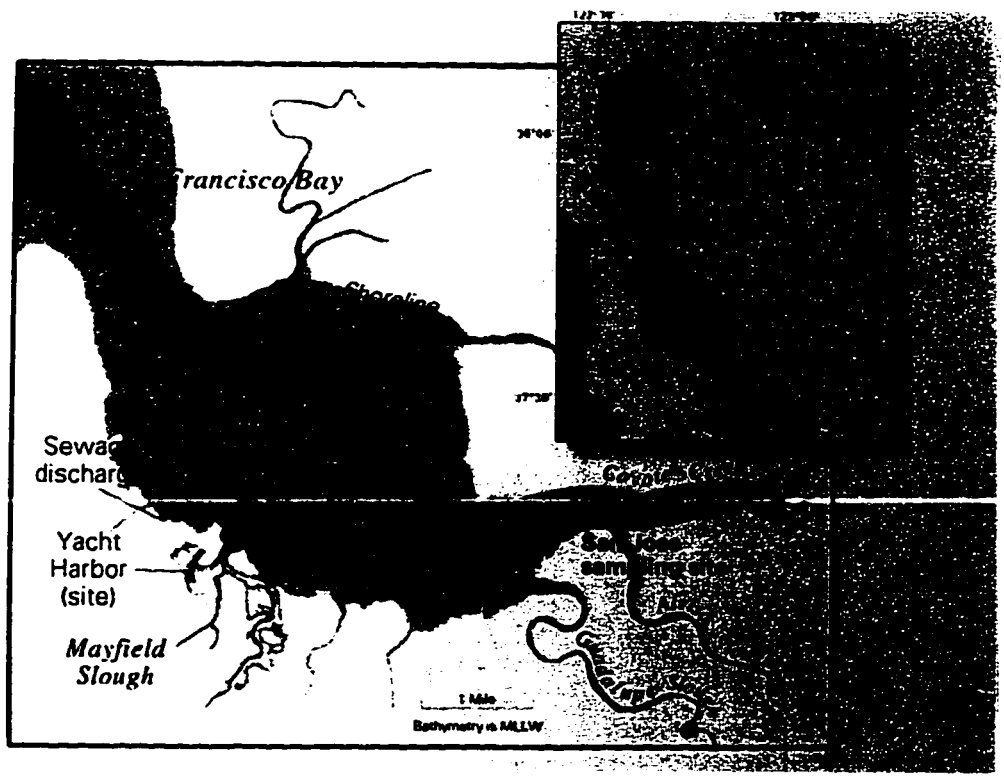

Figure 1. Map of Study Site at Palo Alto Baylands Nature Preserve, Sand Point, San Francisco Bay, California. Courtesy of David Jones, U. S. Geological Survey, Menlo Park, California.

Figure 2. Benthic Microalgal Collection Technique (using Nitex pre-set in embroidery hoops to facilitate handling on windy days).

Figure 3. Benthic Microalgal Collection Technique (using Nitex placed directly on sediment without the use of embroidery hoops).

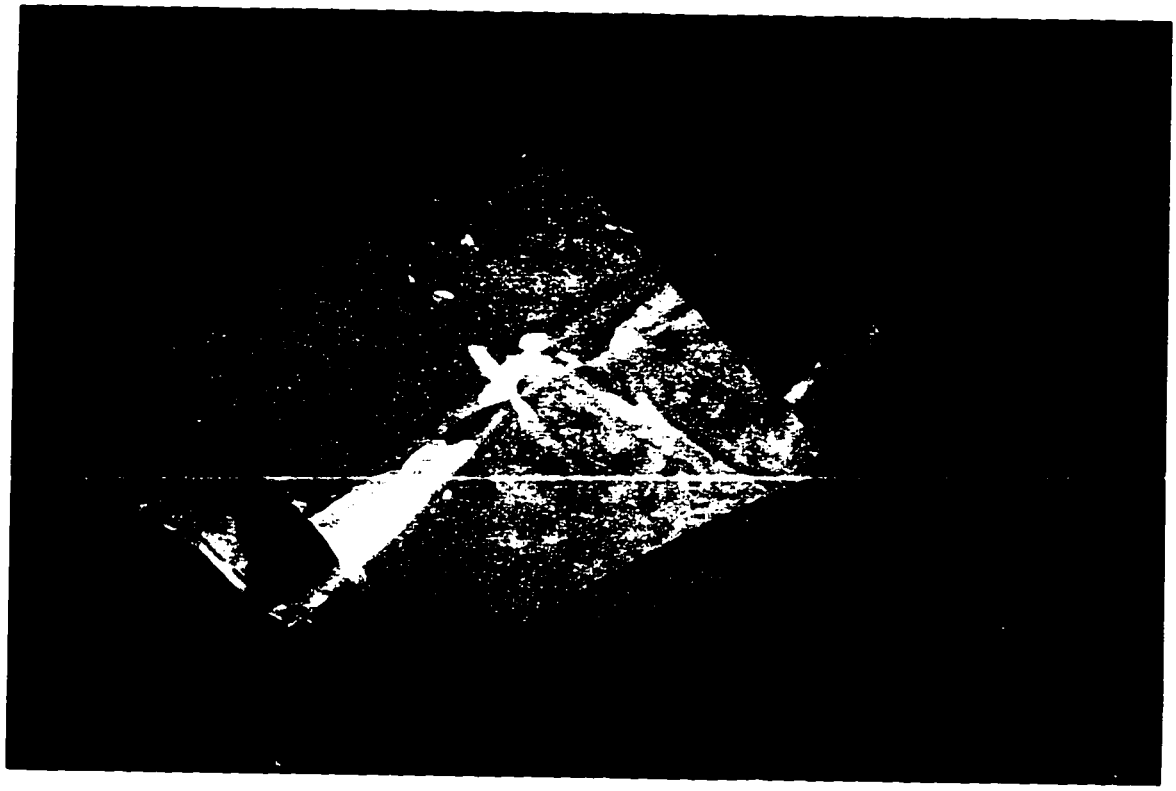

Figure 4. Benthic Microalgae Collecting on Surface of Nitex.

Figure 5. Benthic Microalgal Sampling Area (45' x 20').

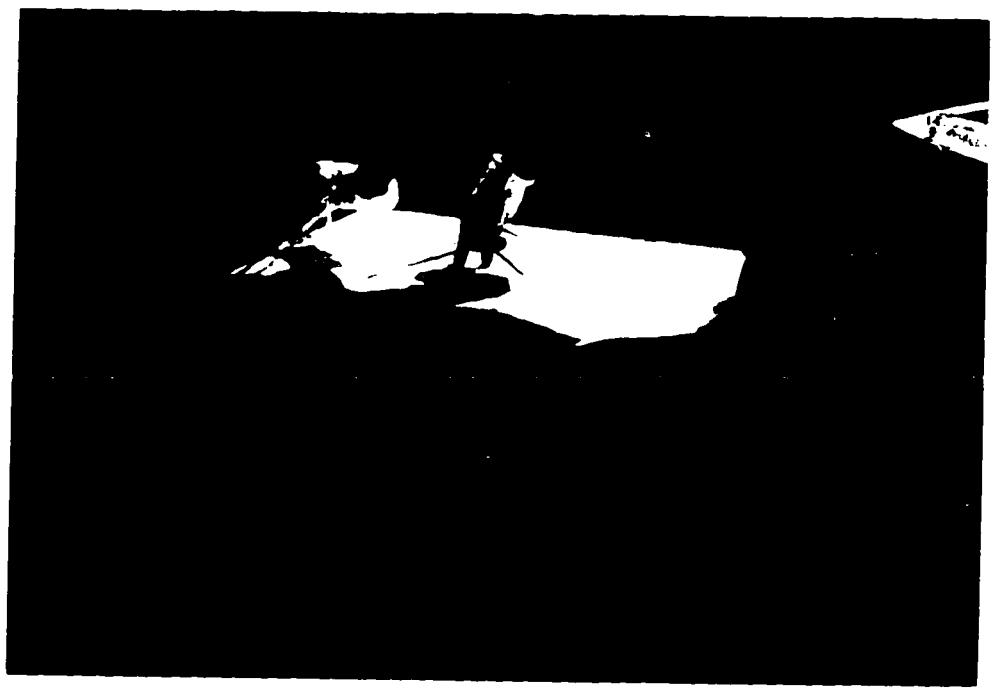

Figure 6. Sampling Technique for Chlorophyll Analysis.

