UNITED STATES

DEPARTMENT OF THE INTERIOR

GEOLOGICAL SURVEY

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Determining the Availability of Sediment-Bound Trace Metals to Aquatic Deposit-Feeding Animals

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ABSTRACT

The laboratory studies of ^{109}Cd, ^{60}Co, ^{110m}Ag and ^{65}Zn uptake from sediment by the deposit-feeding clam Macoma balthica introduced methodology for: (1) Separating solid and solute vectors of uptake by clams in experiments where equilibrium processes resulted in radioisotope backflux from sediment to solution. Animals enclosed in dialysis bags accompanied feeding animals in the experimental aquaria. Uptake from ingestion was determined by the difference between isotope concentrations in enclosed animals and concentrations in feeding animals; (2) Determining the contribution of undigested sediment to total body burdens of nuclide by a graphical subtraction technique; and (3) Separating the effects on isotope availability of binding to different sediment types. Clams were exposed to each of several specific types of laboratory prepared substrates (iron oxides, manganese oxides, decaying plant materials, inorganic carbonates, biogenic carbonates) or to combinations of substrates (iron oxides and bacteria). Internal control on the influences of different feeding rates was provided by simultaneous exposure to the four nuclides.

Results from the experiments showed:

(1) Uptake rates from ingestion of particulates differed by as much as a thousand-fold with ingestion of different particle types. The form of a bound metal substantially affects its availability.

(2) Availability from a specific type of particle differed greatly among metals. Substrates from which some metals are of high availability are low-availability sources of other metals.

(3) The mechanism controlling differences in availability through ingestion is the strength of bonding of a specific metal to a specific substrate. More

strongly bonded metal forms are less available than more weakly bonded forms. If all types of bonding sites in a natural sediment were present in equal abundance, metals would partition dominantly to the sites which bond the metal most strongly. Thus, in natural sediments, the bulk of the metal load may tend to associate with sites of lowest biological availability.

(4) Sediment-water distributions of a metal also differ widely among particle types. Thus, physicochemical form, not the total metal concentration, determines the distribution coefficient (at equilibrium) of a sediment-bound metal. Moreover, weakly bound metals will are more available when ingested and equilibrate to higher concentrations in solution than strongly bound metals, thus enhancing the availability of the metal from both solid and solute vectors, especially where water and sediment are at equilibrium.

(5) Correlations between extractable metal concentrations and uptake by bivalves indicated that no universal extractant is likely to predict the bioavailability of all sediment-bound metals (because of differences in metal chemistry). Ammonium acetate extractability correlated with uptake of ^{60}Co and ^{65}Zn; ethanol extractability correlated with ^{109}Cd uptake; and acid or dithionite-citrate extractability correlated with ^{110m}Ag uptake.

Field studies were designed to test whether the influence of physicochemical form on the availability of sediment-bound metals could be detected in a natural estuarine situation. Animals (bivalves; polychaetes) and oxidized surface sediments were collected from 17 estuaries of widely varying character in southern and western England, and from San Francisco Bay. The sediments were extracted with a variety of extractants including acids (HCl, acetic, oxalic), a reducing agent (hydroxylamine hydrochloride), neutral ammonium acetate and basic extractants for removing humic substances (NaOH; ammonia). The action of extractants on metals and substrates was assessed, and statistical correlations were used to determine predominant metal forms in sediments. A statistical procedure was introduced for determining, in an orderly manner, metal-substrate interactions within subsets of data which contained only predefined concentrations of competing substrates. The studies of sediment chemistry showed:

(1) Few, if any, chemical extractants selectively remove trace metals from specific substrates. Chemical extractability is not a valid measure of the concentration of metal bound to a specific type of substrate.

(2) Extractants used by others to define "anthropogenically-derived", "non-lithogenic" or "adsorbed" quantities of metal extract a constant proportion of total metal over wide concentration ranges. Chemical interactions within sediments, rather than the source of the metal, are the most likely control on metal solubility within such extractants.

(3) Extractants may be highly useful in (a) operational definitions of the partitioning of metals among forms, if patterns in the differences in metal removal among extractants can be found; (b) characterizing the forms of substrates such as iron oxides, manganese oxides, and humic substances in sediments (such information will be essential to modeling metal partitioning in sediments); (c) providing information relevant to predicting the bioavailability of sediment-bound metals and (d) providing information for statistically assessing metal partitioning in sediments.

(4) Metals partition among a variety of substrates within oxidized sediments, and partitioning differs among different sediments. Competition for binding of a metal among substrates was evidenced by improved metal-substrate correlations in data sets with low concentrations of competing substrates, indicating partitioning was controlled by equilibrium processes.

(5) The major and minor metal-substrate associations for Ag, Cd, Co, Cu, Pb and Zn in oxidized sediments.

(6) The method by which sediments are collected and stored may greatly affect the quantity of metal extracted. Effects vary among metals and among extraction

techniques.

The conclusion that metal partitioning in sediments will vary with concentrations of bonding substrates was employed in the development of a method for statistically assessing the bioavailability of sediment-bound metals. Multiple correlations of metal extracted from sediment and various substrate concentrations extracted from sediments with metal concentrations in deposit feeders were calculated. It was assumed that changes in substrate concentration would be a measure of changes in metal partitioning, which could reflect partitioning associated changes in metal availability. The calculations resulted in equations which explained greater than 70 percent of the variance in concentrations of Ag, Cd, Co, Pb, and Mn in the bivalve *Scrobicularia* and the polychaete *Nereis*. Zn and Fe concentrations were also predictable from sediments in *Scrobicularia*, but poor correlations with sediments and a small range of variation suggested physiological regulation was an important control on concentrations of these metals in *Nereis*.

Concentrations of Pb in *Scrobicularia* closely followed the ratio of Pb to acid-soluble Fe in sediments. Comparisons of Pb concentrations predicted by the ratio with Pb concentrations observed in *Scrobicularia* among 5 estuaries suggested the ratio might be widely applicable for predicting biologically available Pb. Concentrations of Zn in *Scrobicularia* correlated best with ammonium acetate-soluble Zn, humic substances (positive) and total organic carbon (TOC-negative). In comparison, concentrations of Zn in *Macoma* balthica from San Francisco Bay were negatively affected where high concentrations of TOC occurred, but were also influenced by the Fe/Mn ratio in sediments. The differences between the two study areas in the controls on Zn availability were consistent with differences in sediment chemistry. The English sediments contained high concentrations of organic materials and low concentrations of Mn, whereas San Francisco Bay sediments were typically high in Fe and Mn, and low in organic carbon.

The multiple regression calculations also showed that the availability of Cd to *Scrobicularia* and to *Nereis* was controlled by factors similar to those controlling the availability of Zn: enhancement by higher concentrations of Mn in sediments and inhibition by TOC. Some contribution by solute sources was also evident. Solute sources also appeared to contribute to Co concentrations in the two species, but the major control was the concentration of acid-soluble Co in sediment. The availability of Mn to the two species was enhanced by Mn associations with humic substances and inhibited where elevated levels of TOC occurred. Differences in Mn concentrations in the two species reflected the greater sensitivity of *Scrobicularia* to acid-soluble Mn and of *Nereis* to humate-bound Mn. Concentrations of Fe in *Scrobicularia* were enhanced by any change that partitioned Fe away from Fe oxides. High concentrations of Fe in the bivalves correlated with elevated levels of Mn oxides and humic substances.

Concentrations of Ag in *Scrobicularia* were controlled primarily by HCl-extractable Ag in sediments and a competitive inhibition of Ag uptake by Cu in sediment. Concentration of Cu in *Scrobicularia*, in contrast, was physiologically enhanced by Ag in the clam. The opposite effect of Cu on Ag uptake and of Ag on Cu uptake resulted in a poor correlation between Cu in *Scrobicularia* and Cu in sediments, although the importance of sediment-bound Cu was evident when the physiological coupling with Ag was statistically removed. Microhabitats with highly enhanced Cu availability to *Scrobicularia* were also found in several estuaries. The enhanced availability of Cu was associated with a combination of anoxia in the surface sediments, and low concentrations of Fe oxides. Other variables, as yet undefined, are also important in these microhabitats.

Spatial and temporal studies of metal interactions in sediments and deposit feeders from San Francisco were conducted. Spartial differences in Cu and Zn concentrations in sediments were generally predictable from concentrations of TOC, Fe oxides and particle size distributions. Data from a variety of benthic molluscs (snails, clams, mussels) indicated Cu and Ag were available in relatively substantial amounts in several locations, despite the apparent lack of large Cu loadings. Apparently the chemistry of the bay provides a greater availability of Cu to molluscs than does the chemistry of other estuaries.

Strong fluctuations, with time, of Cu and Ag in *Macoma balthica* from the southern part of the bay were associated with seasonal changes in hydrologic variables. Early in the rainy season the concentrations in the clams increase, peaking during the period of most intense rainfall and declining to a mimimum in summer. Metal concentrations in *Macoma* were correlated with a discharge index which assumed that metal input (or the input of factors which determined metal availability) followed storm runoff and was balanced by the flushing force of river discharge into the estuary.

Tolerance of a population to a toxin was suggested as an indirect indicator that the toxin was having a deleterious effect upon the species and perhaps the surrounding biological community. Three populations of *Macoma* in San Francisco Bay were shown to have different tolerances to Cu. The differences in sensitivity appeared to be related to Ag burdens in the animals, suggesting a physiological coupling of Ag and Cu (also indicating in *Scrobicularia*) detoxified Cu in *Macoma*.

DETERMINING THE BIOLOGICAL AVAILABILITY OF SEDIMENT-BOUND TRACE METALS

S.N. Luoma

INTRODUCTION

The concentrations of most trace metals in aquatic sediments are orders of magnitude higher than concentrations in water. It has been suggested that sediment-bound trace metals may be an important source of these elements for deposit feeders (Wolfe and Rice, 1972; Lowman, et al., 1971). Yet few studies have either adequately measured the extent to which metals are taken up by deposit-feeding biota through the ingestion of sediments or considered conditions which might facilitate or inhibit uptake of metals directly from the sediments.

Simple relationships are seldom found in natural systems between metal concentrations in deposit feeding organisms and total metal concentrations in either water or sediments. A growing body of evidence suggests these poor correlations reflect the modifying effect of the partitioning of metals among different physicochemical forms in sediments and solution upon the availability of the metals to organisms (Sunda and Guillard, 1976; Pagenkopf, et al, 1974). Chemical models of metal speciation have been used to assess the biological availability of different solute metal forms. Pagenkopf et al (1974) and Andrew (1975) used equilibrium models to suggest that the availability of Cu to fishes was controlled by the concentration of the free Cu ion. Equilibrium models were also used to show that the toxicity of Cu to phytoplankton followed the activity of metals rather than total metal concentrations

(Sunda and Guillard, 1976; Jackson and Morgan, 1978; Anderson and Morel, 1978) and that the concentrations of free Zn ion plus additional factors (e.g. competition from Ca and Mn) may affect the availability of solute Zn to fishes (Pagenkopf, 1975; and Zitko, 1975).

Use of solute speciation models to help predict metal availability to organisms in natural systems has not been attempted to date. Statistical comparisons of calculated Cu activities in various natural waters with concentrations of Cu in resident phytoplankton populations would provide a relatively simple test of the laboratory-based models of Cu availability. For organisms other than phytoplankton, however, the problem of modeling the bioavailability of metals in natural systems will be more complex.

Experiments employing chemical speciation models have dealt only with metal uptake from solution, but food may also be an important source of some metals (Benayoun, et al, 1974; Pentreath, 1973; Pentreath, 1976; Fowler and Benayoun, 1976; Fowler, et al, 1978). The rate of uptake of metals associated with solids is considerably lower than the rate of solute metal forms. However, the concentrations of metals bound to solids are usually orders of magnitude higher than metal concentrations in solution. Models which include both solid and solute sources of metal uptake by animals could be used to show where the low availability of the particulate-bound metal forms is offset by the higher concentrations of these forms (Fig. 1).

Models which include metal uptake from food must deal with the effect on uptake of metal partitioning among different forms within the solids. Some evidence suggests partitioning among ligands may affect the availability of metals from organic food components. Carnivorous fish take up ^{65}Zn more efficiently from synthetic foods than from prey organisms (Penreath, 1973; Merlini, et al, 1976); the availability of ^{65}Zn to fish ingesting labelled snails was lower from snails which were losing their burden of ^{65}Zn than from snails freshly labelled with the nuclide (Merlini et al, 1976). Slowly exchanging biochemical forms of ^{65}Zn would form a larger proportion of the nuclide in the former group of snails than in the latter (Luoma, 1977b;, Cutshall, 1974, suggesting the availability of Zn to the predator varied with biochemical partitioning of the metal within the prey. Similarly, the retention of Hg by plaice varied when the fish were fed four different species of labelled invertebrates (Pentreath, 1976). Mercury originating from different prey organisms apparently took a different biochemical form in the fish. Little is known about metal partitioning among organic ligands, especially within biological tissues. However, the effect of such partitioning the availability of metals to higher trophic-level organisms appears to deserve further study.

The influence of physicochemical sediment characteristics on metal availability of metals to deposit feeding organisms has also been considered. Uptake by both polychaetes (<u>Nereis succinea</u>) and deposit-feeding shrimp (<u>Palaemon debilis</u>) of Hg bound to highly organic estuarine sediment was significantly less than uptake of Hg bound to iron-oxide dominated sediments characteristic of runoff in Hawaii (Luoma, 1977a). In contrast, the availability of ^{65}Zn to polychaetes (<u>Nereis diversicolor</u>) differed little when uptake of organic particulate-bound ^{65}Zn on the surface of marine sediments was compared with uptake of iron-oxide-bound ^{65}Zn on the surface of such sediments (Renfro and Benayoun, 1974).

Several types of substrates which may bind trace metals occur within aquatic sediments (Jenne, 1968). The partitioning of metals among organic matter, carbonates, sulfides and hydrous oxides of iron and manganese may differ widely among sediment types. If differences in metal partitioning among sediments affect uptake by sediment-ingesting organisms, then generalizations concerning the biological availability of sediment-bound metals require consideration of variations both in the partitioning process itself, and in the resultant relationship of partitioning to uptake. Quantifying the influence of physicochemical form on metal uptake from solids is an important prerequisite to developing realistic models of metal dynamics in animals.

The work reported herein is the result of a five year study in which we assessed the influence of physicochemical form on the bioavailability of sediment-bound trace metals (Ag, Cd, Co, Cu, Pb, Zn, Fe, Mn) to estuarine deposit feeding organisms clams and polychaetes). We first tested in the laboratory the hypothesis that the binding of metals to different sediment types affects metal uptake by ingestion. In the laboratory experiments we also studied some of the mechanisms which might determine differences in bioavailability among sediments, and tested techniques for chemical extraction of metals from sediments to determine the feasibility of using extractions for assessing the bioavailability and form of bound metals.

The second stage of the project was a field study whose objective was to determine if metal concentrations in natural populations of deposit feeders could be predicted from the chemical characteristics of sediments from their environments. Animals and sediments were, in one study, sampled from 19 estuaries which differed widely in their physical and chemical characteristics. Sediments were extracted with 9 different extractants. The action of the individual extractant was assessed, statistical methods were used to determine the dominant partitioning of the different metals and methodology was developed and applied to incorporate metal partitioning into correlations between metal concentrations in the animals and the sediments. In a second study animals and sediments were sampled intensively over time at 8 stations in a single estuary. Correlations were sought between temporal fluctuations of metal concentrations in the deposit feeders and fluctuations in physical, chemical and hydrologic variables in the estuary.

The results of the project have proven the importance of physicochemical form in controlling the bioavailability of metals, and illustrated the governing mechanisms. We have demonstrated empirical and statistical methods for assessing metal form in sediments, and used those techniques to illustrate the partitioning processes in oxidized estuarine sediments. We have shown that partitioning also governs availability in natural systems and we have developed indices from which concentrations of Ag, Cd, Co, Cu, Pb, Zn, Fe and Mn in bivalves and polychaetes might be predicted from sediment chemistry. These indices will, no doubt, be modified with further study. They provide an important first step toward directly incorporating metal form of sediment-bound metals into assessments of metal availability in natural systems. More importantly, the indices point to specific hypotheses which might profitably be tested in future studied of metal availability. Our final chapter deals with the testing of one such hypothesis.

The report is divided into sections and chapters. The two sections separate laboratory and field studies. The chapters describe specific studies within each section. Since these studies are often quite autonomous, each chapter will contain its own methods, discussion and conclusion section, and is written to stand alone. The final summary will tie together the conclusions from the various chapters. All the data reported herein have been or will be published in the open literature (see reference list).

This project was funded in part by EPA energy funds.

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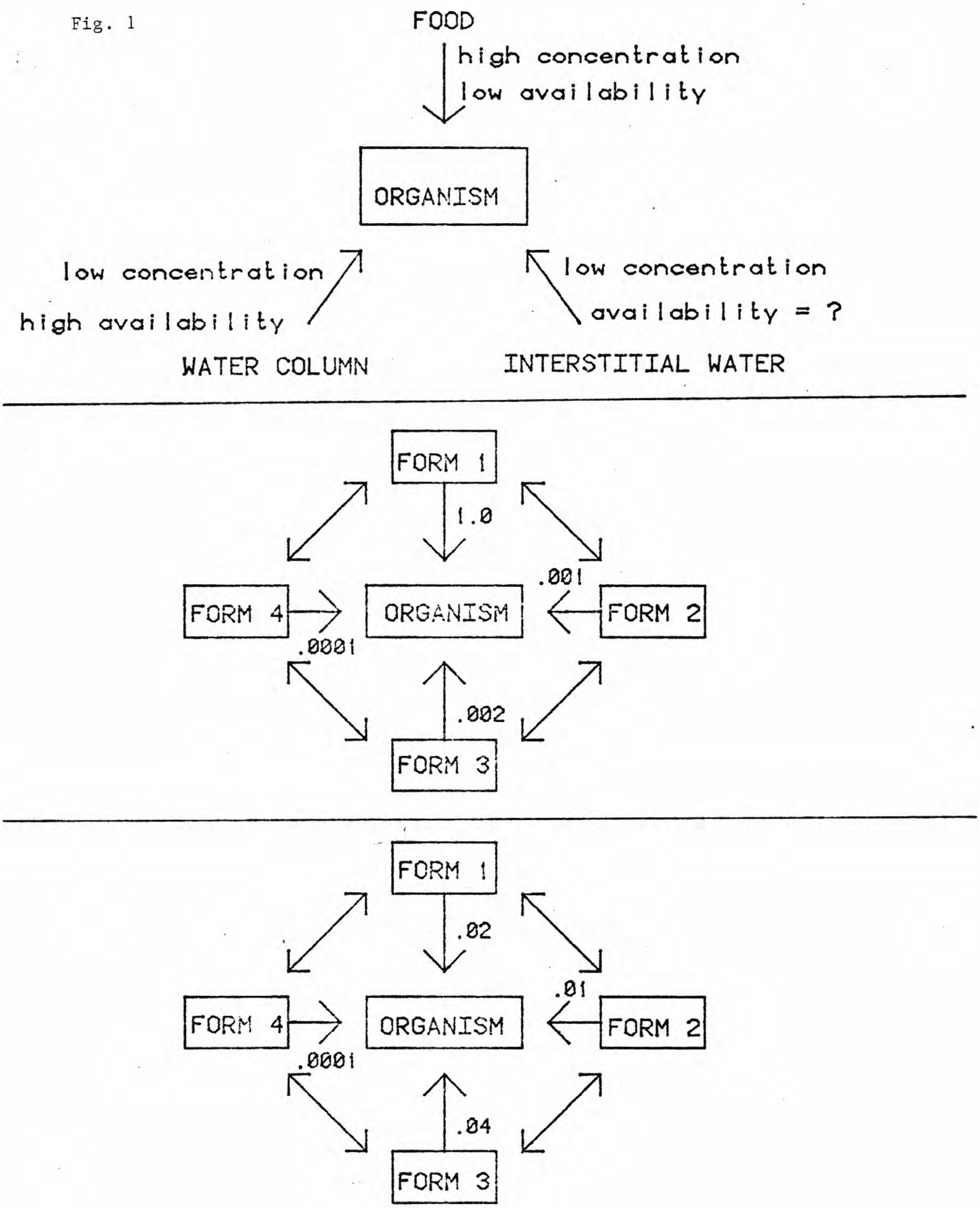
Fig. 1a. Alternative vectors for trace metal uptake. Uptake from food components (solid forms) is characterized by metal forms of low availability occurring in high concentrations (approximately $3X10^4$ - $3X10^6$ μg/Kg). The solute vector is characterized by metal forms in low concentrations (0.01-30 μg/l) but of potentially high availability.

Fig. 1b. Hypothetical uptake of different metal forms from a vector where a single form (e.g. the free ion, form 1) dominates uptake. Forms 2, 3 and 4 are of very low availability relative to form 1. Availability numbers represent proportion of a given form which is assimilated when it reaches the environmental interface of the organism.

Fig. 1c. Hypothetical uptake among metal forms of low availability when highly available forms are not present in significant quantities (as is often true of the free ion). Significant uptake will occur when low availability forms are present in high concentrations, and uptake will be affected by relative abundance of low availability forms.

Fig. 1


FOOD
high concentration
low availability
ORGANISM
low concentration
high availability
WATER COLUMN
low concentration
availability = ?
INTERSTITIAL WATER
FORM 1
1.0
.001
FORM 4
ORGANISM
FORM 2
.0001
.002
FORM 3
FORM 1
.02
.01
FORM 4
ORGANISM
FORM 2
.0001
.04
FORM 3

Chapter 1. Uptake by the clam *Macoma balthica* of sediment-bound Cd from well-defined types of sediment

S.N. Luoma and E.A. Jenne

INTRODUCTION

Several problems may hinder the interpretation of laboratory studies of metal uptake from sediments.

1) Sediment heterogeneity. Natural sediments differ widely in this physicochemical characteristics. If such differences affect partitioning, then conclusions from an uptake study of a natural sediment will be applicable only for the type of partitioning occurring within that sediment. Because metal partitioning in a heterogeneous sediment cannot be identified with any precision, general conclusions from an uptake study with such a sediment are difficult to draw. In order to specifically identify differences in the availability of metals when bound to different binding sites (substrates) within a sediment we studied uptake from well defined substrates which were prepared in the laboratory.

2) Separation of uptake from food and solution. Metal concentrations in a deposit-feeding organism usually represent uptake from both solute and particulate-bound forms. Most solute metals are rapidly accumulated by aquatic biota, relative to the rate of uptake of particulate-bound metal uptake (Luoma, 1975; Dean, 1974a; Renfro, 1973). Even low levels of solute metal may significantly contribute to concentrations in biota and confuse attempts to quantify uptake from ingested forms. In laboratory experiments, where dissolution of metal from labeled sediment is difficult to prevent, determination of the contribution of uptake from the solute pool is a prerequisite to quantitative assessment of direct uptake from the particulate-

bound pool. To separate the two sources of uptake, we enclosed one group of experimental animals in dialysis bags and held them with the feeding animals in each experiment. Uptake from food was calculated as the difference in metal concentrations between feeding and enclosed organisms.

3) Contamination of tissues by undigested intestinal contents. In laboratory experiments the concentration of a metal (or its radioactive tracer) in experimental sediments may be high relative to concentrations in the tissue of bottom-feeding organisms. In such instances, even a small amount of labelled sediment in the intestinal tract of a bottom feeder at the time of whole body analysis may result in overestimation of metal uptake into the tissues of the animal (Chipman, 1967; Renfro, 1973; Bryan and Hummerstone, 1973). We introduce a graphical method for estimating the metals contribution of intestinal contents.

METHODS AND MATERIALS

Radionuclide Analyses

All experiments were conducted using the radiotracer ^{109}Cd-$CdCl_2$. Nuclide analyses of water, sediment and live animals were conducted with a single channel analyzer connected to a NaI well-type detector. The activity in sediments was determined from 50 mg (dry weight) sub-samples. Water samples were filtered (0.2 μm) prior to analysis. Animals were analyzed live in 4 ml of seawater. Digestions of previously analyzed animals in nitric:perchloric acid (17:3) were used to quantify self-absorption of the ^{109}Cd which occurred during analysis of living animals. Counts per minute from each sample were corrected for background, instrument efficiency and decay to determine disintegrations per minute. Because the specific activity of the labeled cadmium varied among the experiments, nanograms per gram (ppb) of labeled Cd was calculated, for each experiment based on the specific activity of original solutions. This correction allows comparison of the different experiments.

Experimental Methodology

The estuarine clams (Macoma balthica) used in these experiments were collected from the intertidal zone of South San Francisco Bay. Previous studies have shown this species is a deposit feeder, ingesting both suspended particulates and surficial sediments (F.H. Nichols, pers. commun.). All animals were collected from the same 50 m^2 area to minimize genetic heterogeneity. Preliminary experiments showed accumulation of ^{109}Cd varied with the size of the clams, and was highly variable in clams kept in the laboratory for longer than 4 wks. Only freshly collected animals (acclimated for 5 d. to laboratory conditions) weighing between 450 and 750 mg (whole body wet weight) were used in the experiments reported here.

Experiments were conducted at 12 ± 2°C using unfiltered seawater (diluted 10 percent with tap water to a salinity of 32°/oo) taken from an offshore pipeline. Thin layers of ^{109}Cd labeled sediment (less than 3 mm in depth) were added to 2 ℓ aquaria with 1 ℓ of seawater. The water was continuously aerated to prevent formation of sulfides. In each experiment eight clams were allowed to feed on labeled sediment. Four additional clams enclosed in separate dialysis bags filled with seawater were also placed within the sediments of the aquarium. The dialysis bags separated the latter group of animals from the sediments, but allowed exchange of solute ^{109}Cd with the waters of the aquarium. Analyses showed ^{109}Cd concentrations within the bags were equal to those outside the bags. Thus, both groups of animals were exposed to solute ^{109}Cd of the same concentration and the same physical-chemical form. Although a very small weight loss occurred in the unfed animals over the course of an experiment (<3 percent in 14 days), it was not sufficient to detectably bias the uptake studies. At each sampling interval whole body analyses for ^{109}Cd were conducted on all 12 animals. The feces of feeding animals were collected occasionally and analyzed for ^{109}Cd to assure these animals were feeding on a labeled fraction of the sediments.

In the 42-d. experiment with San Francisco Bay sediment the water in the experimental aquarium was changed every 3 d. This resulted in fluctuating levels of solute ^{109}Cd. In later 10 to 13-d. studies the seawater was not changed. Tap water was added when necessary to maintain a salinity of $32^{o}/oo$. The concentration of solute ^{109}Cd increased to a steady state in these experimental aquaria. The characteristics of solute ^{109}Cd bioaccumulation in aquaria where the seawater was not frequently changed indicated that the concentration of the biologically available fraction of the solute nuclide remained more constant than when the water was changed frequently.

Only one type of sediment was used in each experiment. The experimental sediments were collected and labelled using the following procedures:

1. Sediment from San Francisco Bay was collected from the surficial 5 mm in the habitat of the clams. Particles greater than 2 mm in diameter were separated by wet sieving and discarded. A small volume of seawater (200 ml) containing ^{109}Cd was mixed with the sediment, the mixture was allowed to equilibrate for 24 hours, then the labelled sediment was washed and placed in an aquarium.
2. To form amorphic iron oxides, 100 g of $FeCl_3$ was dissolved in 2 l of distilled water. A 400 ml aliquot of this solution was taken for each experiment and ^{109}Cd was added. The slow addition of 0.1N NaOH, while stirring, precipitated amorphous iron oxide (Fe_xO_y) and the ^{109}Cd was sorbed to the precipitate. The precipitate was washed twice with seawater before being added to an aquarium. Freshly precipitated ^{109}Cd-Fe_xO_y was used in each uptake experiment.

3. Organic coating of the ^{109}Cd-Fe_xO_y was accomplished by adding yeast extract and glucose in seawater to the precipitate. This mixture was aerated for 3 days to allow reproduction of bacteria. The organic-rich supernatant was discarded and the precipitate was washed in seawater prior to experimentation.
4. Organic detritus was obtained by collecting marsh grass fragments from the sedimentary habitat of the clams. The fragments were washed, then homogenized in a blender. Seawater containing yeast extract, glucose and ^{109}Cd was added to the homogenate, and the mixture was aerated for 3 days to permit the development of a microflora. The supernatant was discarded and the labelled detritus was washed in seawater prior to experimentation.

RESULTS AND DISCUSSION

A Method for Direct Determination of Uptake from Sediment

When clams were fed San Francisco Bay sediment labelled with ^{109}Cd, the concentration of the nuclide in the animals which resulted from ingestion of sediment reached an apparent steady state within 8 d. Analyses of feeding clams showed a continuous accumulation of ^{109}Cd during the 42 d. study period (Fig. 1-1, curve A). Control clams enclosed in dialysis bags and placed in the sediments with the feeding animals also showed a continuous accumulation of the nuclide over the 42-day period (Fig. 1-1, curve B). Subtraction of nuclide concentrations in the latter group of animals (curve B) from nuclide concentrations in feeding animals (curve A) at each sampling point illustrated that accumulation of ^{109}Cd from food (sediment) alone reached an apparent equilibrium relatively early in the study period (Fig. 1-1, curve C). After the 8th d. of the experiment, increasing nuclide levels in feeding clams were solely the result of uptake of ^{109}Cd. Renfro and Benayoun, 1974, obtained results similar to curve A when they exposed the polychaete *Nereis diversicolor* to ^{109}Cd labelled Mediterranean sediments. Our experiments indicate that, unless the solute component of ^{109}Cd uptake is considered, such results may overestimate the availability of ingested, sediment-bound Cd to deposit feeders.

From the data in Fig. 1-1 it is also possible to estimate the contribution to whole-body nuclide concentrations of unassimilated, labeled sediment present in the intestinal tract of the animals. In all experiments feeding animals (curve A) rapidly accumulated ^{109}Cd during the initial 12-24 hr of uptake. This initial, rapid phase of accumulation was not observed in animals exposed only to the solute nuclide (see also Fig. 1-2, curves A and B). The initially high ^{109}Cd concentrations observed only in feeding animals probably resulted from the initial ingestion of radioactive sediments by these organisms. The activity of the unassimilated sediment-bound ^{109}Cd in the clams was estimated from the magnitude of this initial component of uptake, by extrapolating curves A and C to the ordinate using a linear fit to the points in the first five days of uptake. (The Y-intercept then represents the concentration of ^{109}Cd attributable to unassimilated sediment in the gut.) The similarity in the Y-intercepts of curves A and C was observed in all experiments and would be expected if ingestion of labeled sediment were responsible for the initial high concentrations in the feeding animals. Independent estimates of unassimilated ^{109}Cd in the digestive tract of the clam based upon loss studies also agree closely with graphical estimates (S.N. Luoma and E.A. Jenne, unpub. data). The advantage of the graphical method is that it allows following nuclide assimilation in the same individuals over the course of an experiment. The variance around a model based upon the difference between two mean concentrations (such as curve C) is less if the concentrations are drawn from the same individuals than if the concentrations came from different individuals sacrificed at each point in time. Thus, while the error bars in

Figure 1-1 illustrate typical examples of the standard error of the mean for any one point on curves A or B; such bars overestimate the variance around an entire curve or the variance in the model based upon the difference between the two curves. Using the method described above whole body assimilation of ^{109}Cd from ingested sediment may then be determined by subtraction of the Y-intercept from each data point in the curve (C) which was derived from the difference in nuclide concentrations between feeding and non-feeding clams.

Uptake of ^{109}Cd from Different Sediment Types

The physicochemical partitioning of metals within aquatic sediments may differ with sediment type. Uptake studies using sediments from nature characterize metal availability from the specific sub-sample of sediment employed in the experiments, and from the types of binding processes resulting from labeling that sediment in the laboratory. Investigation of metal uptake from specifically defined metal-sediment bonding sites (substrates) may allow development of broader generalizations. Although studies of bioaccumulation from single substrates (as described below) probably depart significantly from natural conditions, comparisons of uptake from the different substrates which clams could encounter in estuarine sediments may illustrate sources of variation in the biological availability of sediment-bound metals.

Uptake of Organically Bound ^{109}Cd

No detectable uptake of ^{109}Cd resulted from ingestion of 3 concentrations of organic detritus by M. balthica (Fig. 1-2, curve C; Table 1). In all three experiments homogenized marsh grass fragments were treated to stimulate microfloral growth at the time the organics were labelled. Thus ^{109}Cd was probably bound by both microflora and by non-viable organic fragments. Particulate organics and their associated microflora are one of the most important sinks for trace metals in aquatic environments. The results of our experiments indicate that the bioavailability of Cd from at least some types of metal-organic associations is very low.

In the experiments with organic detritus, nuclide concentrations in the unfed clams in dialysis bags increased more rapidly than did ^{109}Cd concentrations in feeding clams (Fig. 1-2, curves A and B). This suggested the unfed clams were accumulating solute ^{109}Cd more rapidly than were the feeding clams. Because feeding clam animals had a higher initial concentration of ^{109}Cd (attributable to unassimulated nuclide in their digestive tracts) the difference in concentrations between feeding and unfed clams decreased over time as ^{109}Cd concentrations in the tissues of unfed clams approached ^{109}Cd levels in the tissues and the digestive tract of feeding clams. The slope of this decreasing curve C (Fig. 1-2) function represents the differences in rate of solute ^{109}Cd uptake between the two groups of organisms. In the three experiments with organic detritus this slope was a function of the uptake rate of ^{109}Cd by the unfed clams (Fig. 1-3).* At higher concentrations of biologically available ^{109}Cd (i.e., at higher initial rates of nuclide uptake by unfed clams) the difference in the rate of solute ^{109}Cd uptake between feeding and unfed clams was greater. Different rate constants of ^{109}Cd loss between the two groups of animals is one explanation for such results. Bryan (1967) and Cross, et al. (1969) have observed a faster rate of ^{65}Zn loss from feeding animals than from unfed animals. Results from Fowler and Benayoun (1974) imply such differences may also exist with Cd.

The relationship between the two variables in Fig. 3 was fitted by the equation

$$D = -0.83 + 0.82R \tag{1}$$

where D = the rate of increase in the difference in ^{109}Cd uptake between feeding and unfed animals in ng/g/da and

R = the rate of ^{109}Cd uptake by non-feeding animals in ng/g/da. The standard deviation of the slope of the curve was ± 0.57 (ng/g)/da and the fit of the line was highly significant (p 0.001). The regression equation describing the relationship was used in later experiments to calculate the contribution of solute ^{109}Cd to nuclide concentrations in feeding animals from the uptake rate of the solute nuclide by unfed animals.

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*The comparisons between rates were made over the first 2 days of each experiment and also in two instances where the uptake of solute ^{109}Cd increased sharply as a result of changes in the aquarium water.

Uptake from Iron Oxides

When feeding clams were exposed to ^{109}Cd sorbed to amorphous iron oxides, significant nuclide uptake resulted from ingestion of the Fe_xO_y particulates (Fig. 1-4). However, when the ^{109}Cd-Fe_xO_y precipitate was coated with organics by stimulating microfloral growth, no detectable uptake of ^{109}Cd due to ingestion of the precipitate occurred (Fig. 1-4, curve IV). The characteristics of uptake from organically coated Fe_xO_y were similar to those observed when clams were exposed to organic detritus (the data point from this experiment is included in Fig. 1-3). The concentrations of ^{109}Cd sorbed to the Fe_xO_y precipitates ranged from 149.3 dpm/mg (34.0 μg/g) to 17.4 dpm/mg (2.8 μg/g), as shown in Table 1-1. Nuclide concentrations in organically coated Fe_xO_y sediments were 6.22 dpm/mg (0.99 μg/g). Under certain conditions, adsorption of trace metals to hydrous oxides of iron may be an important mechanism for binding metals to aquatic sediments (Jenne, 1968). In such instances Cd may be available to deposit feeders directly from ingestion of those sediments. However, if the iron oxides develop an organic coating the availability of the Cd will decline significantly.

Table 1-1 compares the biological availability of particulate-bound ^{109}Cd (the ratio of apparent steady state concentrations of the nuclide assimilated by clams from ingestion of sediment to ^{109}Cd concentrations in the sediment) among the types of sediment studied. All values were converted to ng/g to allow comparisons among experiments where slightly different specific activities were employed. In general, uptake from all sediment types was relatively inefficient. However, at high sedimentary concentrations of Cd, some organismic contamination might result from ingestion of either San Francisco Bay sediments or sediments where the metal is primarily bound by hydrous oxides of iron.

Differences in stable Cd concentrations may have had some influence on our determinations of differences in availability among sediment types. A higher background concentration of Cd undoubtedly occurred in both the San Francisco Bay sediment and the organic detritus than in the synthetic Fe_xO_y precipitate. A decreased specific activity (pCi ^{109}Cd/ng Cd) resulting from such background Cd could result in a lower rate of nuclide uptake. However, when Fe_xO_y was coated with bacteria (without a significant change in specific activity) ^{109}Cd uptake closely resembled uptake from organic detritus. Uptake from lower specific activity San Francisco Bay sediment exceeded bioaccumulation from higher specific activity Fe_xO_y. Thus, it appeared that the physicochemical form of the Cd-sediment association had a greater influence on ^{109}Cd bioaccumulation than did the specific activity of the Cd within the sediments. The usefulness of measurements of specific activity is usually limited by an inability to determine the nuclide:stable metal ratio available for transport by biota. Our experiments illustrate that large pools of non-crystalline Cd in natural sediments (e.g., at least some forms of the organically complexed metal) may be unavailable to deposit-feeding organisms. Similar constraints on the availability of metals with environmentally significant radionuclides would suggest that physicochemical partitioning of such nuclides and their stable isotopes is highly significant in determining actual specific activities to which aquatic deposit feeders are exposed.

SUMMARY

Chapter 1

The uptake of ^{109}Cd by the deposit-feeding clam, *Macoma balthica* varied significantly among several types of sediments. To directly estimate the amount of ^{109}Cd taken up through ingestion of sediment experimental techniques were developed for determining the gut content of labeled sediment and the contribution of solute ^{109}Cd to the total body burden of the nuclide in the clams. No detectable uptake of ^{109}Cd occurred through ingestion of either labeled organic detritus or particulate hydrous-iron oxide which had been coprecipitated with the nuclide and coated with organic material. Detectable uptake of ^{109}Cd was observed when clams were exposed to either hydrous-iron oxide particulates which lacked the organic coating or San Francisco Bay sediment labeled with the nuclide. Uptake of ^{109}Cd through ingestion of labeled sediment was relatively inefficient for all sediment types. Apparent steady state concentrations of ^{109}Cd in the clam were never greater than 15% of the concentration of the nuclide in any type of sediment studied. The results from several preliminary chemical extractions of sediments indicate that the amount of ^{109}Cd removed by selected extractants does not correlate in a simple manner with the bioavailability of this trace metal.

Table 1-1. Steady state ^{109}Cd concentrations assimilated by clams due to ingestion of sediment-bound ^{109}Cd as a function of sediment type.

Sediment type	Concentration of ^{109}Cd Clams (whole body steady-state) (ng/g dry wt)	 Sediment (ng/g dry wt)	Bioavailability of sediment-bound ^{109}Cd (Clam/sediment)
S. F. Bay	1250	10,600	0.131
Fe_xO_yI	1057	34,011	0.030
Fe_xO_yII	841	27,690	0.031
Fe_xO_yIII	135	2,770	0.049
*Fe_xO_yIV	N.D.	989	N.D.
Detritus I	N.D.	1,876	N.D.
Detritus II	N.D.	30,663	N.D.
**Detritus I	N.D.	1,876	N.D.

* coated with bacteria

** aged 65 days

N.D. not detectable

I, II, III, IV show replicate experiment number

Table 1-2. Comparisons between sediment extractions of ^{109}Cd and the bioavailability (as defined in Table 1) of the sediment-bound ^{109}Cd. CF = concentration factor; the ratio of ^{109}Cd in clams to ^{109}Cd extracted from sediment by a given technique.

	Dithionite/citrate		Hydroxylamine		0.5N HCl		70% Ethanol	
	ng/g ext.	CF	ng/g ext.	CF	ng/g ext.	CF	ng/g ext.	CF
S. F. Bay	4357	0.29	5279	0.24	8395	0.15	5321	0.23
*Fe_xO_y I	26222	0.04	22447	0.05	18944	0.06	476	2.22
*Fe_xO_y II	26444	0.03	23025	0.04	17749	0.05	748	1.12
**Fe_xO_y III	859	0.16	1283	0.11	1147	0.12	374	0.36
***Fe_xO_y IV	310	<0.05	194	<0.05	339	<0.05	16	<1.00
****Detritus I	951	<0.01	1276	<0.01	1446	<0.01	32	<0.01
*Detritus II	15638	<0.01	20391	<0.01	20299	<0.01	1012	<0.01

* extracted after 10-15 days in seawater

** extracted after 45 days in seawater

*** organically coated

**** extracted after 65 days in seawater

LIST OF FIGURES

Figure 1-1 Uptake of ^{109}Cd from San Francisco Bay sediment by the clam, *Macoma balthica*. A) represents uptake by feeding clams, B) uptake by clams exposed only to solute ^{109}Cd, and C) represents the difference between A and B, i.e., the contribution of ingested sediment to nuclide activity in the feeding clams. Data points represent means, and vertical bars show representative examples of ± one standard error of the mean (SEM).

Figure 1-2 Uptake of ^{109}Cd from labeled detritus by feeding clams (Curve A) and by clams exposed only to the solute nuclide (Curve B). Curve C represents the difference between Curves A and B. Data points represent means and vertical bars show representative examples of ± one SEM.

Figure 1-3 The rate of change in the difference in ^{109}Cd levels between feeding clams and starved clams, as a function of the rate of uptake of solute ^{109}Cd by the unfed animals, from experiments with organically associated ^{109}Cd. Line was fit by linear regression ($p<0.001$).

Figure 1-4 Uptake of ^{109}Cd from ingestion of sediment versus time by clams exposed to labeled iron oxide sediments (experiments I, II, and III) and by clams exposed to labeled iron oxide sediments coated with organic matter (experiment IV).