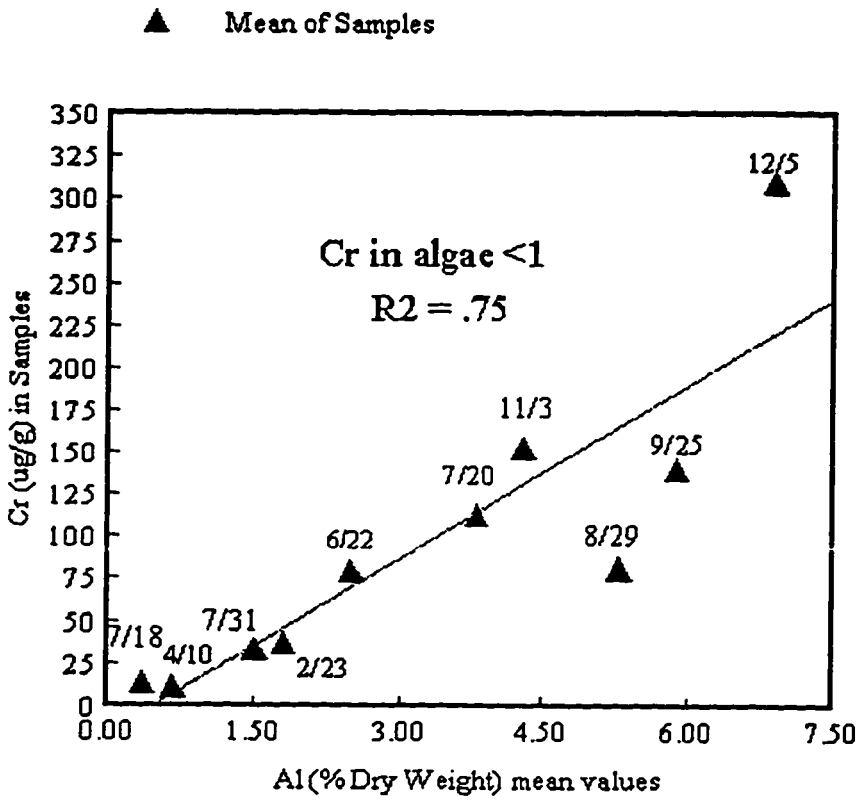

Figure 12. Algal Sample Aluminum and Chromium Correlation.

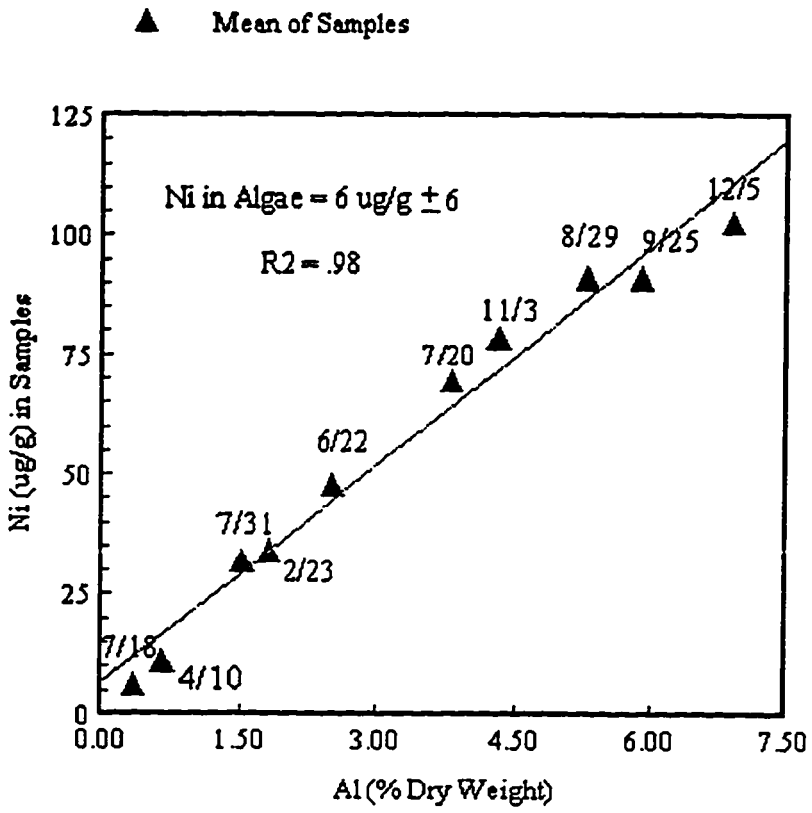

Figure 13. Algal Sample Aluminum and Nickel Correlation.

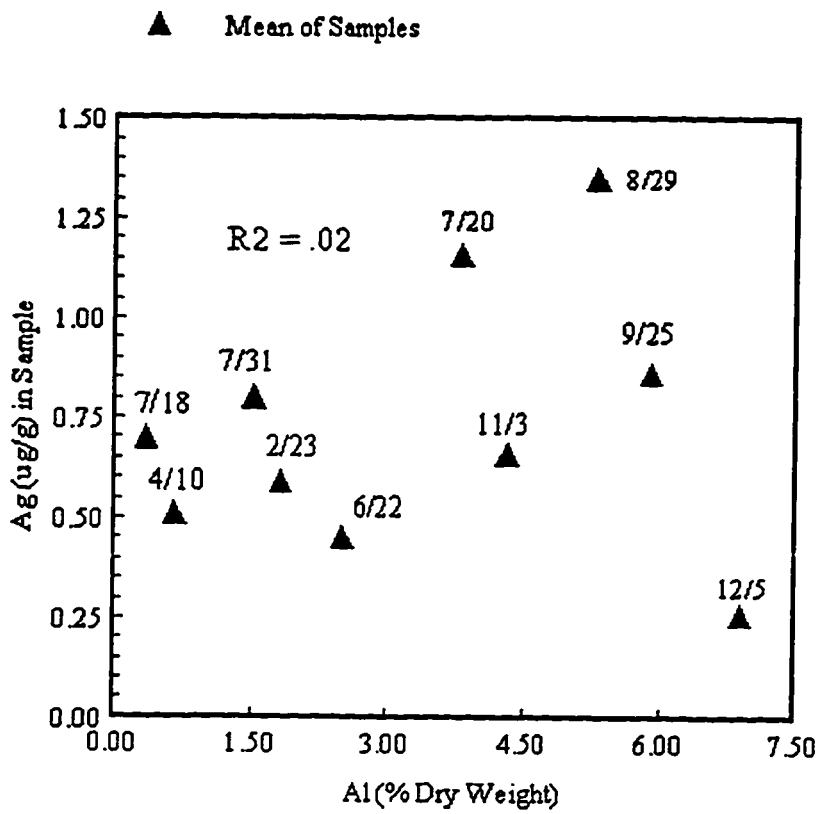

Figure 15. Algal Sample Aluminum and Silver Correlation.