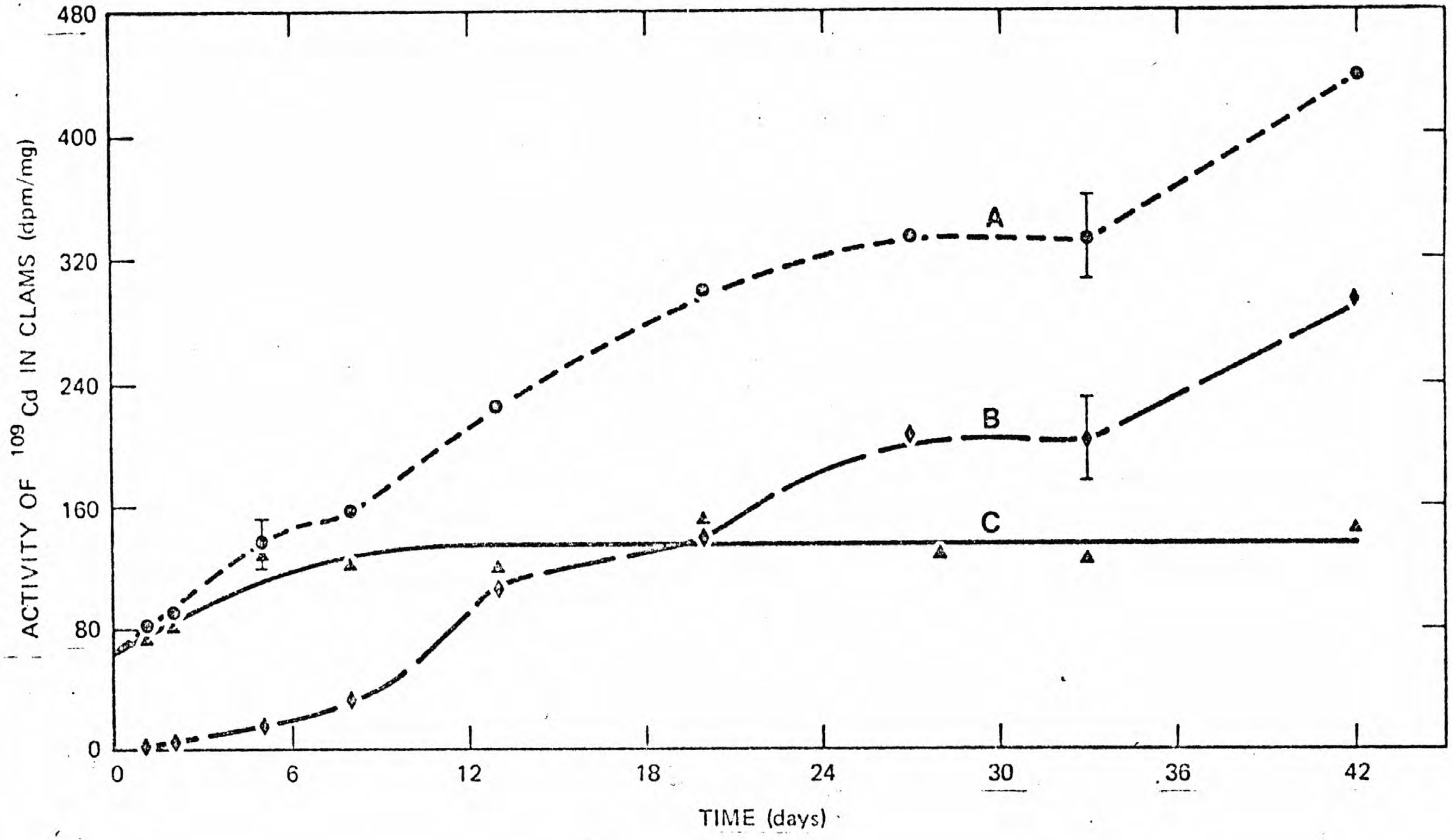
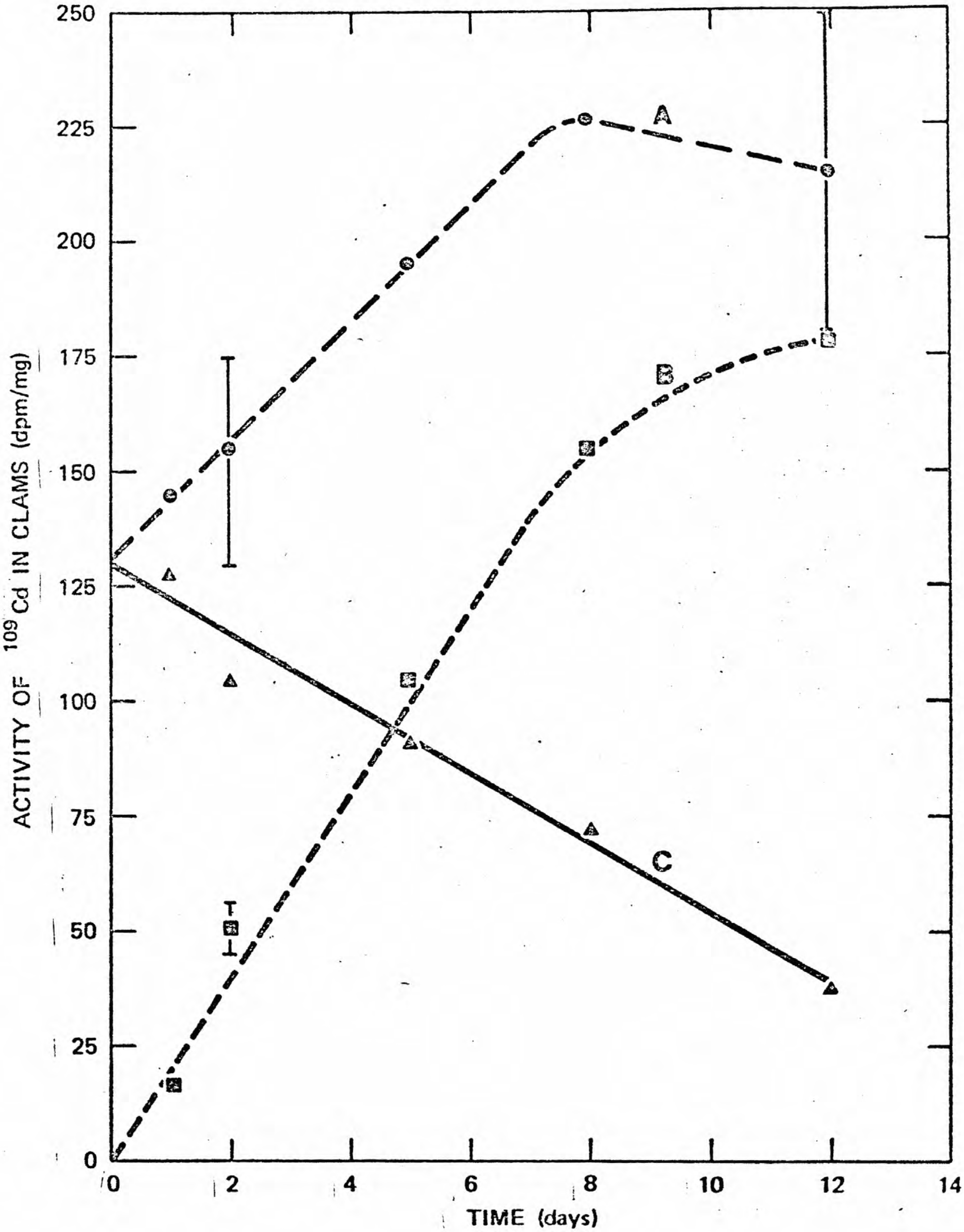

480
400
320
240
160
80
0
ACTIVITY OF ^{109}Cd IN CLAMS (dpm/mg)
A
B
C
0
6
12
18
24
30
36
42
TIME (days)

Figure 1-1

Figure 1-2



250
225
200
175
150
125
100
75
50
25
0
ACTIVITY OF ^{109}Cd IN CLAMS (dpm/mg)
0
2
4
6
8
10
12
14
TIME (days)
A
B
C

Figure 1-3

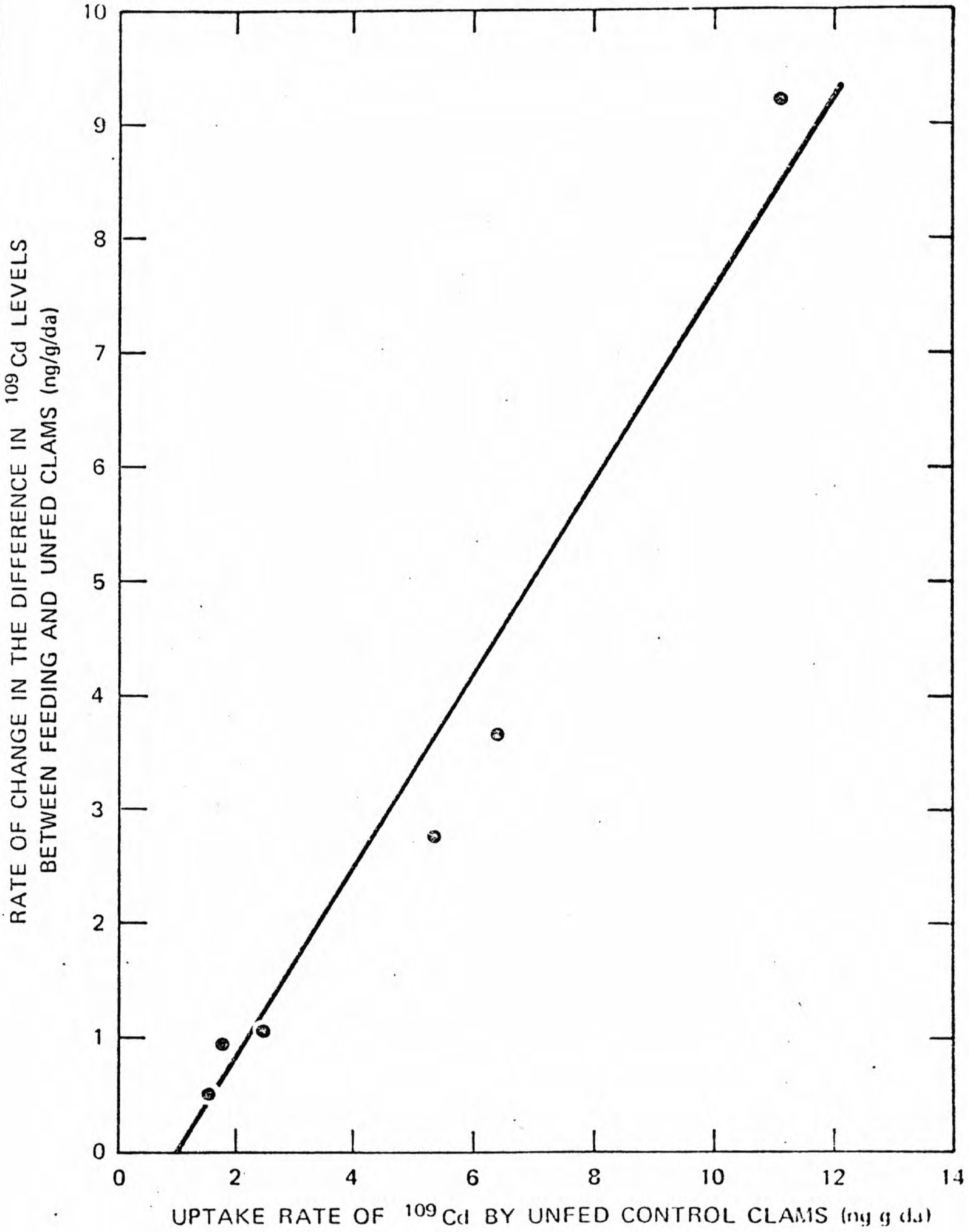
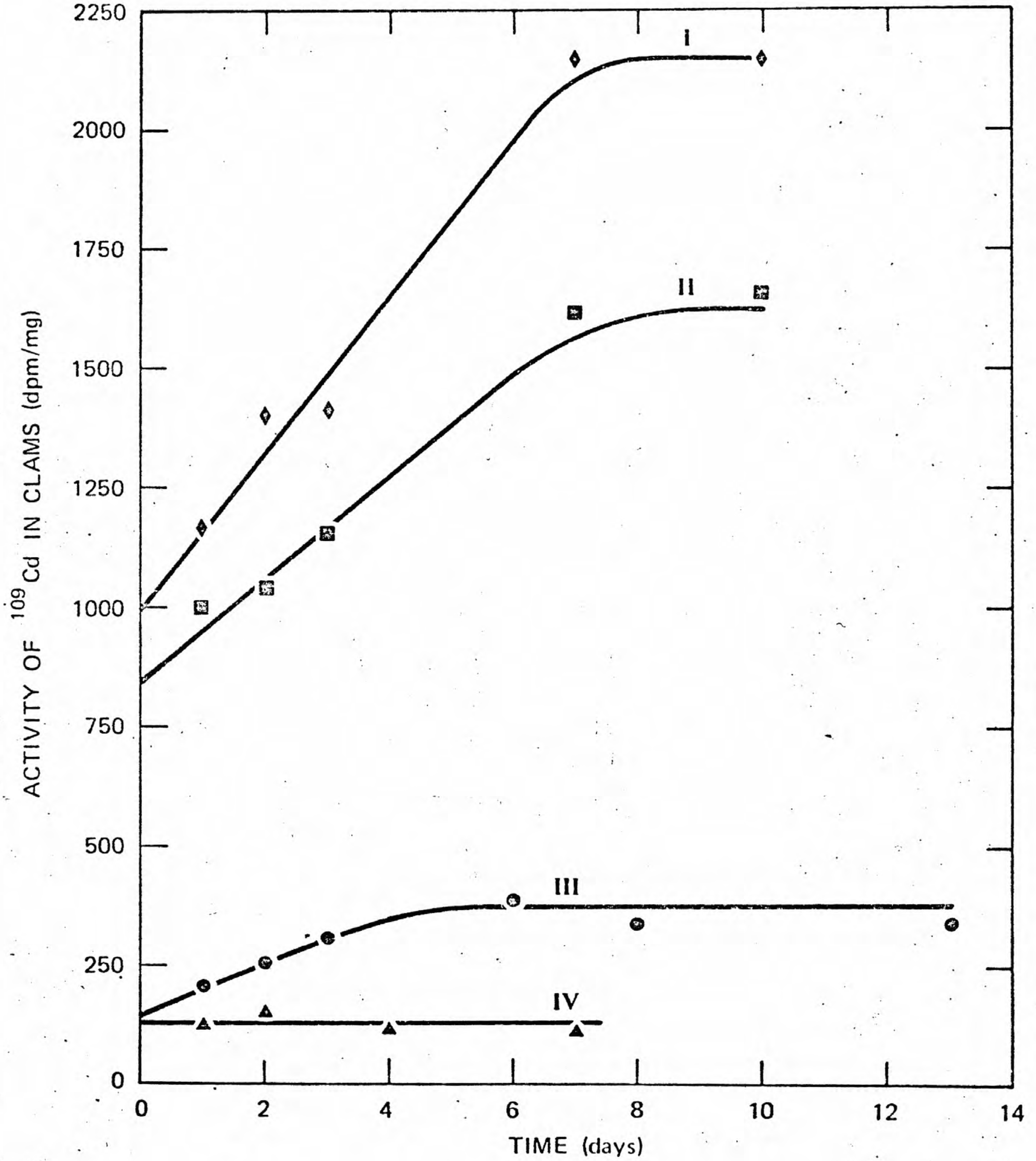
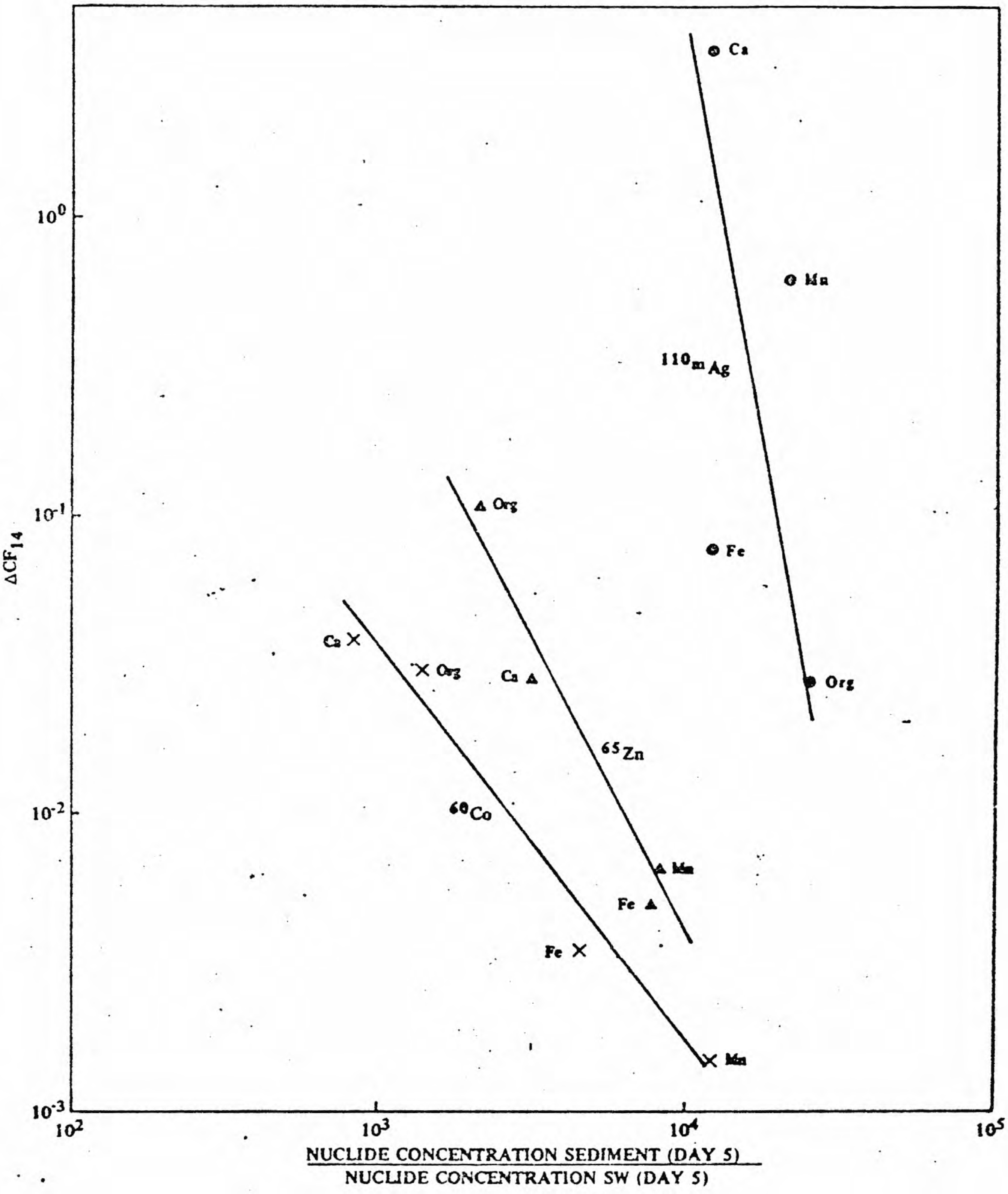

RATE OF CHANGE IN THE DIFFERENCE IN ^{109}Cd LEVELS BETWEEN FEEDING AND UNFED CLAMS (ng/g/da)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
UPTAKE RATE OF ^{109}Cd BY UNFED CONTROL CLAMS (ng/g/da)

Figure 1-4



2250
2000
1750
1500
1250
1000
750
500
250
0
ACTIVITY OF ^{109}Cd IN CLAMS (dpm/mg)
I
II
III
IV
0
2
4
6
8
10
12
14
TIME (days)

Figure 1-5



ΔCF14
10^0
10^-1
10^-2
10^-3
10^2
10^3
10^4
10^5
NUCLIDE CONCENTRATION SEDIMENT (DAY 5)
NUCLIDE CONCENTRATION SW (DAY 5)
Ca
Mn
110m Ag
Fe
Org
Org
Ca
65 Zn
Mn
Fe
Ca
Org
60 Co
Fe
Mn

Chapter 2. Uptake of ^{60}Co, ^{110m}Ag and ^{65}Zn from well-defined sediments by *Macoma balthica*

S.N. Luoma and E.A. Jenne

INTRODUCTION

In this experiment the simultaneous uptake of sediment-bound Ag, Co, and Zn by Macoma balthica was compared among several types of binding substrates which clams might encounter in estuarine sediments. Uptake from each substrate was studied individually. The three metals were chosen because of potential differences in affinities for different sediment types. Zinc and Ag are also characterized by wide anthropogenic dissemination and relatively high toxicity. The radionuclides ^{60}Co, ^{65}An and ^{110m}Ag are potentially important waste products of nuclear technology (Huckabee, et al., 1975).

METHODS AND MATERIALS

Experimental Methodology

Freshly collected clams (Macoma balthica) of similar weight (750-1200 mg whole body) were acclimated in the laboratory for not more than 5 d before each experiment. Periodic inspection indicated the clams extended their siphons at a similar frequency in all types of sediment, and otherwise appeared to behave "normally" over the course of their 19 d captivity. No clam mortality was observed in any experiment. Periodic fecal analyses indicated the clams readily ingested all types of laboratory-prepared sediments. All sediments were of similar particle size, and a similar sediment mass was used in each experiment, thus minimizing particle size and particle concentration-dependent differences in siphoning rates among experiments.

For each experiment, a thin layer of labelled sediment (approximately 15 g) of a single type was placed in an aquarium with 1.6 ℓ of previously unlabelled, aerated seawater (32 $^{o}/oo$) at 12 ± 2°C. The seawater was not changed during the course of a 14 d experiment. Eight clams were placed in the aquarium and allowed to feed on the sediments. Four additional clams were enclosed individually in dialysis bags and placed within the sediments with the feeding animals, as in Chapter I. At each sampling interval whole body radionuclide analyses were conducted on all 12 animals. Nuclide uptake attributable to ingestion of sediment (U_{sed}) was determined at each interval by subtracting the mean nuclide concentration in the clams in dialysis bags (U_{solute}) from the mean concentration in feeding clams ($U_{sed + solute}$).

At the end of each experiment the feeding clams were analyzed, then placed in unlabelled San Francisco Bay sediment for 48 hours to purge their intestines of radioactive sediment. To account for nuclide loss from tissues over this period the clams previously in dialysis bags were removed from their bags and also placed in unlabelled sediment. The mean concentration of nuclide lost from the later group of clams over the 48 hour period was subtracted from the mean concentration lost by clams which had previously been feeding on the radioactive sediments. This difference (the intestinal content of unassimilated radioactive sediment was subtracted from U_{sed} to determine the concentration of nuclide assimilated into the tissues of the clams due to ingestion of sediment (AU_{sed}). After the purging period each animal was dissected into soft parts and shell and dissolved in 17:3 nitric; perchloric acid for determination of tissue:shell nuclide partitioning.

Preparation of Sediments

Amorphic iron oxides and organic detritus was prepared as in Chapter I. Synthetic calcium carbonate was formed by labelling a solution of 4 M $CaCl_2$ in 0.01 N HNO_3, then adding 0.1 M Na_2CO_3 until a precipitate formed. The "biogenic calcium carbonate" substrate was made by exposing 50 to 75 clams to solute nuclides for 10 days. Soft parts were dissected out of the clam shells and discarded. The shells were dried at 12°C then ground to a fine powder for the experiment. Manganese oxides (MnO_x) were formed by adding 15 ml of 0.4 M $NaMnO_4$, 15 ml of 0.1N NaOH, and 15 ml of 0.1 M $HClO_4$ to 600 ml of labelled distilled water. Then 0.6 M $MnSO_4$ and 0.95 M NaOH were slowly added (simultaneously) to the mixture until a precipitate formed. Unless otherwise noted, nuclide sorption onto a substrate was allowed to proceed for 16 to 24 hours. The sediment was then washed twice with unlabelled seawater and added to the experimental aquarium.

Analytical Methods

Nuclide analyses were conducted on a 7 cm diameter GeLi detection. A mini-computer was used for peak separation and data reduction. Analytical efficiency was determined for each type of sample by counting 10 μℓ of a sample type. Counting efficiency and geometry effects were determined for live clams by comparing count rates before and after dissolution of clams in 2 ml of nitric perchloric acid. Live clams were counted for 10 minutes (counting error seldom exceeded 5 percent. Other samples were counted for a sufficient time to provide a counting error of <3 percent. Water samples (25 ml) were filtered (0.45 μm) before analysis. Self-absorption in sediment samples was determined by internal standardization to a 2 mg sample labelled with the 1 μCi standard solution. Disintegrations per min from all samples were corrected for decay to the date of synthesis to allow comparisons among experiments. The concentrations of ^{110m}Ag and ^{65}Zn in ng/g were calculated from the specific activities of the stock solutions with which the sediments were labelled.

Stable metal concentrations in experimental sediments (after extraction with dithionite/citrate, Luoma and Jenne, 1976a) were determined by atomic absorption spectrophotometry. The results of previous radionuclide analyses of the same samples were used to calculate total specific activities (pCi/pg).

To compare equilibrium sediment-water nuclide distribution among experiments a desorption (or leaching) distribution coefficient (K_{des}) was calculated for each experiment,

$$K_{des}^{\cdot} = \frac{C_{sed}}{C_{sw}} \qquad (1)$$

where C_{sw} = the mean of nuclide concentrations in aquarium water on day 14 (in ng/ml or dpm/ml),

and C_{sed} = nuclide concentration in sediment (in ng/g or dpm/g), on day 14. Volume considerations, normally included in distribution coefficient calculations, (Harrison, 1971) cancelled themselves in the comparisons since a similar water volume to sediment mass was used in each experiment.

Sediment Sorption Study

To compare the affinities of the different metals for the different types of sediment, three sub-samples (≃ 10 mg each) of unlabelled sediment of each type were placed in small dialysis bags. These bags were placed in 500 ml of labelled seawater (0.2 μCi/ml of each nuclide), and analyzed daily for 5 d for the radionuclides. Three "control" bags, containing only seawater, were also placed in the aquarium and counted daily to determine nuclide sorption to the bags themselves. Sediments were removed from the bags, dried and weighed at the end of the experiment. Significant self-absorption was observed for the 10 mg samples of calcium carbonate, relative to the other sediments. Because of the large correction factor necessary to make counts on calcite sediments compared to other sediments the distribution coefficients for the calcite are tenuous.

RESULTS

Silver

Uptake of ^{110m}Ag from ingested sediment by whole clams (AU_{sed}) varied by three orders of magnitude when normalized to the concentration of nuclide in a given sediment type (Fig. 2-1 - representative curves). Similar results were observed when soft tissue ^{110m}Ag concentrations were normalized to concentrations in sediment (ΔCF in Table 2-1). Uptake resulting from ingestion of either ^{110m}Ag-calcite (ΔCF=3.7-6.1) or ^{110m}Ag-MnO_x (ΔCF=0.39-0.85) was considerably greater than uptake from other substrate types. Soft tissue ^{110m}Ag levels resulting from ingestion of other sediment types (except Fe_xO_yIII) were less than 10 percent of the concentration in the substrate. Fe_xO_yIII) was equilibrated with the nuclides only 3 h before clams were introduced to the aquarium. The rapid uptake from Fe_xO_yIII, relative to experiments in which Fe precipitates were aged 16 - 24 h, suggests the availability of Ag - Fe_xO_y is influenced by the morphological characteristics of the oxides.

The low ^{110m}Ag concentrations sorbed during preparation of MnO_x and calcite may have contributed to the variability in ΔCF observed in replicate experiments with these sinks and may have enhanced their high concentration factors. However, the lack of a concentration dependence effect on ΔCF in replicate experiments with Fe_xO_y, organics and biogenic $CaCO_3$ suggests the type of substrate was also important in the rapid Ag uptake from calcite and MnO_x.

About one half of the ^{110m}Ag taken up by feeding clams was found in the shell of the animals (mean of 10 experiments - 54.4 percent - Table 1). Subtraction of ^{110m}Ag concentrations in the shells of dialysis bag-enclosed animals, from levels in the shells of feeding animals indicated sediment-bound and solute Ag contributed proportionately similar quantities of Ag to the shell of M. balthica.

Zinc

The availability of ^{65}Zn from ingested sediments by the clam varied by nearly two orders of magnitude among substrates (Fig. 2-2). In contrast to ^{110m}Ag, the soft tissue AU_{sed} of ^{65}Zn never exceeded 20 percent of Zn concentrations in any substrate (Table 2-2). The ΔCf of ^{65}Zn uptake from biogenic carbonates and organic particulates showed some variability in replicate experiments. However, all concentration factors from these substrates (0.084 - 0.186) were significantly higher than concentration factors from hydrous oxides of either Fe (0.003 - 0.009) or Mn (0.006 - 0.0007).

Little ^{65}Zn was partitioned into the shell of feeding clams during the 14 d. course of this experiment. The concentration of ^{65}Zn in the shell of the clams made up more than 10 percent of whole-body concentrations in only two instances.

In all experiments except MnO_xII and Fe_xO_yII sediments were also spiked with Cd. The presence of Cd did not influence ^{65}Zn uptake.

Effects of physiological condition and specific activity

Comparisons of the simultaneous uptake of Ag, Co and Zn suggest that variations in biological parameters had a minimal effect on our results. Because of their comparative nature the experiments were especially susceptible to influence from variations among sediment-types in feeding rate, stress on clams associated with exposure to artificial substrates, or to anomalous bacterial or algal growth. Some of the variance among replicate experiments in ΔCF may be attributable to such factors. However, in nearly all instances, relative availability from a given substrate varied among the three metals. Moreover, the variance in ΔCF also differed among metals for most sediment-types. Since the effects of variations in biological parameters should be common to all metals, the differences among metals suggest a metal-specific variable (the influence of physical-chemical form) controlled our results.

Specific activities (pCi radioisotope/pg stable metal) varied as much as two orders of magnitude among substrates, for a given metal, and among metals (Table 2-4). However, concentration factors were not detectably influenced by specific activity in any replicate experiments, nor could differences among metals for a given substrate be explained on the basis of specific activity.

Bioavailability and Metal-Sediment Affinity

The equilibrium desorption distribution coefficients (K_{des}) of Ag, Co and Zn varied for each metal by as much as 3 orders of magnitude among the different sediment-types (Table 2-5). Likewise K_{des} varied among metals for a given type of sediment by as much as two orders of magnitude (Table 2-5). However, variations in K_{des} in replicate experiments with the same sediment type and the same metal were small (with the exception of Fe_xO_y III, where sediments were only allowed 3 hours after precipitation for equilibration with the nuclides). The physicochemical form of metal-sediment binding was obviously highly significant in determining the distribution coefficient of any given metal.

Sediment-types for which the distribution coefficients of a metal were highest (i.e., sediments from which the least metal was desorbed back into solution) were the sediments from which metal availability to the clams was lowest (Fig. 2-4). Significant log-log correlations between desorption distribution coefficients and 14-d soft tissue concentration factors (determined from AU_{sed}) were observed for ^{65}Zn ($p<0.005$; $r = -0.840$), ^{110m}Ag ($p<0.005$; $r = -0.817$), and ^{60}Co ($p<0.001$; $r = -0.925$). The slopes of the three regressions were different ($b = -0.44$, Zn; $b = -0.55$, Ag; $b = -0.51$, Co), but the differences were not statistically significant ($p>0.25$, analysis of covariance). Thus, the concentration factors of the three metals appeared to change at a similar rate with the K_{des}. The y-intercept of the ^{110m}Ag regression was significantly greater than that of either ^{65}Zn ($p<0.025$) or ^{60}Co ($p<0.005$), illustrating the generally higher concentration factors observed for ^{110m}Ag. The calculated y-intercepts for the ^{65}Zn and ^{60}Co regressions were not significantly different, indicating the general similarity with which these two metals were concentrated by the soft tissues of the clam. Although the linear log-log regression for Ag was highly significant, parametrically the curve appears to have some exponential character. The variability in the data prevent resolution of the exact nature of this relationship. The most anomalous point in both the Zn and Co data occurs in experiment Fe_xO_yIII, which had ad higher K_{des} than expected.

When the 5-day sediment sorption of metals was compared among 4 of the 5 types of sediments studied (all except biogenic $CaCo_3$) results were similar to those observed when the distribution coefficient was measured by desorption (Fig 2-5). The experiment was designed to provide a qualitative comparison of sorption potential among sediment types, thus it was terminated before any sediment type had reached equilibrium. For each metal, an inverse relationship was observed between the biological availability of the metal and the sediment sorption potential.

DISCUSSION

Both the biological availability of sediment-bound Ag, Co and Zn and the degree of desorption of these metals from sediment to seawater vary with the physicochemical form of the bound metal. Sediment-water distribution coefficients reflect the strength of binding between metals and sediments. Thus, the ability of clams to assimilate Ag, Co and Zn from ingested sediments declined as the strength of the binding between metals and sediments increased. Dependence upon the strength of metal-sediment associations may, in general, limit the bioavailability of metals from mixed sediments. Partitioning of metals within mixed sediments is a function of the stability constants and the abundance of the various sinks. This means, among the most abundant sinks within a given sediment, metals will be selectively partitioned into those sinks with the greatest affinity for metal sorption, i.e., there will be a tendency for metals to partition, in mixed sediments, into those sinks with the lowest bioavailability.

Under conditions where metal concentrations reach equilibrium between sediments and bottom waters (e.g., where residence times of bottom waters are long), desorption of sediment-bound metals will strengthen the correlation between bioavailability and metal-sediment binding stability. If a more biologically available form of a metal is dominant in a given sediment, higher solute metal concentrations will also be present in waters at equilibrium with that sediment. Likewise, if a metal is bound to sediments in a form of minimum availability, solute concentrations of metal resulting from sediment desorption will also be low. Small differences in concentrations in bottom feeders as larger differences among sediments in the availability of ingested forms.

Factors other than binding strength may have also influenced metal uptake by the clam from the different sediment-types. The uptake of Zn may be inhibited by high concentrations of Fe (in Oysters, Romeril, 1971) or Mn (in benthic algae, Bryan, 1969). Iron also reduces the rate of Co transport in rat intestine, although Mn has no effect on Co uptake by rats (Thomson and Valberg, 1972). If some dissolution of Fe_xO_y or MnO_x occurs in the intestine of M. balthica competitive inhibition of transport may influence Zn and Co uptake from these substrates. While one might expect similar effects from Ca (Polikarpov, 1966), the availability of Zn and Co from both biogenic and synthetic $CaCO_3$ substrates was relatively high. The presence of Cd also had no detectable competitive effect on Zn or Co uptake where tested.

In general, the rate of uptake of sediment-bound metals by the clam was slow, relative to the rates of solute metal uptake usually observed. However, the ingestion of metal-contaminated sediment could contribute significantly to metal concentrations in M. balthica if a sufficiently high concentration of a sufficiently available form were present in the sediment. Short-term exposures (i.e., several days) to increased concentrations of most forms of particulate-bound metals would probably have little detectable effect on metal concentrations in deposit feeders such as M. balthica. To determine the effects of increasing metal concentrations in the sedimentary habitat of a deposit feeder (e.g., dumping of sewage sludge or dredging spoils) studies must be conducted over a sufficent period of time after the event to allow detectable bioaccumulation from ingested forms to occur. Even at extremely high concentrations, ingestion of some common forms of some metals (e.g., hydrous oxide-bound Zn and Co) will result in little metal uptake by the deposit feeder studied here.

Although tenuous, extrapolation of our results with *M. balthica* to more general situations may be useful in providing an initial step toward gross predictions of variations in susceptibility among ecosystems to trace metal or radionuclide contamination of deposit-feeding fauna. The abundance of the various forms of sediment-bound trace metals in aquatic ecosystems is not well known. Gibbs, 1973, suggested Fe, Cu, Co, Mn and Cr, were transported primarily in crystalline and coprecipitated forms in two rivers. Leland, et al., 1973, found correlations between Co and Mn concentrations in Lake Michigan sediments, and between Co and clays. Where hydrous oxides of Fe and Mn dominate metal sorption within sediments the availability of sediment-bound Zn and Co to benthic deposit feeders should be minimal. However, the same type of system (especially if rich in manganese oxides) might be highly susceptible to Ag contamination of deposit feeders. The significant differences among metals in availability from a given substrate may produce environments of high susceptibility to biocontamination by some metals, but low susceptibility to other metals.

In coastal waters, estuaries, and eutrophic freshwaters organic complexation plays an important role in binding metals to sediments. Luoma and Jenne (1976), showed that little Cd is available to *M. balthica* from at least some types of organic complexes. Luoma (1974), showed similar results with inorganic mercury, using deposit-feeding polychaetes and shrimp. Organic, particulate-bound Ag is the least available form of this metal included in our study. (Because of the generally high bioavailability of all forms of sediment-bound Ag studied, however, some contamination of deposit feeders through sediment ingestion might be expected wherever this metal is present in nature.) In contract to Cd, Hg and Ag, significant uptake of sediment-bound Zn by deposit feeders might be expected where (organic complexation with organic matter dominates Zn sorption to sediment. Likewise, when organic complexation of Co dominates, this metal will be more available to deposit feeders than when Co is sorbed by hydrous oxides.

Both Zn and Co showed some availability to M. balthica when coprecipitated with calcium carbonate. Calcite-bound Ag was the most available form of this metal studied, and was the only sediment-bound form of any metal included in this study from which bioconcentration was observed. Inorganic calcites may be an important component of the sediments in fluvial systems which drain calcium carbonate sediments, but are not abundant in most estuarine sediments. Biogenic carbonates, however, are abundant in estuarine and marine ecosystems. The high availability of Zn and Co from biogenic carbonates may be partially countered by the relatively low concentrations of these metals usually found in mollusc shells (Wolfe and Rice, 1972). Silver, however, is rapidly partitioned to the shell of M. balthica, and when present in nature, may occur in high concentrations in mollusc shells (Luoma and Jenne, unpub. data). The high availability of Zn and Co, and the relatively high concentrations of Ag associated with biogenic carbonates could make this substrate an important reservoir from which metals are recycled into the food webs of some aquatic systems. Likewise, systems dominated by other types of biogenic carbonate sediments (e.g., areas where coral debris dominates sediments) may also be quite susceptible to trace metal or radionuclide contaminations. Direct study of different types of carbonate sediments and their associated biota is necessary to support such a conclusion.

SUMMARY

Chapter 2

The availability to Macoma balthica of sediment-bound metals was dependent upon the physical-chemical nature of the metal-sediment association. Laboratory studies of bioaccumulation from individual sedimentary trace-element sinks labelled with radioactive tracers of Ag, Co and Zn indicated bioavailability varied among metals within a given substrate and among substrates for a given metal. Little bioaccumulation was observed from several sinks which may be common in nature, e.g., little Zn or Co uptake was observed when those metals were coprecipitated with amorphic iron oxide or manganese oxide. However, Ag, Co, and Zn were all taken up from detrital organics and Ag was accumulated by the clam from the iron oxide precipitate. Even quantitatively minor substrates within aquatic sediments may be important sources of some metals for M. balthica. Uptake rates of Co and Zn from biogenic carbonates (crushed clam shells) were significantly greater than rates of uptake from other sinks. Likewise, Ag uptake from both biogenic carbonates and synthetic calcites was greater than Ag uptake from iron oxides or detrital organics. Substrates from which bioaccumulation of bound metals was greatest also showed the greatest rate of sediment to water desorption of metals. Where such substrates are abundant in nature, bioavailability of sediment-bound metals may be enhanced both through increased uptake from ingested particulates by deposit feeders, and through increased sedimentary desorption, resulting in higher concentrations of solute metals.

Table 2-1 The concentration of ^{110m}Ag in different types of sediments and in the soft tissues of clams exposed to those sediment types. ΔCF_{14} = tissue concentrations of Ag resulting from only sediment ingestion relative to concentration of Ag in sediment.

	Concentration of ^{110m}Ag (ng/g)				
Sediment Type	Sediment (dry)	Tissue Concentration (dry)		ΔCF_{14}	% in Shell
		Feeding clams	Dialysis bag clams	(Dry tissue/ dry sediment)	(Feeding)
Calcite I	18	92	26	3.667	56.1
Calcite II	11	93	26	6.140	59.2
MnO_x I	153	197	67	0.850	67.4
MnO_x II	61	43	19	0.395	
Fe_xO_y I	4,450	234	39	0.043	47.8
Fe_xO_y II	1,600	134	63	0.044	55.3
Fe_xO_y III*	670	232	135	0.146	65.6
Biogenic $CaCO_3$I	396	34	4	0.076	
Biogenic $CaCO_3$ II	1,190	55	15	0.034	58.3
Organics I	7,100	252	56	0.028	44.0
Organics II	4,660	144	15	0.028	47.4
Organics III	16,010	552	76	0.030	43.0

* Allowed only 3 h for equilibration between sediment and water during labelling.

Table 2-2 The concentration of ^{65}Zn in different types of sediment and in the soft tissues of clams exposed to those sediment types. ΔCF_{14} = tissue concentrations of ^{65}Zn resulting from ingestion of sediment relative to concentration of ^{65}Zn in sediment.

	Concentration of ^{65}Zn (ng/g)				
Sediment Type	Sediment (dry)	Tissue Concentration (dry)		ΔCF_{14}	% in Shell
		Feeding clams	Dialysis bag clams	(Dry tissue/ dry sediment)	(Feeding)
Biogenic $CaCO_3$ I	454	256	172	0.186	
Biogenic $CaCO_3$ II	350	121	76	0.130	19.0
Organics I	6,270	1,144	415	0.116	7.0
Organics II	2,350	418	132	0.122	nd
Organics III	7,129	843	214	0.084	11.2
Calcite I	6,540	243	18	0.034	2.4
Calcite II	3,050	92	22	0.023	nd
MnO_x I	2,790	15	nd	0.006	nd
MnO_x II	470	3	nd	0.007	
Fe_xO_y I	17,710	154	nd	0.009	nd
Fe_xO_y II	10,180	36	nd	0.004	nd
Fe_xO_y III*	2,650	8	nd	0.003	nd

* Allowed only 3 h equilibration between sediment and water during labelling.

Table 2-3 The activity of ^{60}Co in different types of sediment and in the soft tissues of clams exposed to those sediment types. ΔCF_{14} = tissue concentration of ^{60}Co resulting from ingestion of sediment relative to ^{60}Co concentrations in sediment.

	Activity of ^{60}Co (dpm/mg)				
Sediment Type	Sediment (dry)	Tissue Concentration (dry)		ΔCF_{14}	% in Shell
		Feeding clams	Dialysis bag clams	(Dry tissue/ dry sediment)	(Feeding)
Biogenic $CaCO_3$ I	725	308	256	0.071	31.5
Biogenic $CaCO_3$ II	3,308	959	410	0.103	
Calcite I	13,400	444	420	0.039	10.1
Calcite II	7,026	497	251	0.035	12.3
Organics I	31,060	3,267	1,784	0.048	20.7
Organics II	21,353	1,005	533	0.022	45.7
Organics III	41,406	1,805	995	0.020	61.1
Fe_xO_y I	166,926	1,071	174	0.005	29.4
Fe_xO_y II	92,818	605	190	0.004	15.4
Fe_xO_y III*	11,710	36	10	0.002	nd
MnO_x	114,843	113	nd	0.001	12.0
MnO_x II	27,922	51	nd	0.002	

* Allowed only 3 h equilibration between sediment and water during labelling.

Table 2-4 Specific activities of Ag, Co and Zn in experimental sediments

	Sediments		
	Ag (pCi/pg)	Zn (pCi/pg)	Co (pCi/pg)
Organics I	1.14	2.00	31.21
Organics II	0.45	0.38	11.90
Organics III	1.59	1.27	
Fe_xO_y I	0.45	3.69	80.57
Fe_xO_y II	0.38	1.63	47.14
Fe_xO_y III	0.07	0.43	5.92
Mn_xO_y		0.38	
Calcite I	0.07	32.70	0.17
Biogenic $CaCO_3$ II	0.32	0.59	16.43

Table 2-5 The concentration (^{110m}Ag, ^{65}Zn) or activity (^{60}Co) of labelled metal in the different types of sediment and in seawater at equilibrium with that sediment. K_{des} = the distribution coefficient = concentration in sediment relative to concentration, in comparable units, in seawater.

Sediment Type	^{110m}Ag			^{65}Zn			^{60}Co		
	Sed (μg/g)	Solute (μg/ℓ)	K_{des}	Sed (μg/g)	Solute (μg/ℓ)	K_{des}	Sed (dpm/g)	Solute (dpm/ℓ)	K_{des}
Biogenic $CaCO_3$ I	0.40	0.021	2.00×10^4	0.45	0.52	8.65×10^2	0.73	0.70	1.03×10^3
Biogenic $CaCO_3$ II	1.19	0.109	1.08×10^4	0.35	0.28	1.26×10^3	5.31	16.24	3.27×10^2
Calcite I	0.018	0.014	1.29×10^3	6.54	0.05	1.30×10^5	13.40	8.25	1.62×10^3
Fe_xO_y I	4.45	0.014	3.17×10^5	17.71	0.004	4.39×10^6	166.92	1.36	1.23×10^5
Fe_xO_y II	1.60	0.018	8.94×10^5	10.18	0.002	4.81×10^6	92.8	1.02	9.15×10^4
Fe_xO_y III*	0.67	0.430	1.56×10^3	2.65	0.04	7.10×10^4	11.71	.77	1.52×10^4
Mn_xO_y I	0.15	0.090	1.67×10^3	2.79	0.01	2.79×10^5	114.84	0.08	1.53×10^6
Mn_xO_y II	0.06	0.015	3.90×10^3	0.47	0.0002	2.35×10^6	27.92	0.03	1.09×10^6
Organic I	4.66	0.012	3.96×10^5	6.27	0.56	1.13×10^4	31.06	42.58	7.30×10^2
Organic II	7.10	0.031	2.28×10^5	2.35	0.19	1.23×10^4	21.35	7.61	2.81×10^3
Organic III	16.01	0.022	7.34×10^5	7.13	0.66	1.09×10^4	41.41	13.45	3.08×10^3

* Allowed only 3 h equilibration between water and sediment during labelling.

LIST OF FIGURES

Figure 1. Uptake of ^{110m}Ag by the clam M. balthica from ingestion of sediments. Wet weight whole body concentrations of ^{110m}Ag were normalized by dry weight sediment concentrations of the nuclide at each point. 0-0 precipitated $CaCo_3$; Δ-▲ MnO_x; Δ-Δ Fe_xO_y; X-X biogenic $CaCO_3$; 0-0 organic particulates. Roman numerals represent experiment number.

Figure 2. Uptake of ^{65}Zn by the clam M. balthica from intestion of sediments. Wet weight whole body concentrations of ^{65}Zn were normalized by dry weight sediment concentrations of the nuclide at each point. 0-0 precipitated $CaCo_3$; Δ-Δ MnO_x; Δ-Δ Fe_xO_y; X-X biogenic $CaCo_3$; 0-0 organic particulates. Roman numerals represent experiment number.

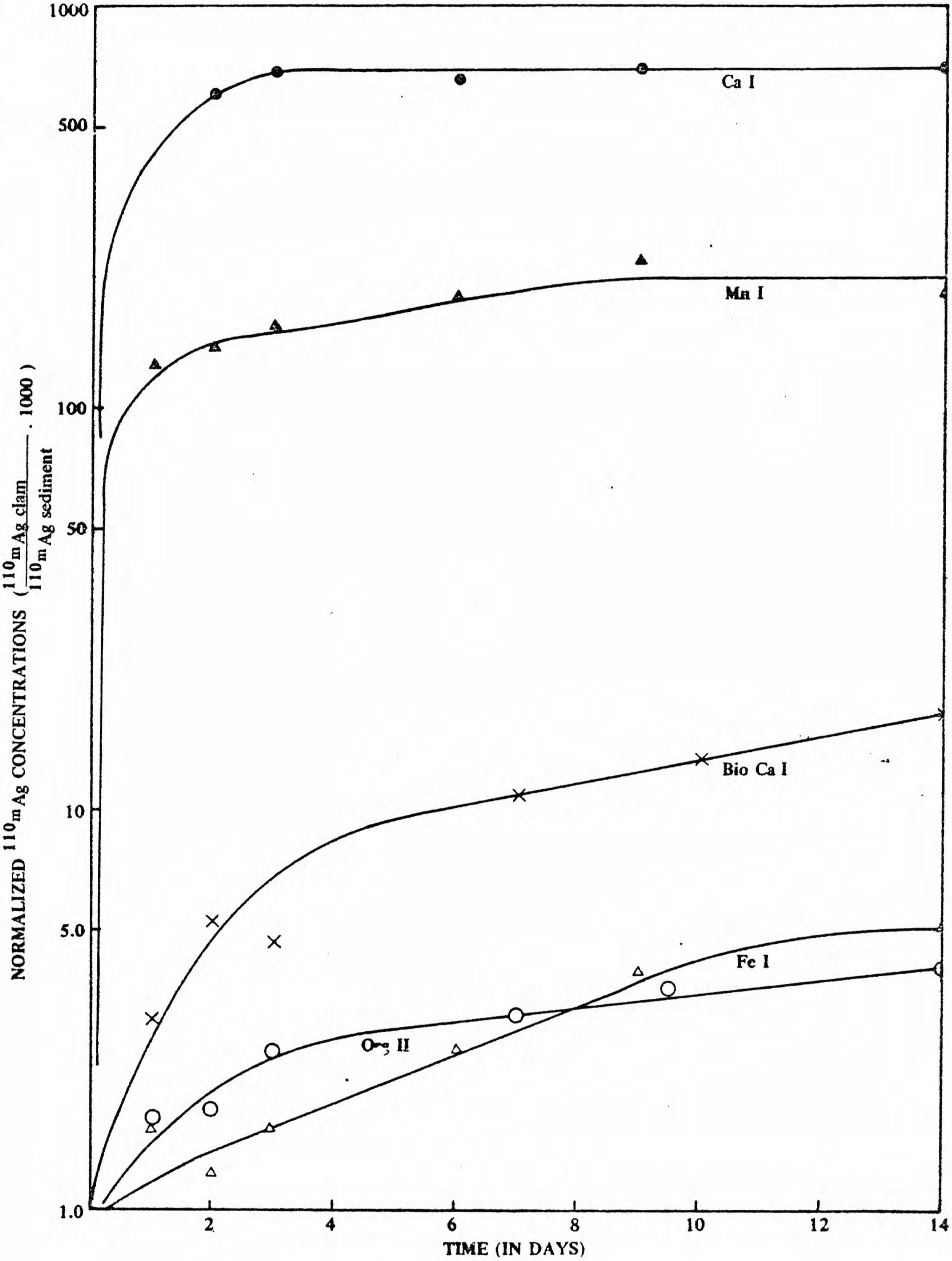
Figure 3. Uptake of ^{60}Co by the clam M. balthica from ingestion of sediments. Wet weight whole body concentration of ^{60}Co were normalized by dry weight sediment concentrations of the nuclide at each point. 0-0 precipitated $CaCO_3$; Δ-Δ Fe_xO_y; X-X biogenic $CaCO_3$; 0-0 organic particulates. Roman numerals represent experiment number.

Figure 4. The 14 day concentration factor in clam (Macoma balthica) soft parts (ΔCF_{14}) as a function of the desorption distribution coefficient (k_{des}) for ^{110m}Ag, ^{65}Zn and ^{60}Co. Lines were fit by regression analysis.

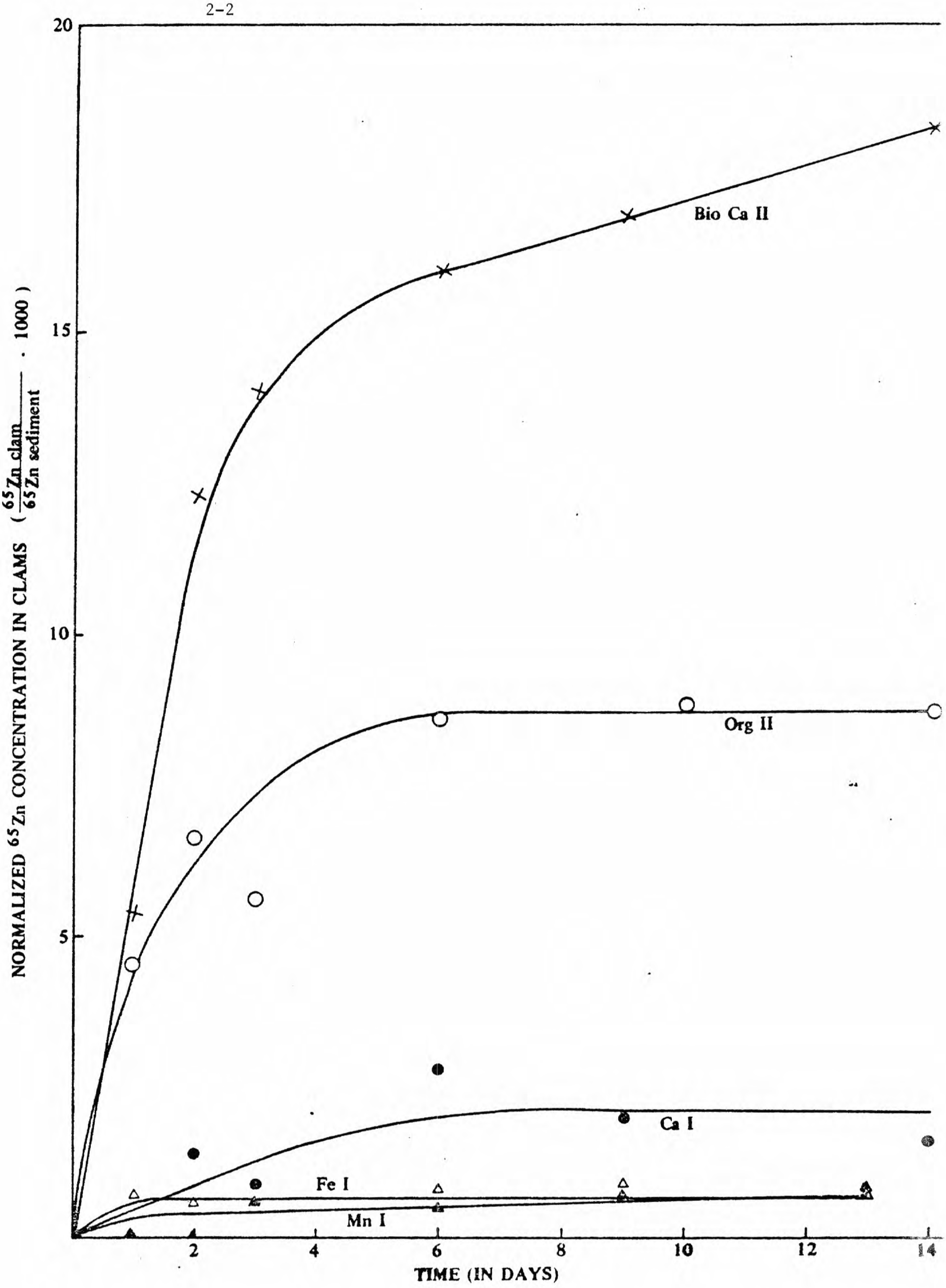
Figure 5. The 14 day concentration factor in clam (Macoma balthica) soft parts (ΔCF_{14}) as a function of the relative sorption of ^{110m}Ag, ^{65}Zn and ^{60}Co to $CaCO_3$, organic particulates, Fe_xO_y or MnO_x. Relative sorption was deter-

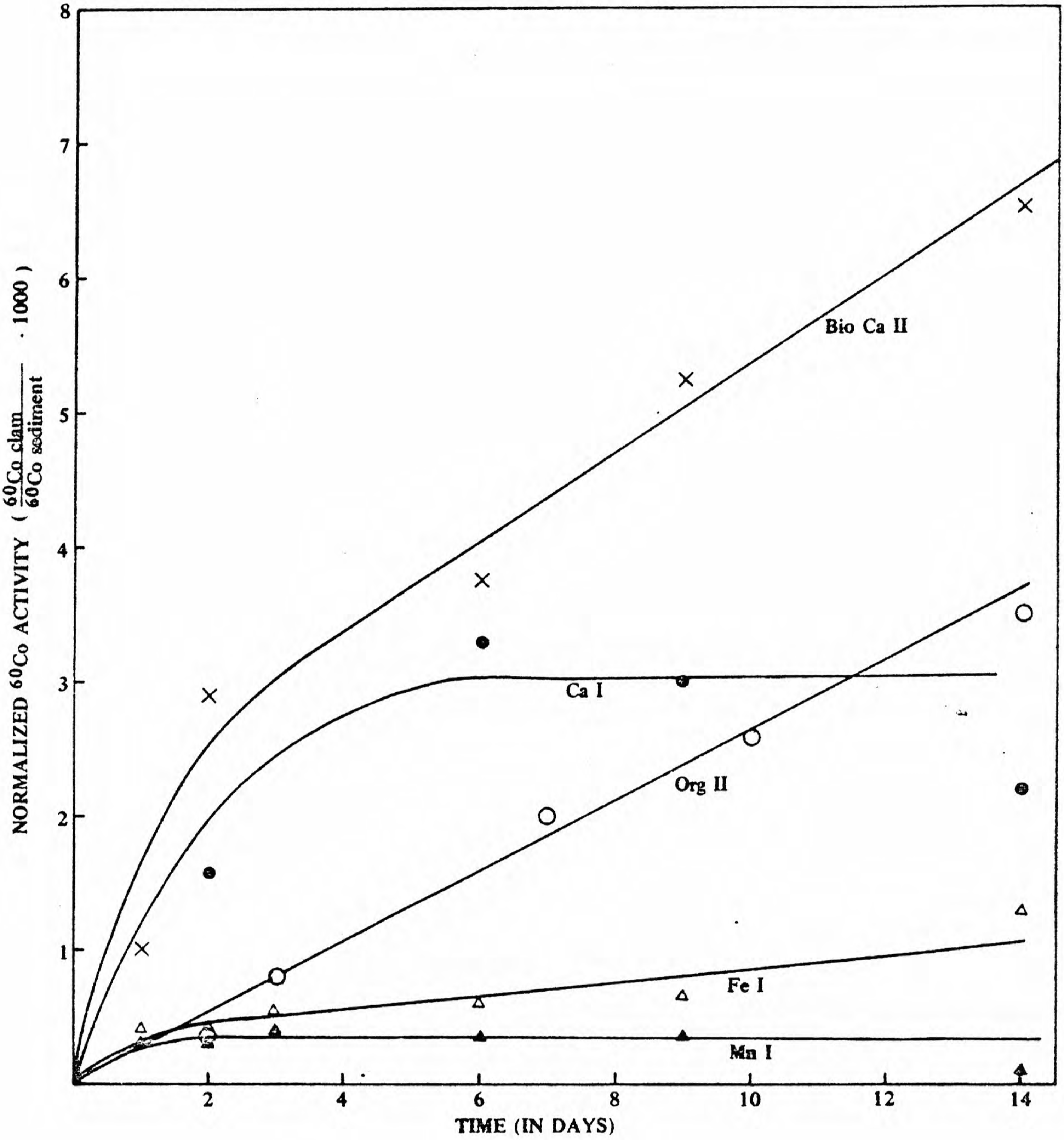
mined by dividing nuclide concentrations in sediment (after 5 days exposure to labelled water) by nuclide concentrations in the water on day 5. The values for ΔCF_{14} represent the mean concentration factor from experiments with each sediment-type.

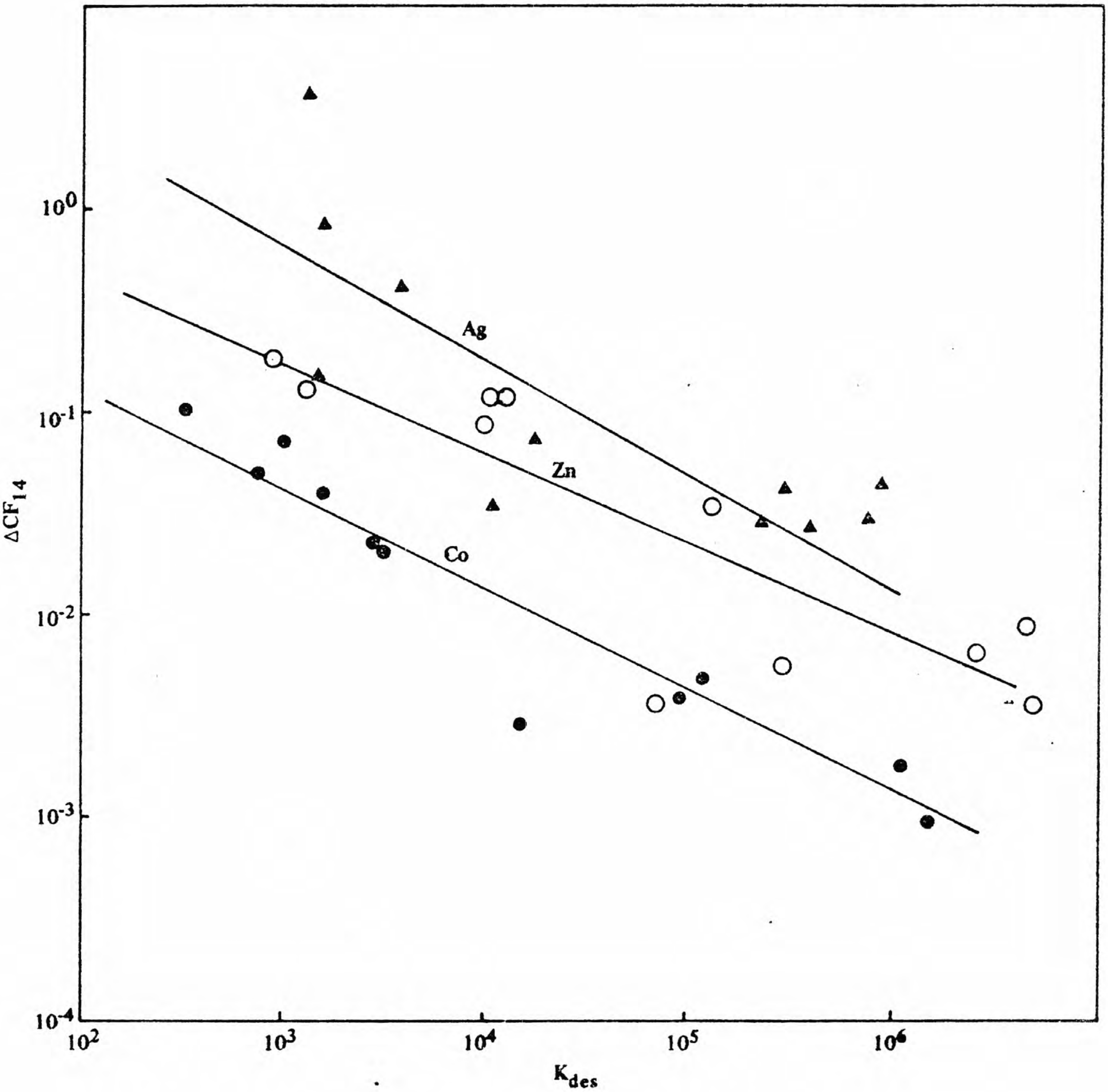
2-1



1000
500
100
50
10
5.0
1.0
Ca I
Mn I
Bio Ca I
Fe I
Org II
NORMALIZED ^{110m}Ag CONCENTRATIONS $(\frac{^{110m}\text{Ag clam}}{^{110m}\text{Ag sediment}} \cdot 1000)$
2
4
6
8
10
12
14
TIME (IN DAYS)


20
15
10
5
2
4
6
8
10
12
14
TIME (IN DAYS)
NORMALIZED ^{65}Zn CONCENTRATION IN CLAMS ($\frac{^{65}Zn\ clam}{^{65}Zn\ sediment} \cdot 1000$)
Bio Ca II
Org II
Ca I
Fe I
Mn I


NORMALIZED ^{60}Co ACTIVITY ($\frac{^{60}\text{Co clam}}{^{60}\text{Co sediment}}$ · 1000)
8
7
6
5
4
3
2
1
2
4
6
8
10
12
14
TIME (IN DAYS)
Bio Ca II
Ca I
Org II
Fe I
Mn I


10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
ΔCF_{14}
10^2
10^3
10^4
10^5
10^6
K_{des}
Ag
Zn
Co

Chapter 3. Estimating bioavailability using chemical extractants: Laboratory studies with well-defined sediment types.

S.N. Luoma and E.A. Jenne

INTRODUCTION

This experiment compares bioaccumulation of sediment-bound Ag, Cd, Co and Zn from physicochemically well-defined sediments, reported previously (Chapter 1 and 2), with the amount of metal extracted from the sediments by several different chemical techniques. Correlations between uptake and extractability are used to suggest methods for chemically estimating metal bioavailability from sediments of different physicochemical character. The selectivity of metal extraction from specific substrates is also considered.

METHODS AND MATERIALS

In the previously reported bioaccumulation experiments, deposit-feeding clams (Macoma balthica) were fed six different types of laboratory-prepared sediment. Each sediment was an analog of a sedimentary trace metal sink common to estuaries, including organic detritus (decaying marsh grass fragments), iron oxide, iron oxide coated with bacteria, manganese oxide, inorganic carbonate and biogenic carbonate (crushed clam shells). The sediments were equilibrated with ^{110m}Ag, ^{109}Cd, ^{60}Co and ^{65}Zn in seawater, then rinsed with unlabelled seawater. One group of clams was allowed to feed ad libitum on the sediments while a second group of clams was enclosed in dialysis bags. Uptake from particle ingestion was determined at the end of each experiment by subtracting nuclide concentrations in the tissues of dialysis bag-enclosed animals from concentrations in feeding animals.

At the end of each uptake experiment, sub-samples of sediments (≈100 mg) were analyzed for radionuclides by multichannel gamma spectrometry, then subjected to chemical extraction. Extractants were chosen to both simulate a wide range of possible digestive processes and to allow chemical characterization of the sediments (Table 3-1). After extraction and filtration (0.45 μm), radioanalysis of the extractant was conducted. A different sub-sample was used for each extraction, and each extraction was done in duplicate. Unless otherwise noted, extractions were carried out for 2 h in 10 ml of extractant at room temperature. Details of radioanalysis and sediment preparation methodology as in chapters 1 and 2.

RESULTS

Metal Extraction from Single Substrates

Most of the extractants removed at least a small proportion of all four metals from all sediment types studied (Table 3-2a-d). Weak acids (0.1 N hydrochloric acid = HCl, and 25% acetic acid = HAc), an oxidizing agent (3% hydrogen peroxide plus citrate = H_2O_2 + $C_6H_8O_7$) and reducing agents (Hydroxylamine hydrochloride = $NH_2OH \cdot HCl$, and sodium dithionite + citrate = $Na_2S_2O_4$ + $C_6H_8O_7$) were especially efficient in metal removal.

Hydrochloric acid and HAc extracted large proportions of Cd, Co and Zn from all metal-sediment associations except Co-manganese oxide. Extractions of calcium carbonate with HCl were inefficient due to neutralization of the HCl during carbonate dissolution. Silver was less easily extracted by acids than were Cd, Co and Zn, expecially from the biogenic calcium carbonate and organic particulate sinks.

Neither the reducing agents nor H_2O_2 demonstrated the selective metal removal suggested in recent extraction schemes (Engler, et al.,

agents removed Cd, Co and Zn from the organic sinks and the carbonates as effectively as from iron and manganese oxides. Dithionite + citrate was the most effective method for removing Ag and Zn from all types of sediment. Likewise, H_2O_2 + $C_6H_8O_8$ removed more Ag, Cd, Co and Zn from iron and manganese oxides than from the organic sinks.

Extractants relying upon exchange, mild dissolution and/or complexation (ammonium acetate = NH_4Ac; ethanol = EtOH; and EDTA in sodium hydroxide = EDTA + NaOH) removed widely varying proportions of metals from different sediments. Ammonium acetate removed 10 times more Co and Zn from organic material than from iron oxide and 10 times more from iron oxide than from manganese oxide. The combination of EDTA + NaOH extracted Cd, Co and Zn more efficiently from organic particulates and iron oxide than from manganese oxide and biogenic carbonate. A relatively large proportion of Cd was extracted by both NH_4Ac and EDTA + NaOH from organics, iron oxide and biogenic carbonate, but little Cd was removed from manganese oxide by NH_4Ac. In contrast, NH_4Ac and EDTA + NaOH removed a significant amount of Ag from manganese oxide but little Ag from any other sink.

Ethanol extracted little Ag or Zn from any sediment type. The proportions of total Cd and Co extracted by this procedure were also low, but the relative amounts extracted from the different substrates appeared to be significantly different.

Replicate experiments with iron oxide and organic sediments generally indicated good (± 10%) precision for all extractants. However, variations in the quantity of metal extracted in replicate manganese oxide experiments were often large (as great as 100% of the mean shown in Table 3-2). Small differences in manganese oxide data must be interpreted with this in mind. Preliminary experiments indicated metal extraction from iron oxide was influenced by the age (and thus crystallinity) of the precipitate. Undetected differences in oxide crystallinity may have been responsible for the variance in the manganese oxide data.

Correlations with Bioavailability

The quantities of metal removed by some of the extractants showed significant correlations with the amount taken up by clams when comparisons were made over all experiments (Table 3-3). The concentration of ^{65}Zn concentration by NH_4Ac correlated at a high level of significance ($p<0.01$) with ^{65}Zn concentrations in the feeding clams (Table 3-3a). Ethanol-extractable Cd also correlated remarkably well ($p<0.01$) with Cd concentrations in the feeding animals. Significant correlations ($p<0.05$) were observed between EDTA + NaOH extractable Zn and Zn in the clams, and for Co uptake vs. both NH_4Ac extraction and EtOH extraction. Uptake of Cd, Co or Zn by the feeding animals did not correlate significantly with any extractions by mild acids, reducing agents or the oxidizing agent.

Several of the relationships between extractability and uptake of Cd, Co and Zn were improved (Table 3-3b) when uptake from only food was considered (determined by subtracting radioisotope concentrations of clams in dialysis bags from concentrations in feeding clams). Uptake of all three metals correlated significantly with NH_4Ac extractability, and correlation of Co uptake with EDTA + NaOH extraction became significant, when the influence of solute metal uptake was removed. Correlations between the mild extractions and uptake from food alone were significant for all instances except EDTA + NaOH extraction of Cd and EtOH extraction of Zn.

A preliminary test of the applicability of the substrate data to natural sediments was conducted using Cd and surface sediments from San Francisco Bay (Tables 3-2 and 3-3). Uptake from the natural sediments was high and significantly improved the correlation with EtOH extraction of Cd. When the Bay sediment values were excluded, the correlation coefficient for EtOH extraction vs. Cd uptake by feeding clams was 0.721 ($p<0.05$). When the natural sediment data were included in the regression r was 0.987 ($p<0.01$). Weakly significant correlations between uptake by feeding clams and both HCl extractable Cd and total Cd became insignificant when the natural sediment data were considered. When the solute component of uptake was removed, correlation of EtOH extraction from only the single sinks with bioaccumulation was improved ($r=0.895$), and correlations of uptake with both total Cd and HCl extractable Cd dropped well below the level of significance. The strong influence of the solute component of Cd uptake on the correlations with extraction of the single sinks resulted from an anomalously large desorption of Cd from sediment to solution in the experiments with particulate organics. Uptake from organic detritus by feeding animals was high but most of this uptake was from solution; uptake from food alone was lower from the organic material than from any other phase studied.

DISCUSSION

Our data strongly suggest that weak acids, reducing agents and oxidizing agents will remove significant quantities of Ag, Cd, Co and Zn from nearly every type of physicochemical sink present in natural sediments. These extractants do not appear at all selective in removing a single physicochemical form of any of these metals. Moreover, none of these extractants removed quantities of Cd, Co or Zn from the different sinks which correlated with the quantities accumulated by deposit-feeding clams. Despite superficial similarities with the apparent chemical mechanisms involved in digestion, weak acids and weak reducing agents appear to extract a different fraction of bound Cd, Co or Zn than do digestive processes.

The chemical selectivity of NH_4Ac, EDTA + NaOH and EtOH appeared to more closely follow the selectivity of the digestive processes of the clam. Correlations between NH_4Ac extractability and uptake of Cd, Co and Zn from food alone suggest mild exchange conditions and complexation may be important physiological mechanisms of metal assimilation in *M. balthica*. Weak exchange is consistent with the mild chemical conditions characteristic of deposit-feeder digestive fluids (Owen, 1966). Complexation may be more important in binding metals to transport sites responsible for carrying polar compounds and elements across nonpolar biological membranes.

The remarkable correlation between Cd uptake and ethanol extractability is more difficult to explain. Several authors have observed that Cd is more readily desorbed from sediment to solution, or more weakly bound to sediment than are most metals (Ramamoorthy and Kushner, 1975, J. D. Hem unpublished data). The fraction of Cd extracted by EtOH and the mechanisms which control bioavailability may both be sensitive to such mobility. The correlation of Ag and Co uptake with EtOH extraction suggests that EtOH may simulate a biological mechanism of more general significance in trace metal assimilation. The occurrence of Ag, Cd and Co compounds or complexes of low polarity would enhance solubility in EtOH and facilitate penetration of biological membranes. Other effects common to EtOH treatment and digestive processes are also possible. The low quantities of Ag and Co removed by EtOH may limit its usefulness as an extractant of these metals from natural sediments. However, sufficient quantities of Cd are EtOH soluble to make EtOH extraction a potentially useful indicator of biologically available Cd in sediments.

Although uptake of Ag varied with physicochemical form, significant quantities of Ag were taken-up from all sediment-types. Uptake by the clam was strongly correlated with total ^{110m}Ag concentrations in sediment, suggesting the generally high bioavailability of this metal overrides the influence of form. Extractants which remove significant Ag from all forms in sediments should be good indicators of Ag bioavailability.

The optimum chemical methods for defining bioavailability varied among the four metals studied, as did the efficiency with which many of the methods extracted metals. Small differences in metal chemistry appeared to significantly influence metal interactions with sediment phases, extractants and clams. The behavior of Ag was especially anomalous relative to that of Cd, Co and Zn. Both bioaccumulation and extraction data indicated Ag was weakly bound by manganese oxides. In contrast, Cd, Zn and especially Co appeared to be strongly sorbed to manganese oxide. Unlike the other metals, Ag uptake correlated poorly with NH_4Ac extractability. The relatively inefficient extraction of most Ag forms by NH_4Ac may result from the low stability of the Ag-acetate ligand (pK=0.73) relative to acetate complexation of Cd (pK=1.7), Co (pK=1.5) and Zn (pK=1.6). (Comparisons are of acetate in acetic acid, under similar stoichiometric conditions, from Sillen and Martel, 1964). The efficient removal of Cd by NH_4Ac and EDTA + NaOH from all sediments except manganese oxides, and the anomalous desorption of Cd from organic particulates were both indicative of the low stability of Cd binding to most substrates (with the possible exception of manganese oxides). The low stability of the Cd-citrate complex (pK=3.3), relative to Co (pK=4.5) and Zn (pK=4.6) may also explain the unexpectedly inefficient extraction of Cd by $Na_2S_2O_4$ + $C_6H_8O_7$. The importance of specific aspects of metal chemistry in determining extractability and bioavailability suggest no "universal" chemical extractant will adequately estimate the bioavailability of numerous trace metals from the quantitatively important physicochemical forms in sediments.

Despite a lack of absolute specificity, several of the extractants removed proportionately different quantities of metals from major substrate. The relative specificity of such extractants may be useful in devising improved schemes for characterizing metal forms in sediments.

CONCLUSIONS

Despite limitations, these comparative experiments with physicochemically well defined systems allow several useful conclusions: 1) differences in trace metal chemistry appear to result in important differences among metals in the biological availability and chemical extractability of different physicochemical forms. It is unlikely that any single chemical extractant will adequately describe the biologically available fraction of numerous trace metals; 2) despite the complexity of metal-sediment-biota interactions, several specific methods (e.g. EtOH extraction of Cd; NH_4Ac extraction of Zn and Co; $Na_5S_2O_4$ + $C_6H_8O_7$ extraction of Ag) show promise as indicators of the bioavailability of individual trace elements from sediments; 3) many of the semi-selective extraction schemes employed recently to study metal partitioning in sediment (Gupta and Chen, 1975; Serne and Mercer, 1975) may lack the absolute specificity implied by the authors; and 4) the relative specificities of milder chemical extractants may facilitate a more precise definition of the physicochemical form of sediment-bound metals.

SUMMARY

Chapter 3

Chemical extraction from laboratory-prepared sediments of the biologically available fraction of Ag, Cd, Co and Zn is best accomplished by complexation and/or mild dissolution procedures. Deposit-feeding clams (Macoma balthica) were exposed in separate experiments to radio-isotopes of the four elements bound to six physiocochemically different types of sediment (organic detritus, iron oxides, organically coated iron oxides, manganese oxides, inorganic carbonates and biogenic carbonates). At the end of each experiment, tracer concentrations in clam soft tissues were compared with the concentration of tracer chemically extracted from experimental sediments by several geochemical techniques. Because Ag was highly available from all types of sediment, uptake by the clam correlated well with most indicators of non-crystalline Ag concentrations. However, uptake of Cd, Co and Zn was strongly influenced by physicochemical form. Concentrations of Cd or Co accumulated by the clam from the different sediments correlated significantly only with the concentration of the metal extracted by 70% ethanol or 1 N ammonium acetate. Bioaccumulation of Zn was most closely estimated by 1 N ammonium acetate extraction or 1 N NaOH plus EDTA extraction of the different sediments. Concentrations of Cd, Co and Zn extracted by weak acids (0.1 N HCl; 25% acetic acid), reducing agents (1 N hydroxylamine hydrochloride in 0.01 N HNO_3; sodium dithionite plus citrate), or oxidizing agents (3% H_2O_2 plus citrate) correlated poorly with bioaccumulation.

Table 3-1 Extraction techniques employed and modes of action of the extractants.

Extractant	Mode of Action
1 N Ammonium Acetate (NH_4Ac)	Exchange; COO^- complexation
0.1 M Ethylene diaminetetraacetic acid in 1 N sodium hydroxide (EDTA + NaOH)	Dissolution (of organic compounds); complexation
70% Ethanol (24 h) (EtOH)	Viable cell breakdown; dissolution low polarity compounds
0.1 N Hydrochloric acid (HCl)	Hydrogen exchange
25% Acetic acid (HAc)	Hydrogen exchange
3% Hydrogen peroxide + citrate buffer (95.6 g/l sodium citrate + 36.8 g/l citric acid ($H_2O_2 + C_6H_8O_7$)	Oxidation; complexation
0.1 M Hydroxylamine hydrochloride in 0.01 N HNO_3 (15 ml for 30 min) ($NH_2OH \cdot HCl$)	Reduction
Sodium Dithionite (0.5 g) + citrate buffer. (4 hr at 80°C after which 5 ml sodium nitrate - 529 g/l was added) ($Na_2S_2O_4 + C_6H_8O_7$)	Reduction; complexation

Table 3-2 The proportion of total nuclide extracted from each type of sediment by various techniques. [Numbers in parentheses indicate the number of experiments with each sediment type. Blanks in table represent unextracted sediments.]

	Proportion of total nuclide extracted							
Sediment Type	NH_4Ac	EDTA + NaOH	EtOH	HCl	HAc	H_2O_2 + $C_6H_8O_7$	$NH_2 \cdot OH \cdot HCl$	$Na_2S_2O_4$ + $C_6H_8O_7$
			a) ^{60}Co					
Biogenic $CaCO_3$(1)	0.086	0.076	0.065	0.217	0.403	0.298	0.185	0.783
$CaCO_3$(1)	0.046	-----	0.001	0.225	0.851	-----	0.253	0.914
Organics(3)	0.117	0.188	0.012	0.585	0.559	0.245	0.572	0.807
Fe_xO_y + Org(1)	0.035	0.117	0.006	0.354	0.398	0.133	0.210	0.773
Fe_xO_y(3)	0.070	0.262	0.002	0.542	0.716	0.870	0.614	0.880
MnO_x(2)	0.003	0.003	-----	0.076	0.009	0.655	0.711	0.364
			b) ^{109}Cd					
S.F. Bay(1)	0.350	-----	0.502	0.784	0.709	-----	0.498	0.411
Biogenic $CaCO_3$(1)	0.399	0.210	0.083	0.486	0.552	0.011	0.444	0.138
$CaCO_3$(1)	0.025	-----	<0.005	0.093	0.829	-----	0.206	0.251
Organics(3)	0.377	0.483	0.008	0.607	0.732	0.201	0.556	0.592
Fe_xO_y + Org(1)	0.139	0.359	0.016	0.560	0.587	0.007	0.672	0.429
Fe_xO_y(3)	0.211	0.515	0.070	0.630	0.707	0.405	0.706	0.727
MnO_x(2)	0.005	0.218	-----	0.427	0.203	0.561	0.514	0.955

Sediment Type	Proportion of total nuclide extracted							
	NH_4Ac	EDTA + NaOH	EtOH	HCl	HAc	H_2O_2 + $C_6H_8O_7$	$NH_2 \cdot OH \cdot HCl$	$Na_2S_2O_4$ + $C_6H_8O_7$
			c) ^{65}Zn					
Biogenic $CaCO_3$(1)	0.328	0.079	0.002	0.496	0.584	0.021	0.438	0.761
$CaCO_3$(1)	0.025	-----	<0.001	0.064	0.821	-----	0.148	0.852
Organics(3)	0.308	0.231	0.004	0.683	0.667	0.100	0.696	0.738
Fe_xO_y + Org(1)	0.034	0.044	0.012	0.267	0.435	0.007	0.228	0.806
Fe_xO_y(3)	0.039	0.107	<0.001	0.400	0.640	0.814	0.385	0.832
MnO_x(2)	0.001	0.015	-----	0.601	0.475	0.774	0.833	0.665
			d) ^{110m}Ag					
Biogenic $CaCo_3$(1)	0.002	0.002	0.001	0.047	0.002	0.012	0.006	0.742
$CaCO_3$(1)	<0.03	-----	0.044	<0.030	<0.030	-----	0.500	0.745
Organics(3)	0.002	0.007	<0.001	0.114	0.026	0.014	0.048	0.577
Fe_xO_y + Org(1)	0.009	0.020	0.003	0.218	0.072	0.171	0.060	0.636
Fe_xO_y(3)	0.007	0.008	0.002	0.451	0.471	0.300	0.299	0.896
MnO_x(2)	0.575	0.066		0.443	0.350	0.135	0.285	0.438

Table 3-3 Correlation coefficients (r) describing relationship of radionuclide concentrations in soft tissues of clams feeding on labeled sediments with radionuclide concentrations removed from those sediments by designated chemical extractant. [n=8-11. *=the probability (p) that r=0 is <0.05. ** = $p < 0.01$.]

	Total Nuclide	NH_4Ac	EDTA + NaOH	EtOH	HCl	HAc	H_2O_2 + $C_6H_8O_7$	$NH_2OH \cdot HCl$	$Na_2S_2O_4$ + $C_6H_6O_7$
a) Correlation with uptake by feeding clams (r)									
Ag	0.902**	0.114	0.791**	0.878**	0.650*	0.349	0.683	0.789**	0.935**
Cd	0.054	0.544	0.490	0.987**	0.104	0.432	0.110	0.045	0.032
Co	0.032	0.664*	0.409	0.784*	0.173	0.173	0.281	0.095	0.134
Zn	0.158	0.927**	0.703*	0.557	0.288	0.241	0.389	0.531	0.167
b) Correlation with uptake from food alone (r)									
Ag	0.936**	0.077	0.812**	0.903**	0.651*	0.327	0.637	0.752**	0.965**
Cd	0.022	0.744*	0.534	0.993**	0.071	0.383	0.389	0.006	0.055
Co	0.173	0.788*	0.683*	0.793*	0.401	0.400	0.257	0.089	0.374
Zn	0.228	0.947**	0.752*	0.551	0.328	0.317	0.352	0.570	0.007

Chapter 4. Determining the Form of Metals in Oxidized Estuarine Sediments: Assessing the Action of Chemical Extractants

S.N. Luoma and G.W. Bryan

The physiocochemical forms of sediment-bound trace metals (determined by metal partitioning among different substrates within the sediments) may strongly influence trace metal bioavailability and metal distributions between sediment and solution (Luoma and Jenne, 1977; Jenne and Luoma, 1977). Understanding metal partitioning in sediments is thus essential to understanding the biological impact and the cycling of metals in aquatic environments. Determining metal partitioning in the oxidized fraction of the bed sediment is especially important in estuaries.

The oxidized layer of sediment in most estuaries lies at the interface of the sediment bed and the water column. This interface plays several roles in the chemistry of trace metals: (1) The surficial oxidation zone in the sediments acts as a diffusion barrier for solutes migrating upward from reducing zones of sediment. Precipitation of manganese oxides and ferric hydroxides (with an associated coprecipitation of available trace metals) at the interface will control the exchange of trace elements between sediments and the water column (Hem, 1979, 1978). (2) Surface sediments on the bed of many estuaries exchange readily with suspended sediments (Conomos and Peterson, 1977); and (3) The sediments at the water-sediment interface are probably more important to biological macrofauna than are subsurface sediments. Suspension-feeding and epibenthic organisms are exposed directly to metals in the environment of the sediment-water interface; while most macroscopic infauna use tubes, burrows or siphons to obtain the bulk of their food and oxygen (and thus their metal burden) from the oxidized sediment surface.

The trace metal chemistry of oxidized sediments such as those at the sediment surface is more complex than that of anoxic sediments. Sulfides dominate the processes controlling metal form in reducing sediments (Morel, et al., 1975). However, in oxidized sediments, organic materials, carbonates and hydrous oxides of both Fe and Mn may all compete for the sorption of trace metals. (For the purposes of this discussion we will assume the term sorption encompasses adsorption, complexation, coprecipitation and ion exchange). The sorption substrates themselves occur in a variety of forms. The forms of the substrates in any given sediment greatly influence the strength of metal-substrate sorption processes, and thus the relative importance of the different substrates in metal partitioning. In estuarine sediments, major classes of organic materials include humic substances, bacteria, bacterial metabolites and refractory, non-viable organic material (e.g. cellulose and lignins). Carbonates may occur as $CaCO_3$, $MgCO_3$, or quantitatively less important metal carbonates. Oxides of Fe exist in layers on particle surfaces or as particles of mineralogic Fe. The crystallinity of the Fe oxides varies over a continium from highly amorphous to highly crystalline. Manganese may occur as Mn oxides of varying structure or Mn carbonate. In turn, both Fe and Mn may also occur associated with each other or with other sorption substrates. Clays and other silicate mineral surfaces also have some capacity for binding metals. However, the strength of metal association with clay surfaces are weak relative to metal association with substrates which would compete for metal sorption in oxidized estuarine sediments. Thus, the most likely role of the clays in such sediments is that of a carrier for higher stability binding substrates (Jenne, 1968; 1977).

Realistic mathematical models predicting metal partitioning in oxidized sediments of varying character (analogous to current models of metal speciation in solution) have not been developed. Such models require methods for quantifying the relative abundances of the different forms of the different substrates present in natural sediments, and determination of metal-substrate "stability" constants. The complexity of the substrates involved in metal sorption has retarded the development of realistic stability constants. However, established geochemical extraction techniques may be useful in assessing the abundances of substrate forms most important in reactions with trace metals.

Most recent studies employing chemical extractions have attempted to define metal partitioning in natural aquatic sediments by specifically extracting metals from single substrates. Some authors have treated sediments sequentially with a series of extractants. (Gibbs, 1973; Brannon, et al, 1976; Gupta and Chen, 1975; Serne, 1977) while others have treated each sub-sample with one extractant (Chester and Hughes, 1967; Loring, 1976). The selectivity attributed to different extractants in removing specific forms of metals has varied among the authors (Gibbs, 1973; Khalid, et al, 1977); Stover, et al, 1976; Serne, 1977). However, studies with chemically well-defined sediments suggest extractants which selectively remove metals from a single substrate (much less a given form of a substrate) are the exception rather than the rule (Luoma and Jenne, 1976; Guy, et al 1978). Most extraction studies in aquatic sediments have considered substrate characterization a secondary goal, if they have considered it at all.

Statistical correlations have also been used to define chemical relationships between substrates and metals in sediments. Chemical relationships have been inferred from significant correlations between total metal concentrations and concentrations of organic carbon (Edgington, 1976; Gong, et al, 1977; Jaffe and Walters, 1977; Willey, 1976) or total Fe (Foster and Hunt, 1975; Korte, et al, 1975). In many instances such correlations may be ambiguous, reflecting either simultaneous deposition of a metal and the associated substrate, or a correlation of both variables with a third variable, such as particle size distributions in the sediment samples. Correlations resulting from depositional or statistically spurious associations are more probable where the data cover a narrow range of concentrations, or are collected from a single body of water (as is often the case). Moreover, total metal concentrations and total substrate concentrations are not necessarily the best measures of the reactive phases of either sorbed metals or their associated substrates.

In this paper we compare metal extraction and substrate characterization in oxidized sediments among 9 commonly employed geochemical extractants. The nature of the extractants included strong acids (concentrated nitric), weak acids (1N hydrochloric), weak acids with complexing agents (25 percent acetic acid; acid ammonium oxalate), an acidic reducing agent (1 N hydroxylamine hydrochloride in 0.01 N nitric acid), basic extractants with complexation (0.1 N sodium pyrophosphate) and without strong complexation (0.1 N sodium hydroxide, 1 N ammonia), and a neutral extractant with exchanging cations (1 N ammonium acetate). Our objectives were to assess the action of the various extractants on metals and substrates in oxidized sediments, and to test the selectivity of the extractants in terms of removing specific forms of both metals and substrates. The data obtained in the analyses of the extracts were also used to provide a base for statistical correlations designed to test the role in metal partitioning played by the metal and substrate forms extracted by the various techniques. The correlations are described in the next chapter.

METHODS AND MATERIALS

Sample Collection and Treatment

Sediment samples were collected from 50 stations in 19 estuaries in south and west England (for precise geographical locations see Luoma and Bryan, 1978). Estuaries and stations were chosen to give the widest possible variability in physicochemical conditions. Metal concentrations differed among stations by 1-3 orders of magnitude (Table 4-1); substrate concentrations (Fe, Mn, humic substances, total organic carbon, carbonates) varied by 1-3 orders of magnitude; metal inputs included agricultural, urban, industrial and mining wastes; and the nature of the estuaries varied from broad, sandy sediment estuaries dominated by marine processes (e.g., Gannel River) to more protected estuaries with silt-clay sediments (e.g. Tamar River).

Samples of oxidized sediment were scraped from the surface layers (∿5mm deep) of intertidal sediments at low tide. For comparison, anaerobic subsurface sediments were collected at 5 cm depth at 7 of the stations. Within 4 hours of collection sediments were sieved through 100μm polyethylene mesh using diluted seawater, to remove particle size biases caused by large sand grains (de Groot, et al, 1976). Following overnight settlement (within 24 hours of collection) the <100μm sediments were either extracted wet or dried at room temperature.

In the interest of developing sediment characterization schemes that were of maximum use in correlations with biological variables and that allowed collection of statistically useful quantity of data, every effort was made to keep chemical treatments as simple as possible. Subsamples of wet sediment were collected from a slurry using a pipette sampler while swirling. Each subsample was treated with a different extractant, i.e., samples were not extracted sequentially. Duplicate subsamples were washed with distilled water then dried at 80^{0} C to obtain salt-free weights. Dry weights (250-550 mg) seldom varied by more than 10 mg between replicates. Salt-free weights were calculated for the dried samples from the salinity of the water used in the sieving procedure. Extractions were carried out in 20 ml glass scintillation-counting vials, which were shaken at frequent intervals. The extract was separated from the sediment by filtration under pressure through a 0.45 µm membrane filter. The filtrates were analyzed for Ag, Cd, Co, Cu, Fe, Mn, Pb and Zn by atomic absorption spectrophotometry. Background correction was used in Ag, Cd, Co and Pb analyses. Because of low concentrations in sediments Cd is reported only in HCl (HCl-soluble Cd equalled totals) while Ag is reported only in total, HCl, ammonia and NaOH extracts.

Extractions

Concentrated nitric acid (Total). Samples were wet ashed using the procedure of Bryan and Uysal (1978). Oxidation with concentrated nitric acid destroys all substrates of significance to trace metal sorption in oxidized sediments and extracts the total metal, as evidenced by the 100 percent extraction of Cu, Pb and Zn, and over 90 percent extraction of Mn and Fe compared to total sample dissolution in hydrofluoric acid (Bryan and Hummerstone, 1971).

1N hydrochloric acid (HCl). Subsamples of 1 g air-dried sediments were extracted for 2 hours with 10 ml of 1 N HCl. Preliminary experiments showed similar concentrations of metal were extracted from air-dried and wet sediment. Weaker solutions of HCl (0.1 N) were neutralized when carbonate concentrations in the sediments exceeded 1 percent, which greatly inhibited the action of the extractant. The 1N HCl solutions were sufficiently acidic to extract samples having up to 30 percent $CaCo_3$. As a strong acid, 1 N HCl extracts metals by destroying carbonates and hydroxides of Fe and Mn, and removes metals from organic ligands by exchange with hydrogen ions (Agemian and Chau, 1977). Other authors have suggested weak HCl solutions (0.3 -.5N) remove the "absorbed" metal forms in sediments (Agemian and Chau, 1977; Malo, 1977).

25 percent acetic acid. Subsamples (2 ml) of wet sediment were extracted for 2 hours in 20 ml of acetic acid. A slight neutralization of the acid (from pH 2.2 to pH 2.6 in the most extreme cases) was observed in sediments with the highest concentrations of carbonates. Dried subsamples of these sediments were re-extracted and titrated back to pH 2.2 with concentrated nitric acid shortly after initiation of the extraction. Only the extraction of Cu was detectably affected by the slight neutralization of the acetic acid. The copper values from the re-extraction were used in our data analysis. Acetic acid is weaker than HCl, but its strong buffering capacity and moderately low pH (pH = 2.2) should release metals by destroying carbonates and some hydroxide forms. Some complexing with metals and reduction of Mn oxide are also likely. In single phase experimental sediment acetic acid also desorbed metals weakly complexed to organic ligands (Chapter 3).

0.4N ammonium oxalate in 0.4N oxalic acid at pH 3.3 (acid ammonium oxalate-Schwertmann, 1964). Subsamples of wet sediment were extracted for 2 hours in 20 ml of extract and preserved for analysis by injecting 25 µl of concentrated HNO_3 into the filtrate. Acid ammonium oxalate desorbs cations, complexes trace metals and reduces oxides of Fe and Mn. At the mild pH of this extractant (pH = 3.3) resorption of freed metals to undestroyed substrates is possible during the extraction. Oxalate is commonly used to quantify amorphous iron oxide concentrations in sediments (Jenne, 1977).

0.1 N hydroxylamine hydrochloride in 0.01 N Nitric acid at pH 2 (hydroxylamine-Chao, 1972). Subsamples were extracted for 30 minutes in 20 ml of extractant. Hydroxylamine was extremely sensitive to the presence of any $CaCO_3$ in sediments (due to the poor buffering capacity of 0.01N nitric acid). Neutralization dramatically affected extraction of at least Cu, Zn, and Fe (Luoma, unpublished data), as did drying the sediments (Chapter 6), thus our hydroxylamine data was meaningful only at stations where carbonates were present in very low concentrations. Hydroxylamine may complex some metals, it reduces oxides of Mn (Chao, 1972) and it dissolves some amorphous Fe oxide. Hydroxylamine also desorbed metals weakly associated with organic ligands in single phase experimental sediments (Chapter 3).

1 N ammonium acetate at pH 7. Subsamples of wet sediment were extracted for 2 hours. The filtrate was preserved for analysis with 25 µl concentrated HNO_3. Ammonium acetate desorbs cations from surface exchange sites, may weakly complex some metals and may reduce some hydrous oxides.

0.1 N sodium pyrophosphate. Subsamples were extracted for 2 hours. Pyrophsophate is a strong complexing agent, and it dissolves and/or dis perses (as hydrosols) organic compqnds and associated metals.

1 N ammonia and 0.1 N sodium hydroxide. Both of these extractants are strong bases which will form anionic hydroxide metal complexes and will dissolve or disperse some types of organic materials (operationally defined "humic substances") and their associated metal complexes. Ammonia (present as ammonium hydroxide) may also complex metals directly, and NaOH will attack silica and clays.

Preliminary experiments were conducted to determine the optimum time of extraction, strength of extractant and extractant volume/sediment mass ratios for ammonia and NaOH.

The concentrations of Fe, Cu, Ag and humic substance extracted by both NaOH and ammonia increased rapidly with time toward a plateau at approximately 3 d, with only a slight increase in concentration occurring between 3 d and 7 d (Fig. 4-1). Concentrations of Zn and Mn in the basic extracts increased slowly over the entire period of the experiment. The pattern of extraction as a function of extractant molarity was similar for ammonia and NaOH. Humic substances, Cu, Fe and Mn all were maximally extracted at intermediate strengths of both extractants, but the peaks of extractability did not coincide (Fig. 4-2). In contrast the extraction of Zn increased dramatically in the higher pH extractants. Although variations in the ratio of extractant volume/sediment weight did not affect the action of other extractants, it was a variable of interest for ammonia and NaOH. A dilution ratio of 22 resulted in a 3 to 4 fold greater extraction of Fe, Mn and Zn than did a ratio of 7; but similar quantities of Cu and humic substances were extracted at the two dilutions.

The different effects on different metals, of time, extractant molarity and variations in the dilution factor suggested no one procedure was optimal for extraction of all metals by NaOH and ammonia. One week extractions with 1 N ammonia and 0.1 N sodium hydroxide were chosen so as to optimize the extraction of the humic substance and the metals most soluble in the basic extracts--Cu and Ag. All sediments were extracted wet and the dilution factor was kept as near 15:1 as possible.

Concentrations of humic substances extracted by ammonia and NaOH were measured by light absorbance (450nm) in 1 cm cells containing the filtered extract. Calibration with sediments from the Looe estuary indicated 1 unit of absorbance/g sediment equalled 0.0005 g humic acid/g sediment (determined by repeated precipitation at pH 1 and weighing the residue). Addition of $FeCl_2$ up to twice the highest Fe concentration found in any extract did not affect absorbance, suggesting all the absorbance was due to organic materials.

Carbon Determination

Calcium carbonate. Concentrations of Ca were determined in the HCl extract by atomic absorption spectrophotometry, with 1 percent lanthanum chloride added to samples and standards. Carbonate was determined as inorganic carbon on a carbon analyzer.

Total organic carbon (TOC). Organic carbon was determined on a carbon analyzer by the difference between total carbon and inorganic carbon. The TOC results were compared with concentrations of organic materials determined from either ashing at 400° C for 6 hours (Jaffe and Walters, 1977) or ashing for 1 hour at 500° C (Dean, 1974b). The comparison indicated the former method is a satisfactory substitute for TOC analyses when an organic carbon analyzer is not available (Fig. latter method removes approximately 3 percent of the sediment weight that is not organic carbon.

Particle Size

Particle size distributions were determined as "percent particles less than 14 µm" by settling for 15 minutes in an Andreason pipette in water of less than 0.3 salinity.

RESULTS

SAMPLE COLLECTION

Extraction of metals from surface, oxidized sediments differed substantially from extractions of subsurface, reduced sediments. Significantly higher concentrations of Cu, Pb, Zn and Co were extracted from the oxidized sediments by all extractants than from the reduced sediment (Table 4-2). The difference in metal extractability by acetic acid from the two sediments generally followed the order Cu >> Pb > Zn = Co (Table 4-3). The concentration of Fe removed by ammonium acetate, HCl and acetic acid was higher in the subsurface sediments than in surface sediments, while Fe solubility in oxalate, pyrophospate and ammonia was higher in the surface sediments. Manganese was not included in the comparisons because total Mn concentrations were lower in subsurface than in surface sediments.

Substrate Characterizations

Organic materials, carbonates, particle size

Concentrations of total organic carbon in the oxidized sediments varied from 1.14 percent to 6.31 percent (Table 1). Sediment particle size distributions (after sieving the sediment through 100 μm mesh) ranged from 7.0 percent to 47.7 percent less than 14 μm in diameter. Organic carbon and particle size were significantly correlated among all stations (Chapter 6).

Ammonia and NaOH were used to separate the "humic substance" fraction of the organic substrate. Ammonia and NaOH removed similar quantities of humic substances from the sediments (as measured by absorbance).

Concentrations of humic substances were weakly correlated ($r = 0.46$) with concentrations of total organic carbon. At the station where the weight of the humic materials was calibrated against absorbance, the humic substances constituted 11 percent of the total organic material in the sediment (assuming C/total organics = 0.5). If this calibration held throughout the study area the humic materials extracted by ammonia and NaOH varied from 0.5 percent (Bristol Channel; Restronguet Creek) to 11 percent (East Looe) of the organic matter among the different stations.