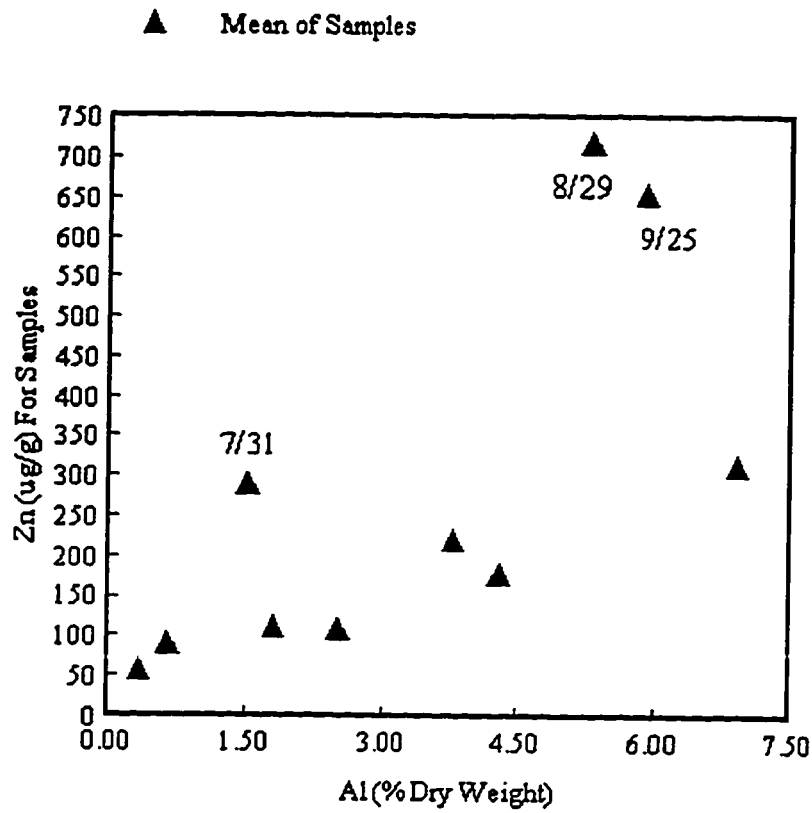

Figure 16. Algal Sample Aluminum and Zinc Correlation.

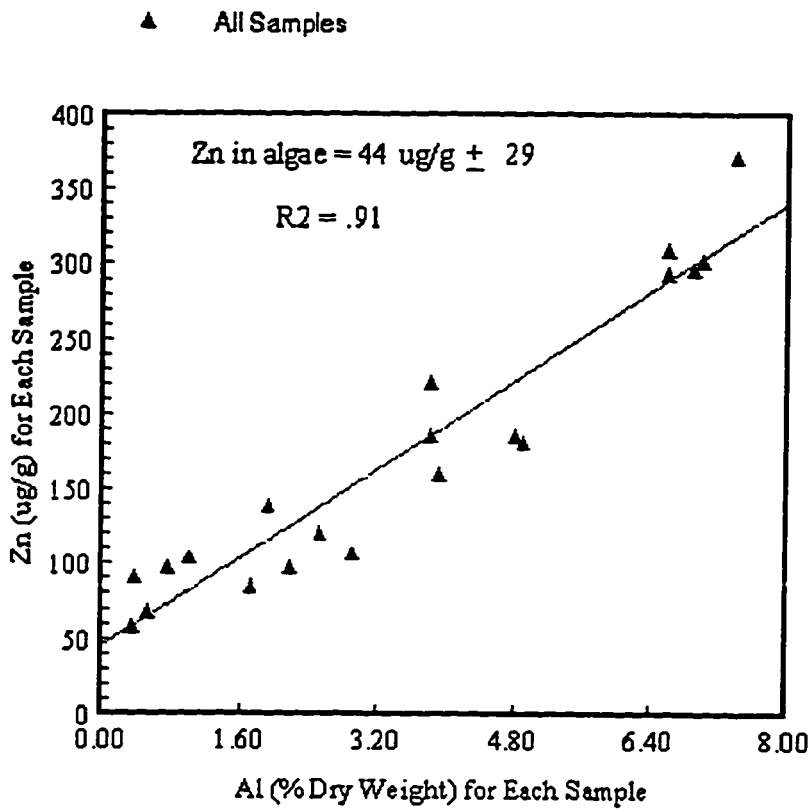

Figure 17. Algal Sample Aluminum and Zinc Correlation Excluding Data Points 7/31, 8/29, and 9/25 of 1995.

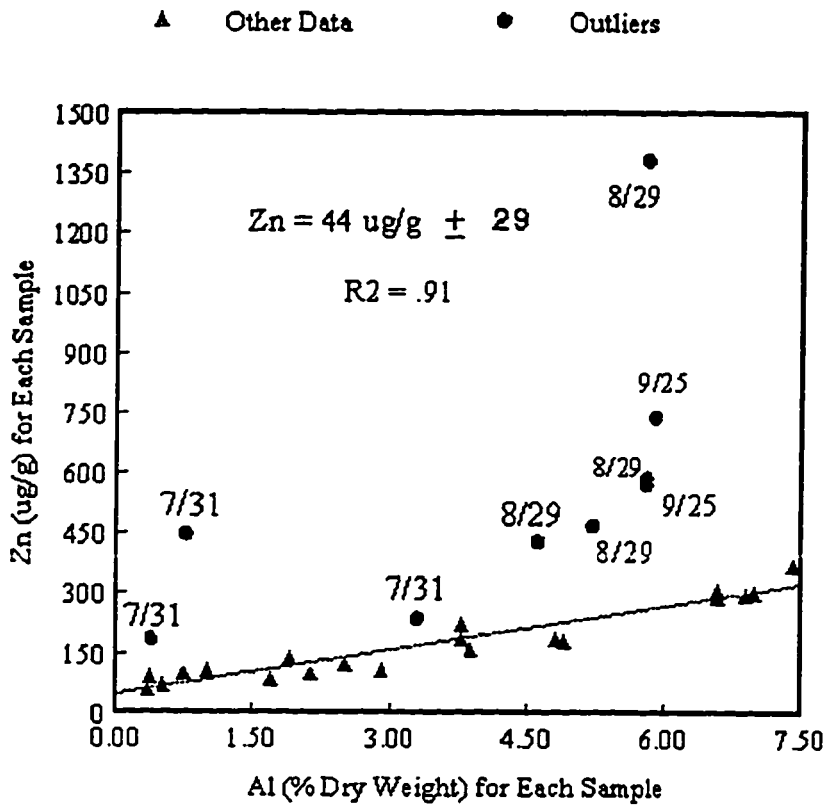

Figure 18. Algal Sample Aluminum and Zinc Correlation Including All Data Points.