A substantial fraction of the humic substances in the oxidized sediments appeared to be sorbed to amorphous Fe oxides (as suggested by the work of Sholkovitz, 1976). The humic substances most closely associated with the iron oxides were not extracted in a single treatment with ammonia or NaOH. Sediment subsamples from 4 stations were first extracted with acid ammonium oxalate to remove amorphous Fe, then extracted with ammonia. In all four cases the concentrations of humic substances extracted from the oxalate pretreated subsamples were higher than concentrations removed from subsamples extracted without pretreatment (Table 4-4).

Concentrations of $CaCO_3$ ranged from <1 percent to 30 percent of sediment weight (Table 4-1). Concentrations of Ca correlated strongly ($r=0.98$) with concentrations of inorganic carbon (fig. 4-4). The ratio of Ca/inorganic C, calculated from the regression, varied from 3.30 at 1 percent Ca, to 3.42 to 10 percent Ca. The close agreement with Ca/inorganic C in pure $CaCo_3$ (3.34) indicated the bulk of both the Ca and the CO_3 in the sediment was $CaCo_3$.

Iron Oxides

Acid ammonium oxalate removed the highest proportion of total Fe (median = 35.9 percent) from sediments among the techniques tested, although at most stations the concentration of Fe removed by 1 N HCl was similar to that in the oxalate extract (Table 4-5). The median concentration of Fe removed by 25 per cent acetic acid was less than half of that removed by oxalate. Earlier work using only Tamar sediments (Bryan, unpublished data) indicated a mixture of 0.1 N hydroxylamine in 25 percent acetic acid at room temperature (Chester and Hughes, 1967) extracted slightly more Fe from oxidized sediments than did acetic acid alone (19.8 percent *vs*. 11.6 percent, respectively), but considerably less than the 35.9 percent we found extracted by oxalate. Extraction of Fe by hydroxylamine hydrochloride in dilute nitric acid was severely affected by neutralization at stations where carbonates were present. In the upper Tamar, where carbonates were very low, the hydroxylamine-nitric acid mixture extracted an average of 6.2 percent of the total Fe (comparable to the 8.9 percent of total Fe removed from low carbonate San Francisco Bay sediments by hydroxylamine-nitric acid).

Correlations between weak acid extractable (HCl, acetic acid), and total Fe were substantially weaker than correlations between the acid-extractable fraction and total concentrations of other metals (Table 4-6). The concentration of Fe insoluble in HCl (total Fe minus HCl-soluble Fe) varied little among stations within estuaries and was much less variable than acid-soluble Fe among all stations. Acid-soluble Fe varied 11 fold in concentration over the entire study area (0.16 to 1.73 percent of sediment weight). Acid insoluble Fe varied only 4 fold (0.77 to 3.14 percent). If the Plym estuary, where sediments were primarily china clay waste, were excluded the variation in HCl-insoluble Fe was only 1.8 fold (1.71-3.14 percent).

Manganese oxides.--From the comparison, chemical extractions appeared to separate 3 fractions of Mn: total Mn, a Mn fraction similarly soluble in most acids and reducing agents, and an ammonium acetate-soluble fraction of Mn. A similar concentration of Mn was removed from sediments at most stations by the different acids (HCl, acetic acid, acid ammonium oxalate). In the Tamar estuary (Bryan, unpublished) a proportion of Mn similar to that removed by acetic acid (42 percent) was removed by a mixture of acetic acid/hydroxylamine (46 percent) and hydroxylamine in nitric acid (42 percent). Among all stations the median proportion of Mn extracted by acetic acid was slightly higher than that of the other extracts (Table 4-5), but this reflected a very high acetic acid solubility of Mn (100 percent of total) in the Bristol Channel. Carbonate neutralization had substantially less effect on hydroxylamine extraction of Mn than was observed for any other metal, emphasizing the importance of reduction, rather than pH, in the extraction of Mn by hydroxylamine.

Substantial concentrations of Mn were solubilized by neutral ammonium acetate (up to 21.6 percent of the total Mn; Table 4-5), and the ammonium acetate-extractable Mn correlated more weakly with the total Mn in the sediment than did the acid-soluble Mn fractions (Table 4-6).

As with Fe, the concentrations of Mn insoluble in HCl varied less widely (6.5 fold; 79-515 µg/g) among stations than did HCl-soluble Mn (17.5 fold; 58-1013 µg/g).

Extraction of Trace Metals

Alkaline extraction

Extraction of metals associated with the humic fraction of the organic substrate was compared among ammonia, sodium hydroxide and sodium pyrophosphate. Pyrophosphate extracted less Cu, but more Fe, Mn and Zn from the sediments than did either ammonia or NaOH (Table 4-5). All three extractants have been used to estimate concentrations of organically complexed Fe. However, an average of 10.5 times more Fe was extracted by pyrophosphates than by NaOH or ammonia. At one station (not included in calculations of the average) the difference in Fe extractability was 250 times. The concentration of Fe extracted by pyrophosphate was similar to Fe concentrations removed by NaOH after pretreatment of samples with oxalate to remove amorphous Fe (Table 4-4, 4-5). However, the concentrations of Cu removed by NaOH after pretreatments with oxalate were not low like the Cu concentrations extracted by pyrophosphate.

A high proportion, relative to other metals, of total Cu and Ag was removed from the sediments by NaOH and ammonia (Table 4-5). The importance of humic substances as a substrate for binding Cu and Ag was emphasized by the very high concentrations of these metals extracted per g humic material (Table 4-7).

Ammonia appeared to selectively extract the fraction of hydrochloric acid-soluble copper that was not extracted by acetic acid, i.e., the sum of acetic acid-Cu and ammonia-Cu was similar to the concentrations of HCl-Cu (Table 4-8). Strong deviations from this relationship were observed at 2 stations where the samples included some reduced sediments due to sampling difficulties (thus inhibiting the extraction of Cu by acetic acid) and at stations near the freshwater-seawater interface in 3 estuaries. Excluding the 5 anomalous stations the regression of HCl-Cu vs acetic acid Cu + ammonia-Cu had a correlation coefficient (r) of 0.98, a slope of 0.98 and an intercept of 1.07 μg/g Cu. Hydrochloric acid desorbs Cu from humic materials and dissolves most inorganic substrates. At most stations ammonia extracted the humic substances containing the HCl-soluble Cu. The carbonates and amorphous oxides dissolved by acetic acid also appeared to contain only HCl-soluble Cu (although desorption of Cu from non-humic organic material by acetic acid cannot be discounted). It was not possible to objectively test the selectivity of ammonia and acetic acid with other metals because metal concentrations extracted by ammonia were such a small proportion of the HCl-soluble fraction.

The concentrations of Ag, Cu and Zn extracted by the alkaline extractants were strongly correlated with total Ag, Cu and Zn in the sediments, but were not correlated with the abundance of sedimentary humic materials (Table 4-9). The proportion of Cu and Ag extracted by ammonia declined with total Cu enrichment in the sediments (slopes <1) while the extractability of Zn increased with Zn enrichment. In contrast, the alkaline-soluble fraction of Mn and Fe correlated strongly with the concentration of humic substances in the sediment (Table 4-9); fig. 4-5a,b) and insignificantly with total Mn and Fe. The relationship between Mn and humic materials was not as strong as the Fe-humic substance correlation.

<u>Ammonium acetate</u>.--As observed by others (Cox and Kamprath, 1972; Brannon, et al., 1976; Serne, 1977; Khalid, et al, 1977), the very low concen-

trations of Fe, Cu, Pb and Co extractable by ammonium acetate from most stations suggested little of these metals usually occurred in an exchangeable form in sediments (Table 4-5). Elevated concentrations of Fe, were extracted by ammonium acetate from a few stations near the freshwater-seawater interface of a few estuaries (although this effect was not observed in all estuaries studied), and elevated levels of Cu were extracted from very sandy sediments. Relative to the other metals, a high proportion of Zn was extracted by ammonium acetate, and the ammonium acetate-soluble fraction of Zn correlated more strongly with total Zn (Table 4-6) than was the case for the other metals.

Acid extracts.--Hydrochloric acid removed a greater concentration of trace metal from the sediments than the other acid extractants (Table 4-5). This was not surprising since 1 N HCl is highly effective in dissolving oxides and should have been the most effective extractant in desorbing metals from undissolved substrates. Greater than 60 percent of the Ag, Cu, Pb and Zn in the oxidized sediments was removed by HCl, although only 38 percent of the Co was HCl-soluble. (The latter is consistent with an apparent association of Co with HCl-insoluble forms of Fe oxide- (Chapter 5).
In contrast to Fe and Mn, the HCl-insoluble concentrations of the trace metals were not constant, varying to the same degree as the acid-soluble concentrations.

Both HCl and acetic acid extracted substantially more Cu and Pb than acid ammonium oxalate, but all three extractants removed roughly similar concentrations of Co and Zn. In the Tamar sediments (Bryan, unpublished data) the mixture of a reducing agent with acetic acid (i.e., acetic acid/hydroxylamine) resulted in an extraction similar to acetic acid alone for Cu and Zn (34 vs. 44 percent and 70 vs. 66 percent, respectively), but extracted more Pb than acetic acid (68 vs. 42 percent).

Metal concentrations extracted by HCl and acetic acid were highly correlated with the total metal concentrations in the sediment (Table 4-6). The slopes of the relationships for Co, Cu, Pb and Zn were greater than 1, indicating the proportion of the metal that was extractable was higher where total metal concentrations were enriched. The extractability of Ag by HCl declined with Ag enrichment (slope <1). The concentrations of Zn and Co extracted by acid ammonium oxlate were also correlated closely with total metal concentrations. However, those metals least soluble in oxalate (Pb and Cu), showed the weakest correlation between oxalate-metal and total metal concentrations.

DISCUSSION

Extracting oxidized sediments

Chemical characterization of the oxidized sediments in estuaries will be grossly affected by procedures used to collect and store sediments, prior to extraction. Since the oxidized sediments in most estuaries lie in a thin layer at the sediment-water interface, samples collected by procedures (grabs, mixed cores, etc.) which include subsurface sediments will show a significantly lower extractability of trace metals than would be observed if only surface sediments were collected. Storage procedures which allow sediments to become anoxic will yield similar results. A comparison with our results indicates that most studies which have attempted, to date, to characterize sediment-bound metal forms with chemical extractants have considered anoxic sediments (Gupta and Chen, 1975; Serne, 1977; Brannon, et al., 1976). The suppression of metal extractability from our subsurface sediments follow the order of metal-sulfide solubility in seawater (Krauskopf, 1956), indicating (not surprisingly), that metal-sulfide forms dominate in the subsurface. The increased acid-solubility of Fe in the subsurface sediments is also consistent with the presence of predominantly FeS. Iron hydroxide is more crystalline than iron sulfide (Jenne, 1977), and thus less acid-soluble; although subsurface forms of Fe do seem slightly less susceptible to extraction by complexing agents than Fe oxides.

Extraction of Metals

Hydrochloric acid is the best choice of an extractant for removing a variety of sorbed "non-detrital" metal forms from oxidized sediments (Agemian and Chau, 1977; Malo, 1977). Metals (e.g., Cu), strongly bound to organic materials such as humic substances are desorbed by HCl, and HCl is highly effective in dissolving carbonates (provided a sufficiently strong solution is employed), Fe, and Mn. Acetic acid and acetic acid-hydroxylamine have been employed by others to extract "non-detrital" (Loring, 1976) or "non-lattice" metals (Chester, et al, 1976). Our evidence suggests these extractants are less effective in dissolving Fe oxides than either HCl or acid ammonium oxalate (thus possibly missing metal sorbed to less amorphous Fe forms) and do not desorb metals strongly complexed by some organic materials (e.g. Cu). Despite its strong dissolution of oxides, acid ammonium oxlate extracts even less of the metals which complex most strongly with organics (Cu and Pb) than does acetic acid. Either desorption of Cu and Pb is ineffective at pH 3.3, or some resorption of metals to undissolved substrates (or precipitation in the case of Pb) occurs during the extraction.

The fraction of metal removed from sediments by the weak acid extractants was a constant function of the total metal in the sediment. Although the proportion of weak acid-soluble metal increased with enrichment (except for Ag), the correlation with total metal, and the high variability of metal concentrations insoluble in weak acid, suggested no acid extractant successfully removed all the non-lithogenic metal. The formation of sedimentary particles by successive layering of adsorption substrates (Jenne, 1977), the solid state diffusion of metals into the interstices of substrates such as manganese oxides (Jenne, 1977), and the movement of metals into lattices after adsorption (Farrah and Pickering, 1978) may limit the efficiency of acid extractions. A single extraction with a weak acid may aid in assessing the biological availability of some metals from sediments (Luoma and Bryan, 1978; in prep.b) but out data suggests no extractant will satisfactorily define the anthrophogenically-derived metal in a sediment (as proposed, for example, by Agemian and Chau, 1977).

Extraction of Humic Substances and Associated Metals

The quantity of humic substance extracted by a single treatment with NaOH or ammonia comprised only a small fraction of the total organic material in the estuarine sediments and less than 2 percent of the total sediment weight. The high proportion of total Cu and Ag extracted with this quantitatively small humic substrate is evidence of the strong reaction of humic substances with at least some trace metals.

Several lines of evidence suggested ammonia and NaOH may selectively extract humate-bound Cu and Ag: 1) the concentration of Ag (where measured) and Cu in these extractants closely followed the concentration of organics extracted as a function of time; 2) Variations in sediment mass/ extractant volume had similar effects on Cu and organics; and 3) ammonia selectively extracted the fraction of HCl-soluble Cu not attacked by acetic acid. However, the extraction of Cu as a function of pH varied slightly from the extraction of humic substances; and Cu concentrations in the NaOH and ammonia were a function of total Cu, not extractable humic substances, leaving open some possibility that Cu and Ag removal was partly influenced by a mechanism other than simultaneous dispersion or dissolution with the organic material.

Ammonia and NaOH extractions obviously did not provide reliable estimates of the concentration of Zn complexed by humic substances. Speciation models (E.A. Jenne and J. Ball, unpublished data) indicate an increasing precipitation of Zn at lower molarities, and the increasing formation of solute hydroxyl complexes of Zn at higher molarities in NaOH. Precipitation of Zn may have stripped weakly complexed Zn from organic ligands in dilute solutions of NaOH and ammonia (resulting in an underestimate of humate-bound Zn); or solute hydroxyl complexation desorbed Zn from substrates other than organics in the more alkaline solutions (resulting in an overestimate of humate-bound Zn). In either case, the results indicated undefined chemical interactions within the extractant played a dominant role in Zn dissolution by the alkaline extractants.

The addition of the complexing agent pyrophosphate to the alkaline extraction greatly enhanced the concentration of Fe extracted, compared to NaOH and ammonia, and reduced the extraction of Cu. In San Francisco Bay sediments sodium pyrophosphate extracted slightly higher concentrations of humic substances than did NaOH (Luoma, et al, unpublished). The concentrations of Fe, Cu and Zn extracted by pyrophosphate did not correlate significantly with the concentration of humic substances in the Bay; but pyrophosphate-Cu and Zn did correlate significantly with pyrophosphate-Fe (Luoma and Jenne, in prep.). The latter relationship suggests pyrophosphate extracts some trace metal-reactive amorphous Fe oxide from sediments (probably by complexation) - a conclusion suggested by Jenne, (1977). In contrast, the correlation of Fe and Mn with humic substances in ammonia and NaOH suggests these extractants remove only humate-bound Fe and Mn from sediment.

Extraction of Oxide Substrates

The efficiency with which Fe was removed from oxidized sediments varied widely among extractants, reflecting the action of each extractant upon the continium of amorphous to crystalline Fe oxide forms occurring in such sediments. Acid ammonium oxalate and HCl removed the largest quantity of Fe. Statistical correlations indicated this was the fraction of the Fe oxide most strongly involved in most trace metal interactions (Chapter 5).

The combination of low pH and reduction by hydroxylamine-nitric acid removed a proportionately small, but possibly significant concentration of Fe from the oxidized sediments. The dissolution of hydroxylamine-Fe is highly pH dependent (as evidenced by the strong effect of carbonate neutralization) and is strongly influenced by the age of the Fe oxide (Luoma and Bryan, 1979). Moreover, the Fe extracted by hydroxylamine-nitric is not associated with Mn oxide, since extraction of the latter is not greatly affected by pH. The suggestion that only very amorphous Fe is soluble in hydroxylamine-nitric acid and the strong correlation of Zn with hydroxylamine-Fe in San Francisco Bay sediments (Luoma and Bryan, 1979) raise the possibility that hydroxylamine-nitric acid may extract freshly precipitated Fe oxide active in coprecipitation of trace metals at redox interfaces (Hem, 1977).

Statistical evidence suggests Mn occurred as both Mn oxide and Mn carbonate in the sediments of these estuaries (Luoma and Bryan, in prep. b).

However, no separation of the two Mn forms was evident in HCl, acetic acid, acid ammonium oxalate or hydroxylamine-nitric acid. Apparently all four extractants were effective at reducing Mn oxides and of sufficent acidity to dissolve Mn carbonate. All four extracts also removed a similar quantity of Mn from the sediments. In any scheme, extraction of approximately 50 percent of the Mn substrate will occur whenever a reducing environment and/or low pH are employed. The small fraction of Mn removed by ammonium acetate apparently did not include any Mn carbonate (Chapter 5). The different forms of ammonium acetate-Mn and acid-soluble Mn may explain the different effects of the two on the bioavailability of Zn (Chapter 8).

The above discussion indicates that selective separation of amorphous Fe oxides from amorphous Mn oxides (and separation of their associated trace metals) in oxidized sediments is not accomplished by the chemical extractants we have considered. Evidence from other studies indicates separation of metals bound to Fe and Mn oxides from metals bound to other substrates in oxidized sediments may also be difficult using low pH chemical extractants (Chapter 3, Guy, et al, 1978). We have shown that acetic acid apparently separates a fraction of HCl-soluble Cu not bound to humic materials in most (but not all) oxidized estuarine sediments. The desorption of Cu from humic substances by acetic acid is apparently minimal, due to the very strong association of Cu with organic ligands. For metals which complex less strongly with organic materials selective extraction of inorganic forms by acetic acid is unlikely.

We conclude that chemical extractants which selectively remove metals from specific substrates in oxidized sediments are rare. This is not surprising given the complexity of the substrates involved, the differences among metals in strengths of metal-substrate associations and the basically similar modes (i.e., desorption and/or substrate destruction) by which most extractants remove metals. At best, extraction schemes may reduce the number of substrates affected to less than the total number in the sediment. Results of extractions must be interpreted with such limitations in mind.

The most useful role of chemical extractions may be characterization of the substrates involved in trace metal sorption. Determining what forms of different substrates are most important in trace metal sorption in oxidized sediments is essential to realistic modeling of metal partitioning in such sediments (even if "form" is only defined operationally) as is quantifying the relative abundances of different substrate forms in different environments. Statistical analyses of relationships between operationally defined forms of both metals and substrates may also be a valuable aid in development of conceptual models of metal partitioning. Such statistical analyses are the subject of Chapter 5.

SUMMARY

Chapter 4

The thin layer of oxidized sediment at the sediment-water interface plays an important role in the chemistry of trace metals in estuaries. Characterization of oxidized sediments from 19 estuaries in south and west England by acidic, alkaline and neutral chemical extractants indicate:

(1) Collection procedures which allow mixing of oxidized sediment with reduced sediments will greatly affect the results of metal extraction from sediments.

(2) Among acidic extractants, 1 N HCl is preferable to either acetic acid or acid ammonium oxalate for a partial fractionation of metals. However, it is unlikely that any extract specifically removes "adsorbed", "non-lithogenic" or "anthropogenically-derived" metal.

(3) Either 0.1 N NaOH or 1 N ammonia are preferable to sodium pyrophosphate in extracting metals largely from humic substances. Pyrophosphate extracts 10-fold more Fe than NaOH or ammonia; and correlations with Zn and Cu indicate pyrophosphate-Fe is largely amorphous, oxidic Fe.

(4) Selective separation of metals by chemical extractants from specific binding sites (substrates) in oxidized sediments is rare. Results of extractions must be interpreted with the limited selectivity of the extractants in mind.

Table 4-1 Representative data from selected stations in 19 estuaries of southwest England. Upper reach - salinity - 10‰, lower reach - salinity [illegible] Geographic location of each estuary in Luoma and Bryan (1978). *Concentration in HCl.

Estuary	Reach	Organic Carbon (%)	Inorganic Carbon (%)	Humic Material (absorbance/g) Ammonia	Particle size (% < 14μ)	------Fe------ Total	Amorphic (percent)	Mn Total (μg/g)	----Total Metal Concentrations (μg/g)------ Ag	Cd*	Co	Cu	Pb	Zn
Bristol Channel	lower	3.92	1.36	1.77	27.3	2.60	0.53	869	0.38	0.20	17.4	39	99	278
Torridge	lower	2.04	1.37	4.02	24.9	2.32	0.48	512	0.24	0.37	12.2	26	50	137
Camel	mid	2.17	2.78	6.20	19.5	2.34	0.54	512	0.49	0.33	10.9	88	53	190
Gannel	upper	1.40	1.69	3.46	14.0	2.71	0.62	1259	1.75	0.61	36.1	176	1602	812
	lower	1.98	3.00	3.68	15.2	2.42	0.44	1314	0.65	0.32	25.0	109	422	346
Hayle	lower	2.01	0.91	4.25	15.4	3.61	1.55	767	3.60	0.91	24.7	1170	188	943
Restronguet Creek	mid	2.49	0.10	1.79	7.0	4.84	2.65	680	4.52	0.83	23.2	3052	323	3544
Fowey	upper	2.46	0.007	17.35	14.9	2.23	0.32	360	0.52	0,32	9.1	142	75	207
	lower	2.22	0.12	8.75	13.9	2.19	0.34	370	0.31	0.27	8.9	120	70	180
Looe (West)	upper	2.34	0.06	18.90	15.8	3.13	0.36	534	1.61	0.24	12.9	64	408	176
	lower	1.26	0.41	5.06	15.9	2.68	0.25	410	0.26	0.16	10.4	43	123	131
Looe (East)	upper	2.24	0.05	20.03	10.0	3.15	0.33	502	1.71	0.35	14.2	35	92	186
	lower	1.14	1.02	3.55	18.4	2.57	0.20	390	0.21	0.17	10.3	37	64	108
Tamar	upper	4.32	0.04	20.66	27.8	3.23	1.02	944	1.22	1.03	26.9	410	186	456
	lower	3.10	0.02	11.08	27.4	3.63	0.86	671	0.44	0.45	18.0	282	179	363
Tavy	mid	2.77	0,09	13.54	35.1	3.79	0.72	616	0.35	0.42	18.0	314	190	370
Plym	upper	2.29	0.05	5.43	21.8	1.07	0.35	181	0.52	1.86	4.68	73	177	68
Erme	mid	3.85	0.85	19.22	22.0	3.25	0.71	575	0.21	0.19	13.5	31	66	141
Poole Harbor	lower	3.78	0.58	6.58	34.1	3.57	0.87	180	3.81	10.80	15.6	98	145	386
Beaulieu	upper	2.94	0.05	17.3	46.5	2.92	1.55	415	0.29	0.06	16.0	24	57	142
Test	upper	6.31	2.23	39.2	47.7	2.53	0.86	301	0.50	1.52	15.2	42	171	510
Itchen	mid	4.51	1.25	12.1	35.8	3.09	0.97	245	1.51	0.63	9.4	131	253	266
Hamble	mid	2.02	0.58	6.98	35.5	2.92	1.55	415	0.29	0.06	16.0	24	57	142
Elorn	upper	3.28	1.15	9.29	35.8	2.50	0.78	186	0.56	1.32	7.0	64	85	309
	lower	2.31	2.00	5.69	29.7	2.31	0.69	216	0.50	0.26	8.1	39	67	175

Table 4-2 The ratio surface concentrations/subsurface concentrations for five metals as extracted from sediments of seven estuaries (mean ± one standard deviation). Manganese was not considered because total Mn concentrations in surface sediment were greater than in subsurface sediments.

	AmAc	HAC	HCl	Oxalate	Pyrophosphate	Ammonia
Co	bd	2.2±.0.7	9.9±14.1	2.1±0.5	bd	bd
Cu	25+35	382±500	85±80	19.7±13	17.6±22	5.4±5.7
Fe	0.2±0.2	0.8±.0.2	0.4±0.1	1.3±0.3	1.3±0.7	4.4±4.6
Pb	bd	17.5±21.0	8.8±15.9	2.1±3.2	28.1±2.4	bd
Zn	3.8±3.7	2.5±1.1	2.5±3.0	1.2±0.3	5.7±3.3	1.0±0.3

bd = Below detection

Table 4-3 A comparison of Cu, Pb, Zn, Co and Fe extraction from oxidized surface sediments and anoxic subsurface sediments in three estuaries with varying metal enrichment.

	TOTAL METAL		ACETIC ACID METAL	
	Surface Sediment	Subsurface Sediment	Surface Sediment	Subsurface Sediment
<u>Copper</u>				
Restronguet	3052	3648	1638	10
Tamar	282	306	95	0.04
Looe	36	49	5	0.19
<u>Lead</u>				
Restronguet	323	385	126	2.0
Tamar	179	222	97	7.5
Looe	78	117	48	8
<u>Zinc</u>				
Restronguet	3544	3110	2627	901
Tamar	363	386	222	101
Looe	115	137	40	25
<u>Cobalt</u>				
Restronguet	23.2	27.3	12.6	8.0
Tamar	18.0	16.5	6.3	3.1
Looe	11.1	11.1	0.8	0.3
<u>Iron</u>				
Restronguet	48,383	46,531	10,802	12,096
Tamar	36,330	37,031	3,869	6,438
Looe	27,739	28,000	919	1,086

Table 4-4 A comparison of the extraction of humic substances, Cu and Fe by ammonia from untreated sediments and from sediments pre-treated with ammonium oxalate to remove amorphic Fe.

Station	Concentration of humic substance (abs./g)		ammonia-extractable metal conc.			
			Cu (ug/g)		Fe (ug/g)	
	untreated	after pre-treatment	untreated	after pre-treatment	untreated	after pre-treatment
Poole Harbor	6.85	36.6	19.9	46.7	10	246
Beaulieu R.	22.3	31.1	13.0	7.3	156	313
Looe I	4.8	11.3	4.9	5.4	26	149
Looe II	3.6	8.7	4.2	8.1	19	126

Table 4-5 The range of metal concentrations and the percent of total metal extracted by various extractants.

Extractants	Ag	Co	Cu	Fe	Mn	Pb	Zn
Concentrated nitric acid							
range of concentrations (μg/g)	<0.2-10.0	3.1-36.1	17-3079	2.00-[d] 4.84	138-1448	41-6006	95-3544
proportion of total (%)	100	100	100	100	100	100	100
Hydrochloric acid							
range of concentrations (%)	0.10-1.69	1.4-14.9	8-1860	0.16[d]-1.73	58-1013	22-4760	43-2656
proportion of total: range (%)	6.8-100	9.3-65.6	38.1-91.0	7.5-39.6	15.3-78.1	51.9-92.0	35.1-92.3
median (%)	63.4	37.5	64.6	23.6	46.7	72.0	63.7
Acetic acid							
range of concentrations (μg/g)		0.3-12.6	3-1638	0.10-[d] 1.08	40-1092	13-4291	40-2627
proportion of total: range (%)		1.0-64.0	5.5-90.0	3.9-25.3	10.7-100	20.0-73.6	29.0-91.1
median (%)		32.6	47.8	14.2	55.4	46.8	60.1
Acid ammonium oxalate							
range of concentrations (μg/g)		0.7-15.9	<0.2-619	0.20-[d] 279	47-958	1-505	30-2786
proportion of total: range (%)		9.5-63.6	0.1-62.5	7.9-63.9	14.1-67.7	0.7-42.5	24.1-83.4
median (%)		37.0	31.3	35.9	40.9	21.6	53.8
Hydroxylamine hydrochloride-nitric acid[a]							
range of concentrations		0.3-13.4	0.2-379	180-2185	37-958	1-120	15-1555
proportion of total: range (μg/g		6.1-77.6	0.1-48.8	0.70-14.4	8.3-66.5	0.8-70.8	10.6-70.1
median (%)		41.9	24.4	7.6	37.4	35.8	40.4

Table 4-5 - continued

Extractant	AG	Co	Cu	Fe	Mn	Pb	Zn
Ammonium acetate							
range of concentrations (μg/g)		<0.1-2.6	<0.1-131	<1-347	3-245	<0.8-443	0.7-295
proportion of total: range (%)		1.2-3.2	0.4-4.9	0.005-0.40	2.4-21.6	0.4-7.3	1.0-10.7
median (%)		4.2	2.5	0.03	12.0	3.8	5.9
Pyrophospate[b]							
range of concentrations (μg/g)		<0.1-9.3	0.8-202	109-1618	14-159	1-13	5-753
proportion of total: range (%)		0.8-44.1	0.4-9.7	0.4-4.5	3.5-27.2	0.8-11.7	3.8-27.2
median (%)		6.2	5.1	2.5	15.6	6.3	15.5
Ammonia							
range of concentrations	<0.02-0.77		1-399	0.5-642	0.1-17.5		0.2-137
proportion of total: range (%)	9.0-45.0		7.1 33.3	0.02-0.9	0.1-1.2		0.1-7.6
median (%)	27.0		20.2	0.46	0.61		3.8
Sodium hydroxide							
range of concentrations	0.07-0.51		6.2-360	1.0-286	0.3-6.8		0.33-11.5
proportion of total: range (%)	8.0-41.4		8.9-54.2	0.004-1.27	0.05-1.04		0.17-2.30
median	24.7		31.6	0.64	0.55		1.24

a = hydroxylamine data includes 18 samples unaffected by carbonates.
b = pyrophosphate extraction were conducted on 17 samples
c = sodium hydroxide extractions were conducted on 27 samples
d = concentration in percent

Table 4-6 Correlation of extractable metal concentration with total metal concentration. r = correlation coefficient. b = slope of regression. n = 45 for all except pyrophosphate where n = 17 and Ag-ammonia, where n = 21. a = insignificant correlation, p > 0.05. nd = not determined.

Extractant		Ag	Co	Cu	Fe	Mn	Pb	Zn
Hydrochloric Acid	r	0.70	0.77	0.88	0.62	0.84	1.00	1.00
	b	0.53	1.66	1.08	1.38	1.39	1.06	1.16
Acetic Acid	r	nd	0.81	1.00	0.67	0.83	1.00	1.00
	b		2.04	1.11	1.61	1.43	1.12	1.21
Oxalate	r	nd	0.86	0.52	0.69	0.81	0.69	1.00
	b		1.68	0.80	1.68	1.35	1.04	1.24
Ammonium Acetate	r	nd	0.33	0.55	0.40	0.45	0.62	0.90
	b		0.86	0.81	2.08	0.87	1.09	1.49
Pyrophosphate	r	nd	0.11[a]	0.90	0.22[a]	0.48	0.67	0.95
	b		0.23	0.96	0.41	0.89	0.82	1.17

Table 4-7 Extraction of metals from sediment in upper E. Looe Estuary with 1 N ammonia. In March extractable humic substances were 1.12 percent of sediment weight. In August humic substances were 0.34 percent of sediment weight.

	μg/g				
	Ag	Cu	Fe	Mn	Zn
March 1977					
Total metal	1.71	34.5	31500	502	186
Ammonia-soluble metal	1.00	9.9	149	1.56	2.30
Concentration associated with humics (μg/g humic substance)	89	881	13261	216	205
August 1977					
Total metal	1.5	36.0	24390	433	149
Ammonia-soluble metal	0.174	5.7	33.3	0.76	0.34
Concentration associated with humics* (μg/g humic substance)	52	1696	9910	226	101

*Weight of humics estimated from absorbance of extract. For upper E. Looe sediment in August. 1 absorbance unit per g sediment (1 cm cuvette)= 0.0005g/ml of humics.

Table 4-8 A comparison of HCl-Cu with the sum of (acetic acid-Cu) + (ammonia-Cu) at selected stations and along the salinity gradient of 4 estuaries.

Estuary		A acetic acid - Cu (µg/g)	B ammonia - Cu (µg/g)	(A)+(B) (µg/g)	HCl - Cu (µg/g)
Plym		25.1	23.7	48.8	49.1
Erme		9.3	8.2	17.5	14.5
W. Looe		13.7	12.6	26.3	27.8
Fal	(mouth)	1423	367	1790	1770
		1638	399	2037	1823
	(head)	426	240	666	1860**
Tamar	(mouth)	154	59	213	194
		95	50	145	168
		154	55	209	233
		127	53	180	253
		165	60	225	260
		149	47	196	250
	(head)	700	237	937	605**
Fowey	(mouth)	26	13	39	49
		22	15	37	44
		23	17	37	50
		33	20	53	50
		35	24	59	65
	(head)	11	18	29	55**
Gannel	(mouth)	28	11	39	41
		35	11	46	41
		71	30	101	87
	(head)	135	49	184	197
Beaulieu		10	7	17	12
Poole		16	23	39	90**
Hayle		493	121	614	645
Bristol Channel		17	5	22	23

** Stations with substantial deviations between HCl-Cu and Acetic Acid Cu + Ammonia-Cu.

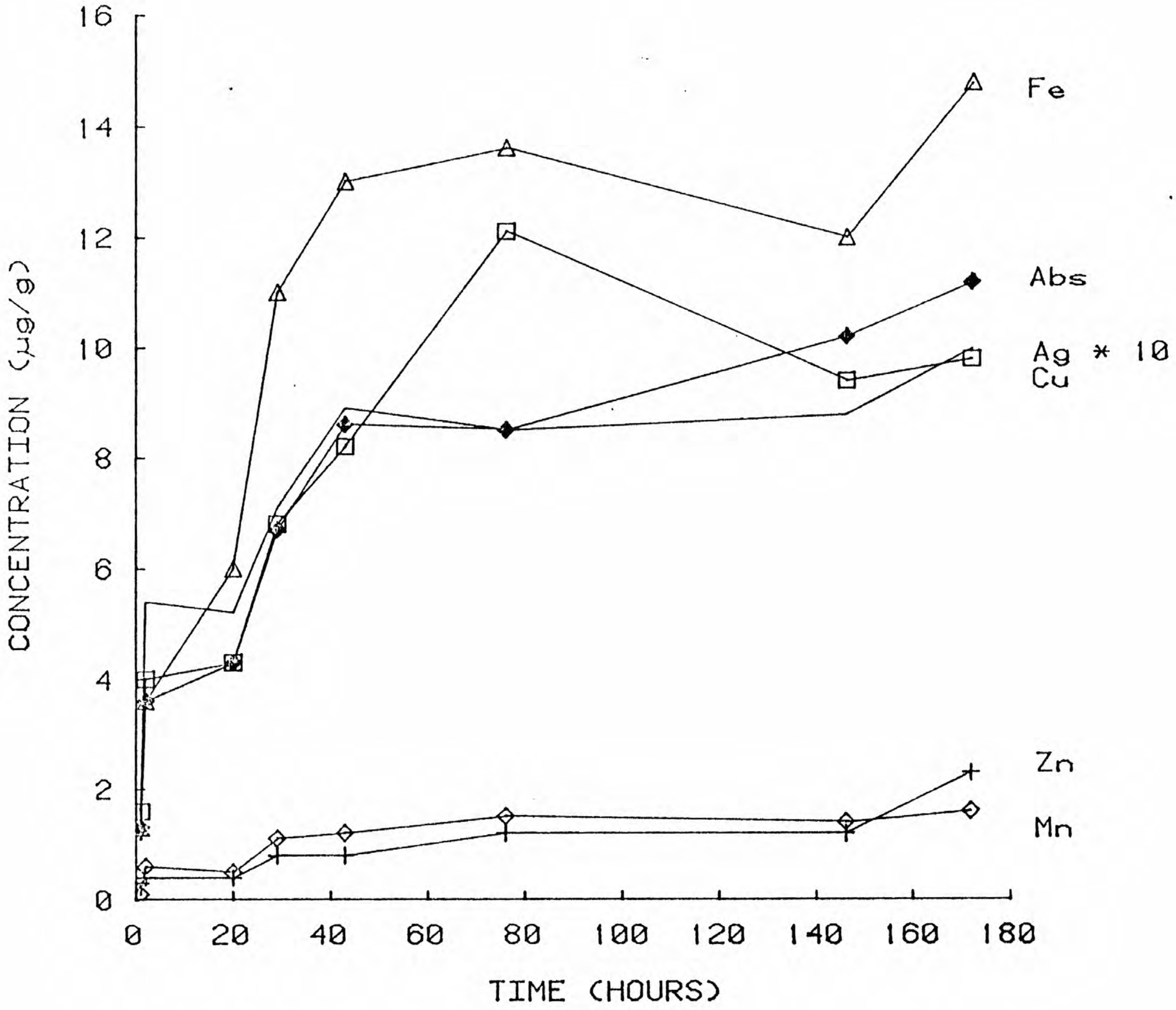
Table 4-9 Correlation coefficients for the regression of Cu, Ag, Zn, Fe and Mn, extracted by 1 N ammonia with total concentrations of these metals and with concentrations of extractable humic materials in sediments.

	------------Correlation with	---------------		
	Total Metal		Concentration of humic materials	
	r	slope	r	slope
Cu - ammonia	1.00**	0.88	0.23	0.31
Ag - ammonia	0.81**	0.73	0.01	0.19
Zn - ammonia	0.90**	1.72	0.09	0.17
Fe - ammonia	0.04	0.21	0.89**	1.47
Mn - ammonia	0.30*	0.66	0.72**	1.01

*p<0.01
**p<0.001

List of Figures

Fig. 4-1



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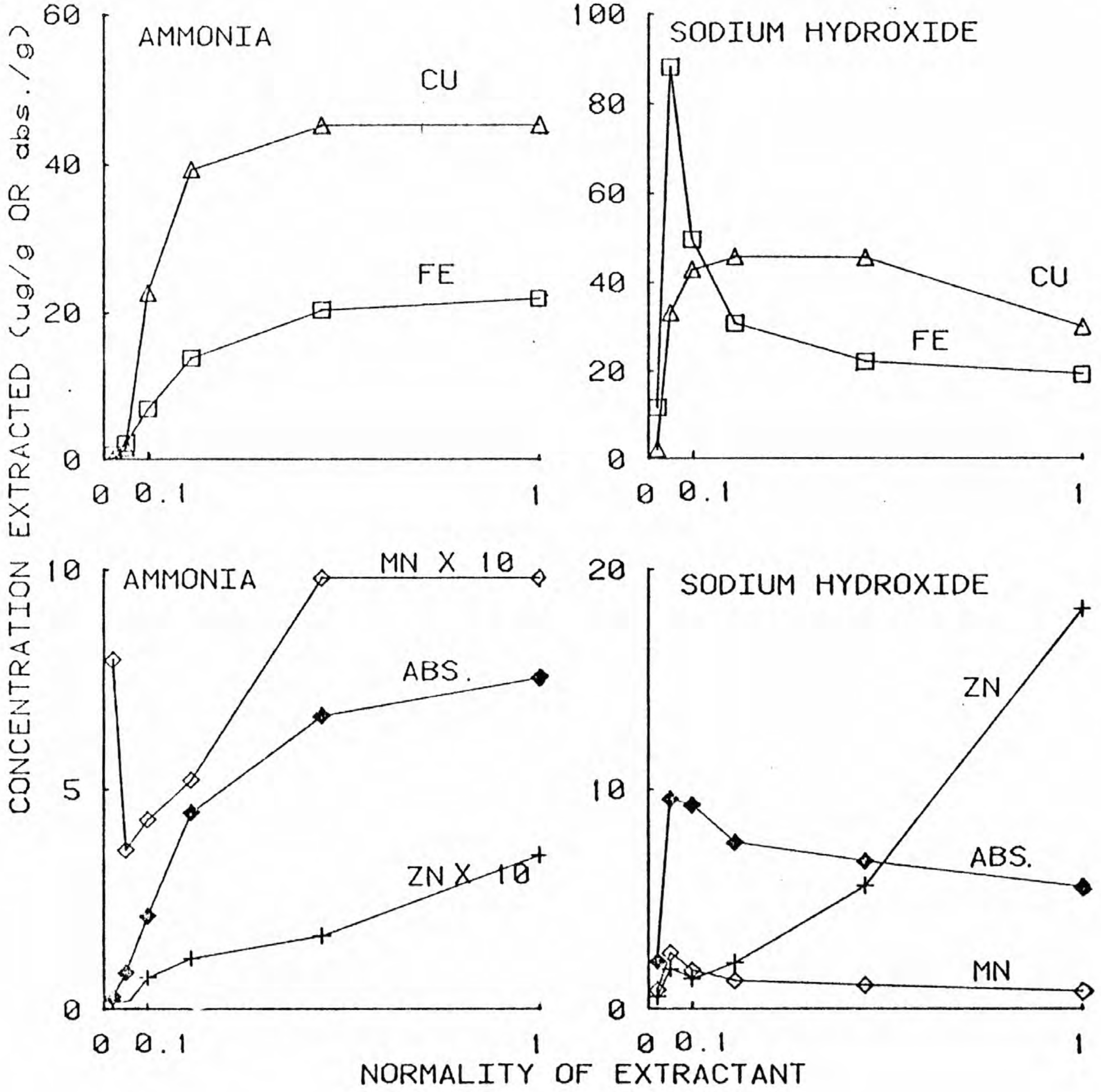
14
12
10
8
6
4
2
0
CONCENTRATION (µg/g)
0
20
40
60
80
100
120
140
160
180
TIME (HOURS)
Fe
Abs
Ag * 10
Cu
Zn
Mn

Fig. 4-2



AMMONIA
CU
FE
SODIUM HYDROXIDE
CU
FE
AMMONIA
MN X 10
ABS.
ZN X 10
SODIUM HYDROXIDE
ZN
ABS.
MN
CONCENTRATION EXTRACTED (ug/g OR abs./g)
NORMALITY OF EXTRACTANT
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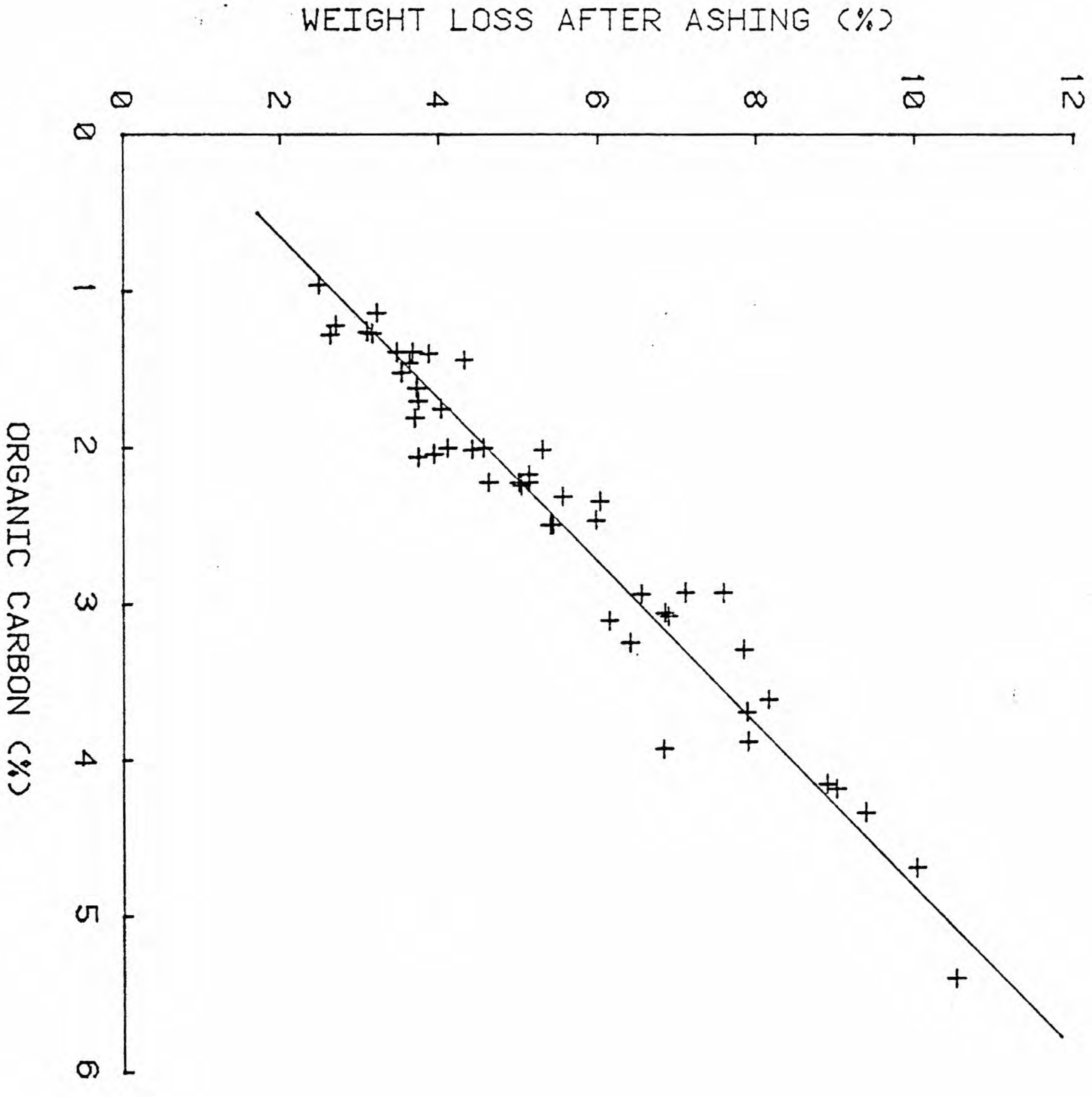
40
60
80
100
5
10
0.1
1

Fig. 4-3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2
4
6
8
10
12
ORGANIC CARBON (%)
0
1
2
3
4
5
6

Fig. 4-4

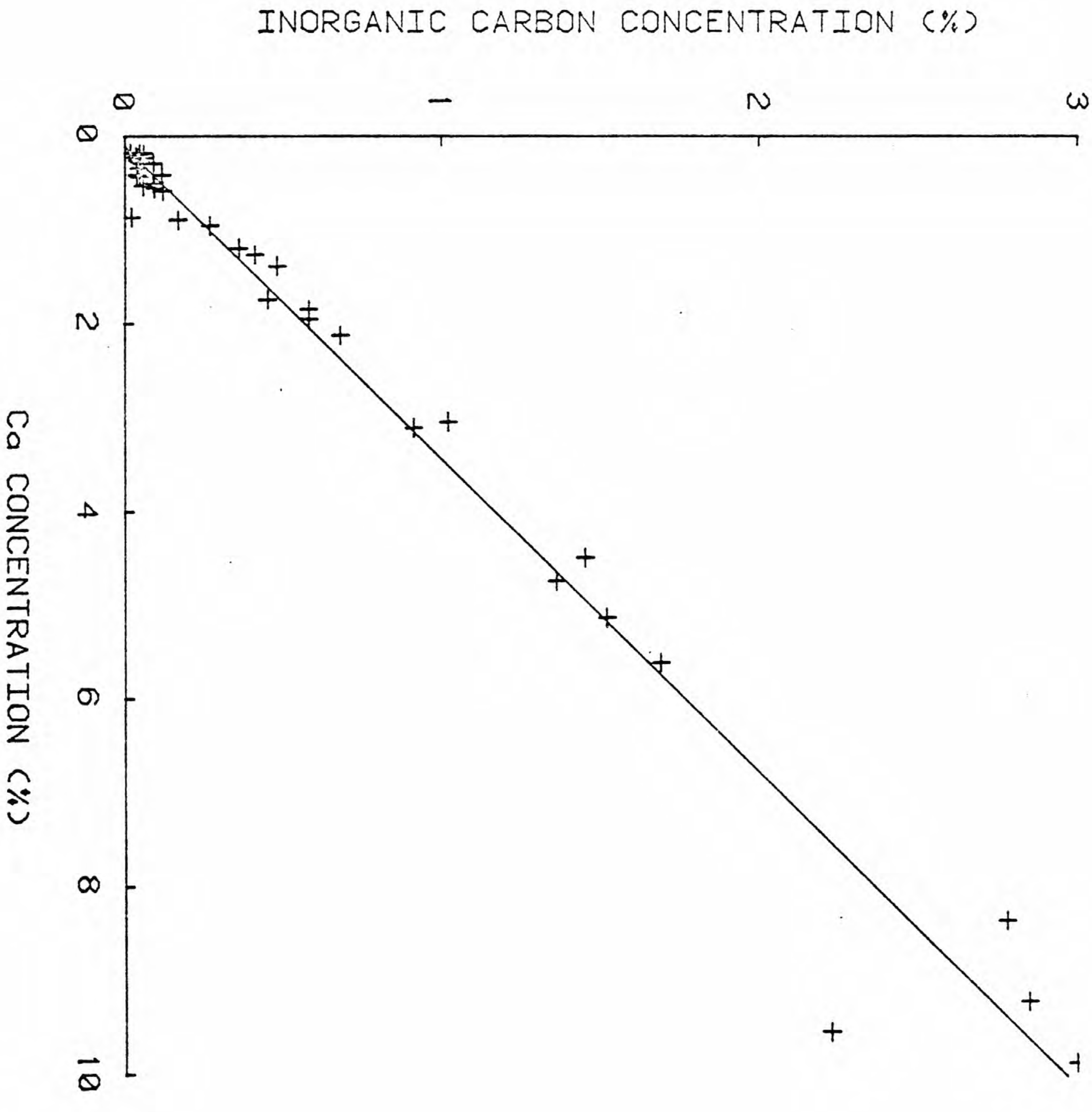

INORGANIC CARBON CONCENTRATION (%)
0
1
2
3
Ca CONCENTRATION (%)
0
2
4
6
8
10

Fig. 4-5a

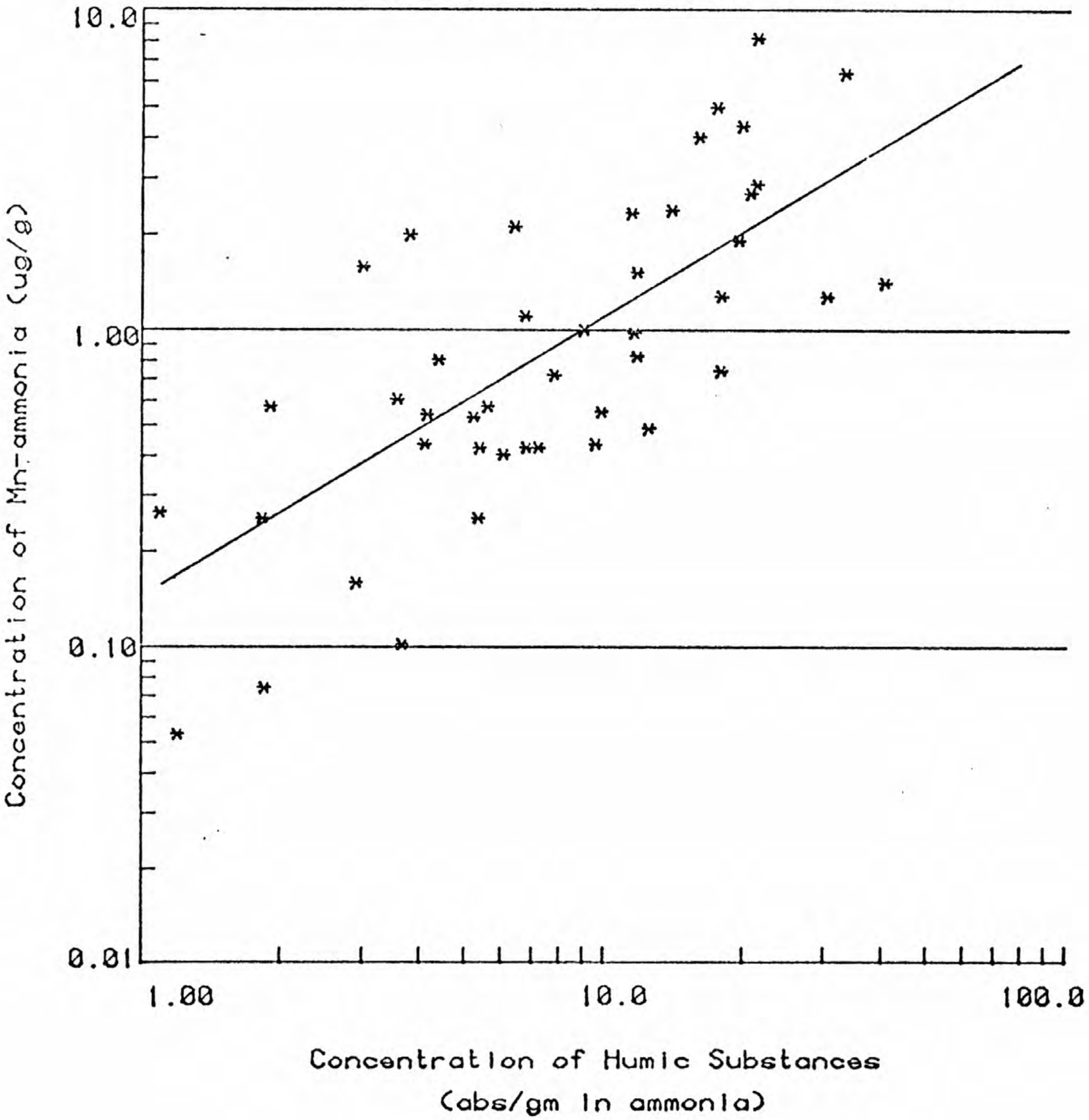
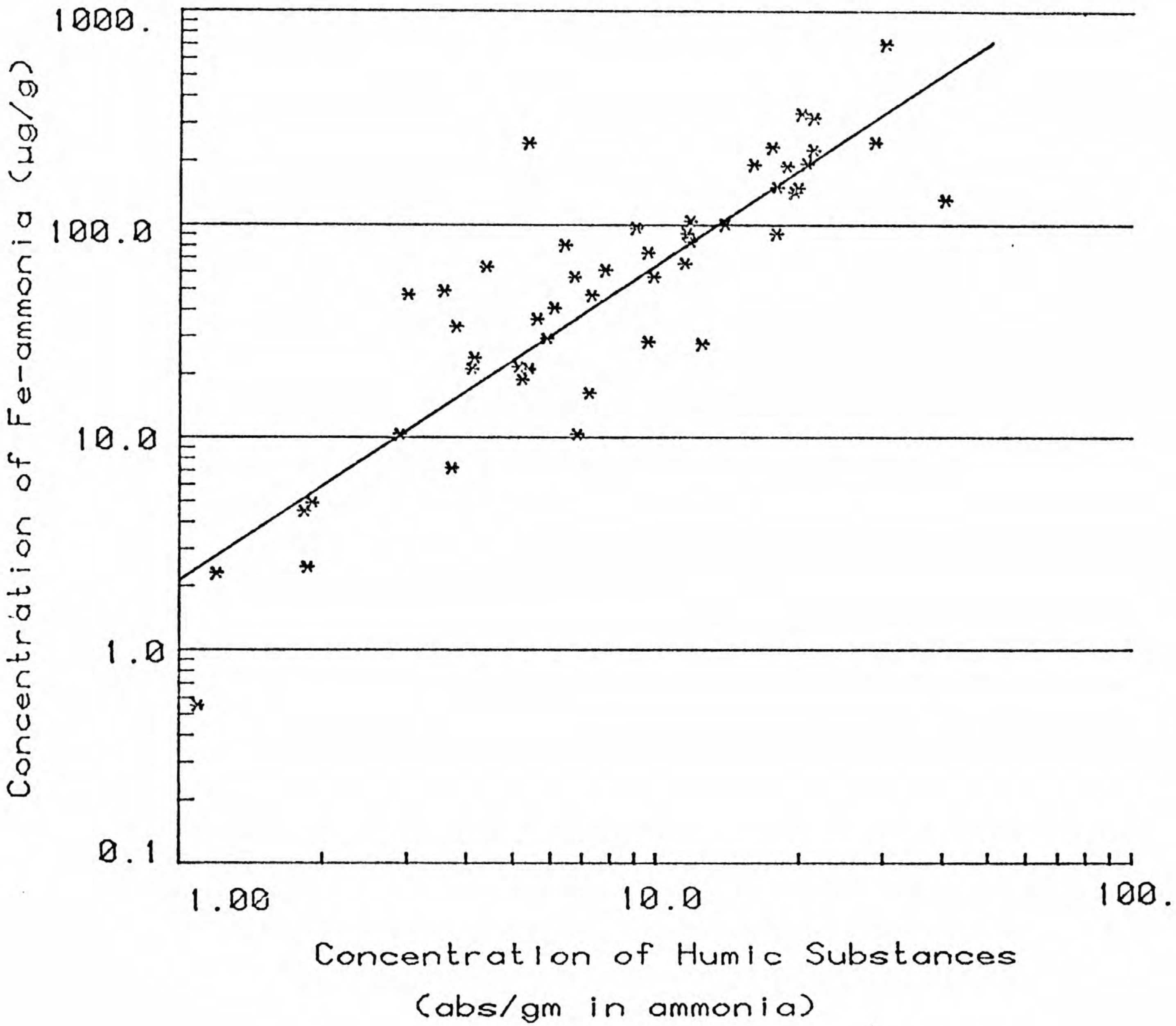

10.0
1.00
0.10
0.01
Concentration of Mn-ammonia (ug/g)
1.00
10.0
100.0
Concentration of Humic Substances
(abs/gm in ammonia)

Fig. 4-5b


1000.
100.0
10.0
1.0
0.1
1.00
10.0
100.
Concentration of Fe-ammonia (µg/g)
Concentration of Humic Substances
(abs/gm in ammonia)

Chapter 5. Determining the form of Metals in Oxidized Estuarine Sediments: Statistical Assessments

S.N. Luoma and G.W. Bryan

In the oxidized sediments of estuaries a number of substrates compete in the partitioning of trace metals (Jenne, 1968; 1977). Hydrous oxides of Fe and Mn, various forms of organic materials, and carbonates are all capable of trace metal sorption (where we define sorption to include adsorption, coprecipitation, complexation or ion exchange). The partitioning of metals among these substrates is a function of the strength of bonding of individual metal-substrate associations and the relative abundance of the different substrates. The substrate which bond a metal most strongly should dominate in the partitioning of that metal (provided they are present in sufficient concentration); and statistical correlations between metal concentrations and substrate concentrations should be observed among different sediment samples. Aside from the standard limitations to statistical methodology (Luoma and Bryan, in prep.), interpretations of correlations between metal and substrate concentrations may be affected by a number of variables, including: (1) the form, (3) intercorrelations between fractions of metal removed by different extractants, and (4) the effect of equilibrium processes on metal partitioning in different sediments.

(1) Substrate form. All substrates occur in a variety of forms in oxidized sediments (Luoma and Bryan, in prep. a). The different forms may vary in their strengths of metal sorption. Chemical associations between metals and substrates may be obscured in regression calculations of total metal *vs* total substrate if the concentrations of substrate includes fractions (whose sizes are independent of total substrate concentration) not

involved in metal partitioning. If partial chemical extractions are employed to characterize substrate forms, stronger statistical correlations between metal and substrate will occur in those extractants which most selectively remove only the fraction of the substrate most reactive with the trace metal. The poorer the selectivity of the extractant for the reactive form of the substrate the greater the "dilution" of the metal substrate correlation.

(2) Incongruent dissolution. Resorption of extracted metals to undissolved substrates or precipitation of extracted metals may occur within extractants such as ammonium acetate (pH7), sodium hydroxide, or acid ammonium oxalate (pH 3.3). In such instances the concentration of metal in the extract itself will not correlate with the concentration of substrate dissolved by the extract even if a chemical association existed in the sediments. Correlations between substrate concentrations in an extractant which does not hold metals in solution and metal concentrations in a second extractant might be expected if the latter extractant removes, and holds in solution, metal removed from a fraction of the substrate best described by the former extractant.

(3) Intercorrelation of extractable fractions. Concentrations of metals removed by many acid extractants correlate closely with total concentrations (Luoma and Bryan in prep. a). In such instances the most reactive fraction of the metal will not be separable from statistical calculations.

(4) Equilibrium effects. If equilibrium processes control metal partitioning, substrates with similar bonding strengths for a metal should compete in the partitioning of that metal, with the relative abundances of

the competing substrates determining how the metal is partitioned. Statistically, such competition would be evidenced by stronger correlations between a metal and one substrate in environments where competing substrates are in low concentrations. However, within a set of samples with widely varying concentrations of competing substrates (and thus wide variations in metal partitioning) metal-substrate associations which occur in only some sediments within data set (due to competition) will not be evident in either simple or multiple regressions calculations which include the entire data set.

In this study we use statistical calculations to assess the partitioning of metals in oxidized estuarine sediments. We employ six chemical extractants to operationally define the forms of metals and substrates, and use correlations to determine which extractants best define metal-substrate interactions. All combinations of extractable metals are correlated with all combinations of extractable substrates to assess the importance of incongruent dissolution. To test if competition among substrates affects metal partitioning or metal-substrate correlations over wide data ranges a statistical "filtering" technique is introduced. The statistical filter requires a large data base, but where sufficient data was available it provides an orderly, objective method for study of interactions within specific subsets of data. Finally, to circumvent the limitations of conducting statistical studies either over narrow data ranges or within single water bodies we compare a number of estuaries which vary widely in their physicochemical characteristics.

Study Area

Samples were collected from 50 stations in 19 estuaries. The stations varied widely in metal concentrations, substrate concentrations and chemical-physical-geologic characteristics. The wide data ranges should reduce ambiguities in interpreting the statistical calculations. Inclusion of data from throughout the salinity gradients in several estuaries reduced the probability that metal-substrate correlations resulted from the action of a third variable common to the processes within a single estuary or estuarine reach. Coincidental enrichment of metals and substrates was less likely over a wide range of data; as were cross-correlations among controlling variables such as substrate concentrations or particle size. Concentrations of some substrates correlated with physical characteristics of the estuaries, but these correlations are considered in interpretations of the results.

Methods and Materials

The data from Chapter 4 were employed in the statistical calculations. Fifty samples of oxidized sediment were each extracted as described in Chapter 4. Data from 6 of the extractions are considered here: 25% acetic acid; 0.4N acid ammonium oxalate; IN ammonium acetate; IN ammonia, IN HCl and "total" metal digestion. Concentrations of metals (Ag, Cd, Co, Cu, Pb and Zn) and substrates (Fe, Mn and, in basic extractants, humic materials) were determined in each extract; and both total organic carbon and inorganic carbon were analyzed in each sediment sample. Particle size was defined as percent, by weight, less than 14 μm and determined by settling for 15 minutes in an Andreason pipette (Bryan and Hummerstone, 1977). All data were transformed to logarithmns for the statistical analyses.

A statistical "filter" was used to determine if the correlation between a metal and one substrate varied among subsets of data in which the concentration of a second substrate fell within defined limits; the assumption being that if the two substrates were competing for sorption of the metal, stronger associations between the metal and the first substrate would be observed if only sediments with low concentrations of the competing substrate were considered in the calculation. Regressions were first calculated for all metal-substrate combinations in all extractants using all the data. Then, regressions were calculated using data sets which excluded the stations where the "filtered" substrate was above a pre-defined concentration. In additional regressions the substrate level above which data were excluded (filtered out) from the calculation was progressively lowered. From 3 to 5 "filter" steps were progressively employed for each substrate (fewer filters were used for substrates with narrow data ranges). Concentrations were chosen as filter levels (Table 5-1) on the basis of a maximum change in sample size between filter steps. Sample size was never less than 12 in any regression at any filtration step. A significant competitive effect of the filter substrate on a metal-substrate association was concluded only were a progressive increase in the correlation coefficient of the relationship occurred at each filtration step. An increase in the level of significance of the relationship indicated a highly significant effect. Because of the number of regressions calculated (>15,000) only statistically significant results are reported here. Although filtering reduced the overall variance in the data set in some cases by progressively reducing the number of estuaries included in the calculation, the overwhelming majority of the correlations remained insignificant.

RESULTS

Inter-substrate relationships

Chemical relationships between a metal and a substrate cannot be inferred from a statistical association unless correlations among substrates in the data set are well understood. In our data set intersubstrate correlations were generally weak. The strongest relationship was the correlation of amorphous Fe with organic carbon; probably resulting from the increased concentration of both substrates in fine-grained sediments (Table 5-2). Concentrations of humic substance showed a weak relationship to total organic carbon, and a significant negative relationship with carbonates. The latter was not the result of a chemical interaction, however. Humic materials declined in concentration from the head to the mouth of most estuaries (Fig. 5-1), and occurred in low concentrations at stations heavily influenced by marine processes. Carbonates occurred in high concentrations where marine influences were greatest, and increased from the head to the mouth of most estuaries. Samples with low concentrations of humic substances and/or high concentrations of carbonates were generally from the seaward reaches of estuaries, while the opposite was true of the landward estuarine reaches.

Manganese

Manganese may partition in sediments among Mn carbonate, Mn oxide and Mn sorbed to other substrates. All three forms appeared to exist in oxidized sediments from the estuaries of southwest England. Competition between carbonates and other substrates was evident in subsets of data. Strong correlations between Mn and carbonate suggested Mn carbonate was an important form in sediments low in either organic materials or total Fe (Table 5-3). The strong correlation of Mn with organic materials in sediment low in carbonates indicated complexation of Mn was important where Mn carbonate was least likely to form in abundance. Manganese also correlated significantly with particle size (increasing in concentration in finer grained sediments) in low carbonate sediments, either as a secondary result of complexation with organic materials or due to an increase in the relative abundance of Mn oxide coatings on particle surfaces in environments where Mn carbonate was less abundant.

Acidic extractants (HCl, acetic acid, acid ammonium oxalate, hydroxylamine-nitric acid) appeared to solubilize all three forms of Mn, since no correlation of Mn with any substrate was evident in acid extractants when all samples were considered. Ammonium acetate, however, did not solubilize Mn carbonate (as might be expected from a neutral extractant). Among all samples ammonium acetate-Mn correlated poorly with total Mn (total Mn included Mn carbonate, Mn oxide and sorbed Mn when all sediments were considered). However, where total Mn included only Mn oxide and sorbed Mn (low carbonate sediments) ammonium acetate-Mn correlated strongly with total Mn (Table 5-3). Desorption of organically complexed Mn and dissolution of Mn oxides by ion exchange are consistent with the action of neutral ammonium acetate.

Evidence from calculations with Co, Zn, Pb, Cu and Cd indicated ammonium acetate selectively extracted Mn oxide in low carbon sediments. Correlations of all 5 metals with ammonium acetate-Mn were weak among all sediments but very strong among sediments low in organic carbon (see Tables for individual metals). Hydrous oxide is the form of Mn which sorbs metals most strongly in sediments. Where little Mn was expected in complexed form (sediments low in organic carbon) the association of metals with ammonium acetate-soluble Mn was strong. Metal correlations with acid-soluble Mn also improved slightly in low carbon sediments (since less organically complexed Mn was extracted the association of the trace metals with the form of Mn involved in sorption was more evident), but the improvements were not as dramatic as with ammonium acetate-Mn (since the acids were extracting both Mn carbonate and Mn oxide).

Common Effects Among Metals

All metals except Cd correlated insignificantly with particle size distributions, either among all samples or in any subset of samples. As expected, both amorphous Fe and organic carbon increased in concentration in fine grained sediments substantiating the validity of the particle size measurements. Particle size is an important variable affecting trace metals within samples (Bryan and Hummerstone, 1977). Due to the wide data range, however, anthropogenic/geologic enrichment and chemical associations swamped out any statistically detectable particle size effects among samples.

All metals except Co, also correlated negatively with carbonates in sediments low in humic substances (i.e., sediments most influenced by marine processes-see Tables for individual metals). Either carbonates substantially diluted metal concentrations in the seaward reaches of the estuaries, or some net desorption of metals increasingly occurred with marine influence in seaward estuarine reaches. The former explanation seems most likely, since calcium carbonates constituted up to 30 percent of the sediment weight in some areas. If so, comparisons of trace metal enrichments within and among estuarine stations should be calculated on a carbonate-free basis.

Cobalt

Among all samples, Co correlated most strongly with total Fe, total Mn and oxalate-soluble Mn (Table 5-4). The residuals of the correlation between total Co and total Fe (Fig. 5-2a) generally showed a positive relationship with both extractable and total Mn (Fig. 5-2b). A multiple regression of Co vs. Fe and Mn explained 88 percent of the variance in total sedimentary concentrations of Co.

The dominance of Fe and Mn in the partitioning of Co was also evident within subsets of data. Improvements in the Fe-Co correlation were observed when stations with high Mn concentrations were progressively excluded from the calculation (Table 5-5). Improvements in the correlation between Mn and Co were evident when samples with either high oxalate-soluble Fe or high total Fe were filtered out of the Mn-Co regression. Cobalt was dominantly partitioned to Mn oxides where concentrations of Fe oxide where low, and where concentrations of Mn oxide were low, Co was dominantly partitioned to Fe oxide.

Cobalt correlated significantly with organic carbon in sediments low in carbonates. Most likely, this correlation was a secondary result of the strong association of Co with Mn, since Mn and organic carbon also were closely correlated in low carbonate sediment. Similarly, the improvement of the correlation between Co and Mn in sediments low in organic carbon was consistent with an increased partitioning of Mn in such sediments to forms most reactive in sorption of Co (Mn oxides).

Zinc

Amorphous Fe oxide dominated the partitioning of Zn. Both acid-soluble and total Zn correlated very strongly with concentrations of Fe in the sediments (Table 5-6), and correlations with extractable Fe were substantially stronger than correlations with total Fe. Manganese and organic materials both appeared to compete with Fe in the partitioning of Zn. The correlation of Zn and amorphous Fe was enhanced among samples with low total organic carbon, low concentrations of humic substances or low concentrations of Mn (Table 5-7).

Manganese and organic materials were secondary substrates in Zn partitioning. Among all samples Zn correlated significantly with total Mn (but not with extractable Mn) and with organic carbon, but the correlations were substantially weaker than the correlation with Fe (Table 5-6). In sediments with low concentrations of either Fe or Mn, organic carbon was an important substrate for Zn partitioning. Total Mn was most important in Zn partitioning in sediments with low Fe/Mn ratios, while extractable forms of Mn (especially the Mn fraction soluble in ammonium acetate) were important in sediments low in organic carbon (the more selective extraction of Mn oxides in these sediments may have enhanced the Zn-Mn correlation).

Lead

Concentrations of Pb in sediments correlated significantly with only Fe and Mn among all samples (Table 5-8). The importance of amorphous Fe in the partitioning of Pb was apparent among stations with either low concentrations of Mn or low concentrations of organic carbon (Table 5-9). Partitioning of Pb to the Mn substrate was evidenced by strong Pb-Mn correlations among stations with low Fe/Mn ratios or low concentrations of organic carbon. The correlations of Pb with Fe were generally stronger than correlations of Pb with Mn suggesting amorphous Fe dominated Pb partitioning in general. Moreover, the subset of stations with the lowest concentrations of organic carbon coincidentally had very high Pb/Fe ratios, due to enrichment from Pb mining (Luoma and Bryan, 1978). Thus some saturation of Fe oxides, and "spill-over" of Pb to the Mn oxide substrate may have enhanced the correlation of Pb and Mn in this subset of the data (along with the more selective extraction of Mn oxides).

No significant correlation of Pb with concentrations of organic carbon was observed among all samples (Table 5-8); however, some partitioning of Pb to organic materials was evident among stations where concentrations of Mn were low (Table 5-9). These correlations, and the competition of organic materials with Fe, indicate Pb partitions to organic materials as a secondary substrate, primarily in sediments deficient in hydrous oxides.

The fraction of Pb extracted by acid ammonium oxalate correlated relatively poorly with total Pb (Luoma and Bryan, in prep. a). Acid ammonium oxalate-Pb correlated with humic substances ($r = 0.68$; $p<0.005$) in sediments low in amorphous Fe, and with amorphous Fe ($r = 0.55$; $p<0.05$) in sediments low in humic substances. Lead oxalate is of low aqueous solubility (L. Filipek, U.S.G.S., Denver, personal commun.). Thus any Pb extracted by acid ammonium oxalate must be held in solution or suspension in association with ligands stronger than the Pb oxalate bond. The correlations were consistent with a dissolution or dispersion by acid ammonium oxalate of humic substances which strongly complex Pb; plus colloidal Fe oxides which hold Pb in suspension on particles < 0.45 μm diameter. Where both humic substances and amorphous Fe oxides are in abundance, Pb is dispersed with both and no correlation with either is obvious. Only where one of the two substrates is in low concentration is the control of the other on Pb solubility in the extractant statistically evident.

The dispersion of colloidal Fe was consistent with the appearance of the extract before filtration. It is unlikely, however, that humic substances would be highly soluble at pH 3.3. Thus dispersion of humate-Pb may also have been in association with colloidal Fe, a conclusion consistent with the enhanced release of humic substances from sediments pre-treated with acid ammonium oxalate by Luoma and Bryan (in prep. a).

Copper

The different extractants appeared to provide a general fractionation of the different forms of Cu in the sediments. Total Cu and HCl-Cu were strongly correlated with acid-extractable Fe and with organic carbon (Table 5-10). Acid ammonium oxalate-Cu correlated significantly with Mn. Ammonium acetate and acetic acid-Cu correlated significantly with both Fe and Mn (but did not provide the best correlation with either) and were not significantly correlated with organic carbon. As suggested previously (Luoma and Bryan, in prep.a), HCl appeared to extract both organic and inorganic forms of Cu, acetic acid extracted Cu largely from inorganic sinks while oxalate appeared to extract Cu with Mn oxides.

Employment of statistical filters indicated Cu was partitioned among amorphous Fe oxides, Mn oxides and organic materials (especially humic substances). Amorphous Fe generally dominated the partitioning of Cu as evidenced by the very strong correlations between Cu and extractable Fe in sediments low in humic substances, Mn or total organic carbon (Table 5-11). Total organic materials appeared to play a dominant role in Cu partitioning only in sediments very low in amorphous Fe, although the importance of the total organic substrate was enhanced in sediments low in Mn. The importance of humic substances in the partitioning of Cu to organic complexes was evident from the generally high concentrations of Cu extracted from most sediments by NaOH or ammonia (Luoma and Bryan, in prep. a); the reduced correlation of Cu and organic carbon in sediments low in humic substances, and the apparent strong competition between humic substances and amorphous Fe for Cu partitioning.

Competition of Mn with amorphous Fe for Cu partitioning and enhanced Cu-Mn correlations in more selective extractions of Mn oxide (ammonium acetate data) was also evident from the statistical calculations, but the generally weak correlations between Cu and Mn suggested Mn was a substrate of secondary importance for this metal.

Silver

Due to low concentrations, the only Ag fractions considered were totals, and HCl-Ag. HCl-Ag correlated relatively weakly with total Ag (r = 0.70) (Luoma and Bryan, in prep. a). Dissolution of Ag by HCl must occur through formation of a Ag-chloro complex after either desorption of Ag or dissolution of a substrate and its associated Ag. Undefined effects on the solubility of the chloro complex may have added to the variability of Ag dissolution by HCl. However, much of the total Ag in the sediments also appeared to be associated with inorganic oxides in a manner that did not permit HCl-dissolution. The proportion of Ag solubilized by HCl was lowest in sediments characterized by either high concentrations of Fe (Fal, Tamar estuaries) or high concentrations of Mn (Gannel estuary). Total Ag correlated strongly with Fe (Table competition between Fe and Mn for the partitioning of total Ag was strongly evident (Table 5-13). Evidence for competition between Mn and Fe for partitioning of HCl-Ag was less convincing than that for total Ag, suggesting HCl-insoluble forms of Mn were most important in partitioning of Ag to the Mn oxide substrate. The importance of humic substances in the partitioning of Ag was also indicated by the correlation of total Ag with humic substances in sediments low in amorphous Fe, and by the substantial fraction of Ag dissolved by ammonia or NaOH from sediments along with humic materials (Luoma and Bryan, in prep. a).

In summary, the chemistry of Ag in the oxidized sediments was very similar to that of Cu. Partitioning was primarily between Fe oxides and the humic fraction of the organic materials, with a shift to dominance by one substrate occurring in sediments where the other was in low concentration.

Cadmium

Only total and HCl-soluble Cd were determined in the sediments, because of the generally low Cd concentrations. Similar concentrations of Cd were removed by both techniques, but the HCl data were considered more reliable because a larger sediment mass was extracted.

No unambiguous conclusions were clear regarding the primary substrate in Cd partitioning. Among all samples Cd correlated significantly with amorphous Fe, organic carbon and particle size (Table 5-12); and among sediments low in Mn the correlations of Cd with all three variables was enhanced. However, amorphous Fe, organic carbon and particle size are intercorrelated (Table 5-2), thus the relative importance of the three variables in Cd partitioning could not be separated.

There was some evidence of a secondary partitioning of Cd to Mn oxides. Cadmium correlated strongly with ammonium acetate-Mn in low carbonate sediments, and Mn appeared to compete with the partitioning of Cd to either Fe or organic materials.

Discussion

Partitioning

Statistical evidence indicated Ag, Cd, Co, Cu, Pb and Zn are all partitioned among a variety of substrates in oxidized estuarine sediments (Fig. 5-3). Partitioning appeared to be characterized by equilibrium processes which resulted in changes in substrate-metal interactions when changes in the physicochemical characteristics of the sediments occurred. Hydrous oxides of Fe dominated the partitioning of Zn, Pb and, possibly, Cd in most sediments; oxides of Fe and humic substances were both generally important in the partitioning of Cu and Ag; and Co appeared to be equally partitioned between hydrous oxides of Fe and Mn. Organic materials and oxides of Mn were generally most important in partitioning where sediments contained low concentrations of Fe, except in the case of Co.

The equilibrium-controlled multi-substrate partitioning of metals suggests that simple generalizations which infer metal associations with only one type of substrate are not valid. Metal partitioning in sediments must be viewed as a dynamic process subject to influence by the physicochemical environment, analogous to the solute speciation of metals (although kinetics certainly vary between the two processes). Thus the dominant form of any metal may vary substantially at any one time among sediments, or within sediments over time. Methods which rely upon the correlation of a metal and one substrate to detect anthropogenic metal enrichment in sediments (Trefrey and Presley, 1976) may yield erroneous conclusions if partitioning varies among the sediment samples (e.g., see Co results in

Fig. 5-2). Multiple regression must be employed in such assessment to account for multi-substrate partitioning (Bradford and Luoma, in press), and the sediments used in the comparisons should be relatively homogeneous in their physical and chemical characteristics.

Optimal extraction of metals and substrates

The strong correlations among concentrations of metal extracted by acid treatments and total concentrations made it difficult to determine which extractants best defined the reactive phase of metals in sediments. In general the strongest metal-substrate correlations were observed with either HCl-soluble or total metal. This substantiates our conclusion (Luoma and Bryan, in prep. a) that HCl is superior to acetic acid or other methods for generally removing "non-detrital" metal forms. The strong correlations of totals with HCl-metal prevented conclusions about the proportion of the total metal in the sediments involved in partitioning.

In some instances partial extractants were useful in depicting specific aspects of the partitioning of metals. For example, partial extractions of Pb and Cu with acid ammonium oxalate demonstrated the reaction of these metals with secondary substrates (humic materials and manganese oxides, respectively); the extraction of Ag with HCl allowed demonstration of statistical relationship between Ag and humic materials, and extractions of metals with NaOH or ammonia appeared to remove metals largely from (or with) humic substances (Luoma and Bryan, in prep. a). In general, however, there was no statistical evidence any extractants except, perhaps, ammonia and NaOH selectively removed metals from single substrates.

Partial extractions were most useful in describing the fractions of substrates which reacted most strongly with trace metals. The alkalai-soluble fraction of the organic substrate dominated organic complexation of at least Cu and Ag. The amorphous fraction of Fe oxides showed a stronger association with Ag, Cd, Cu, Pb and Zn (but not Co) than did total Fe, reflecting the higher density of sorption sites in more amorphous Fe phases (Jenne, 1977). Acid ammonium oxalate and HCl (which dissolved similar quantities of Fe) provided a better measure of the fraction of amorphous Fe oxide most reactive with trace metals than did the smaller quantity of Fe removed by acetic acid.

The low solubility in acid extracts of both Co and Fe relative to other metals (Luoma and Bryan, in prep.a) substantiated the statistical inference that Co associated with a more crystalline fraction of Fe than did other metals. The association of Co with total Fe indicated either specific sorption sites of low density, but with a high affinity for Co, may be characteristic of crystalline Fe forms; or much of the Co in these estuaries originated in geologic association with some crystalline Fe mineral. The latter would suggest that nearly all the non-detrital Co was partitioned to Mn.

The extractants operationally defined three fractions of Mn (Luoma and Bryan, in prep. a), but did not clearly resolve the partitioning of Mn in the sediments. Among all stations, Co, Zn, Pb and Cu generally correlated more strongly with total Mn than with any of the extractable fractions. If we assume Mn oxides are much more important in metal partitioning than Mn carbonates or sorbed Mn, the strong correlations of metals with total Mn suggested most extractants contain proportionately more of the less reactive Mn forms, and less Mn oxide than does the concentrated nitric acid digest (i.e., much of the trace metal-reactive Mn oxide in sediments is not solubilized by weak acid extraction). The presence of Mn carbonates in the sediments is evidenced by the correlations of Mn and carbonate. However, the improvement of Mn-metal correlations primarily in sediments low in organic carbon (rather than in low carbonate sediments) suggested complexed forms of Mn made-up a larger fraction of the Mn extracted by acids and especially ammonium acetate, than did Mn carbonates. In sediments low in organic carbon, acid ammonium oxalate or ammonium acetate were the preferable methods of extracting metal-reactive Mn forms (most likely, the higher pH of these extracts reduces the quantity of Mn carbonate extracted).

In summary, descriptions of metal partitioning in oxidized sediments may be optimally obtained from simple partial extraction schemes which:

1) Employ HCl of sufficient acidity to buffer carbonate neutralization. HCl-based schemes are preferable to those employing acetic acid, based both upon stronger metal-substrate correlations in HCl and upon the inefficiency of acetic acid in extracting some organically complexed metals (Luoma and Bryan, in prep. a).

2) Employ an alkaline extractant to characterize concentrations of humic substances and

3) Characterize amorphous Fe and Mn oxides with acid ammonium oxalate. Correlations suggest IN HCl may be employed in place of acid ammonium oxalate with only a small loss of information. An additional extraction with ammonium acetate could be added to gain information about parti-

titioning to Mn oxides. If acid ammonium oxalate is used, statistical correlations between HCl-metals and oxalate-substrates would be expected where chemical associations occur, due to the incongruent dissolution of some metals in the oxalate extract. Moreover, all such calculations should be interpreted only after considering the effect of intersample variations in substrate concentrations on metal partitioning.

SUMMARY

Chapter 5

Statistical evidence shows that Ag, Cd, Co, Cu, Pb and Zn are each partitioned among a variety of binding sites (substrates) in oxidized estuarine sediments. The partitioning among substrates of each metal changes as the physical and chemical characteristics of the sediments change. Partitioning processes are demonstrated by partial chemical extractions of metals and substrates from sediments followed by statistical comparisons of extractable concentrations. A statistical filtering technique is employed to demonstrate enhanced correlations between a substrate and its associated metal in sediments with low concentrations of competing substrates. The most useful extractants in depicting metal partitioning include N HCl for partial extraction of metals, acid ammonium oxalate for characterization of Fe oxides and Mn forms in sediments, ammonium acetate for selective extraction of Mn. oxides in sediments with low concentrations of organic carbon and an alkaline extraction to quantify the concentrations of humic substances.

Table 5-1 Samples with higher substrate concentrations than shown here were excluded (filtered out) from the regression calculation for each respective "filter". The correlation of each metal-substrate combination was calculated employing each set of filtered samples shown. Numbers in parentheses are the sample size (n) in each calculation. Total sample size was 50.

Filter Substrate Fraction		Filter_1	Filter_2	Filter_3	Filter_4	Filter_5
Fe	oxalate (μg/g)	10,000 (37)	7,000 (27)	4,000 (16)		
Fe	total (μg/g)	35,000 (37)	30,000 (27)	26,000 (16)		
Mn	acetic acid (μg/g)	600 (41)	400 (38)	300 (32)	200 (27)	120 (17)
Mn	total (μg/g)	900 (38)	500 (22)	400 (15)		
Humic Substances (absorbance/g)		15 (36)	9 (27)	6 (20)	5 (14)	
Organic Carbon (percent)		4.0 (42)	3.5 (35)	2.5 (26)	2.0 (12)	
Carbonate carbon (percent)		2.0 (42)	1.0 (33)	0.5 (28)	0.1 (20)	
Fe/Mn	acetic acid	150 (40)	50 (30)	30 (21)	22 (15)	

Table 5-2 A matrix of correlation coefficients, showing interrelationships among sedimentary substrates. Within element correlations for Fe and Mn as extracted by various techniques were reported elsewhere (Luoma and Bryan). Sample size (n) = 50. (-) indicates a negatively sloped relationship.

	$Fe_{Acet\ Ac.}$	Fe_{HCl}	Fe_{Total}	Mn_{AmAc}	$Mn_{Acet.\ Ac.}$	Mn_{Total}	Particle size	Humic substances	Organic Carbon
Mn_{AmAc}	0.12	0.15	0.13						
$Mn_{Ace.Ac.}$	0.00	0.20	0.26						
Mn_{Total}	0.19	0.34	0.43**						
Particle Size	0.47**	0.39*	0.09	(-)0.05	0.01	0.08			
Humic substances	0.15	0.06	0.12	0.06	(-)0.23	(-)0.19	0.29		
Organic carbon	0.72***	0.64***	0.35	0.05	0.07	0.10	0.65***	0.46**	
Carbonate carbon	0.01	(-)0.04	(-)0.09	0.01	0.16	0.15	0.15	(-)0.56***	(-)0.19

*$p<0.01$

**$p<0.005$

***$p<0.001$

Table 5-3 Correlation coefficients describing the relationships of Mn with carbonate, particle size and organic carbon as affected by excluding (filtering) from the calculation all samples with concentrations of "filter" substrate above a progressively lower filter level. Filter levels and sample sizes for the corresponding regression are shown in Table 1.

Filters		Correlation Coefficients (r)				
Substrate	Fraction	All Samples	Filter$_1$	Filter$_2$	Filter$_3$	Filter$_4$
		[a]Mn_{HCl} vs Carbonate				
Fe	Total	0.15	0.34^{+}	0.67**	0.72**	
Total organic carbon		0.15	0.35^{+}	0.37^{+}	0.59**	0.81**
Humic Substances		0.15	0.39^{+}	0.55*	0.53^{+}	0.54^{+}
		[a]Mn_{HCl} vs particle size				
Carbonate		0.06	0.14	0.18	0.34	0.52^{+}
		[a]Mn_{HCl} vs TOC				
Carbonate		0.09	0.18	0.27	0.49^{++}	0.62*
		[a]Mn_{AmAc} vs Mn_{Total}				
Carbonate		0.44*	0.42*	0.65**	0.69**	0.78**

+p<0.05
++p<0.01
*p<0.005
**p<0.001

[a] similar effects, but weaker correlations were observed with total and oxalate-Mn

Table 5-4 Correlation of extractable fractions of Co with organic carbon and selected fractions of Fe and Mn. Numbers are correlation coefficients (r).

Cobalt Extraction	Mn_{HCl}	$Mn_{oxal.}$	Mn_{total}	Fe_{HCl}	Fe_{total}	Organic Carbon
Ammonium Acetate	0.08	0.15	-0.01	0.19	0.00	(-)0.06
Acetic Acid	0.61**	0.63**	0.55**	0.46**	0.56**	0.12
HCl	0.61**	0.67**	0.60**	0.48**	0.43**	0.23
Oxalate	0.68**	0.75**	0.75**	0.54**	0.51**	0.18
Total	0.69**	0.71**	0.75**	0.58**	0.70**	0.19

$^{**}p<0.001$

Table 5-5 Correlation coefficients for the relationship of Co with organic carbon, Fe and fractions of Mn, as affected by excluding (filtering out) from the regression samples with concentrations of "filter" substrate above a progressively lower filter level. "Filter levels" and sample size for the corresponding regression as shown in Table 1.

Filter		Correlation Coefficients (r)					
Substrate	Fraction	All samples	$Filter_1$	$Filter_2$	$Filter_3$	$Filter_4$	$Filter_5$
		Fe_{total} vs $^{a}CO_{total}$					
Mn	acetic acid	0.71**	0.81**	0.83**	0.97**	0.99**	1.00**
		Mn_{total} vs $^{a}CO_{total}$					
Fe	oxalate	0.75**	0.80**	0.98**	0.97**		
Fe	total	0.75**	0.82**	0.82**	0.90**		
Organic carbon		0.75**	0.72**	0.86**	0.88**	0.82**	
		Mn_{oxal} vs $^{a}Co_{total}$					
Organic carbon		0.71**	0.65**	0.75**	0.74**	0.83**	
		$Mn_{am.\ acet}$ vs $^{a}Co_{total}$					
Organic carbon		0.43*	0.33^{+}	0.59**	0.64**	0.84**	
		Organic carbon vs Co_{total}					
Carbonate		0.19	0.25	0.40^{+}	0.46^{+}	0.68**	

+$p<0.05$
++$p<0.01$
*$p<0.005$
**$p<0.001$

[a] Similar results were obtained with all forms of acid extractable Co.

Table 5-6 Correlation of various Zn fractions with organic carbon and selected fractions of Mn and Fe. Numbers are correlated coefficients (r).

Zinc Extraction	Mn_{AmAc}	Mn_{HCl}	Mn_{Total}	Fe_{HCl}	Fe_{Total}	Organic Carton
Ammonium Acetate	0.30^{+}	0.21	0.38^{++}	0.64**	0.41*	0.19
Acetic Acid	0.30^{+}	0.15	0.36^{++}	0.79**	0.52**	0.41*
HCl	0.25	0.10	0.35^{+}	0.82**	0.52**	0.44^{*}
Oxalate	0.27	0.15	0.38^{++}	0.80**	0.50**	0.42*
Total	0.26	0.14	0.37^{++}	0.79**	0.58**	0.36^{+}

+$p<0.05$
++$p<0.01$
*$p<0.005$
**$p<0.001$

Table 5-7 Correlation coefficients for the relationship of Zn with carbonate, organic carbon, Fe and fractions of Mn, as affected by excluding (filtering out) from the regression samples with concentrations of filter substrate above a progressively lower filter level. "Filter levels" and sample size for the corresponding regression are shown in Table 1.

Filter Substrate	Fraction	Correlation coefficients (r) All Samples	Filter$_1$	Filter$_2$	Filter$_3$	Filter$_4$	Filter$_5$
		$^{a}Zn_{HCl}$ vs Fe_{oxal}					
Mn	acetic acid	0.82**	0.84**	0.85**	0.91**	0.94**	0.92**
Organic carbon		0.82**	0.81**	0.80**	0.83**	0.95**	
Humic substances		0.82**	0.85**	0.89**	0.93**	0.91**	
		$^{a}Zn_{HCl}$ vs Organic carbon					
Fe	oxalate	0.44*	0.47*	0.33	0.81**		
Mn	total	0.44*	0.47*	0.77**	0.92**		
Mn	acetic acid	0.44*	0.50*	0.53**	0.63**	0.60**	0.79**
		$^{a}Zn_{HCl}$ vs Mn_{total}					
Fe/Mn		0.35^{+}	0.49*	0.74**	0.84**	0.92**	
Organic carbon		0.35^{+}	0.32^{+}	0.46^{+}	0.45^{+}	0.55	
		$^{a}Zn_{HCl}$ vs Mn_{oxal}					
organic carbon		0.16	0.11	0.24	0.38	0.68^{+}	
		$^{a}Zn_{HCl}$ vs Mn_{amac}					
Organic carbon		0.25	0.22	0.49	0.58*	0.85**	

^{a}Similar results obtained with total Zn.
+$p<0.05$
++$p<0.01$
*$p<0.005$
**$p<0.001$

Table 5-8 Correlation coefficients describing correlation of various fractions of Pb with organic carbon, fractions of Mn, and fractions of Fe. Concentrations of Pb extracted by ammonium acetate were too close to detection limits to be reliable for statistical calculations.

Pb Extractions	$Mn_{Am\ Ac}$	Mn_{oxal}	Mn_{Total}	Fe_{HCl}	Fe_{Total}	$Fe_{Acet\ Ac}$	Organic carbon
Acetic Acid	0.35^{+}	0.31^{+}	0.37^{++}	0.35^{+}	0.37^{++}	0.25	0.03
HCl	0.36^{+}	0.33^{+}	0.40^{++}	0.46**	0.42*	0.34^{+}	0.07
Oxalate	0.32^{+}	0.35^{+}	0.22	0.30^{+}	0.27	0.18	0.06
Total	0.38^{++}	0.31^{+}	0.30^{+}	0.48**	0.40^{++}	0.37^{++}	0.05

+$p<0.05$
++$p<0.01$
*$p<0.005$
**$p<0.001$

Table 5-9 Correlation coefficients for the relationship of Pb with organic carbon, Fe and fractions of Mn, as affected by excluding (filtering out) from the regression samples with concentrations of filter substrate above a progressively lower filter level. "Filter levels" and sample size for the corresponding regression are shown in Table 5-1.

Filters		Correlation Coefficients (r)					
Substrate	Fraction	All Samples	$Filter_1$	$Filter_2$	$Filter_3$	$Filter_4$	$Filter_5$
		$^{b}Pb_{total}$ vs Fe_{oxal}					
Mn	acetic acid[a]	0.48**	0.56**	0.56**	0.91**	0.99**	1.00**
Organic carbon		0.48**	0.46*	0.46^{++}	0.48^{++}	0.91**	
		$^{b}Pb_{total}$ vs Mn_{total}					
Fe/Mn	acetic acid	0.38^{++}	0.43^{++}	0.50*	0.76**	0.74*	
Organic carbon		0.38^{++}	0.44	0.56**	0.55*	0.59^{+}	
		$^{b}Pb_{total}$ vs Mn_{ox}					
Organic carbon		0.31^{+}	0.34^{+}	0.48*	0.58*	0.67^{++}	
		$^{b}Pb_{total}$ vs $Mn_{am.ac.}$					
Organic carbon		0.38^{++}	0.38^{+}	0.60**	0.62**	0.85**	
		$^{b}Pb_{total}$ vs organic carbon					
Mn	total	0.05	0.19	0.64*	0.78**		
Mn	acetic acid	0.05	0.20	0.21	0.59**	0.56*	0.63^{++}

+p<0.05
++p<0.01
*p<0.005
**p<0.001

[a] A slightly weaker but similar response was observed to the total Mn filter.

[b] Slightly weaker but similar responses were observed with HCl-soluble Pb.

Table 5-10 Correlation coefficients describing relationship of various Cu fractions to organic carbon, carbonates and selected fractions of Fe and Mn.

Cu extractions	$Mn_{am\ ac}$	Mn_{ox}	Mn_{total}	Fe_{HCl}	Fe_{total}	Organic carbon	Carbonate
Ammonium acetate	0.34^{+}	0.37^{++}	0.51**	0.42*	0.33^{+}	0.00	0.19
Acetic acid	0.36^{+}	0.40*	0.41*	0.49**	0.41*	0.13	(-)0.41*
HCl	0.28^{+}	0.13	0.27	0.71**	0.54**	0.39*	(-)0.36^{++}
Oxalate	0.25	0.59**	0.50**	0.28^{+}	0.29	(-)0.10	(-)0.27
Total	0.29^{+}	0.10	0.30^{+}	0.69**	0.52**	0.33^{+}	(-)0.36^{++}

$^{+}p<0.05$
$^{++}p<0.01$
$^{*}p<0.005$
$^{**}p<0.001$

Table 5-11 Correlation coefficients for the relationship of Cu with carbonate, organic carbon, Fe and fractions of Mn, as affected by excluding (filtering out) from the regression samples with concentrations of filter substrates above a progressively lower filter level. "Filter levels" and sample size for the corresponding regression are shown in Table 5-1.