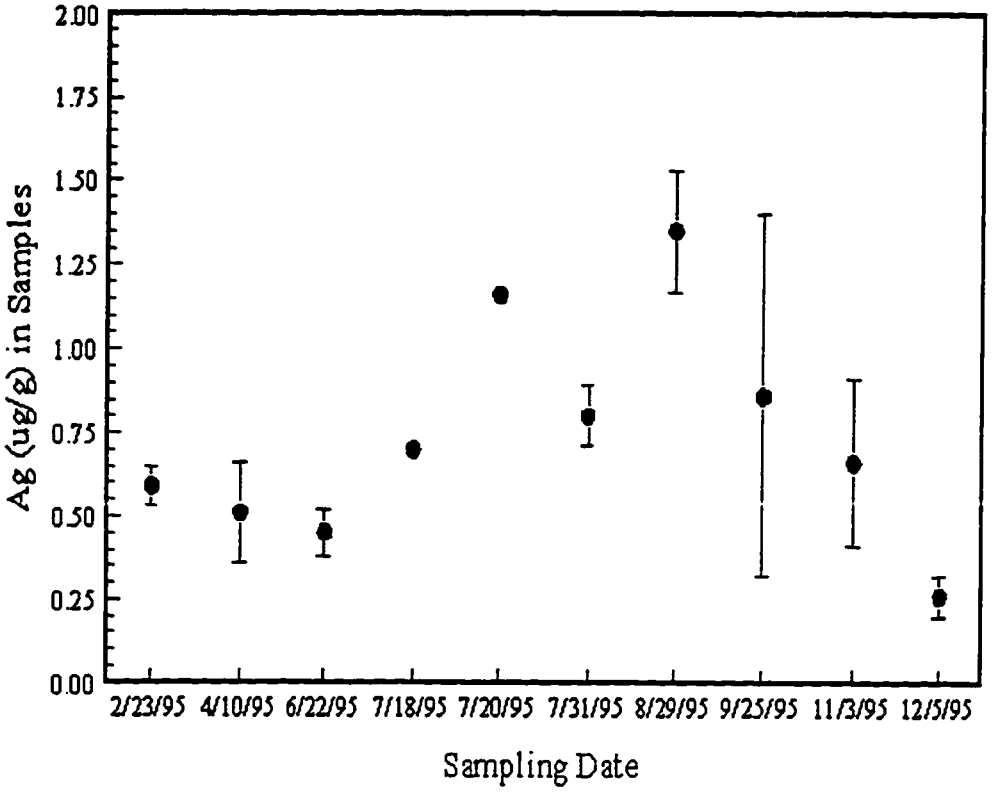

Figure 19. Silver Concentration in Concentrate of Benthic Microalgae.

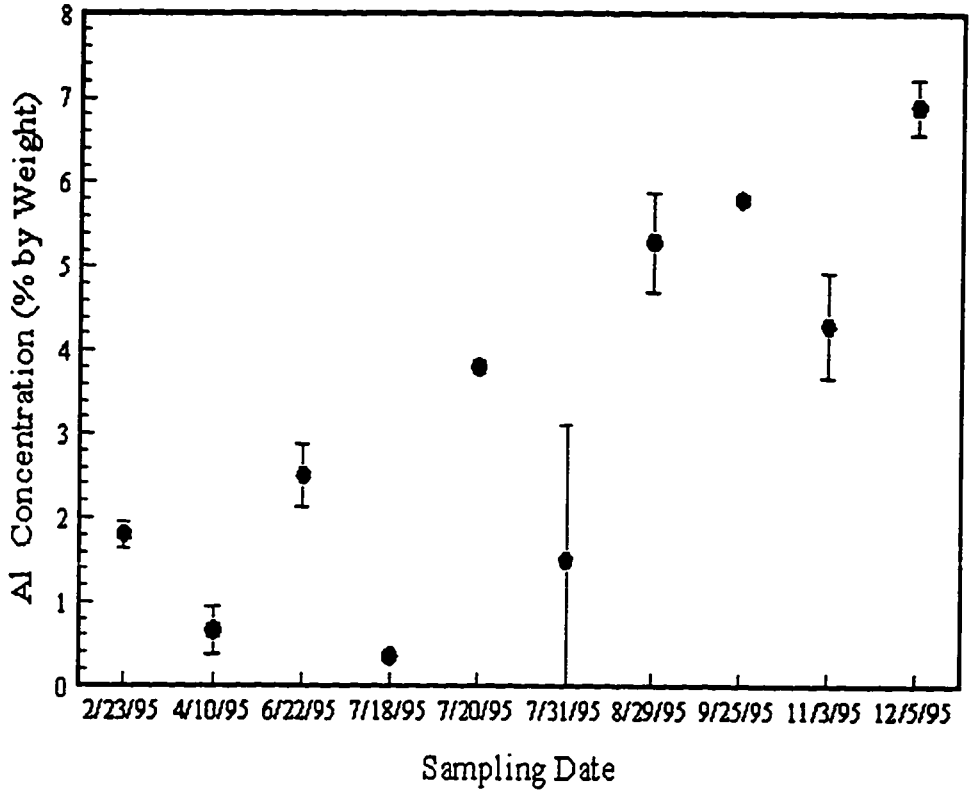

Figure 20. Aluminum Concentration in Concentrate of Benthic Microalgae.

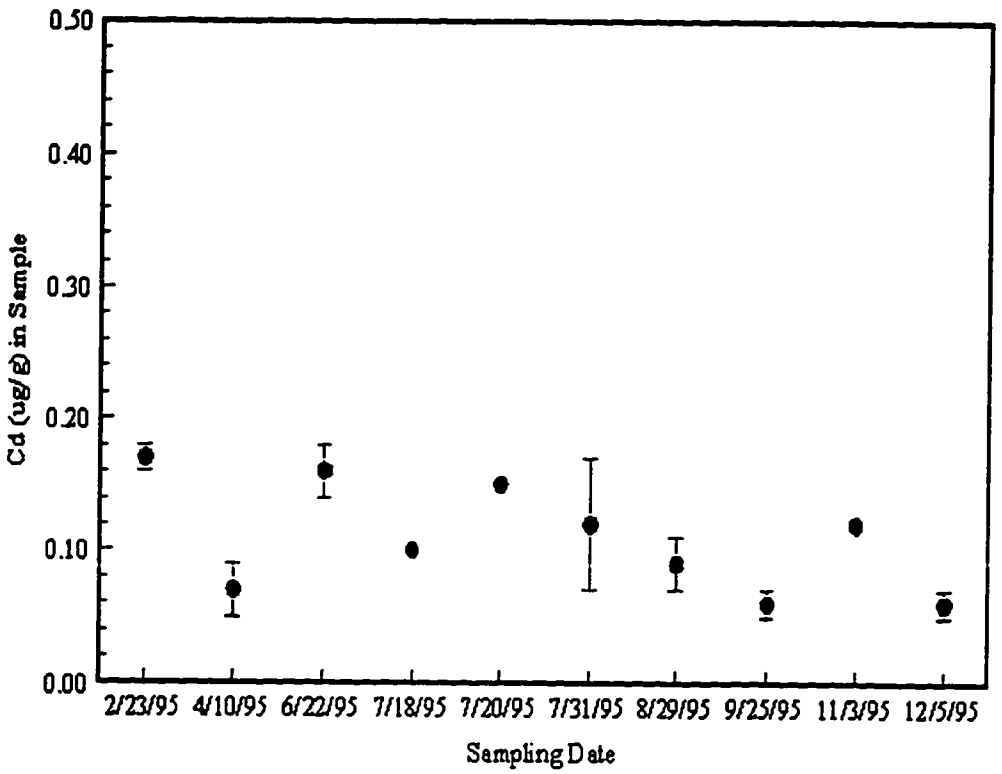

Figure 21. Cadmium Concentration in Concentrate of Benthic Microalgae.