Filter		Correlation Coefficients (r)					
		All Samples	Filter_1	Filter_2	Filter_3	Filter_4	Filter_5
Substrate	Fraction						
		[a]Cu_{total} vs Fe_{oxal}					
Humic substances		0.69**	0.78**	0.84**	0.95**	0.96**	
Mn	acetic acid	0.69**	0.71**	0.72**	0.81**	0.89**	0.77**
Organic carbon		0.69**	0.72**	0.71**	0.75**	0.80**	
		[a]Cu_{total} vs Organic carbon					
Mn	acetic acid	0.33^{+}	0.33^{+}	0.33^{+}	0.40^{+}	0.34	0.55^{+}
Fe	oxalate	0.33^{+}	0.36^{+}	0.30	0.71*		
Humic substances		0.33^{+}	0.31	0.27	0.25	0.10	
		[a]Cu_{total} vs am.acet.					
Organic carbon		0.29^{+}	0.21	0.34	0.36	0.72^{++}	
		[a]Cu_{total} vs carbonate					
Humic substances		$(-)0.36^{+}$	$(-)0.35^{+}$	$(-)0.44^{+}$	$(-)0.57^{++}$	(-)0.83**	

+p 0.05
++p 0.01
*p 0.005
**p 0.001

[a] HCl-soluble Cu shows a slightly weaker but similar trend.

Table 5-12 Correlation coefficients (r) for the relationship of Ag or Cd with particle size (percent particles <14μm), organic carbon and fractions of Fe and Mn.

Extraction	Mn_{AmAc}	Mn_{ox}	Mn_{total}	Fe_{HCl}	Fe_{total}	Particle size	Organic carbon
HCl-Ag	0.01	-012	0.17	0.29^{+}	0.49**	(-)0.30^{+}	-0.07
Total-Ag	0.12	-0.05	0.09	0.57**	0.47**	(-)0.19	0.21
HCl-Cd	0.10	-0.21	-0.21	0.50**	0.15	0.34^{+}	0.49**

+$p<0.05$
**$p<0.001$

Table 5-13 Correlation coefficients for the relationship of Ag with carbonate, organic carbon, Fe and humic substances, as affected by excluding (filtering out) from the regression samples with concentrations of filter substrate above a progressively lower filter level. "Filter levels" and sample size for the corresponding regression are shown in Table 5-1.

Filter		Correlation Coefficients (r)					
		All Samples	$Filter_1$	$Filter_2$	$Filter_3$	$Filter_4$	$Filter_5$
Substrate	Fraction						
		Ag_{HCl} vs Fe_{oxal}					
Humic substances		0.31^{+}	0.51*	0.63**	0.67**	0.77*	
Mn	acetic acid	0.31^{+}	0.31^{+}	0.35^{+}	0.43^{+}	0.54*	0.50^{+}
		Ag_{total} vs Fe_{oxal}					
Humic substances		0.58**	0.67**	0.74**	0.79**	0.75*	
Mn	acetic acid	0.58**	0.58**	0.60**	0.73**	0.84**	0.94**
		Ag_{total} vs Humic substance					
Fe	oxalate	0.08	0.16	0.37	0.57^{+}		
		Ag_{total} vs Co_3					
Humic substances		(-)0.17	(-)0.11	(-)0.26	(-)0.41	(-)0.76*	
		Ag_{total} vs Organic carbon					
Mn	acetic acid	0.21	0.26	0.30	0.47^{++}	0.46^{+}	0.59^{+}

+p<0.05
++p<0.01
*p<0.005
**p<0.001

Table 5-14 Correlation coefficients for the relationship of Cd with carbonate, organic carbon, Fe, particle size and Mn, as affected by excluding (filtering out) from the regression samples with concentrations of filter substrate above a progressively lower filter level. "Filter levels" and sample size for the corresponding regression are shown in Table 5-1.

Filter		Correlation Coefficients (r)					
Substrate	Fraction	All Samples	Filter$_1$	Filter$_2$	Filter$_3$	Filter$_4$	Filter$_5$
		Cd_{HCl} vs organic carbon					
Mn	acetic acid	0.49**	0.51**	0.58**	0.68**	0.67**	0.68**
Humic substances		0.49**	0.50*	0.44^{+}	0.30	0.09	
		Cd_{HCl} vs Fe_{oxal}					
Mn	acetic acid	0.49**	0.47*	0.47*	0.60**	0.63**	0.66**
Organic carbon		0.49**	0.45*	0.41^{+}	0.51^{++}	0.65^{+}	
		Cd_{HCl} vs $Mn_{am.acet.}$					
Carbonate		0.10	0.18	0.14	0.43^{+}	0.74**	
		Cd_{HCl} vs Carbonate					
Humic substances		0.07	0.04	(-)0.10	(-)0.32	(-)0.51^{++}	
		Cd_{HCl} vs Particle size					
Mn	acetic acid	0.34^{+}	0.35^{+}	0.35^{+}	0.54*	0.63**	0.75**

+$p<0.05$
++$p<0.01$
*$p<0.005$
**$p<0.001$

LIST OF FIGURES

Figure 5-1

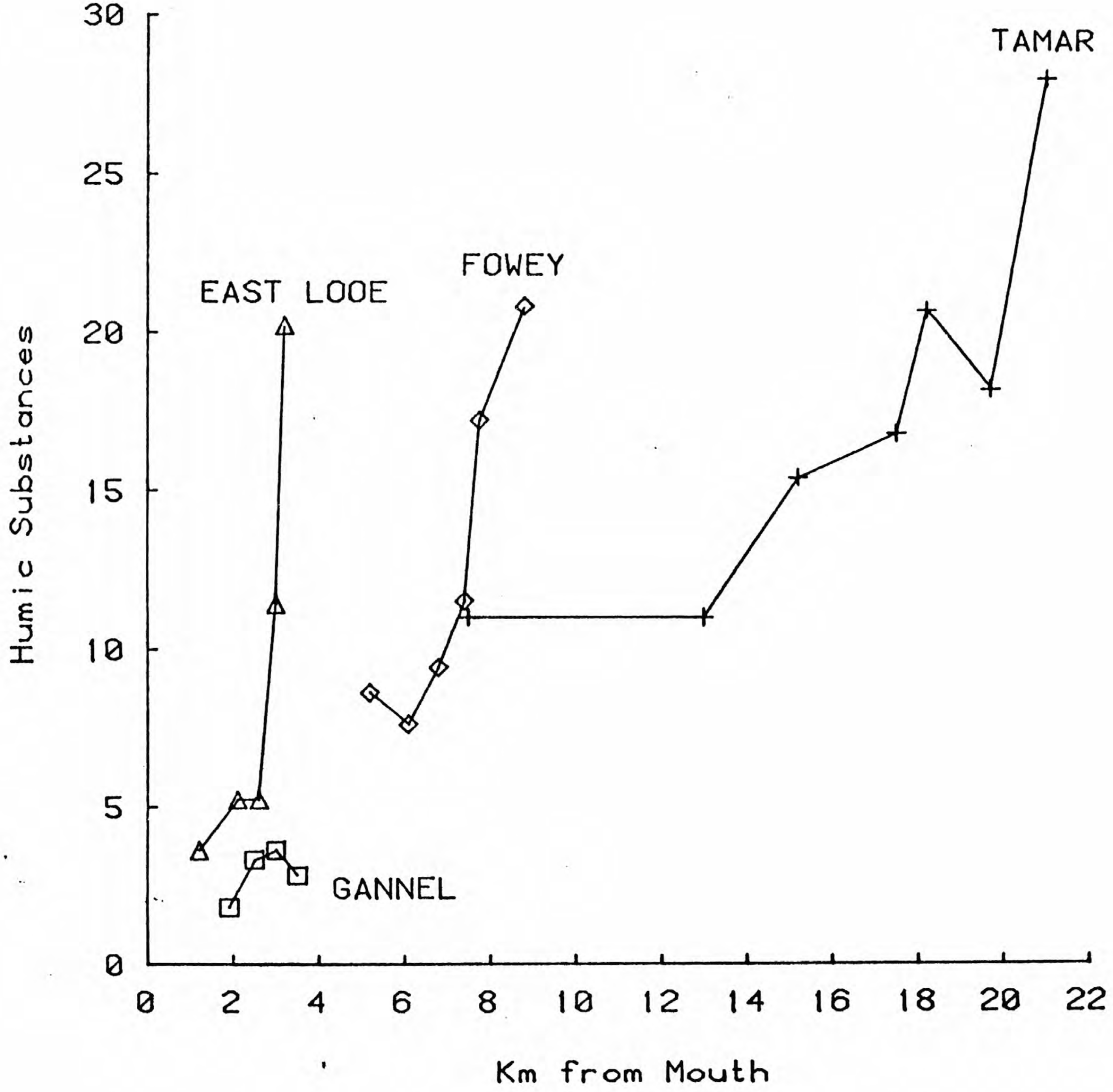
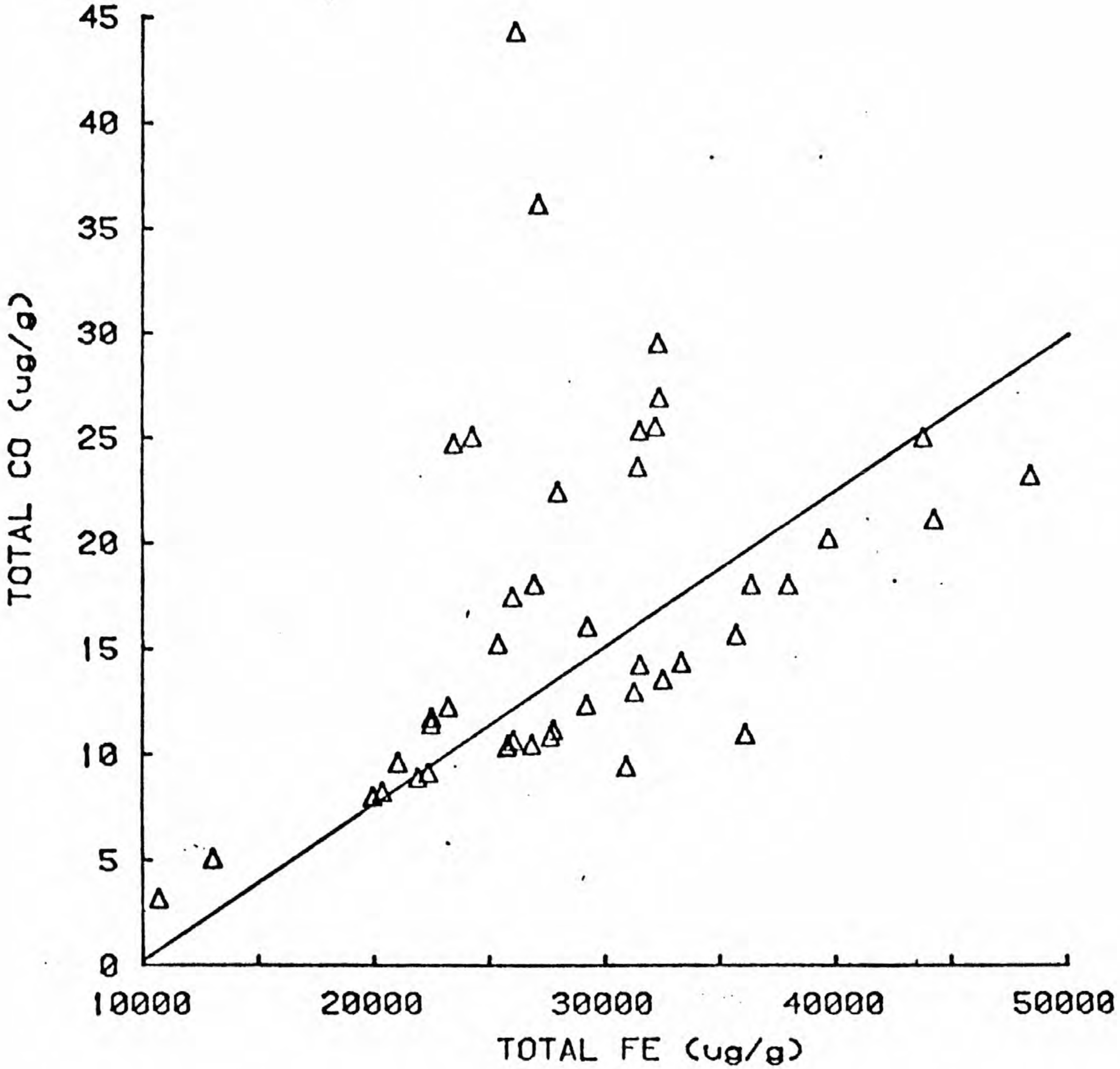
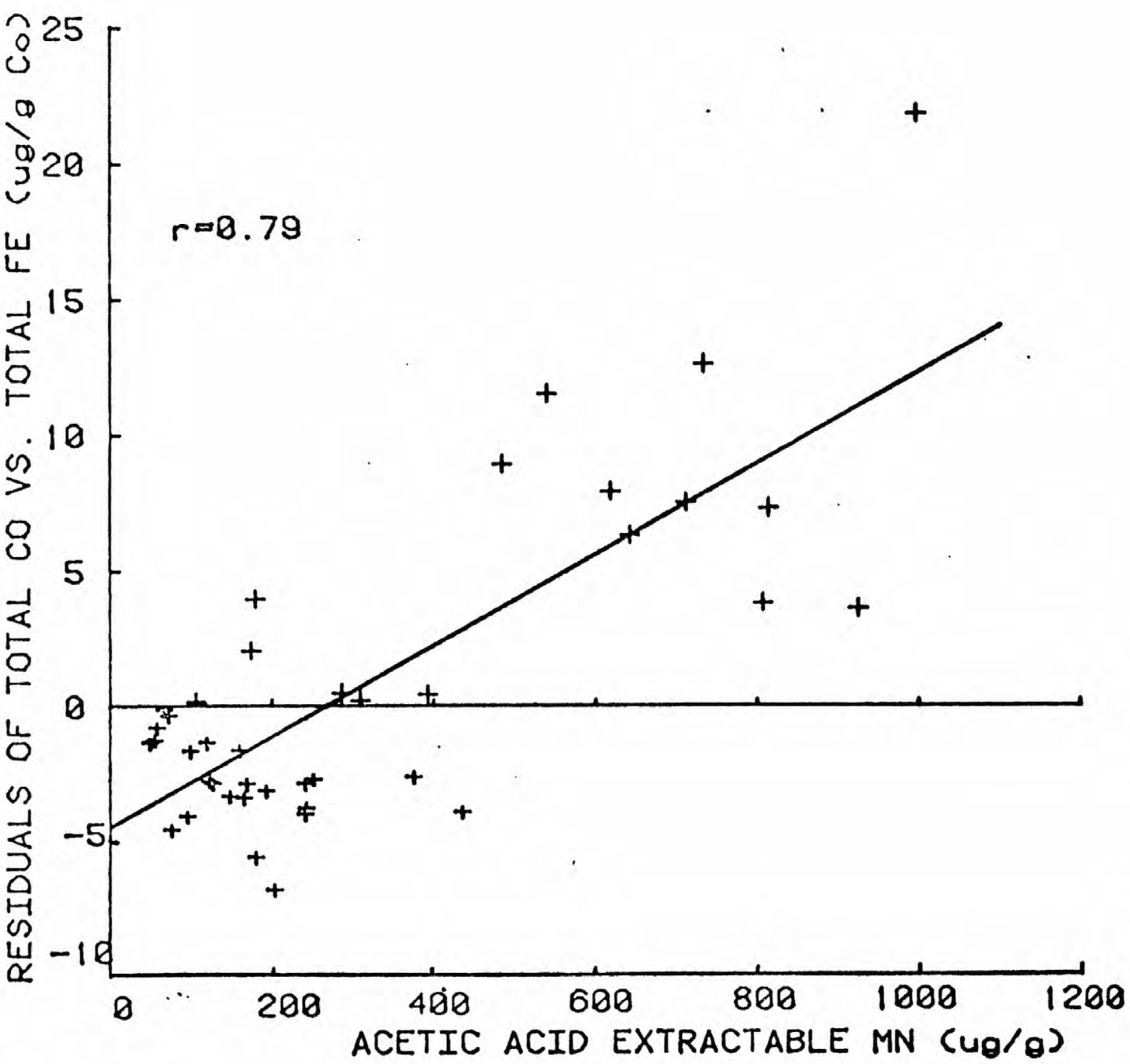

30
25
20
15
10
5
0
Humic Substances
0
2
4
6
8
10
12
14
16
18
20
22
Km from Mouth
TAMAR
FOWEY
EAST LOOE
GANNEL

Figure 5-2a



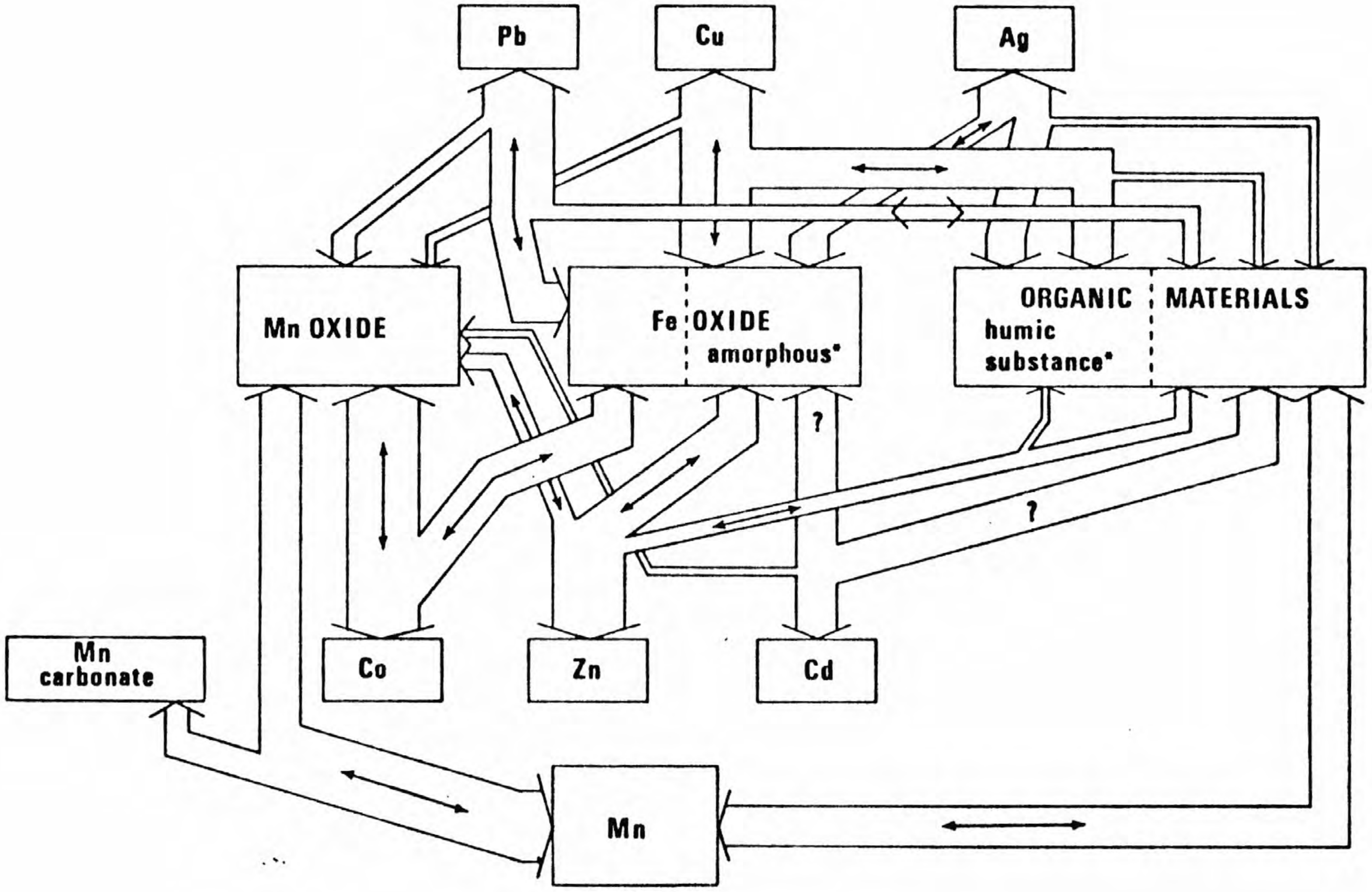
TOTAL CO (ug/g)
45
40
35
30
25
20
15
10
5
0
10000
20000
30000
40000
50000
TOTAL FE (ug/g)

Figure 5-2b



r=0.79
RESIDUALS OF TOTAL CO VS. TOTAL FE (ug/g Co)
25
20
15
10
5
0
-5
-10
0
200
400
600
800
1000
1200
ACETIC ACID EXTRACTABLE MN (ug/g)

Figure 5-3



*Operationally-defined

Chapter 6. Effect of Sample Storage on Metal Extractability from Oxidized Estuarine Sediments

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Trace metal contaminants tend to be highly concentrated in the sediments of aquatic environments, in contrast to the overlying water column. However, neither chemical interactions nor biological impacts are predictable from knowledge of metal concentrations in sediments (Luoma and Jenne, 1977; Luoma and Bryan, in prep. c). Chemical extraction schemes have become a popular mechanism for assessing metal form in sediments (Gibbs, 1973; Brannon, et al, 1976; Tessler, et al, 1979; Luoma and Bryan, in prep. a & b) and for assisting in definition of the biological availability of sediment-bound metals (Loring, 1976; Lee, 1978; Luoma and Bryan, 1978, 1979, in prep. c).

The fraction of metal removed from sediments by chemical extractants may be greatly influenced by physical and chemical characteristics, such as redox state, which themselves may be affected by the manner in which the sediments are treated between the time of collection and extraction. Although problems of sample handling are recognized (LaFleur, 1976), the time and method of sediment storage before extraction are often not mentioned in trace metal extraction studies, despite the fact that many types of storage may result in changes which would greatly alter the chemistry of the sediments.

In this paper we determine the effects of different methods of storing sediment samples on the subsequent extraction of Fe, Mn, Cu, Zn and organic materials from oxidized estuarine sediments. Oxidized sediments were chosen for study because of their importance in the biology and chemistry of trace metals in estuaries (Luoma and Bryan, in prep. a). Oxidized sediments at the interface of the water column and the sediment bed control the exchange of metals between sediment and water in many aquatic environments (Hem, 1977, 1978). Oxidized sediments, either suspended in the water column or at the sediment-water interface, also provide an important source of trace metals for benthic organisms (Luoma and Bryan, 1978, 1979). Because of the complexity of metal partitioning in oxidized sediments (Luoma and Bryan, in prep. a) preservation of the integrity of the samples collected from the field may be especially difficult. Optimum methods of storage would then be worth careful study.

Extraction of Fe, Mn and organic materials is considered because of the potential value in using extractable phases of these binding substrates in conceptual or quantitative models of metal partitioning in oxidized sediments. Copper and zinc were chosen for study because of contrasts in their chemistry which might characterize the range of responses to storage expected from a variety of metals. The two metals show strong differences in partitioning in oxidized sediments (inorganic oxides are more important in binding Zn than Cu and organic materials appear to be more important in the partitioning of Cu than Zn - Luoma and Bryan, in prep. b). Moreover, Cu and Zn are potential pollutants of significance in aquatic environments.

Sediment treatments chosen for comparison included:

(1) Control. Extraction within 24 hours of collection was chosen as the control, since prompt extraction is the most likely method of assuring preservation of the integrity of the sediments (Luoma and Bryan, 1978, in prep. a).

(2) Wet storage. Storage of wet sediment in sealed containers results in a minimum of oxygen exchange. Potential effects include a depletion of oxygen due to microbial activity and changes in the microbial community of the sample. Microbial succession could affect metal chemistry by processes such as sulfide depletion through selection for thiobacilli, or changes in the nature of the organic material from viable to non-viable and back again as microbial populations die and are replaced by other species. To determine if the slowing

of microbial activity at low temperatures would preserve the integrity of the sediments, we compared wet storage at 25° C and at 4° C. We included reduced subsurface sediment for an end-point comparison, based on the assumption that sediment in the sealed containers would approach a reduced state over time.

(3) Freezing. Freezing will stop microbial activity completely. However, the process of freezing will break down (lyse) cells and may free intracellular organic materials, along with any associated trace metals. Freezing also results in flocculation of amorphous inorganic oxides (Luoma, unpublished data) and perhaps some organic material. To determine if flocculation affected extraction results, we sieved sediments before freezing in one treatment, and after freezing (thus removing any large flocs) in a second treatment.

(4) Drying. Drying of oxidized sediment lyses cells and should enhance the rate of crystallization of (hydrous) oxides. We compared drying at 90° C with drying at 25° C.

METHODS AND MATERIALS

Sample Treatment

Silt-clay sediments were collected from an intertidal mudflat in San Francisco Bay in the summer (expt. 1) and winter (expt. 2). Oxidized sediments were scraped from the upper 5mm of the surface of the mudflat and subsurface sediments were collected from the reduced zone 5-10cm below the surface. The sediment was treated as shown in Fig. 6-1. Immediately after collection, sediments were wet sieved through 110µm polyethylene mesh with 1 liter of seawater (salinity 32 0/00) then allowed to settle overnight. The following day excess water was decanted and the remaining slurry was split into representative subsamples. The "control" subsample was extracted immediately. The remaining subsamples of "summer" sediment were used to test the effect on metal extractability of drying (90^{0} C and 25^{0} C) and freezing (Fig. 6-1). The "winter" sediments were used to repeat the freezing experiment and to test the effects of wet storage at 25^{0} C and 4^{0} C.

Two subsamples were employed in each freezing experiment. One was sieved before freezing, and the other was sieved after freezing. In the second experiment both subsamples were allowed to settle in 1 liter of seawater for 24 hours after thawing although only the second sample was sieved. In the first experiment only the unsieved sample contacted the seawater after thawing.

The wet storage experiments were conducted in six sealed plastic containers held in the dark. A different container was sampled at either 15 days, 43 days or 70 days.

Extraction Procedures

For each extraction, a subsample of wet sediment (100-200mg) was pipetted from a slurry into a glass scintillation vial using a pipette sampler. Dried sediment was ground, then a sediment mass similar to the control was weighed into a vial. The ratio of extractant volume: sediment weight was always greater than 25:1. Five replicates of each extraction were conducted for each treatment and the control. A representative subsample, pipetted in duplicate from each slurry, was washed with distilled water then dried at 80° C to obtain an estimate of the salt-free dry weight of sediment used in each extraction. Subsample sediment weights varied less than 7% between duplicates in 10 out of 14 cases; in the other 4 cases weights varied between 7 and 12%.

Trace metal extraction by 9 different techniques was studied (Table 6-1). Except where noted, extractions were conducted for 2 hours at room temperature in scintillation vials following procedures described by Luoma and Bryan (in prep. a). The extract was then separated from the sediment by filtration through a 0.45μm membrane filter. For the ammonium acetate extraction, 10 ml of the sample was passed through the filter before collection in order to reduce metal contamination from the filter membrane.

The filtrate was analyzed for Cu, Zn, Fe and Mn by atomic absorption spectrophotometry. Corrections were made for blanks found in HCl and pyrophosphate reagents. Blanks indicated minimal contamination in the vials or resulting from the filtration procedure relative to metal concentrations in the samples.

Absorbance in the NaOH, DTPA and pyrophosphate filtrates was measured at 480nm to assess the concentration of humic substance extracted. Total organic carbon was determined for controls and wet-storage sediments using a carbon analyzer.

Statistical Analysis

Analysis of variance (ANOVA) was used to test whether or not treatments significantly affected metal extractability. Bartlett's test (Sokal and Rohlf, 1969) was used to determine whether or not the assumption of homogeneous variance among treatment means, which is implicit in ANOVA tests of significance, was valid, and to determine whether or not any treatment affected the degree of variability in metal extraction from a sediment.

Total metal concentrations in some treatments varied significantly from the control, as shown in Tables 6-3 - 6-6. In some cases the difference may be explained by the effect of treatment, but in others the differences result from small unexplained variations among treatments. Thus, we have been conservative in accepting differences as being statistically significant, using $p<0.1$ to indicate significance. Given the inherent variability of sediments, we felt that $p<0.05$ would not always be a meaningful indicator of significant differences.

RESULTS

Comparison of control sediments

Concentrations of metal extracted within 24 hours of collection (used as treatment controls) from oxidized sediments are shown in Table 6-2. The variability among the five replicate extractions was less than 15% (coefficient of variation), in all but 11 cases. Only in four of the ammonium acetate and four of the pyrophosphate extracts, plus one each of the HCl, acetic acid, and hydroxylamine hydrochloride extracts, did the coefficient of variation exceed 15%. Concentration of metals, Fe, Mn and humic substances in the winter sediments (expt. 2) were generally higher than in the summer sediments, as is typical in San Francisco Bay.

Wet Storage

The effect of wet storage on metal extractability varied as a function of time, temperature, extractant and metal. In general, storage at 4° C resulted in changes in Cu, Zn, and Fe extractability similar to those observed at 25° C, but the effects were either delayed or of lesser magnitude in the refrigerated samples. In no instance did refrigeration preserve the sediments in their original state. Changes in extractability with time varied among metals, and for any given metal often varied among extractants.

Zinc. The effects of wet storage on Zn extraction varied with the mode of action of the extractant. When an acidic extractant was used there was a substantial increase in mean extractable Zn during the initial phases of storage (although the precise timing of the enhancement varied with extractant and temperature) followed by a rapid decline in the extractability to near or less than that of the control (Fig. 6-2). Maximum enhancement range for hydroxylamine from 9% at 4° C to 150% at 25° C, and from 46% to 77% at both temperatures for HCl, acetic acid and oxalate. An initial enhancement (40-150%) of Zn extractability by DTPA at 25° C, and pyrophosphate and ammonium acetate at both temperatures was also observed. The extraction of Zn by these techniques was substantially inhibited after 70 days storage, however, especially at 25° C (Fig. 6-2; Table 6-3). The reduced extractability of Zn with time by acetic acid, pyrophosphate and hydroxylamine suggests that long-term storage in sealed containers may result in extractability approaching that of subsurface, reduced sediment (Table 6-3); however, this trend is not seen in the extraction of Zn by ammonium acetate, HCl, oxalate and DTPA.

Wet storage also affected the replicability of Zn extraction from sediments. Enhancement of mean Zn concentrations at 15 days and 43 days coincided with large (up to 40 fold) increases in the standard deviation and coefficient of variation (Table 6-4). Bartlett's test indicated significant inhomogeneity of variances in most extractants after 15 days and 43 days compared to the control. Contamination is always a potential problem with Zn analyses. We investigated potential contamination sources in the laboratory procedure but found no evidence of contamination in the blanks. Also, several lines of evidence suggested the increased variability in extractable Zn was a treatment effect:

(1) Large standard deviations were not observed in the extraction of Zn, by any method, from either control sediments or sediments stored for 70 days (Table 6-4). Standard deviations were less than 5 μg/g in 15 cases and less than 12μg/g in 5 cases.

(2) The large standard deviations in the 15 day and 43 day storage experiments did not occur for all extractants on the same day, but did follow similar trends (e.g. a decline in the standard deviation over time for sediments stored at room temperature - Table 6-4) and

(3) A delay in the onset of variability and/or a lower magnitude of variability in Zn extraction was observed at 4^{o} C, compared to room temperature, which is a result consistent with the biological processes hypothesized to cause the effects in this treatment. If the changes in the variance of Zn extractability were treatment effects, then wet storage for short periods of time may have resulted in an initial heterogeneous partitioning of Zn as microbial populations in the sediments changed and the sediment underwent the initial stages of anaerobiosis. An increased abundance with time of black spots and other irregularities in the sediment indicated differences in microbial activity and redox state in different microenvironments were occurring. As the sediment equilibrated toward a more uniform reduced state, the

partitioning of Zn returned to a more homogeneous condition.

Copper. The changes in copper extractability with wet storage were nearly opposite those of Zn. The effects of wet storage on Cu were generally similar for all extractants, and variances were homogeneous with time and extractant.

The extractability of Cu sharply declined during the initial phases of storage, as expected if Cu were being converted to the highly insoluble form characteristic of reduced sediments (Table 6-5; Fig. 6-3). The initial decline was much less substantial at 4° C - also consistent with the sensitivity of Cu to the degree of anaerobiosis in the sediments. Surprisingly, Cu was more extractable after 70 days storage than after the shorter periods, except in HCl and hydroxylamine extracts of sediments stored at 25° C and in NaOH extracts of both sediments. In only a few instances did extractability recover to the level of the control by 70 days, however.

Iron. The extraction of Fe was enhanced by the wet storage of sediments (Fig. 6-4; Table 6-6), with the exception of pyrophosphate extracts at 43 days (both temperatures) and 70 days (25° C) and HCl extracts at 15 days (both temperatures) and 43 days (4° C). After 70 days storage, total Fe concentrations in the sediments were significantly lower than in the control, apparently reflecting a mobilization of some Fe from a solid to a solute in the increasingly reduced sediment. When concentrations of Fe extracted after 70 days' storage were corrected for this decline in "total" Fe (assuming a value of 100 if no loss occurred), the resultant values were similar to the concentrations of Fe extracted from subsurface reduced sediment (Table 6-6).

Bartlett's test indicated that the variance in Fe extractability among treatment means was generally homogeneous for all extractants.

Manganese. The primary effect of wet storage on Mn was a reduction in total Mn observed after 70 days (Table 6-7). Similar to Fe, the decline in total Mn indicated some conversion of Mn to a reduced, solute form. Wet storage had little effect on the extractability of Mn by acids, DTPA or hydroxylamine, when a correction for the loss of total Mn at 70 days was made. A significant and substantial inhibition of pyrophosphate-Mn (as much as 45%) and an initial enhancement of ammonium acetate-Mn (as much as 106%) were observed during wet storage, however. The enhancement of ammonium acetate extractability from refrigeration samples suggests that a biological process was involved.

No wet storage treatment affected the replicability of Mn extraction. Standard deviations were small for Mn; thus minor differences in concentration between treatments and the control were statistically significant.

Exposure to air. Preliminary wet storage experiments were conducted with sediments stored in open beakers inside plastic bags. As expected, effects were generally the same as for sediments stored in sealed containers, but most effects were delayed or inhibited by contact of the sediments with a renewable supply of oxygen. One subsample of wet sediment was also aerated for 16 days in a covered beaker using an air stone and clean air pump. Extractability of Cu, Fe and Mn from the aerated sediment by most methods was similar to that from sediments stored wet for 15 days without aeration (Tables 6-3, 5, 6, 7). In contrast, Zn extractability was generally inhibited in the aerated sediments, compared to sediments stored without aeration.

Freezing

Total concentrations of Cu, Zn, Fe and Mn in frozen sediments were generally lower than total concentrations in control sediments (Table 6-8). The differences were more substantial in the three treatments where sediments were immersed in seawater (if sieving or to simulate sieving effects) after freezing than in the one treatment (Expt. 1, sieving before freezing) where sediments contacted seawater only before freezing. The results suggest freezing enhanced the water solubility of the four metals. Perhaps metals were released when bacterial cells were lysed by freezing and thawing. differences between experiments 1 and 2 in the degree of metal loss suggested different quantities of metal may be freed from sediments with different characteristics. Among the metals, the order in which freezing enhances water solubility was Mn (8-17%) > Cu(9-15%) > Zn(6-12%) > Fe(3-7%). Some unexplained loss of total Mn (10%) was observed in sediments which contacted seawater only before freezing. If a similar loss occurred in sediments sieved after freezing in experiment 1, the 17% loss of total Mn may not be totally due to the effects of freezing the sediment. Although the loss of total metal was relatively small in most cases, it was sufficient to necessitate correcting treatment/control ratios for the extractable metal concentrations.

In general, concentrations of metals removed from the sediments by complexation, reduction or exchange showed some effect from freezing. The chemical characteristics of the sediments influenced these effects, as shown by differences between summer and winter sediments (experiments 1 and 2, respectively). The results of the freezing experiments were generally quite variable. However, very evident effects included in 144-152% enhancement of Zn extraction and 258-301% enhancement of Fe extraction by DTPA in experiment 1; a 27-83% enhancement of Zn extraction by hydroxylamine (observed in both experiments); plus a substantial enhancement (55-73%) and inhibition (32-44%) of Zn extraction by ammonium acetate in experiments 1 and 2, respectively. No differences of greater than 25% were observed between control and treatment sediments extracted by acetic acid, HCl, oxalate or NaOH and most of these concentrations (34 out of 42) were within 10% of the control.

The variability in metal concentrations extracted from frozen sediments was largely the result of flocculation which occurred as a result of freezing. Flocculated particles were visually evident in sediments sieved before freezing, (especially sediments collected in winter, when concentrations of amorphous Fe and humic substances were highest), and may have contributed to the difficulty of obtaining homogeneous subsamples of consistent size from all slurries of frozen sediment. Sieving after freezing visually appeared to remove many of the large flocs from the sediments. However, floc removal had no consistently detectable effect on metal concentrations in either experiment, suggesting the particles comprising the large flocs had the same metal concentrations as particles which passed through the sieve.

Drying

Extraction of metals by 0.5 N HCl was the only technique not affected by drying sediments for storage (Table 6-9). Effects specific to other extractions varied among metals and between drying temperatures. In the course of drying the room-temperature sediment, water was poured off the subsample after several days to facilitate dehydration. Evidence of some loss of Zn (9%) and perhaps Fe and Mn (4 and 6%, respectively) due to the water removal is indicated by the lower "total" metal concentration extracted from the 25° dried sediment than from either the control or 90° C dried subsamples.

Drying the sediments reduced the quantity of Fe extracted by techniques which removed the most amorphous Fe oxides (acetic acid, pyrophosphate, hydroxylamine) by at least 20%, suggesting an increase in the rate of Fe crystallization. This effect was most evident when sediments were dried at 90° C. In contrast the quantity of Fe extracted by complexation with DTPA from dried sediments was more than twice that of the control.

Drying also increased the extractability of Mn by complexation and by ammonium acetate by at least 20%, but had little effect on Mn removal by acids. Most changes in the extractability of Zn from dried sediments were consistent with the partitioning of this metal between Fe and Mn oxides. In 2 cases where the extraction of Fe was inhibited by more than 30% (90° C in pyrophosphate and hydroxylamine), Zn extraction was also inhibited. In other extractions the effects on Zn fell between the effects on Fe and Mn. The extraction of Cu was enhanced in all extractants except HCl and acetic acid, perhaps reflecting a change in the nature of organic ligands caused by drying the sediments.

DISCUSSION

The optimal method for storing oxidized sediments before trace metal analyses will depend upon the type of trace metal extraction planned for the sediments.

Drying of sediments is preferable to either wet storage or freezing for total metal analyses. During wet storage substantial solubilization of Fe and Mn may occur if the sediment becomes reduced. In this study, as observed in nature (Goldberg, et al, 1978), the mobilization of Fe and Mn in wet sediment was not accompanied by a mobilization of trace metals. This was probably due to precipitation of metal sulfides.

Freezing of the sediments mobilizes metals. Manganese, Cu and Zn were proportionately more affected by freezing than was Fe. Freezing also affects the physical characteristics of some sediments. Thus any particle size analyses or sieving to remove large particles should be done before freezing. It is notable, however, that we detect no effect of sieving after freezing on total metal concentrations.

Either freezing or drying are adequate methods of storing sediments for NaOH extraction of Cu or HCl extraction of all metals. Only freezing is satisfactory for acid extraction employing either acetic acid or acid ammonium oxalate, however. Drying sediments inhibits the extraction of Fe by acetic acid by 27 and 37% (25^{o} C and 90^{o} C, respectively), greatly enhances the oxalate extractability of Cu (154 and 264%), and inhibits the oxalate extractability of Zn (18 and 14%). Wet storage of sediments is unsatisfactory for NaOH or acid extraction of trace metals. The possible formation of metal sulfides (especially for metals such as Cu, with highly insoluble sulfides) and other processes which occur during reduction of stored wet sediments may greatly change the extractability of metals and affect precision between replicate extractions. Refrigeration at 4^{o} C delays and/or inhibits the effects of wet storage, but within 15 days many of the characteristics of the refrigerated sediments changed detectably.

Extraction of Organic Materials

Absorbance is a colorimetric measure of concentration of alkali-soluble organic material (primarily humic substances). Absorbance per gram sediment was measured on the sodium hydroxide, DTPA and pyrophosphate extractions of the wet-stored, frozen and aerated sediments of experiment 2, and on the sodium hydroxide extractions of dried sediments and frozen sediments of experiment 1. In control sediments, absorbance varied with extractant in the order NaOH<DTPA<Pyrophosphate although all control values were generally low, less than 9 absorbance units per gram. Only pyrophosphate absorbance was significantly affected by storage of sediment (Table 6-10), and only when sediment was stored wet. Pyrophosphate absorbance for samples stored at both 25° C and 4° C was enhanced with time of storage, most strongly between 15 and 43 days, nearly doubling in value.

Total organic carbon (%) was measured only for the 43 day and the 70 day wet-stored sediment. Wet storage at both 25° C and 4° C resulted in a 7-17% loss of organic carbon with time (Table 6-10).

No method of storage preserved the extractability of all metals by complexation with DTPA or pyrophosphate, reduction at low pH in hydroxylamine or desorption by ammonium acetate. Extraction of fresh sediments by these techniques may aid in determining the fraction of biologically available metal in sediments (Lee, et al, 1978; Luoma and Bryan, 1979; Luoma et al, unpublished data). Storage of such sediments before extraction will greatly compromise the results obtained from such extractions.

In summary, wet storage causes the greatest changes to occur in oxidized sediments held for chemical extraction and trace metal analysis. Freezing and drying of sediments are satisfactory methods for maintaining some of the acid extractability of Cu, Zn, Fe and Mn, with freezing being preferable. However, oxidized sediments must be extracted immediately upon collection from the field (within 24 hours) if the in-situ chemical characteristics defineable by a variety of extractants are to be retained.

Summary - Chapter 6

Concentrations of extractable Cu, Zn, Fe and Mn in oxidized estuarine sediment are influenced by the manner and time of storage between collection and extraction. No storage method tested maintains the initial chemical and physical integrity of the sediment. Extractions should be done immediately to assure accurate results for a variety of extractants.

Collected sediment was wet sieved (110μm mesh) and split into subsamples: control (extracted after 24 hours); wet storage (4^{o} C or 25^{o} C) for 15, 43 or 70 days; frozen storage; and dried storage. Nine extractants were applied to the sediment after storage. Concentrations of Cu, Zn, Fe and Mn extracted from the stored subsamples were compared to those from the control subsample. The largest changes in concentration of trace metals, humic substances, and other organics occurs when sediment is stored wet. Using the control as a standard, drying is an adequate pre-storage treatment if only NaOH extraction of Cu and/or HCl extraction of Cu, Zn, Fe and Mn are to be done. Freezing is a satisfactory storage treatment if Cu, Zn Fe and Mn are to be extracted from sediments using HCl, acetic acid, and acid ammonium oxalate and/or Cu is to be extracted using NaOH. Both drying and freezing affect physical characteristics of the sediment and the extent of metal extraction when using weaker extractants (ammonium acetate, Na pyrophosphate, DTPA, and hydroxylamine hydrochloride).

Table 6-1 Extractants applied to separate subsamples of sediment.

Extractant	Comments	References
Concentrated nitric and sulfuric acid digestion (total)	"Near-total" metal	Luoma and Bryan (in prep. a)
0.5 N HCl[a]	a,b,c: "Non-lithogenic" or "adsorbed" metal	a: Luoma and Bryan (in prep.a) Agemian and Chau (1977); Malo (1977)
25 percent acetic acid,[b] pH 2.2		b: Loring (1976)
0.4 N ammonium oxalate[c] in 0.4 N oxalic acid, pH 3.3		c: acid ammonium oxalate-Schwertmann (1964); Eaton (1979)
0.1 N hydroxylamine hydrochloride[1] in 0.01 N nitric acid, pH 2.0, extracted for ½ hour	low-pH reducing agent	Chao (1972)
0.1 N Na pyrophosphate[d], pH 10, extracted for one week	d and e: Alkaline extracts with complexing agents	
0.004 N DTPA[e], pH7		
0.1 N NaOH, pH 12	Alkaline extract without a complexing agent	Luoma and Bryan (in prep. a)
1 N ammonium acetate, pH7	Ion exchange	Luoma and Bryan (1979; in prep.a)

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[1] To reduce effects of carbonate neutralization, each hydroxylamine extract was titrated back to pH 2.0 after contacting the sediments for several minutes.

Table 6-2 Mean metal concentrations (µg/g dry weight) and concentrations of humic substances (absorbance/g) ± one coefficient of variation (%) extracted from oxidized control sediments, plus the mean total metal concentration ± 1 cv observed among all treatments in which an effect on total metal was not definable (total all). For total all n = 3 in experiment 1 and n = 6 in experiment 2.

Metal	HCl		acetic acid		oxalate		Ammonium acetate		Pyrophosphate		DTPA		Hydroxylamine		NaOH		Total control		Total All	
	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)	µg/g	cv(%)
									Experiment 1											
Cu	38.7	3.7	28.2	4.6	12.5	4.9	nd	-	10.5	18.8	7.44	13.4	8.11	20.3	14.9	5.2	57.8	6.0	57.2	2.3
Zn	56.0	17.2	48.9	8.3	40.5	6.0	0.8	17.8	15.4	42.8	16.9	9.7	25.6	6.4	-	-	120	2.9	117	2.0
Fe	8236	6.0	5451	4.5	8627	6.0	nd	-	1046	4.5	1951	6.9	2491	2.3	-	-	47856	1.3	48407	1.1
Mn	533	5.6	571	4.3	464	2.8	162	2.1	259	3.4	372	6.4	430	5.0	-	-	949	0.05	917	5.8
Humic Substance															2.7	8.1				
									Experiment 2											
Cu	53.9	4.8	47.0	4.8	33.2	6.0	2.85	26.3	27.5	3.0	25.0	4.9	27.7	2.9	32.7	1.9	77.8	3.9	74.0	3.8
Zn	79.5	11.1	74.3	15.4	56.0	13.4	6.2	40.6	32.0	4.2	35.1	11.4	39.0	5.3	-	-	156	5.7	153	3.2
Fe	10436	5.7	6367	3.0	9016	9.4	nd	-	2681	3.0	1436	8.4	2537	5.6	-	-	56850	2.1	56752	2.2
Mn	1063	2.8	1192	2.2	981	4.4	282	3.6	740	4.8	794	4.7	941	3.2	-	-	1463	2.5	1417	1.9
Humic Substance											4.7	6.2	8.8	6.2	4.1	5.0				

Table 6-3 The mean treatment/control ratio (in percent) for concentrations of zinc extracted from sediments after stored wet (expt. 2) in sealed containers for 15 days to 70 days, and aerated at 25^{0} C for 16 days, compared to subsurface reduced sediment (expt. 1), ANOVA was used to test the aerated and both 70 day treatments for all extractants except "total". Inhomogeneous variances in the other treatments prevented testing by ANOVA. All treatments were used for ANOVA for"total" zinc.