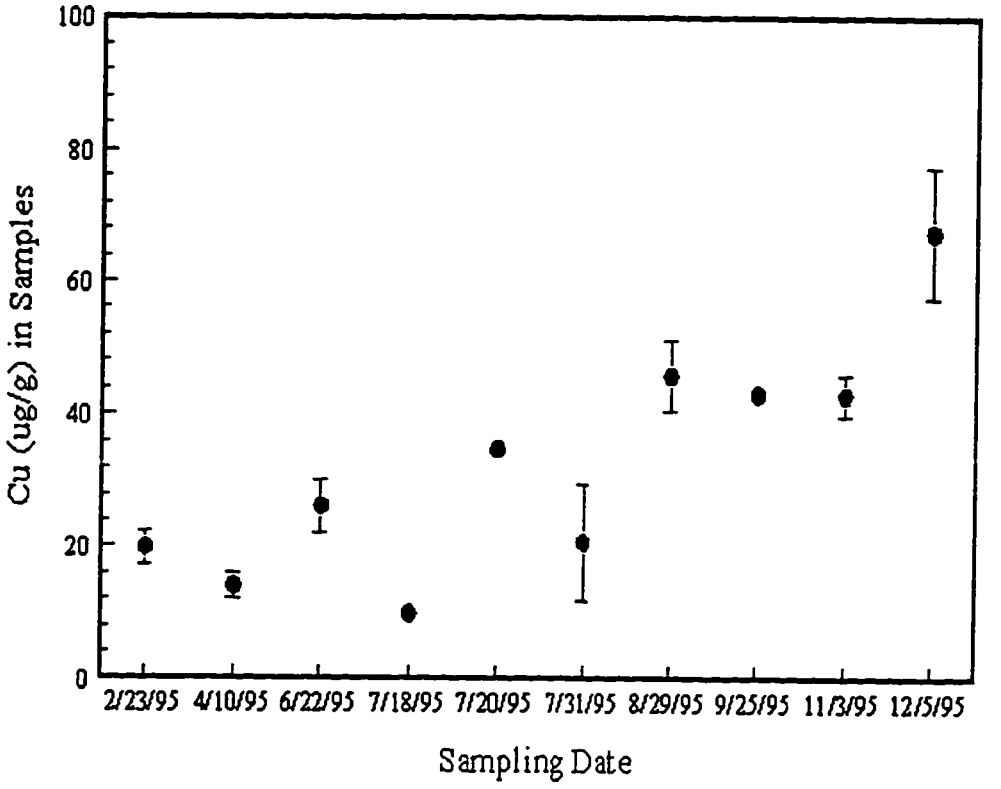

Figure 22. Copper Concentration in Concentration of Benthic Microalgae.

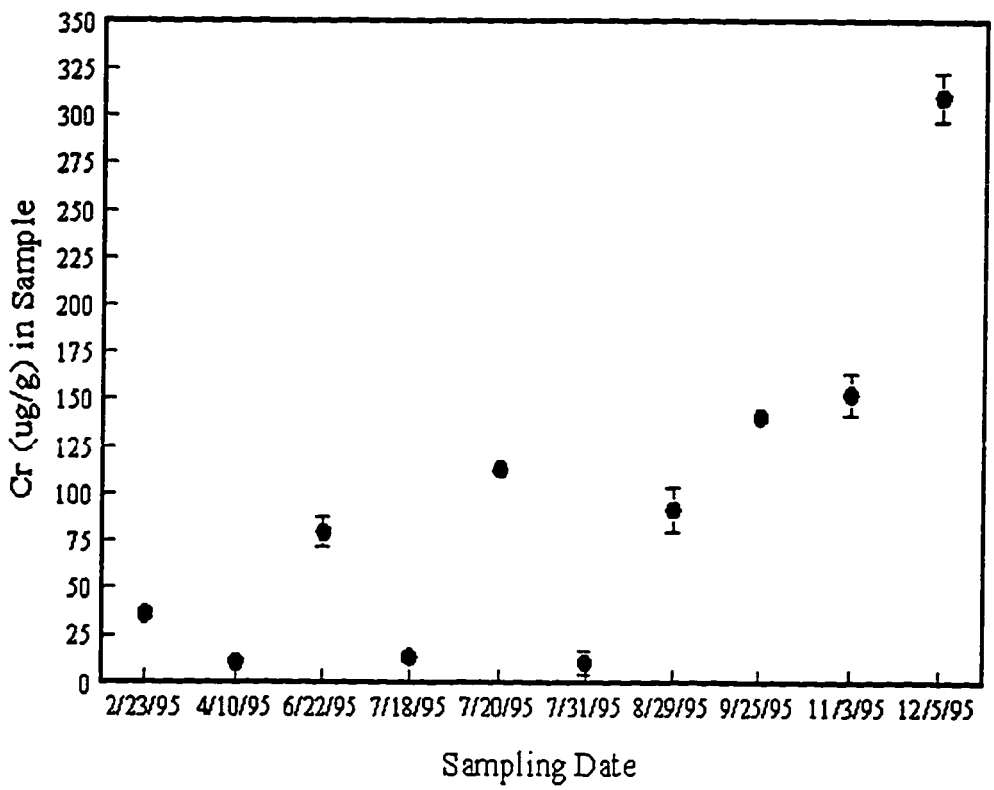

Figure 23. Chromium Concentration in Concentrate of Benthic Microalgae.

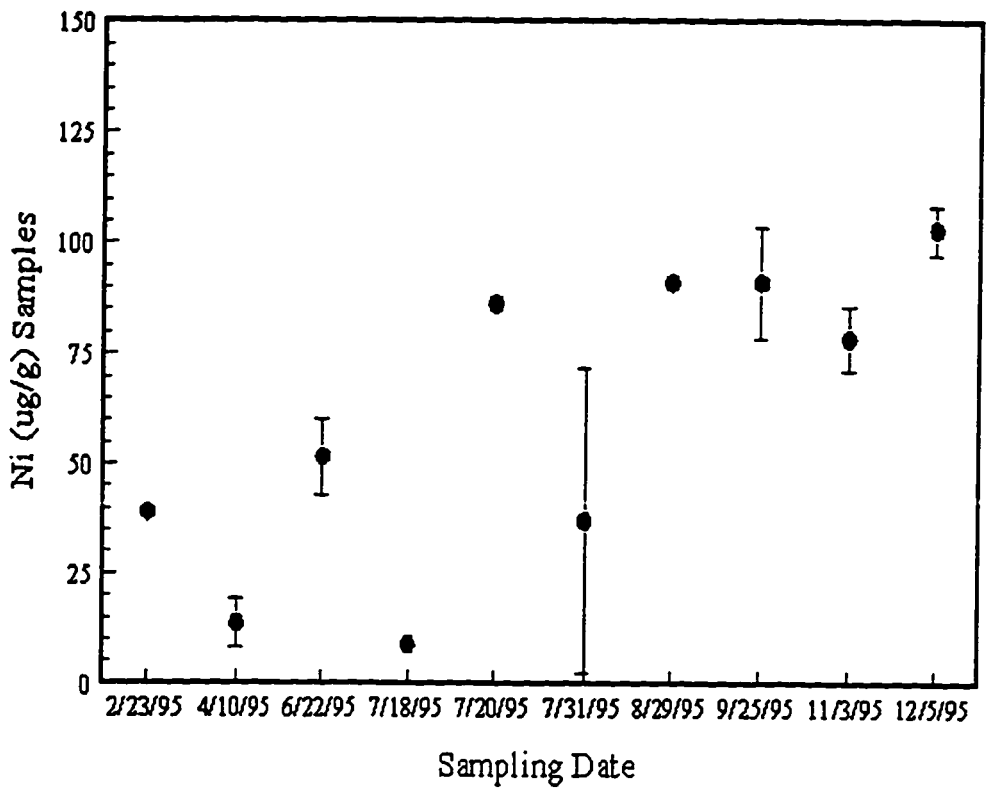

Figure 24. Nickel Concentration in Concentrate of Benthic Microalgae.

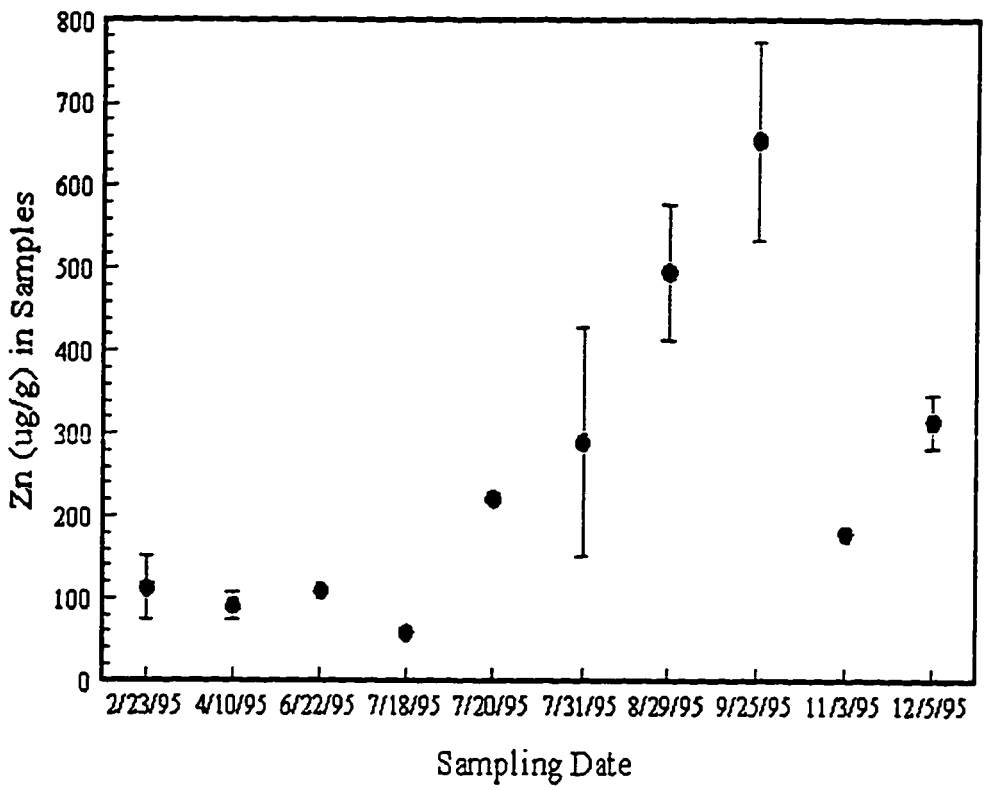

Figure 25. Zinc Concentration in Concentrate of Benthic Microalgae.

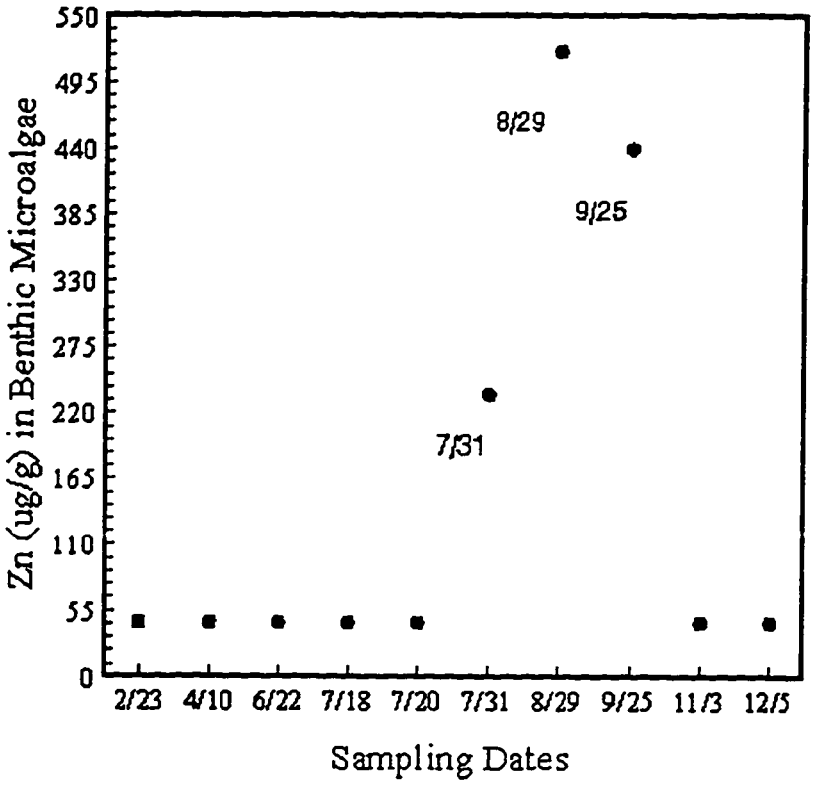

Figure 26. Zinc Concentration in Algal Samples for Outlier Points (7/31, 8/29, and 9/25, 1995).

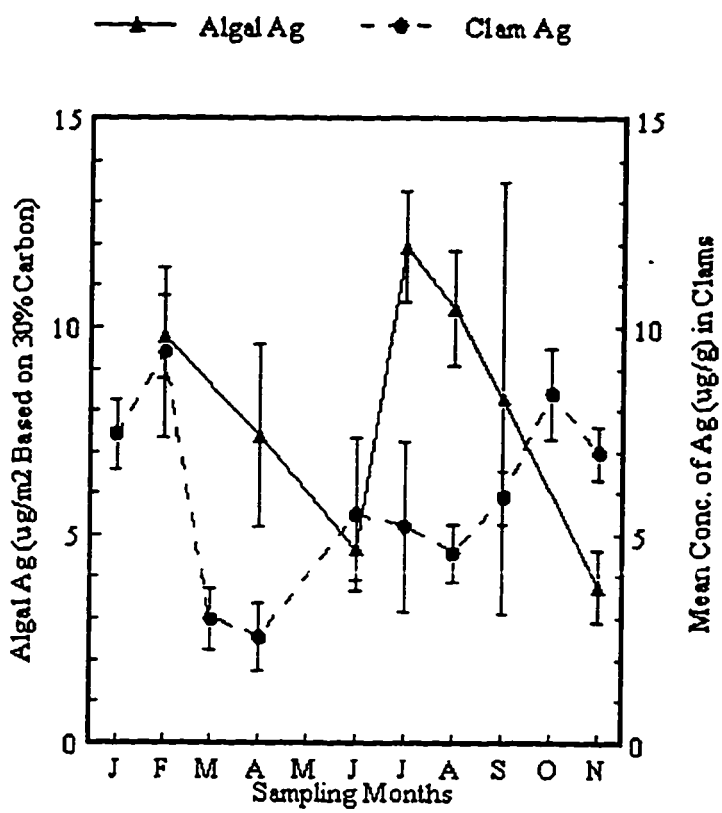

Figure 27. Algal Silver (ug/m^2) vs. Silver Concentration in *M. balthica* (ug/g).