		(Treatment/Control) X 100						
		25^{0} C			4^{0} C			
Extractant	Aerated	15 days	43 days	70 days	15 days	43 days	70 days	subsurface
Ammonium acetate	59	163	51	26**	75	234	103	172
HCl	83**	156	99	82**	97	146	93	136
Acetic acid	84*	122	166	73**	85	150	84*	64
Oxalate	113	177	155	93	177	104	100	113
Pyrophosphate	148a	179	39	34**	93	140	50**	16
DTPA	91	167	161	57**	94	67	72**	251
Hydroxylamine	96	110	250	94	109	107	82**	50
Total	102	93*	96	103	97	98	117a	129

a= inhomogeneous variance
*=p<.01
**=p<.001

Table 6-4 Comparison of the coefficient of variation and the standard deviation for Zn extracted from sediments stored wet in sealed containers (expt. 2) at 25° C or 4° C for 15, 43 or 70 days

Treatment	HCl		Acetic acid		Oxalate		Ammonium acetate		Pyrophosphate		DTPA		Hydroxylamine	
	sd (µg/g)	cv (%)	sd (µg/g)	cv (%)	sd (µg/g)	cv (%)	sd (µg/g)	cv (%)	sd (µg/g)	cv (%)	sd (µg/g)	cv (%)	sd (µg/g)	cv (%)
Control	8.9	11.1	11.4	15.4	7.5	13.4	2.5	40.5	1.34	4.2	4.0	11.5	2.1	5.3
25° C 15 days	44.0	35.5	20.3	22.3	28.7	28.9	5.8	58.0	26.0	45.5	30.5	52.1	4.6	10.7
25° C 43 days	13.7	17.3	30.8	24.9	19.6	22.6	0.4	13.5	7.1	56.4	13.2	23.3	30.8	31.6
25° C 70 days	6.1	9.5	3.1	5.7	2.9	5.7	1.2	74.8	2.8	26.2	3.8	18.6	2.5	6.8
4° C 15 days	7.1	9.2	7.9	12.6	31.4	31.6	0.7	14.3	2.2	7.3	7.7	23.2	1.8	4.3
4° C 43 days	27.7	23.9	32.1	28.8	11.6	19.8	6.1	41.9	46.1	102.8	3.5	14.8	6.0	14.3
4° C 70 days	2.9	3.9	1.1	1.8	3.2	5.8	1.8	28.5	0.7	4.5	5.3	21.1	3.4	10.6

Table 6-5 The mean treatment/control ratio (in percent) for concentrations of copper extracted from sediments stored wet (expt. 2) in sealed containers for 15 days to 70 days, and aerated at 25° C for 16 days, compared to the ratio in subsurface reduced sediment (expt. 1).

		(Treatment/Control) X 100						
		25° C			4° C			
Extractants	Aerated	15 days	43 days	70 days	15 days	43 days	70 days	subsurface
HCl	97	79**	76**	80**	106	84**	93	33
Acetic acid	85**	52**	53**	76**	94	89*	105	nd
Oxalate	40**	14**	8**	34**	52**	64**	68**	nd
Pyrophosphate	10**	13**	34**	47**	39**	77**	84*	11
DTPA	27**	19**	38**	50**	47**	60**	75**	376
Hydroxylamine	57**	49**	44**	35**	73**	90	69**	nd
NaOH	85**	64**	58**	64**	82**	88**	85**	21
Total	94**	92**	97	101	98	91**	101	142

*=p<.01
**=p<.001

Table 6-6 The mean treatment/control ratio (in percent) for iron extracted from sediments stored wet (expt. 2) in sealed containers from 14 days to 70 days, and aerated at 25^{0} C for 16 days compared to subsurface reduced sediment (expt. 1). The numbers in parentheses are corrected for the decline in total Fe assuming a value of 100 if no loss occurred.

	(Treatment/Control) X 100							
		25^{o}C			4^{o}C			
Extractant	Aerated	15 days	43 days	70 days	15 days	43 days	70 days	sub-surface
HCl	100	100	111*	105 (123)	100	100	104 (121)	127
Acetic acid	117*	143**	146**	125** (143)	116*	123**	125** (142)	161
Oxalate	112*	117**	133**	111* (129)	105	128**	124** (141)	129
Pyrophosphate	121**	140**	61**	81** (99)	126**	88*	105 (122)	131
DTPA	158**	213**	144**	157** (175)	165**	198**	215** (232)	348
Hydroxylamine	108	155**	122**	132** (150)	107	143**	129** (146)	217
Total	103	97	99	82**	101	98	83**	122

*=p<.01
**=p<.001

Table 6-7 The mean treatment/control ratio (in percent) for extraction of manganese from sediments stored wet (expt. 2) in sealed containers for 15 days to 70 days at 25° C and 4° C, and aerated at 25° C for 16 days, compared to subsurface reduced sediment (expt. 1). Numbers in parenthesis are values corrected for the loss of total metal (i.e. assuming treatment/control would equal 100 if no loss occurred).

(Treatment/Control) X 100

		25° C			4° C			
Extractant	Aerated	15 days	43 days	70 days	15 days	43 days	70 days	subsurface
Ammonium acetate	157**	206**	148**	71** (92)	172*	105**	65** (81)	32
HCl	84**	95	93*	87** (108)	94	91*	88** (104)	31
Acetic acid	87**	95	93*	87a (108)	90**	93*	96 (112)	29
Oxalate	78**	86**	90**	85** (106)	86**	88a	95 (111)	30
Pyrophosphate	97	108**	80**	48** (69)	108**	55**	47** (63)	25
DTPA	88**	95	86**	73** (94)	100	85**	88** (104)	31
Hydroxylamine	82**	91**	93*	85** (106)	92**	92**	95 (111)	28
Total	95**	96*	97	79**	97	97	84**	63

*=p<.01 **=p<.001

Table 6-8 The mean treatment/control ratio (in percent) for Zn, Cu, Fe and Mn extracted from sediments which were frozen prior to extraction. Experiment 1 was sediment collected in summer (low amorphous Fe, Mn and organic materials) and experiment 2 was sediment collected in winter (higher Mn, organic materials and amorphous Fe). All numbers except totals have been adjusted for loss of total metal, assuming treatment/control for total was 100 if no loss occurred.

	Cu [(treatment/control) X 100]				Zn [(treatment/control) x 100]				Fe [(treatment/control) X 100]				Mn [(treatment/control) X 100]			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1		Experiment 2	
	sieved* before	sieved after	sieved before	sieved after	sieved* before	sieved after	sieved before	sieved after	sieved* before	sieved after	sieved before	sieved after	sieved* before	sieved after	sieved before	sieved after
Ammonium acetate	nd	nd	nd	nd	173	155	68	56	nd	nd	nd	nd	160	115	93	96
HCl	124	108	92	106	120	101	88	120	114	106	95	104	114	106	89	97
Acetic acid	105	93	89	93	112	99	92	89	111	108	97	106	111	105	88	99
Oxalate	81	102	119	105	94	96	92	99	121	96	110	108	107	104	86	91
Pyrophosphate	71	97	124	107	105	109	102	258	131	108	112	123	128	98	92	104
DTPA	115	123	117	102	252	244	104	113	401	358	120	129	124	121	89	98
Hydroxylamine	97	107	85	82	114	110	130	183	127	131	80	83	109	109	90	95
Sodium hydroxide	115	107	103	98												
Total	96	85	91	93	96	88	92	94	101	93	97	93	90	83	92	91

*not immersed in seawater after freezing

Table 6-9 Mean treatment/control ratios (in percent) for Cu, Zn, Fe and Mn extracted from sediment dried at 25° C or 90° C (expt. 1). Means are from 5 replicates. Asterisks indicate statistically significant difference from control.

	Mean treatment/control X 100							
	Cu		Zn		Fe		Mn	
	Dried		Dried		Dried		Dried	
Extractant	25°C	90°C	25°C	90°C	25°C	90°C	25°C	90°C
AmAc			333	282			125**	143**
HCl	110*	112**	106	103	98	98	102	106
Acetic acid	95	97	86*	104	73**	63**	102	106
Oxalate	254**	364**	82	86	112*	82**	108	109*
Pyrophosphate	188**	293**	170*	86	78**	66**	121**	138**
DTPA	227**	328**	212**	131**	285**	250**	124**	125**
Hydroxlamine	144**	145**	98	46**	80**	22**	108*	94
NaOH	104	125**						
Total	98	101	91**	97	96	102	94	100

*=p<.01
**=p<.001

Table 6-10 Concentrations of humic substances (absorbance/g) extracted from control and treated sediments by three extractants, plus percent organic carbon in the sediment.

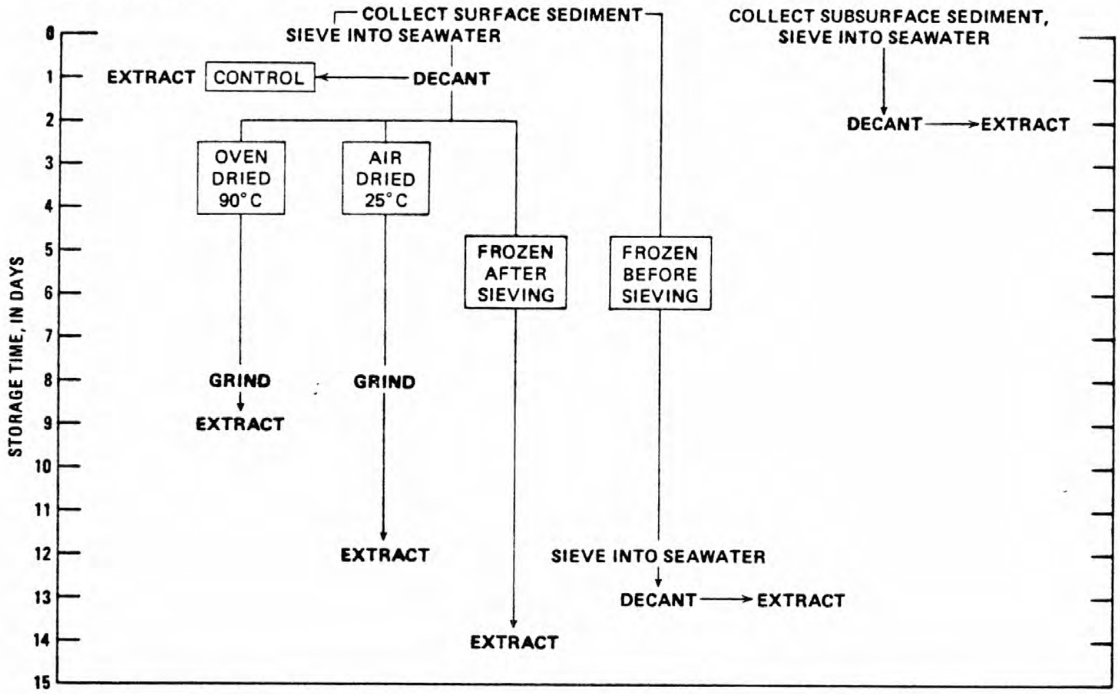
	Humic substances (absorbance/g)			
Treatment	NaOH	DTPA	Pyrophosphate	Total Organic (Carbon %)
Experiment 1:				
Control	2.70			
sieved after freezing	1.6			
sieved before freezing	2.93			
Dried 90° C	3.34			
Dried 25° C	2.67			
Experiment 2:				
Control	4.14	4.7	8.8	1.50
25° C, 15 days	2.9	6.9*	11**	
25° C, 43 days	3.49	5.9	16.0**	1.35
25° C, 70 days	2.8	5.6	15.5**	1.34
4° C, 15 days	3.1	6.1*	10*	
4° C, 43 days	3.97	5.6	16.0**	1.39
4° C, 70 days	2.7	6.9*	17.3**	1.32
sieved after freezing	3.97	4.9	8.96	
sieved before freezing	3.39	4.7	8.7	
aerated	3.97	5.6	10.2*	

*=$p<.01$
**=$p<.001$

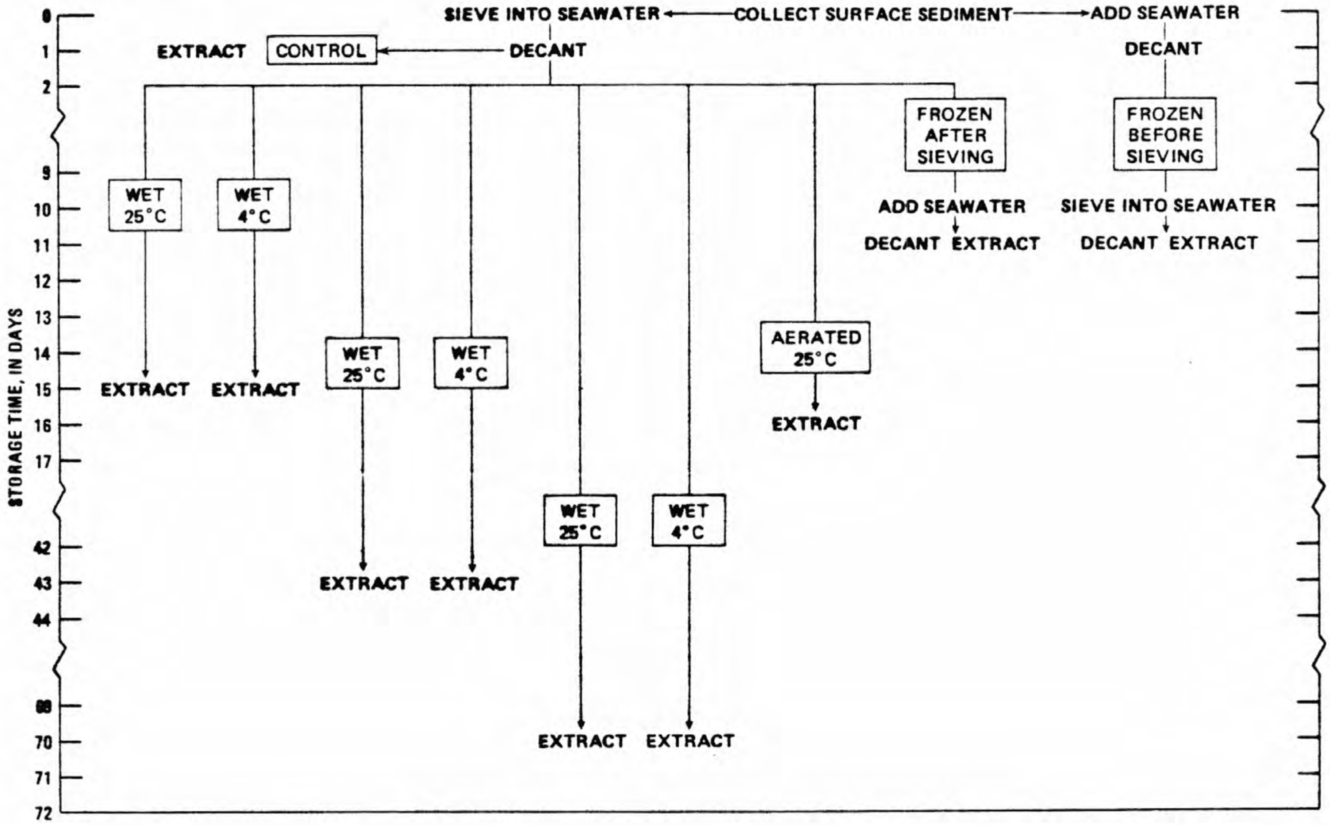
List of Figures

Fig. 6-1

EXPERIMENT 1



EXPERIMENT 2



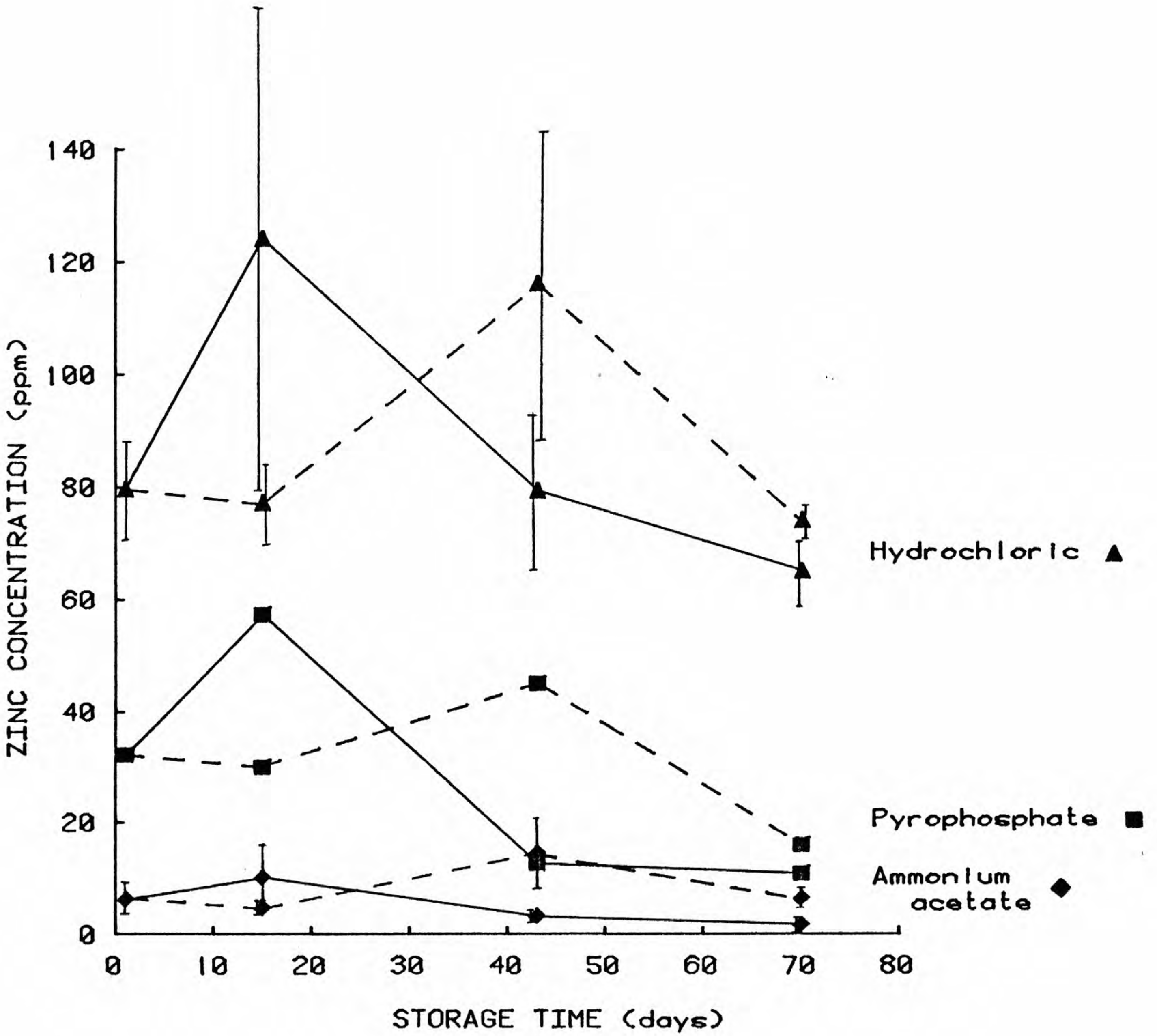
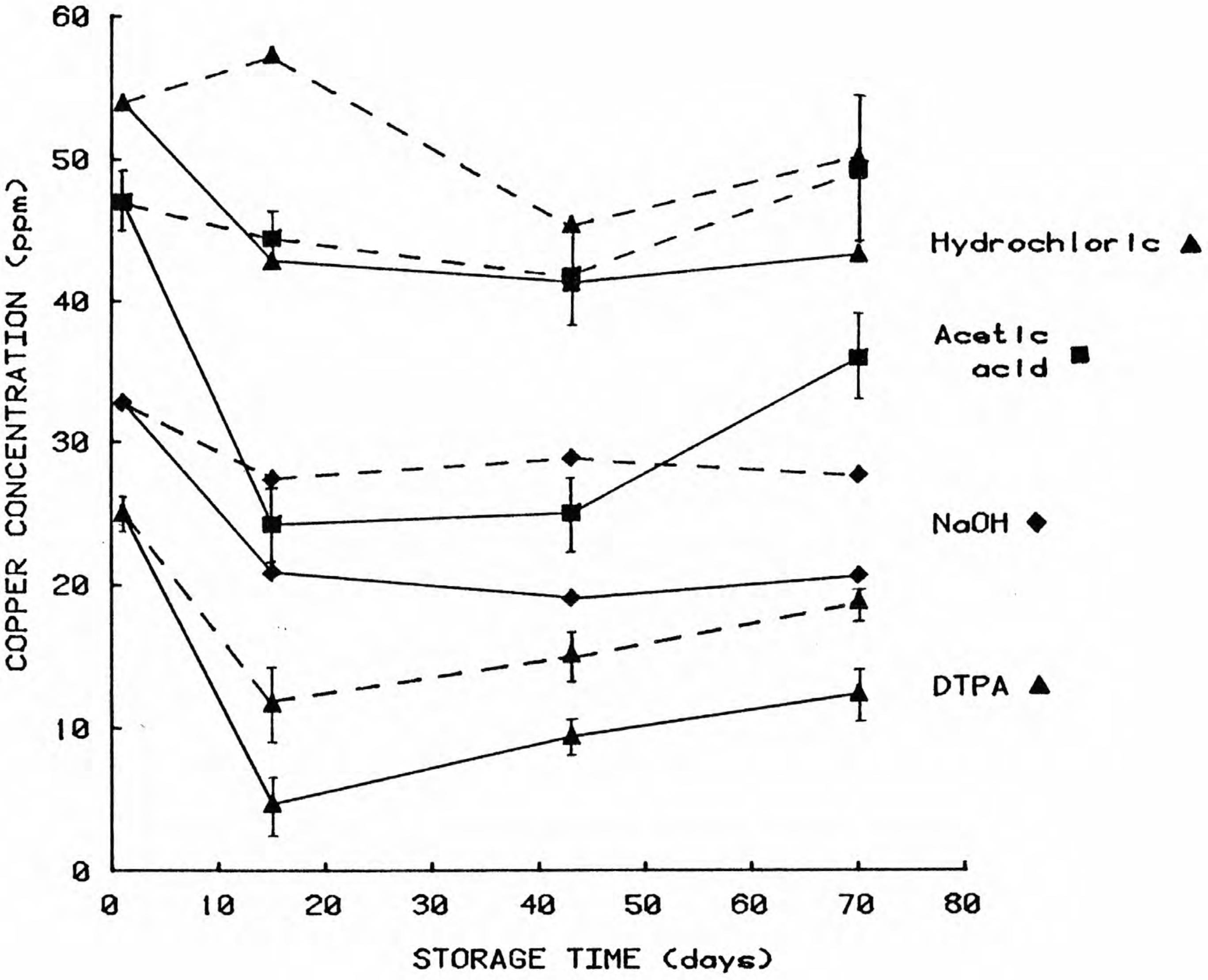

ZINC CONCENTRATION (ppm)
0
20
40
60
80
100
120
140
STORAGE TIME (days)
0
10
20
30
40
50
60
70
80
Hydrochloric ▲
Pyrophosphate ■
Ammonium acetate ◆

Fig. 6-2.



COPPER CONCENTRATION (ppm)
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
STORAGE TIME (days)
Hydrochloric ▲
Acetic acid ■
NaOH ◆
DTPA ▲

Fig. 6-3

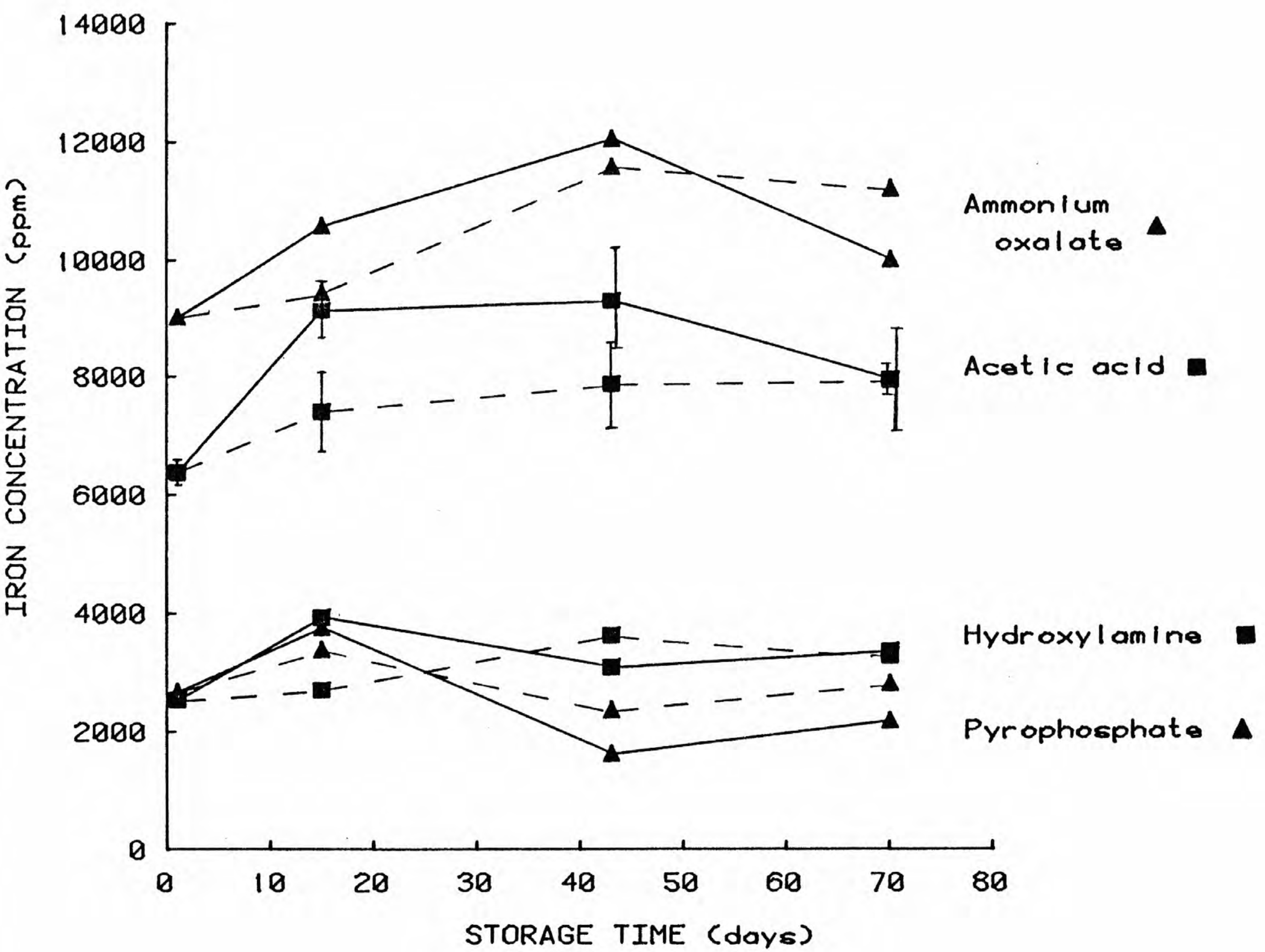

IRON CONCENTRATION (ppm)
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
60
70
80
STORAGE TIME (days)
Ammonium oxalate ▲
Acetic acid ■
Hydroxylamine ■
Pyrophosphate ▲

Fig. 6-4

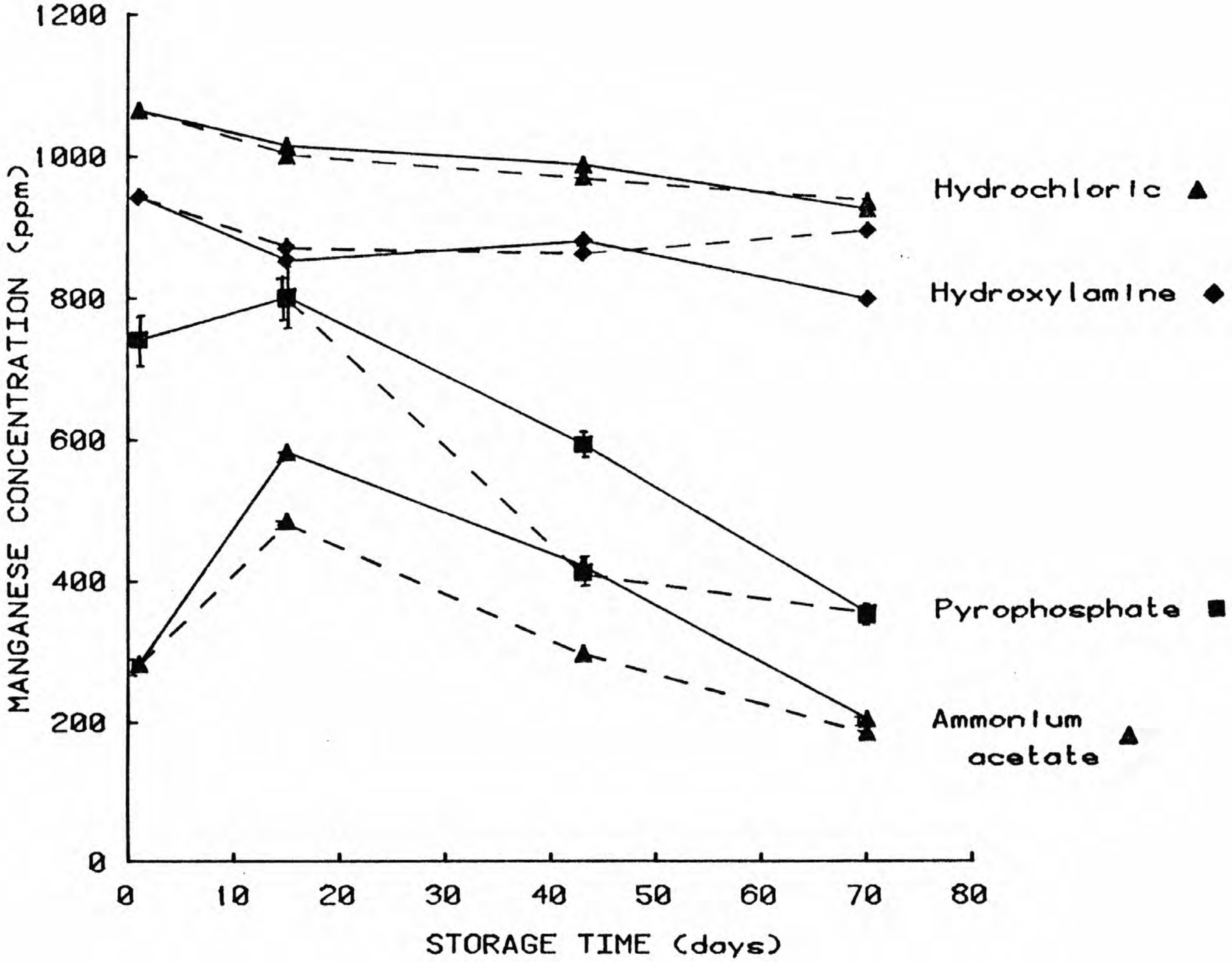

MANGANESE CONCENTRATION (ppm)
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
70
80
STORAGE TIME (days)
Hydrochloric ▲
Hydroxylamine ◆
Pyrophosphate ■
Ammonium acetate ▲

Fig. 6-5

Chapter 7. Correlation of Pb Concentrations and Chemistry of Sediments with Pb in the Bivalve Scrobicularia plana

S.N. Luoma and G.W. Bryan

INTRODUCTION

Realistic prediction of the impact of heavy metals in sediments upon aquatic biological resources will require an understanding of how physical and chemical factors affect metal uptake by organisms. In an attempt to obtain this, we have compared the concentrations of metals in the soft tissues of the deposit-feeding bivalve Scrobicularia plana (da Costa) in various estuaries with metal concentrations in extracts of the sediments. This paper describes our results for lead and shows that its biological availability in the sediment is strongly influenced by the level of readily extractable iron.

MATERIALS AND METHODS

Samples for the statistical analyses were collected from 17 estuaries in south and west England (see Table 1) during the high river-flow period between February and mid-May 1977. Sediment samples were collected and treated as described in Chapters IV and V.

Animals were held in clean 50% seawater for one weak to allow depuration of undigested sediment; soft tissues were then pooled into 3-7 replicate samples, each made up of 3-5 similar sized individuals, and wet-ashed with nitric acid (Bryan and Uysal, 1978). Tissue samples and sediment extracts were analysed for metal using flame atomic absorption spectrophotometry. Background correction was used for all lead analyses but not for iron.

Most of the lead in Scrobicularia lies in the digestive gland, and the concentration is dependent upon the size of the organism (Bryan and Uysal), 1978; Bryan and Hummerstone, 1978). For this study, lead concentrations for each station were averaged from results for animals having lengths within 50% of that of the largest. Size and age data from some estuaries suggested that this method probably compares levels in animals from a similar age range. This technique enabled more animals to be used in the analyses than could be used when comparisons were restricted to animals of about 40 mm shell length (Bryan and Uysal, 1978), but the two systems usually give similar results. Lead concentrations in animals with ripe gonads were about 25% lower than in normal animals from the same station. Corrections for this tissue dilution were applied on the comparatively few occasions when only ripe animals were encountered.

RESULTS AND DISCUSSION

Variations among estuaries

Concentrations of lead in both sediments and bivalves ranged through nearly two orders of magnitude (Table 7-1). The watersheds of the estuaries include agricultural lands, industrial/urban areas and former metal mining areas, thus providing diverse sources of lead contamination. When the concentrations of lead in the animals were compared with those of sediment extracts, significant correlations were found with the lead extracted by 1 N hydrochloric acid (r = 0.69), 25% acetic acid (r = 0.68) and the nitric acid digest (r = 0.61), but not with the lead concentrations in the other extractants. The concentration of lead extracted by hydroxylammonium chloride was affected by the carbonate content of some sediments and the amounts extracted with ammonium acetate were generally too low for accurate analysis. Concentrations extracted from the sediment with 1 N hydrochloric acid are compared with those of the animals in Fig. 7-1. Results for individual estuaries are fairly tightly grouped and within some, such as the Looe and Gannel, there is a clear relationship between levels in the animals and sediments. Between estuaries there is considerable variation. For example, at a sediment concentration of 400 μg/g, animals from Restronguet Creek contain about 60 μg/g whereas those from the Looe and Gannel Estuaries contain about 400 μg/g. These results suggested that reasons for such variation might be found by looking at the characteristics of the sediments in the different estuaries.

The physicochemical characteristics of the estuaries varied widely. Predominant sediment particle size ranged from sand to silt/clay; organic carbon ranged from 1.1 to 6.3 percent and calcium carbonate from less than 1 to 30 percent. There were also considerable differences between the average salinities at different sites. Although any or all of these factors may influence the biological availability of metals, none completely explains the variance in the relationship between acid-soluble lead in sediments and lead concentrations in _Scrobicularia_ and in seaweed _Fucus vesiculosus_ from the same sites (r - 0.04). Assuming that seaweed is an indicator of biologically available lead in the water column (Bryan and Hummerstone, 1973), it appears that concentrations of solute lead had little influence on lead concentrations in _Scrobicularia_.

Influence of iron on the availability of lead

In lieu of a technique for extracting biologically available lead directly, relationships were sought between acid-soluble lead and other components of the sediment which might bind the metal, since the type of substrate to which a metal is bound may strongly affect the availability of the metal to the bivalves (Luoma and Jenne, 1977). Hydrous oxides of iron are an important component of oxidized sediments and are probably an important sink, binding heavy metals in sediments (Jenne, 1968).

The relationship between concentrations of lead and iron in hydrochloric acid extracts is shown in Fig.
acetic acid). Two types of correlation characterized the lead-iron relationship. The regression for the nine stations in the Gannel and Looe estuaries had a significantly different slope from that for the remaining stations. The latter slope probably implies a chemical relationship between lead and iron oxide in the sediments (Hem, 1970). The sediments in the Gannel and Looe Estuaries, which receive wastes from old lead mines, had much higher lead concentrations at a given iron concentration than did the other estuaries, and the slope of the lead-iron relationship implies that significant quantities of lead are bound to substrates other than iron oxide (Hem, 1970). Concentrations of lead in Scrobicularia from the Gannel and Looe Estuaries were also higher than those in other estuaries (Fig.7-1). When the concentrations of lead in animals from all stations were plotted against the Pb/Fe ratios for the 1N hydrochloric acid extracts on logarithmic scales (Fig.7-3) a highly significant correlation was obtained (r = 0.88). Using linear scales, the value of r was increased to 0.99. Depending upon the method of statistical analysis, 78-94 percent of the variance in lead levels of Scrobicularia could be predicted from variations in the Pb/Fe ratio. Some variance remained unexplained among some of the lower lead concentrations in the bivalve (this was the cause of the weaker correlation for the logarithmically transformed data) but, in general, the relationship appears to provide a reliable predictive tool.

When concentrations extracted from the sediment by digestion with nitric acid ('total" extractable metal) were used in the Pb/Fe ratio, the correlation with lead concentration in Scrobicularia was inferior to correlations observed using the 1 N hydrochloric acid or 25 percent acetic acid extracts. The weaker acids remove much of the lead (45-92 percent) from the sediment, but a smaller proportion of the iron (15-40 percent).

Additional iron removed by the nitric acid includes large quantities of the more crystalline iron oxide phases, while the weaker acids remove primarily the more amorphous heavy-metal reactive phases of the oxide (Jenne and Luoma, 1977). This readily extractable iron appears to be mainly responsible for controlling the biological availability of lead to Scrobicularia.

Iron may influence the availability of lead to Scrobicularia in at least two ways. If both metals are released in the digestive tract, then iron may compete with lead for binding or transport sites, thus affecting its uptake. On the other hand, the concentration of iron in the sediment may also affect availability by influencing the physicochemical form of sediment-bound lead. To the extent that mass balance controls the partitioning of lead among different sinks in the sediment, increasing concentrations of iron may result in increasing lead partitioning to the iron oxide sink. If the biological availability of iron oxide-bound lead is low relative to forms such as organically bound lead, then the concentration in the animal could be inversely affected by increasing the iron content of the sediment.

To test the effect of seasonal changes on the usefulness of the relationship, sediments and animals were sampled from the Looe and Gannel estuaries on a second occasion (Table 7-2). Again, the concentrations of lead predicted in the animals by the linear regression agreed reasonably with the observed concentrations, despite the fact that many of the animals had ripe gonads and a correction factor was necessary (see Methods). Results from the Gannel estuary also show that the predicted concentration appears to be independent of whether dry or wet sediment is extracted with 1 N hydrochloric acid (Table 7-2). It is interesting to note that although the sediments of the East Looe and l'Elorn estuaries showed similar degrees of lead contamination, the concentrations of lead in animals from the East Looe were 3-4 times those from the l'Elorn. Higher concentrations of weak acid-soluble iron in the sediments of the l'Elorn (7.1 mg/g) than in the sediments of the East Looe (2.3-3.3 mg/g) appear to greatly reduce the availability of lead to the l'Elorn animals.

In May 1978, _Scrobicularia_ and surface sediments were analysed from the Humber estuary into which large quantities of industrial and domestic wastes are discharged. A 1 N hydrochloric extraction of the sediment gave concentrations of 191 μg/g of lead and 21 mg/g of iron; this latter value is the highest we have observed for iron. The Pb/Fe ratio predicted a lead concentration of 28 μg/g in Scrobicularia: 22 μg/g was observed. This result also contrasts sharply with those for the Looe estuary (Table 7-1). Although the concentrations of extractable lead in the sediments of the two estuaries are of the same order, concentrations in _Scrobicularia_ are an order of magnitude higher in the Looe estuary where the concentration of extractable iron in the sediment is an order of magnitude lower.

In conclusion, the results indicate that the concentration of lead in _Scrobicularia_ is largely controlled by the concentration in the sediment as modified by the inhibitory effects of iron. The Pb/Fe ratio in 1 N hydrochloric acid extracts of sediment appears to reflect the availability of lead to _Scrobicularia_, since the ratio is almost directly proportional to the concentration of lead in the animal. Measurements of this ratio may be a useful way of assessing biological availability of lead in estuarine sediments where _Scrobicularia_ do not occur.

Note added in proof:

In May 1978, Scrobicularia and surface sediment were analysed from the Humber Estuary into which large quantities of industrial and domestic wastes are discharged. A 1 N hydrochloric extraction of the sediment gave concentrations of 191 μg/g of lead and 21 mg/g of iron; this latter value is the highest we have observed for iron. The Pb/Fe ratio predicted a lead concentration of 28 μg/g in Scrobicularia: 22 μg/g was observed. This result contrasts sharply with those for the Looe Estuary (Table 7-1), since although concentrations of extractable lead in the sediments of the two estuaries are of the same order, levels in the animals are an order of magnitude higher in the Looe Estuary where the concentration of extractable iron in the sediment is an order of magnitude lower.

SUMMARY - Chapter 7

Concentrations of lead in the soft tissues of the deposit-feeding bivalve *Scrobicularia plana* have been compared with the physicochemical characteristics of sediments in 17 estuaries in southern and western England and one in north-west France. The results indicate that the biological availability of lead in the sediment is controlled mainly by the concentrations of iron, and that the concentration of lead in the bivalve may be predicted from the Pb/Fe ratio in 1 N hydrochloric acid extracts of surface sediments.

Table 7-1 Characteristics of estuaries sampled in south-west England. Metal concentrations in sediment were measured by extraction with 1N HCl.

Estuary	Month of Sampling	Range of Pb Concentrations (ug/g dry wt.)		Range of Concentrations (mg/g)	Watershed Characteristic
		Sediment	S. plana	Sediment	
Torridge	May	22-26	35-40	4.2	agricultural
Erme	Feb.	22-42	47-60	2.5-4.0	old mines on river
Fowey	May	33-44	20-42	2.7-3.5	old mines in watershed
Camel	Apr.	41	14	3.7	old mines in watershed
Beaulieu	Apr.	42	17	11.6	agricultural
Plym	Feb.	46-60	27-44	2.8-2.9	industrial/urban
S. Bristol Channel	May	80-83	49-54	5.9-6.6	urban/industrial
Poole Harbour	Apr.	103	40	9.8	industrial/urban
Hayle	Apr.	111	26	10.4	old mines on river
Southampton (Test/Itchen/Hamble)	Apr.	78-193	44-132	6.9-7.1	industrial/urban
Tamar/Tavy	Mar.	139-162	40-88	7.2-10.0	old mines on estuaries
Looe	Mar.	52-326	120-428	2.1-3.5	old mines on river
Restronguet Creek (Fal)	Mar.	210-401	58-72	16.1-17.3	old mines on river
Gannel	Apr	264-1134	309-1016	4.2-6.0	old mines on river

Table 7-2 Correlation coefficients describing the relationship between Pb concentrations extracted from sediments by various techniques and Pb concentrations in *Scrobicularia plana*. r is correlation coefficient, and (r^2X 100) is the percent of the variance in the Pb concentrations of the bivalve explained by the concentration of Pb removed by the extractant. For all extractants n-38, except for ammonium acetate, where n-21.

Extractant	Correlation with Pb in *Scrobicularia* (r)	r^2X 100
Hydrochloric acid	0.69*	47.6
Acetic acid	0.68*	46.2
Nitric acid digest	0.61*	37.2
Hydroxylammonium chloride	0.17	2.9
Ammonium oxalate	2.24	5.8
Ammonium acetate	0.28	7.8

Table 7-3 The concentrations of Pb observed in sediments (HCl extraction) and soft tissues of *Scrobicularia plana* from 5 estuaries compared with the concentration of Pb predicted for *S. plana* from the Pb/Fe ratio in the sediments. Station numbers increase from the head to the mouth of the estuary.

Estuary		Month of Sampling	Concentration of HCl-soluble Pb in sediment (μg/g)	Observed Concentration of Pb in *S. plana* (μg/g dry wt.)	Predicted Concentration of Pb in *S. plana* (μg/g dry wt.)
Axe	1	May	25	8	9
	2		23	16	5
	3		22	29	20
Otter	1	May	30	18	22
Thames	1	Nov.	57	25	36
	2		57	29	38
l'Elorn	1	Aug.	71	31*	34
	2		83	37*	42
E. Looe	1	Aug.	93	104*	128
	2		61	176*	97
	3		59	134	116
	4		63	165*	121
Gannel	1	Nov	417 (wet sediment)	326	330
	2		435 (dry sediment)	327	322

*Concentration corrected for ripe gonads.

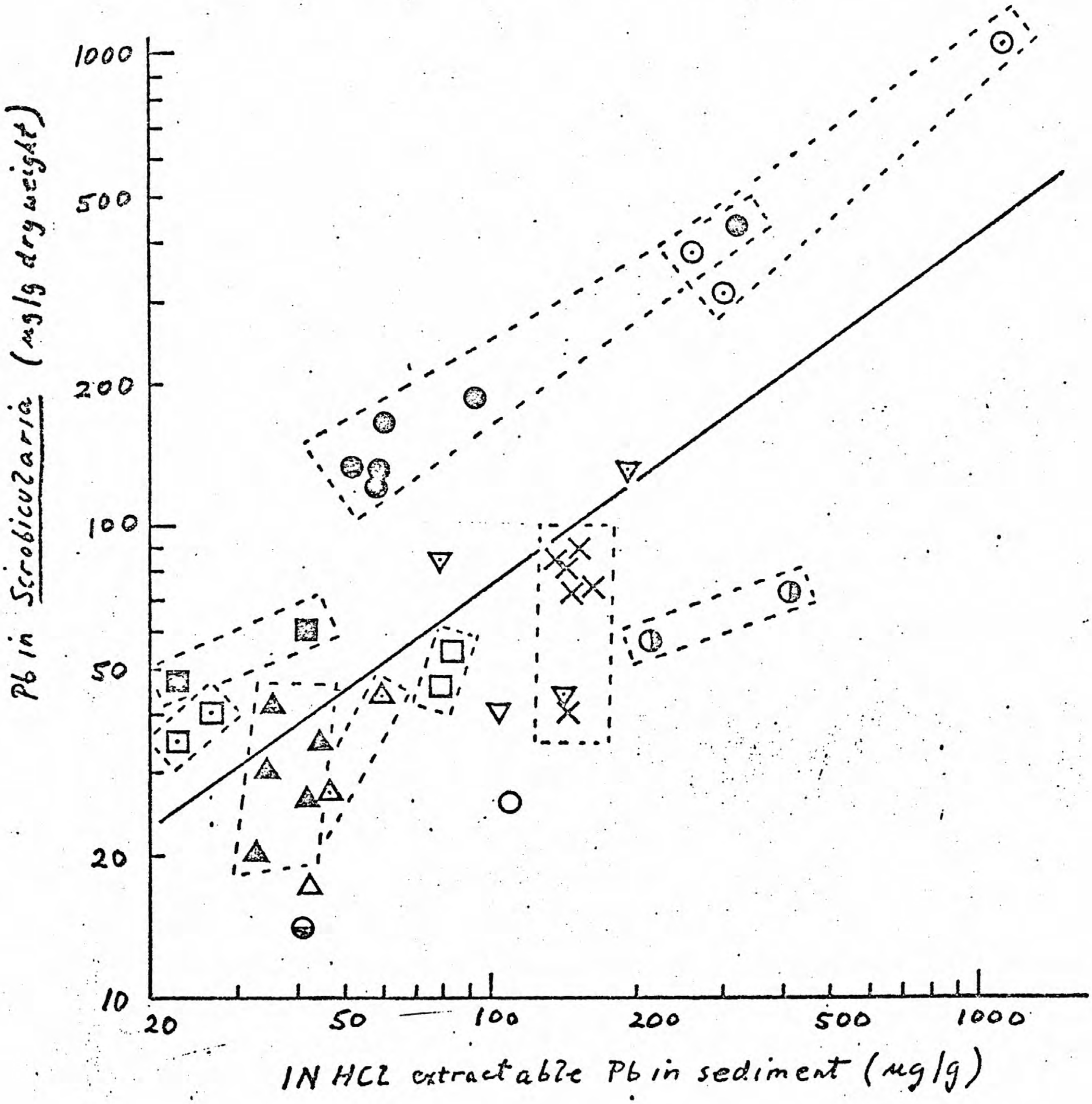
List of Figures - Chapter 7

Correlation between concentration of lead in soft tissues of _Scrobicularia plana_ and concentration extracted from surface sediment with 1 N hydrochloric acid. Boxes enclose stations from each estuary and these are Torridge (△), Erme (■), Fowey (▲), Camel (⬬), Beaulieu (.), Plym (⊡), Bristol Channel (▭), Poole Harbour (○), Hayle (▽), Southampton area (▽), (1 Itchen, 2 Test, 3 Hamble), Tamar/Tavy (X), Looe (●), Restronguet Creek (●), Gannel (⊙). Equation for line is $\log_{10}$Pb animal = 0/728 $\log_{10}$Pb sediment + 0.417 (n = 37, r = 0.69).