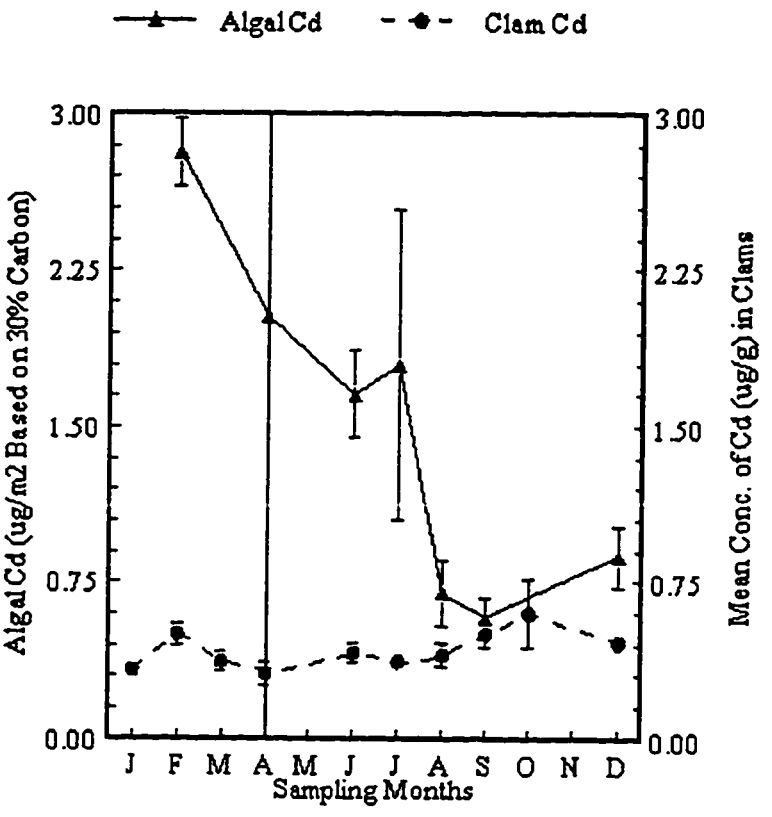

Figure 28. Algal Cadmium (ug/m^2) vs. Cadmium Concentration in *M. balthica* (ug/g).

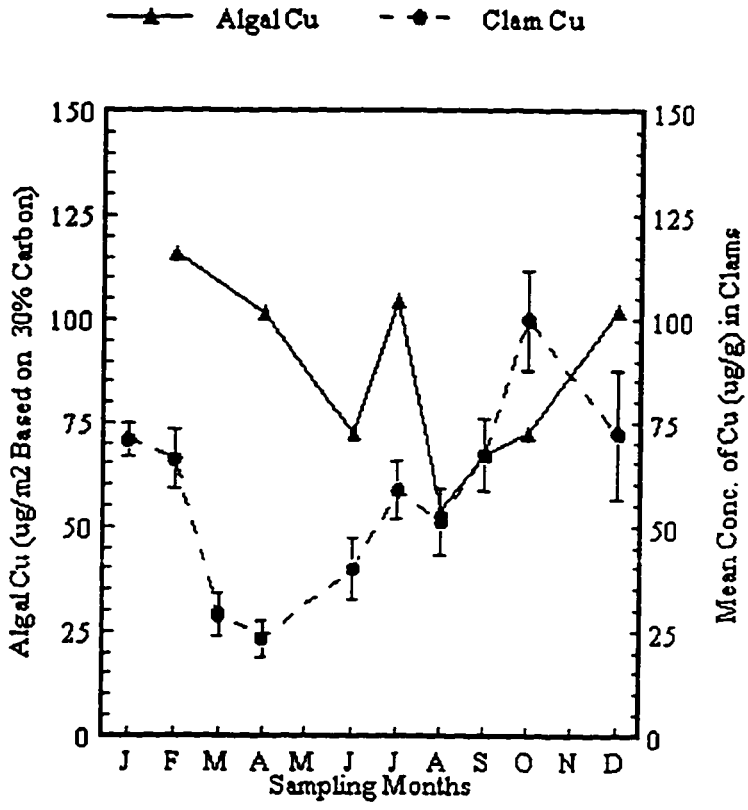

Figure 29. Algal Copper (ug/m^2) vs. Copper Concentration in *M. balthica* (ug/g).

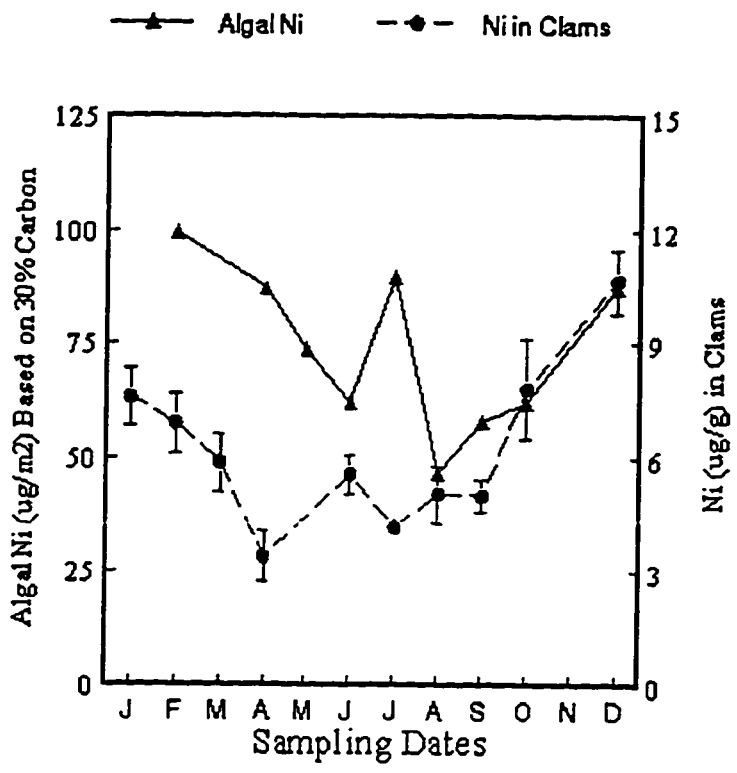

Figure 30. Algal Nickel (ug/m^2) vs. Nickel Concentration in *M. balthica* (ug/g).

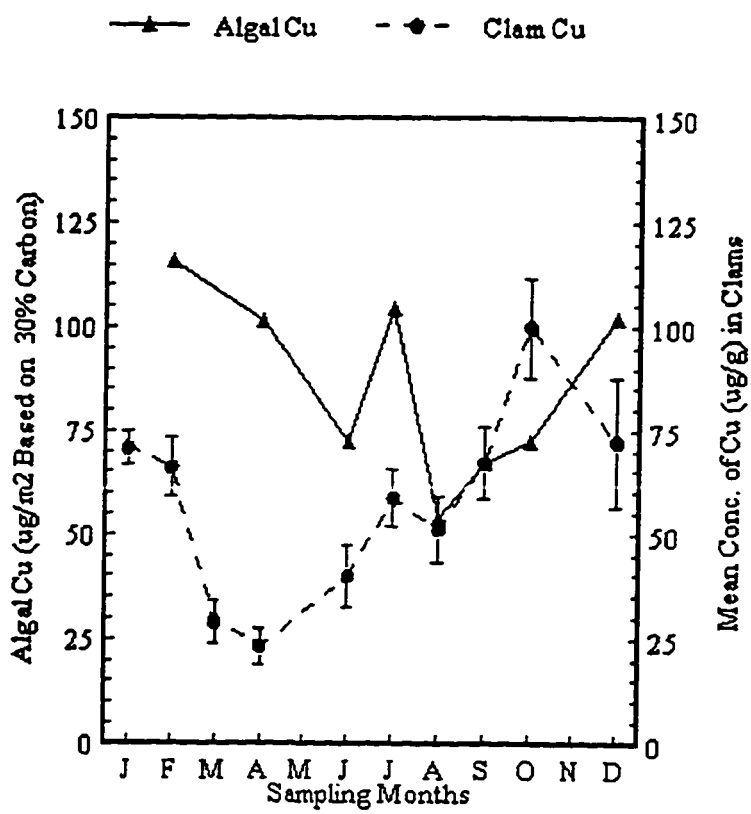

Figure 31. Algal Zinc (ug/m^2) vs. Zinc Concentration in *M. balthica* (ug/g).

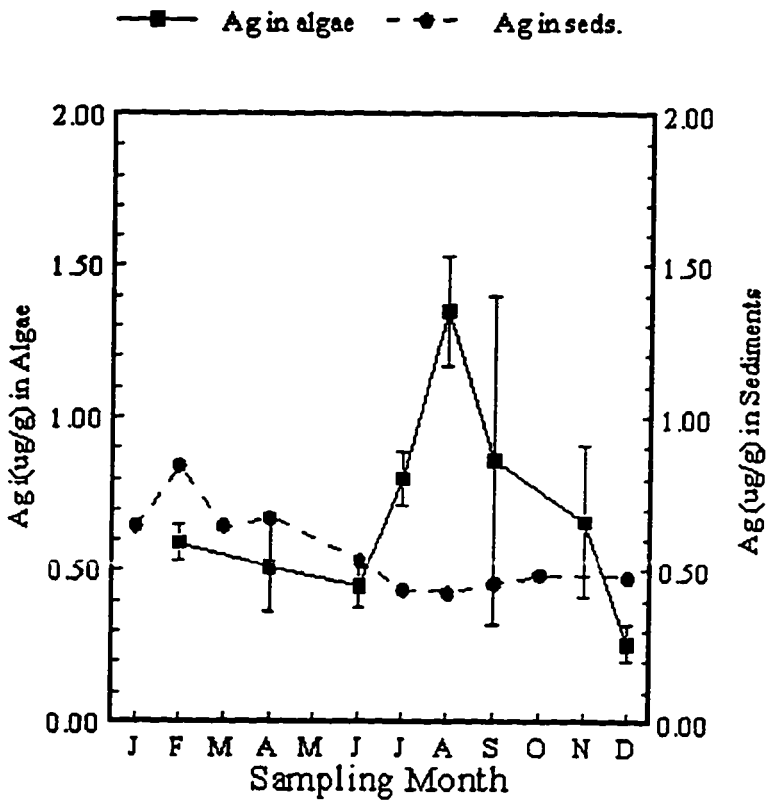

Figure 32. Silver in Algae (ug/g) vs. Silver in Sediments (ug/g).

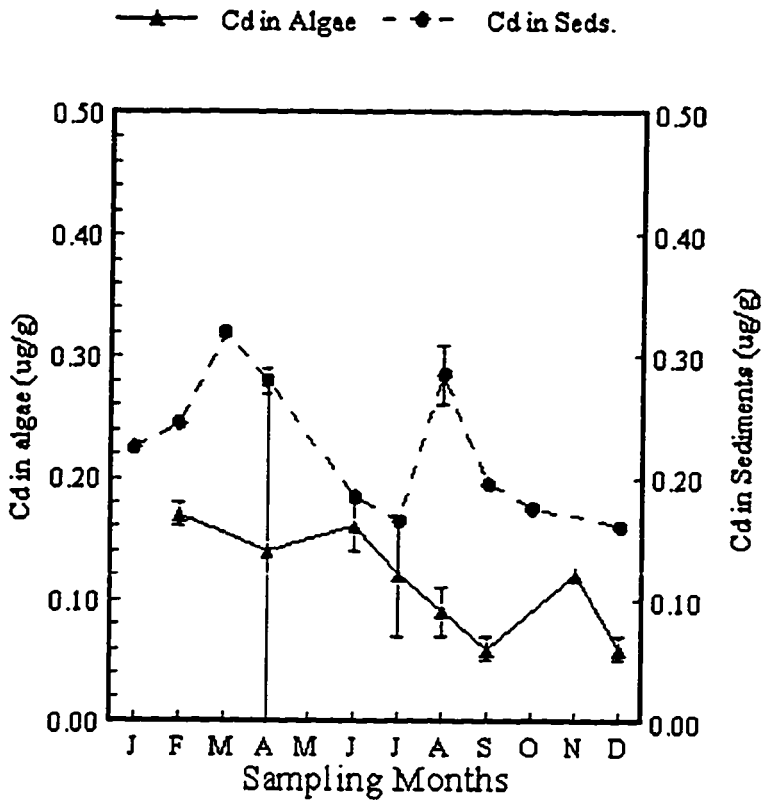

Figure 33. Cadmium in Algae (ug/g) vs. Cadmium in Sediments (ug/g).

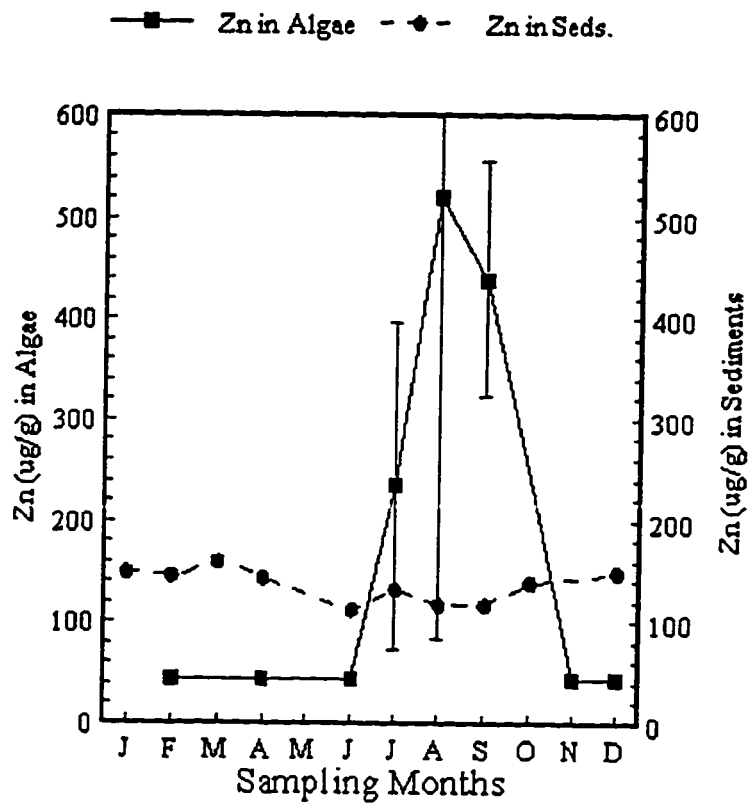

Figure 34. Zinc in Algae (ug/g) vs. Zinc in Sediments (ug/g).