Correlation between concentrations of lead and iron extracted from surface sediments with 1 N hydrochloric acid. Symbols as for Fig. 1. Equation for Gannel and Looe results is $\log_{10}$Pb sediment = 2.79 $\log_{10}$ Fe sediment + 0.801 (n = 9, r= 0.95). Equation for other results is $\log_{10}$ Pb sediment = 1.097 $\log_{10}$ Fe sediment + 1.029 (n = 28, r = 0.82).

Correlation between concentration of lead in soft tissues of _Scrobicularia plana_ and ratio Pb/Fe extracted with 1 N hydrochloric acid. Symbols as for Fig. 1. Equation for results on logarithmic scales is $\log_{10}$ Pb animal = 1.071 $\log_{10}$ Pb/Fe + 0.496 (n = 37, r = 0.88).

Fig. 7-1



Pb in Scrobicularia (µg/g dry weight)
1000
500
200
100
50
20
10
20
50
100
200
500
1000
1N HCl extractable Pb in sediment (µg/g)

Fig. 7-2

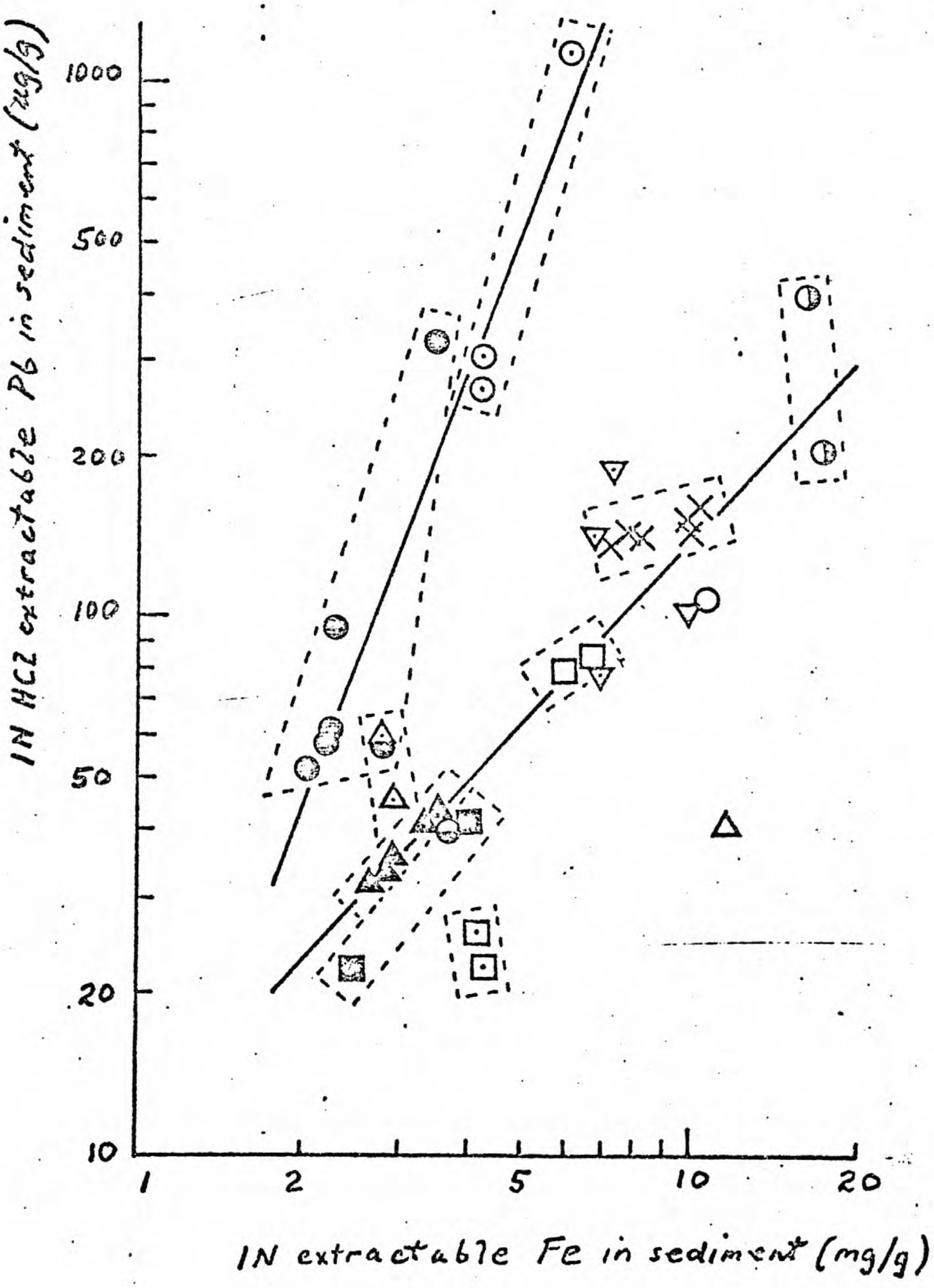
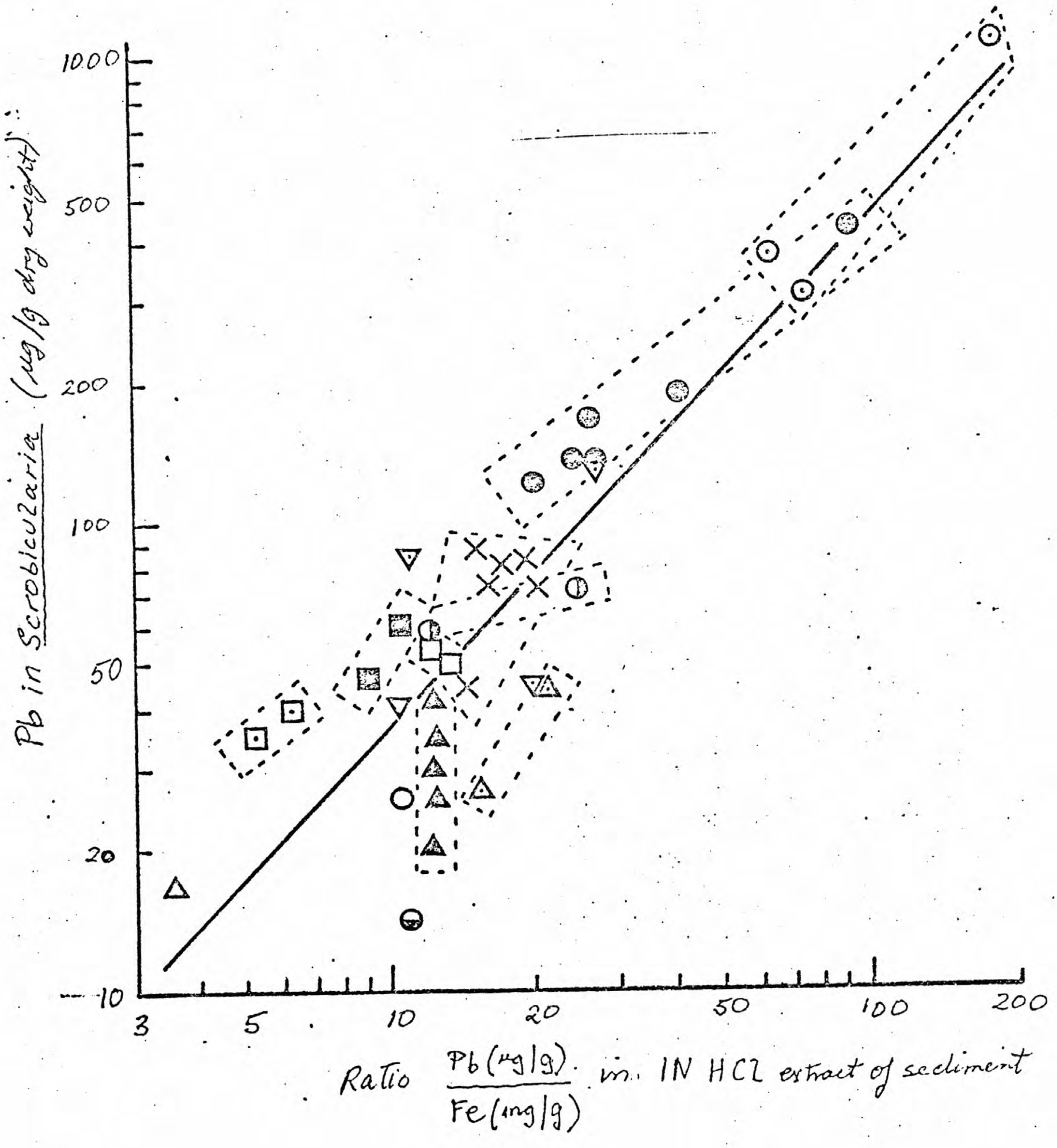

1N HCl extractable Pb in sediment (μg/g)
1000
500
200
100
50
20
10
1
2
5
10
20
1N extractable Fe in sediment (mg/g)

Fig. 7-3



Pb in Scrobicularia (µg/g dry weight)
1000
500
200
100
50
20
10
3
5
10
20
50
100
200
Ratio Pb(µg/g) / Fe(mg/g) in 1N HCl extract of sediment

Chapter 8. Development of Methodology for Incorporating Sediment Chemistry into Predictions of Metal Bioavailability in Sediments. Case Study - Zn

S.N. Luoma and G.W. Bryan

INTRODUCTION

In this chapter we statistically assess the effect of selected chemical characteristics of estuarine sediments on the concentration of Zn in bivalves which ingest those sediments while feeding. Our objective is to determine what proportion of the variation in Zn concentrations of deposit-feeding bivalves from natural systems may be explained by the physicochemical partitioning of Zn in the sedimentary food of the animals.

Methodology for modeling the partitioning of Zn in sediments containing a mixture of sorption substrates has not been developed. To assist our statistical assessments we have made several simplifying assumptions about the chemical and biological interactions of sediment-bound Zn:

1) The relatively thin oxidized layer of sediment at the sediment-water interface is chemically different and biologically more important than are subsurface sediments. Many benthic organisms feed primarily at the sediment-water interface (including the bivalves used in this study). Sulfides dominate metal partitioning in the reducing environment of most estuarine subsurface sediments; however, oxides of iron, oxides of manganese, various organic materials and perhaps carbonates should all be important substrates binding metal under oxidized conditions. Our sediment samples were carefully collected from the oxidized layer of sediment, where, we assumed, sulfides were not important substrate.

2) Total concentrations in the sediment do not necessarily reflect either the biologically or chemically reactive fraction of metals for substrates. Thus

we have used partial extraction techniques to characterize different phases of both metal and substrate. Under different estuarine conditions, hydrous oxides may vary in crystallinity and in their associations with other substrates (e.g., organics). The nature of the organic materials may also vary greatly. Different extractants remove different quantities of these substrates, in response to differences in substrate form (Jenne, 1977). We may not assume that extractants selectively remove trace metals from any single sorption substrate (Luoma, and Jenne, 1977), but differences in trace metal solubility among extractants may be useful to empirically separate metal forms susceptible to different treatments. Statistical association of the substrate forms defined by the partial extractants, with either bioavailability or the empirically defined phases of sediment-bound trace metals should reflect the forms of both substrate and metal which are the most reactive in chemical and/or biological interactions within estuarine sediments.

3) The partitioning of adsorbed metals in sediments is controlled by processes analogous to those controlling the speciation of metals in solution. Thus, the quantity of metal sorbed to competing substrates will be determined by metal-substrate stability constants and the relative abundance of the different substrates. Where physicochemical conditions change we would expect partitioning to change.

4) Too few stability constants are available presently to permit calculation of partitioning in multi-substrate systems. In lieu of stability constants we assume metal partitioning in sediments will change solely in response to changes in the relative abundance of the substrates which bind the metals. Because the availability of Zn to bivalves varies among substrates, changes in the relative abundance of the substrate concentrations should correlate with changes in the biological availability of the metal to organisms whose metal

burden is controlled by sediments. The biological availability of sediment-bound Zn in this case is defined as the concentration of Zn in the bivalves relative to that in the sediment,

$$B = \frac{Zn_{\text{ organism }(\mu g/g)}}{Zn_{\text{ sediment }(\mu g/g)}} \tag{1}$$

The effect on bioavailability of changes in the chemical partitioning of Zn in sediments is assessed from correlations of B with ratios of the concentrations of different substrates removed from sediments by different chemical extractants.

Methods and Materials

Sediments and organisms were collected in two separate studies. In the first study sediments and the deposit-feeding bivalve Macoma balthica were collected intertidally from 19 stations in San Francisco Bay, California. Several stations were sampled during both the rainy and dry seasons. Sediment samples were scraped from the oxidized surface layer of the sediment, and immediately washed through 250 μm polyethylene mesh with seawater (salinity $32^{0}/oo$). Within 24 hours of collection, subsamples (0.10 - 0.35 g dry wt.) were taken from a slurry (using a 0.5 ml micropipette) and extracted in duplicate using methods shown in Table 8-1. Duplicate subsamples from each slurry were washed with distilled water and dried at 80° C to determine the dry weight of solids. Subsample sediment weights varied less than 5 mg between duplicates (<5 percent). After extraction each sample was filtered (0.45 μm), and the filtrate was analyzed for Fe, Mn, and Zn by atomic-absorption spectrophotometry. To reduce contamination from filter membranes in the ammonium acetate extraction, 10 ml of ammonium acetate extract was passed through the filter before any filtrate was collected for analysis. Contamination levels were insignificant in other extractants relative to Zn concentrations in the samples. All extracts were stored at pH<2 prior to analysis. Total carbon and inorganic carbon were determined from solid samples using a Leco carbon analyzer.* Organic carbon was determined by difference between inorganic carbonate and total carbon.

At least 20 individual Macoma were collected in each sample. In most situations, animals of median size (=100 mg soft tissue dry wt.) were pooled for the analyses. Where variations in size were unavoidable, the tissues of

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*(The use of the brand name in this report is for identification purposes only and does not imply endorsement by the U.S. Geological Survey).

individual animals were analyzed separately; however, no strong correlation ($r<0.05$) between Zn concentration and dry tissue weight was found at any station. The Macoma were kept in clean seawater for 24 hours after collection to rid their alimentary tracts of undigested sediment; then the soft tissues were removed and dissolved in hot concentration H_2SO_4 with excess HNO_3. The acid solutions were evaporated just to dryness and reconstituted in 25 percent HCl.

In the second study, sediments and the deposit feeding clam *Scrobicularia plana* were collected from estuaries in southwest England as described in Chapters 4, 5 and 7. Concentrations of Zn in the seaweed, *Fucus vesiculosus*, were used as an indicator of the concentration of biologically available solute Zn.

Scrobicularia and *Macoma* are morphologically and ecologically very similar species. *Scrobicularia* is larger (median dry wt. of soft parts approximately 300 mg) and longer-lived than the *Macoma* found in San Francisco Bay (Bryan and Luoma, unpublished data, 1977). Significant positive correlations were found between concentrations of Zn and soft tissue dry weight in all *Scrobicularia* samples (Bryan, and Hummerstone, 1978). For our purposes mean concentrations of Zn were taken only from animals with lengths within 50 percent of that of the largest animal. This method does not remove all the biases introduced by the size correlation (Bryan and Hummerstone, 1978), but it should allow comparisons of animals of roughly similar ages. The *Scrobicularia* were kept in clean seawater for one week after collection, then soft tissues were dissolved in concentrated HNO_3 and reconstituted in HCl before atomic absorption analysis.

Regression analyses were conducted with both log-transformed and untransformed data. Only the linear regression of untransformed data is shown for the San Francisco Bay data, because concentrations varied over a relatively narrow range and the linear equation yielded the highest correlation coefficient. Sedimentary variables were regressed against the bioavailability ratio in regression with *Macoma*. While intuitively satisfying, the use of the bioabailability ratio as the dependent variable may lead to interpretative difficulties if the denominator of the ratio contributes significantly to the regression. To simplify interpretation of the multiple linear regression techniques employed with the more complex English data (and because Zn levels in the sediment correlated with those in the clam), the concentration of Zn in *Scrobicularia* alone was employed as the dependent variable and Zn concentrations in the sediment were included in the independent variable. The data from England were log-transformed for all statistical analyses because of: 1) the wide range of values, 2) the log-normal distribution of most variables, and 3) the higher correlation coefficients obtained for the log-log regressions than for linear regressions. F-values were calculated for all variables in the multiple regression model.

RESULTS

Sediments

Differences Between Study Areas. The physical differences (e.g. morphology, hydrodynamics, watershed characterisitics and temperatures), between San Francisco Bay and the estuaries of southwest England contributed to major differences in the chemistry of the sediments in the two study areas. San Francisco Bay has a large watershed and is a large physiographically complex estuary, over half of which forms a slowly circulating embayment. The English estuaries were enriched with Zn from mine drainage, and the range of Zn concentrations found in both the bivalves and sediments in southwest England was considerably greater than the range of concentrations in San Francisco Bay (Fig. 8-1a). The English sediments also showed a wider range of Fe concentrations (but a higher frequency of low concentrations) than San Francisco Bay, plus, generally higher concentrations of organic carbon, and lower concentrations of Mn (Fig. 8-1b, c, d). Carbonates were consistently low in San Francisco Bay (less than 0.1 percent carbonate carbon; <1 percent $CaCO_3$) but were as high as 3.0 percent carbonate carbon (18 percent $CaCO_3$) in some English estuaries. Likewise the concentrations of humic substances were much higher throughout the English study than observed at the few stations for which we have data in San Francisco Bay. The significant chemical differences between study areas could result in differences in the partitioning of sediment-bound Zn to be more closely associated with the oxides of iron and manganese in San Francisco Bay, while total organic materials, the humic fraction of the organic and, perhaps, carbonates are likely to be of greater importance in the estuaries of Southwest England.

Biological Availability

Direct Extraction of Available Form. Concentrations of Zn in *Scrobicularia* correlated at high level of significance with the wide range of extractable Zn concentrations in the sediments of the English estuaries (Table 8-2). Within a single estuary (San Francisco Bay) there was no significant correlation between Zn extracted from the sediments and Zn in the animals. The strongest correlation with the animals was observed for the ammonium acetate-soluble fraction of of Zn in the sediments (English data) and for the two extractants which dissolve amorphic oxides of iron and manganese (oxalate in England and hydroxylamine hydrochloride in San Francisco Bay). Direct extraction methods explained less than 12 percent of the variance in the Zn concentrations of *Macoma*, and, despite their statistical significance, less than 40 percent of the variance in *Scrobicularia*.

Influence of Substrate Concentrations.

San Francisco Bay. To assess the influence of Zn partitioning in the sediments on uptake, regressions were calculated between the ratio describing Zn bioavailability (Eq. 1) and concentrations of total organic carbon, iron oxides and manganese oxides (the latter two substrates as extracted by various techniques). Among all combinations of variables, the strongest correlation was observed for the regression

$$y_1 = a + b\,X_1 \qquad (2)$$

where y_1 = the bioavailability ratio (Eq 1), and

$$X_1 = \frac{Fe_{Hydam}}{Mn_{AmAc}}$$

where Fe_{Hydam} is the concentration of Fe removed by hydroxylamine hydrochloride, and Mn_{AmAc} is the concentration of Mn removed by ammonium acetate (Fig. 8-1). The correlation coefficient was highly sensitive to the forms of Mn and Fe employed in the regression. Hydroxylamine-soluble Mn and acetic acid-solube forms of both Fe and Mn showed a weak relationship to the bioavailability of Zn. The variable X_1 increased as the fraction of Mn extracted by ammonium acetate declined toward the head of the estuary. The concentrations of Mn removed by other extractants did not decline rapidly upstream. At the upstream stations the concentrations of Zn in Macoma relative to that in the sediments was also high. Although this suggested the variations in X_1 may be related to salinity, no significant correlation was found between bioavailability and salinity. Moreover, both X_1 and bioavailability were highest at a station outside the mouth of the estuary, where the salinity was near that of seawater.

The inverse correlation between the bioavailability of Zn and Mn_{AmAc} was relatively weak ($r = 0.41$). Positive residuals of this relationship were largely data collected during the winter. The winter influx of fresh water into San Francisco Bay was accompanied by an increase in the sediments of hydroxylamine-extractable Fe (preliminary laboratory experiments indicate hydroxylamine extracts highly amorphous, freshly precipitated Fe) and humic substances (Luoma, and Cain, in press). We have data from too few San Francisco Bay stations to include the humic substances in regression calculations, however, increase in hydroxylamine-Fe generally coincided with increased Zn concentrations in Macoma; thus the combined Fe/Mn ratio in Eq 2 explained 60 percent of the temporal and spatial variance in the Zn concentrations of the bivalve when all the data were considered.

The largest negative residuals in Fig. 8-2 represented stations with high concentrations of total organic carbon, suggesting Zn concentrations in Macoma were lower than expected from the Fe/Mn ratio where total organic carbon concentrations were elevated. Organic carbon concentrations were included in the independent variable of the regression.

$$y_1 = a + b X_2 \qquad (3)$$

where y_1 = the bioavailability ratio (Eq 1), and

$X_2 = (Fe_{Hydam}/Mn_{AmAc}) \times (1/\text{organic}_{\text{carbon}})$.

The correlation coefficient of this regression was slightly higher ($r = 0.83$ vs $r = 0.77$) than when Fe/Mn alone was employed as the X_2 variable. The residuals of the regression suggested an overestimation of Zn bioavailability at the lowest carbon concentrations - i.e., the negative effect of carbon on bioavailability appeared to occur only at high levels of carbon. The effect of excluding low organic carbon concentrations from the relationship was tested by comparing correlation coefficients when carbon values below 0.6% 1.0%, 1.5% or 3.8% were removed from calculation of the X_2 variable. This was accomplished by normalizing all carbon concentrations to one of the above and assuming concentrations lower than the chosen value were to equal one (i.e. all carbon values lower than the normalizing number had no effect on the value of the variable). The maximum correlation coefficient was observed when organic carbon was normalized to 1.0 percent (Fig. 8-3b). Normalizing to carbon levels lower than 1.0 percent progressively detracted from the relationship, while failure to include the higher carbon levels also resulted in a less-than-optimum correlation. When organic carbon was normalized to 1.0 percent the correlation coefficient ($r = 0.96$) suggested 92 percent of the variance in the biological availability of Zn to Macoma could be attributed to variations in concentrations of Fe, Mn and organic carbon in the sediments (Fig. 8-4). The correlation between the denominator of the bioavailability ratio ($Zn_{sediment}$) and variable X_2 was weak ($r = 0.51$); thus, most of the variance in the Zn concentrations of Macoma appeared to be due to variations in the ratio of Fe, Mn and organic carbon. If this ratio is indicative of the partitioning of Zn in sediments then nearly all the variation in Zn concentrations in Macoma may be explained by the form of Zn in the sediments.

Southwest England. The effects of sedimentary variables on Zn concentrations in Scrobicularia were initially tested by multiple regression. Values of the F-statistic were calculated to determine the level of significance at which sedimentary variables explained Zn levels in the clam (Table 8-3). Several different regression equations were calculated to compare the different methods of extracting Zn, Mn and Fe. The multiple regression analyses employed only the 29 stations at which seaweed was found.

In the first run of the multiple regression only the three variables (Fe, Mn, organic carbon) included in the San Francisco Bay study were analyzed (Table 8 -4). The concentration of Zn, Mn and organic carbon in the sediments explained 49 percent of the variance in Scrobicularia. More of the variance in Scrobicularia was explained by ammonium acetate than when other extractants were employed (Table 3). Total organic carbon had a significant negative effect, the concentration of humic substances a positive effect, and solute concentrations of Zn a positive effect. Oxalate-soluble Mn had a weak positive effect, although neither total Mn nor ammonium acetate-soluble Mn significantly affected the regression. Carbonates had a weakly significant effect (negative), in one combination of variables. Neither Cd nor Fe showed any effect detectable by multiple regression. The regression equation yielding the highest correlation coefficient (r = 0.843) was

$$\begin{aligned}\log Zn_{\underline{Scrob.}} &= 0.03 + 0.30 (\log H) - 0.59 (\log C) \\ &+ 0.19 (\log Zn_{AmAc}) + 1.22 (\log Zn_{\ solute}) \\ &+ 0.17 (\log Mn_{oxal.}) - 0.08 (\log Co_3)\end{aligned} \tag{4}$$

where H = the concentration of humic substances (absorbance/g sediment) and C = total organic carbon (percent).

Using data from all 40 stations, simple regressions were also calculated for the equation

$$\log y_2 = \log a + (b)(\log X_3) \quad (5)$$

where y_2 = the concentration of Zn in Scrobicularia and

$$X_3 = (Zn_{sediment}) \times (Substrate_1/Substrate_2)$$

for all combinations of the Fe, Mn, organic carbon, carbonate and humic material substrates. These calculations employed all 36 data points. The results were similar to those calculated by multiple regression (Table 8-4). The correlation was strongest when the fraction of Zn extracted by ammonium acetate was employed (Fig. 8-5a). When the product of ammonium acetate-soluble Zn and the ratio of humic substance concentrations to total organic carbon was employed as X_3, i.e.

$$X_3 = (Zn_{AmAc}) \times (H/C) \quad (6)$$

67.1 percent (r = 0.819) of the variance in Scrobicularia was explained (Fig. 5b). When the ratios Mn/C and Mn/Co_3 were included with Zn_{AmAc} in X_3 the correlation was also improved over that between Zn_{AmAc} and Zn in the bivalve (Table 8-4).Including Mn with the organic ratio in a more complex independent variable did not affect the correlation coefficient. Concentrations of Zn in

$$X_4 = (Zn_{AmAc}) \times (H/C) \times (Mn_{oxal}) \quad (7)$$

Scrobicularia were higher than predicted by the independent variable in Eq 6 at several sites with very high Mn concentrations. A slight improvement in the correlation coefficient (r = 0.842; r^2 = 0.709; Fig. 8-5c) was observed when only the high Mn values were inserted in Eq. 5 by normalizing Mn concentrations to 350 μg/g to include the lower values (assuming all concentrations less than 350 μg/g took the value of 1 in variable X_4, Eq 7).

The residuals of the relationship in Fig. 8-5c suggested salinity also had some effect on Zn concentrations in Scrobicularia. Stations from near the heads of the estuaries generally had negative residuals. Because of evaporation and the transient nature of salinities in the small English estuaries

It was difficult to reliably determine the salinity to which Scrobicularia was exposed on the mudflat. Therefore, the salinity effect will require further investigation.

Discussion

To date, attempts to define the biologically available fraction of particulate-bound metals have largely bypassed any direct consideration of metal partitioning among physicochemical forms. Instead, extractants have been sought that simulate biological processes, directly removing the biologically available fraction of metals. Some correlation between metal concentrations extracted from soils and concentrations in plants have been observed within soil series (e.g. ammonium acetate extraction of Zn; DTPA extraction of Cu), but among different types of soils these relationships break down. The search for a universal extractant for the biologically available fraction of metals has recently been extended to rationalize the development of complex extraction schemes for aquatic sediments (Khalid, et al, 1977; Engler, et al, 1974). In Chapter 3, we used less involved methodology to show some correlation between extractable nuclide concentrations and the uptake by Macoma of ^{65}Zn, ^{109}Cd, ^{60}Co and ^{110m}Ag from laboratory prepared sediments. A close correlation between ammonium acetate-solubility and ^{65}Zn uptake was observed. In nature, ammonium acetate solubility also reflected the bioavailability of Zn to Scrobicularia in the estuaries of southwest England better than did other extractants. The correlation was not sufficiently strong to have predictive value, however, and within the narrower range of Zn concentrations in San Francisco Bay no correlation was observed. The exchangeability of Zn, implied by ammonium acetate extraction, may influence the availability of the metal, but it is not the only factor determining uptake.

To directly assess the effect of Zn partitioning in sediments on uptake, we have assumed partitioning, and, in response, availability will change with changes in the concentrations of substrates which bind Zn. Despite this obvious oversimplification we have found ratios of sedimentary substrate concentrations that explain a very large proportion of the variance in either the bioavailability or the concentration of Zn in two species of bivalves. We reported similar results for lead in Chapter 7 . Although the ratios of substrates which predicted Zn uptake differed between southwest England and San Francisco Bay most of those differences were consistent with chemical differences between the areas. Substrate concentrations suggested inorganic oxides were more dominant than organic materials in the sediments of San Francisco Bay, while organic materials and carbonates occurred in high concentrations in the English estuaries. Distribution of two oxide forms in the sediments explained most changes in the availability of Zn to Macoma in the former estuary, with a negative effect on availability occurring only at higher concentrations (<1 percent) of total organic carbon. The ratio of humic substance concentrations to total organic carbon was an important control on availability in southwest England, with some indications that manganese oxides, solute Zn and low salinities also enhanced Zn uptake. The concentration limit on the effect of total organic carbon in San Francisco Bay (and, possibly, Mn in southwest England) was consistent with the participation of such substrates in partitioning only when present at sufficiently high concentrations to be competitive, although it is also possible the nature of these substrates was different in samples containing low concentrations.

The negative effect of total organic carbon on Zn availability to the bivalves was strongly evident in both San Francisco Bay and southwest England.

If our assumptions are correct about the relationship between substrate concentration, Zn partitioning and the availability of Zn, then the Zn associated with the bulk of organic matter in the sediments may be of low availability to deposit feeders. Alternatively, organic materials could produce a similar statistical effect if total organic carbon concentrations influenced feeding rates in the animals. Whatever the mechanism, the negative effect of total organic carbon on Zn concentrations in the bivalves is a somewhat surprising result and warrants further investigation.

The statistical evidence also suggested that increased concentrations of humic substances enhanced the availability of Zn. The extractable humic materials represent a small, but extremely concentrated pool of trace metals in the sediments. Complexation with humic substances generally reduces the availability of metal in solution (Jenne, and Luoma, 1977), but the possibility that Zn bound to humic substances may be of high availability <u>relative to other bound forms</u> deserves futher investigation.

The role of inorganic oxides in determining the availability of sediment-bound Zn is not entirely clear from our comparisons of the two study areas. Within sediments extractable Zn correlated most strongly with extractable Fe (Chapter 5). Increased availability of Zn to *Macoma* correlated with increased concentrations of hydroxylamine-soluble Fe; but no influence of Fe on Zn levels in *Scrobicularia* was evident (although hydroxylamine-soluble Fe was not determined in the English study due to carbonate interferences with the extraction). In contrast we have previously shown that extractable Fe inhibits the uptake of Pb by *Scrobicularia*. The results also indicated that not only the concentration of hydrous Fe and Mn oxides, but also the characteristics of the oxides themselves (as reflected in their solubility in different extractants) affected Zn uptake. An understanding of the role of oxide form in the physical, chemical and biological interactions in oxidized sediment awaits further study.

The concentration of Zn extracted from sediments by oxalate correlated with Zn levels in Scrobicularia more strongly than did Zn-solubility in other extractants (except, or course, ammonium acetate). Since oxalate dissolves amorphous Fe and Mn oxides (Jenne, 1977), and, presumably, any associated Zn, the correlation is consistent with the positive effect of the oxides on Zn uptake suggested previously. Neither the concentration of Zn extracted by ammonia, nor the ratio of Zn removed by hydroxylamine and ammonium acetate showed any relationship to Zn levels in Scrobicularia or Macoma, however. Such extractants may extract trace metal-reactive fractions of the substrates involved in binding Zn in the sediments, but it is unlikely that they are selective for only the Zn associated with that fraction of those substrates. For example, the fraction of Fe removed from sediments by hydroxylamine may be highly amorphic, with a strong affinity for Zn. However, the concentrations of Zn extracted by hydroxylamine includes not only that associated with Fe, but also Zn associated with Mn oxides and quite possibly some organically-bound Zn (Chapter 3), all of which may vary widely in their bioavailability. Thus, the concentration of Fe extracted by hydroxylamine may be a better indicator of the partitioning of Zn to the highly amorphic oxides of Fe (apparently a high-availability substrate) than is the concentration of Zn in hydroxylamine. Similarly, reactions occurring during the extraction may affect the concentration of substrate removed. Both precipitation of ZnOH and readsorption of Zn to other substrates are likely at the high pH used to extract humic substances (Chapter 4). Thus, again, the concentration of humic substances extracted by alkaline reagents may more closely reflect Zn partitioning to this substrate than does the concentration of Zn extracted. Multi-substrate extraction of trace metals by most extractants and interferences from reactions occurring during the extraction may affect the concentration of Zn removed more strongly than the concentration of substrate removed. Multi-substrate extraction of trace metals by most extractants and interferences from reaction occurring during the extraction process may help explain why the search for universal extractants which remove the biologically available fraction of the metals has met with little success.

Summary

Chapter 8

1) The fraction of sediment-bound Zn extractable in ammonium acetate correlates significantly with Zn concentrations in bivalves when a broad range of Zn concentrations is considered. The relationship is not detectable, however, for a more narrow range of Zn concentrations. While solubility in ammonium acetate reflects a major control on the bioavailability of Zn, other processes are also important.

2) We have assumed the partitioning of Zn among substrates within aquatic sediments is controlled by changes in the relative abundance of the substrates. If so, the the availability of Zn to Macoma in San Francisco Bay is controlled by the competitive partitioning of Zn between two forms of hydrous oxide:hydroxylamine hydrochloride-soluble iron oxide and ammonium acetate-soluble manganese oxide. Organic materials affect the bioavailability of Zn when present in concentrations greater than 1 percent. Concentrations of organic carbon are higher and concentrations of the hydrous oxide are generally lower in the estuaries of southwest England than in San Francisco Bay. The availability of Zn to Scrobicularia appears to be primarily controlled by the ratio of humic substances concentrations to total organic carbon. Solute Zn and low salinities may enhance Zn levels in Scrobicularia at some stations, as may oxalate-solute Mn when present in high concentrations.

3) The primary value of this study is its introduction of a number of new, specific questions about controls on the availability of Zn to deposit feeders. We have also suggested a somewhat new approach to studying chemical and biological interations in natural systems. Such an approach might be useful as a statistical interface between more sophisticated chemical and biological models.

Table 8-1

Extracants applied to separate subsamples of sediment.

Extractant	Conditions[1]		Comments
	pH	Comments	
IN ammonium acetate			dissolves manganese; removes exchangeable metals
0.4 N ammonium oxalate[2] in 0.4 N oxalic acid	3.3		extracts "amorphic iron oxides
25 percent acetic acid	2.2		
IN hydrochloric acid	0.1		
IN ammonia[2]	10;	extract 1 week	extracts "humic substances"[3]
concentrated nitric acid	hot digest		"total" metal[4]
hydroxylamine hydro chloride in 0.01 N nitric	2.0;	extract ½ h	poor buffering capacity

---

[1] All extractions were conducted for two hours at room temperature except where noted, using extractant ratios of approximately 30:1 (v/w) or greater.

[2] Extractants used in southwest England, but not San Francisco Bay.

[3] Alkali-soluble organics comprised less that 5% of the total organic carbon and were not dependent upon total organic concentrations (Luoma and Bryan, unpublished data, 1977).

[4] Some highly crystalline metal forms, soluble only in concentrated hydrofluoric acid are not extracted by concentrated nitric acid (Bryan, unpublished, 1977).

Table 8-2

Correlation coefficients describing the relationship between the concentration of Zn extracted from sediments and the concentration of Zn observed in *Macoma* (San Francisco Bay) and *Scrobicularia* (Southwest England).

Extractant	Correlation with Zn concentrations in clam San Francisco Bay	 Southwest England
Concent. nitric acid	0.09	0.48**
Hydrochloric acid	0.10	0.48**
Oxalate	nd	nd
Hydroxylamine hydrochloride	0.33	
Ammonium acetate	0.20	0.62**
Ammonia	nd	0.37*

**$p<0.01$. For English data ($n=40$), $r>0.39$. For San Francisco Bay ($n=28$) $r>0.46$.

*$p<0.05$. For English data $r>0.30$; for San Francisco Bay $r>0.36$.

nd not determined.

Table 8-3

Values of the F-statistic calculated from a multiple regression of Zn concentration in *Scrobicularia plana* versus the combinations of variables listed. Percent variance (r^2) explained by the regression is also shown.

r^2	F-values											
	Zinc			Iron		Manganese			Organic Carbon	Carbonate Carbon	Humic substances	Extractable Cd	Solute Zn
	Ammonium acetate	Hydrochloric acid.	Oxalate	Oxalate	Total	Ammonium acetate	Oxalate	Total					
	(+)		(+)		(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(+)
71.1	6.53**				0.01		3.66*		9.01**	3.13*	9.22**	2.21	5.80**
66.9	7.10**				0.05			0.40	6.00**	1.58	8.39**	0.91	4.42*
66.6	7.44**				0.03	0.20			5.04*	1.46	6.92**	0.67	5.03*
64.2			1.61		0.00		4.00*		6.09**	2.76	4.69*	2.44	7.50**
62.2				0.50	0.09		4.68*		5.65**	2.73	3.74*	2.78	6.76**
[a]49.0		11.2**			0.01		6.77**		5.44**				

F-values $>3.08 = p < 0.05$*; F-value $>5.29 = p < 0.01$**. (+) and (-) indicate the sign of the slope taken by the variable. [a]Only the variables used in San Francisco Bay study were included in one multiple regression run to facilitate comparison between the study areas.

Table 8-4

Correlation coefficients showing relation of Zn concentrations in <u>Scrobicularia</u> to combinations of sedimentary variables, in the equation

$$\log y = \log a + (b)(\log X_3)$$

where y = Zn concentrations in <u>Scrobicularia</u> and $X_3 = (Zn_x)(\text{substrate}_y/\text{substrate}_z)$.

Table shows factors which make-up X_3 variable.

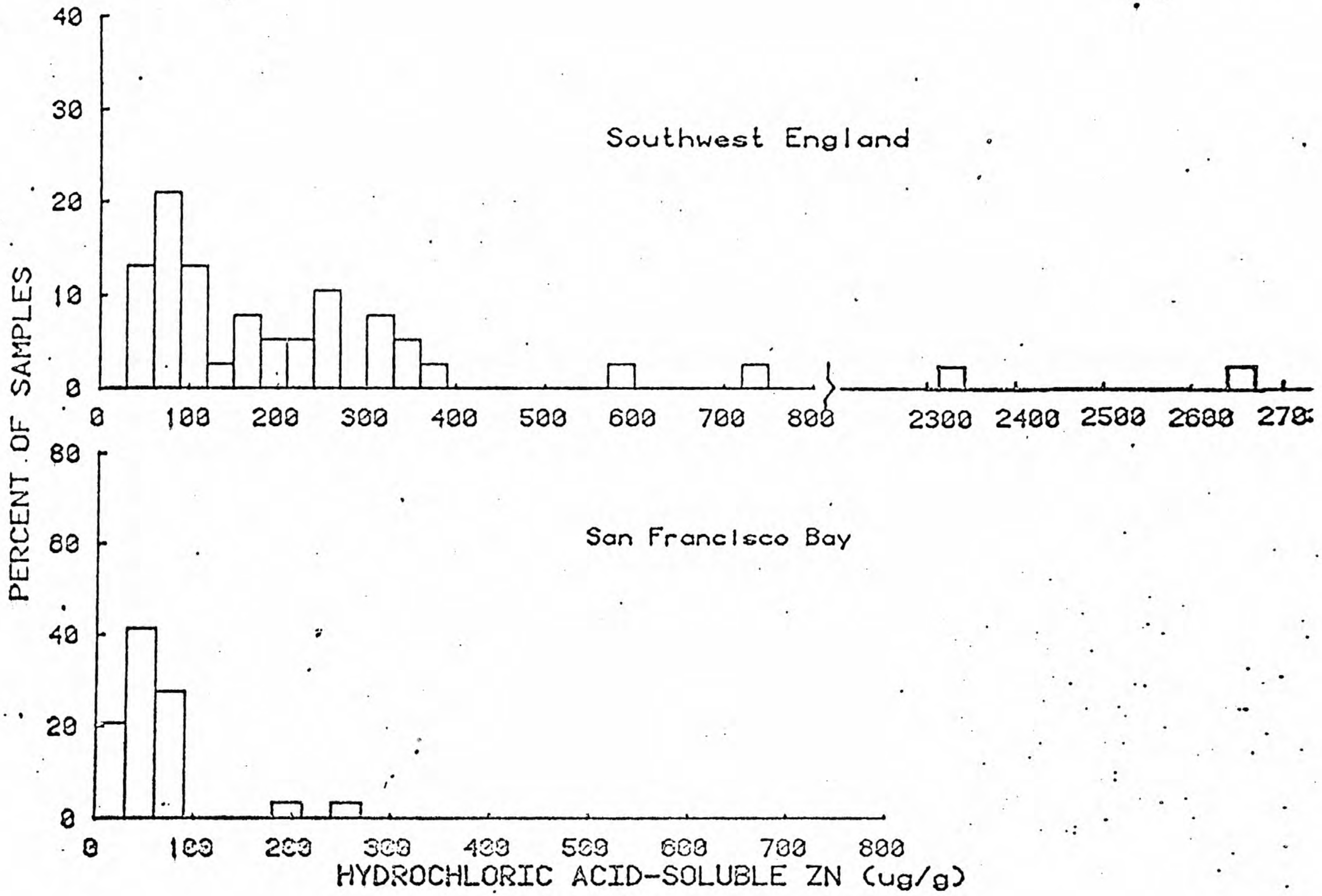
	Zn_1	Zn_4	Zn_3	Zn_6
Fe_4/Mn_1	0.54	0.36	0.39	0.37
Fe_4/Mn_3	0.44	0.22	0.25	0.25
Fe_6/Mn_6	0.58	0.36	0.42	0.32
Fe_4/Carbon	0.61	0.46	0.50*	0.46
Fe_6/Carbon	0.62	0.48	0.53*	0.44
Mn_1/Carbon	0.59	0.47	0.51*	0.43
Mn_4/Carbon	0.66*	0.55*	0.59*	0.52*
Mn_6/Carbon	0.62	0.51	0.55*	0.48
Fe_4/CO_3	0.61	0.48	0.51*	0.45
Fe_6/CO_3	0.60	0.46	0.49*	0.44
Mn_1/CO_3	0.64*	0.52	0.56*	0.50*
Mn_4/CO_3	0.68*	0.55*	0.58*	0.54*
Mn_6/CO_3	0.64*	0.52	0.55*	0.51*
humics/organic carbon	0.82*	0.60*	0.68*	0.50*
humics/carbonate	0.55	0.40	0.44	0.37

*Coefficients exceeding those between <u>Scrobicularia</u> and sedimentary Zn alone (from Table II). Subscript identification numbers are: 1=ammonium acetate; 3=hydrochloric acid; 4=oxalate; 6=concentrated nitric acid.

List of Figures

Fig. 8-1

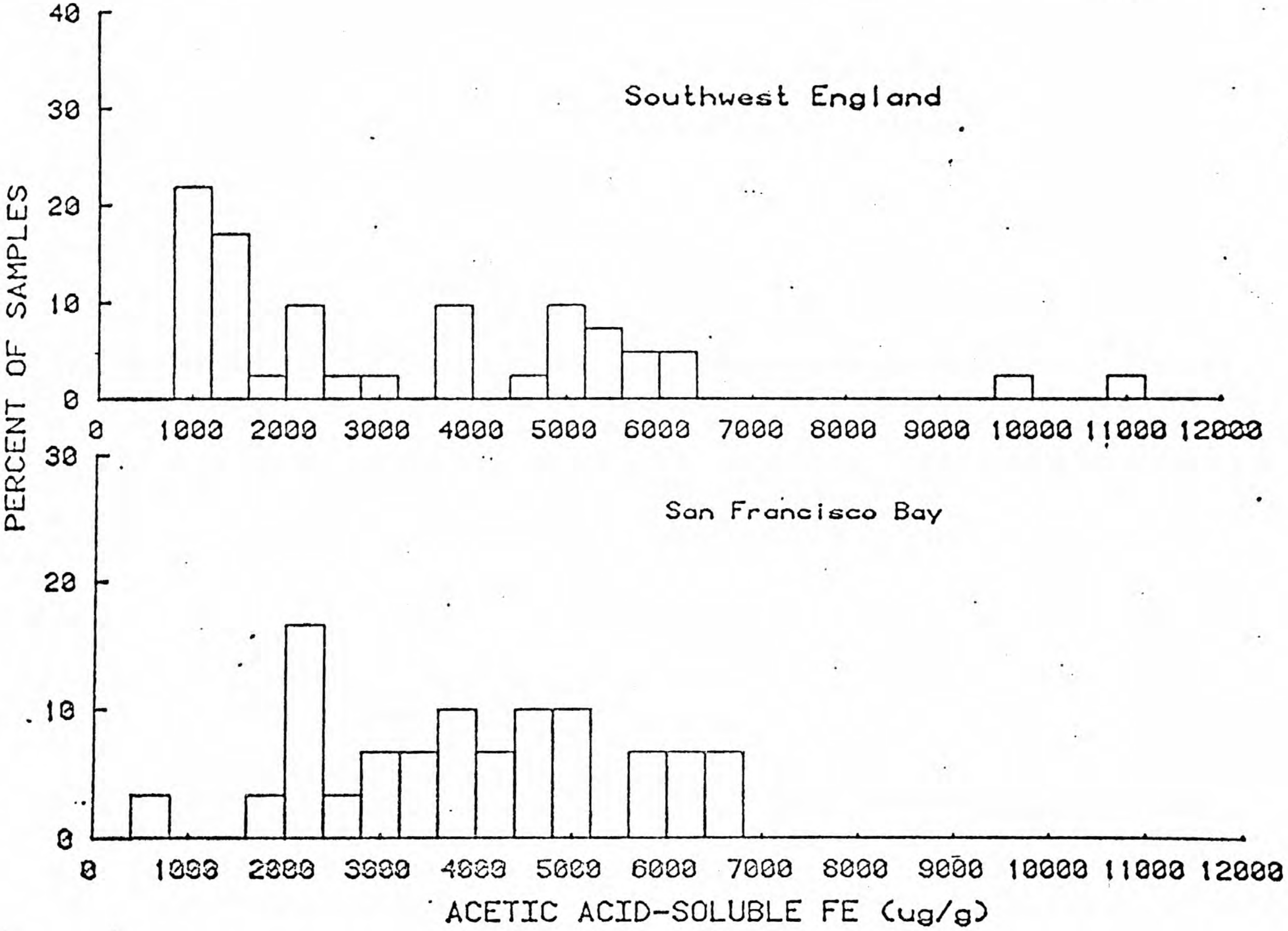
(2a)



Southwest England
San Francisco Bay
PERCENT OF SAMPLES
HYDROCHLORIC ACID-SOLUBLE ZN (ug/g)
40
30
20
10
0
80
60
40
20
0
0
100
200
300
400
500
600
700
800
2300
2400
2500
2600
270
0
100
200
300
400
500
600
700
800

Fig. 8-1

(2b)



PERCENT OF SAMPLES
40
30
20
10
0
Southwest England
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
12000
30
20
10
0
San Francisco Bay
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
12000
ACETIC ACID-SOLUBLE FE (ug/g)

Fig. 8-1

(2c)

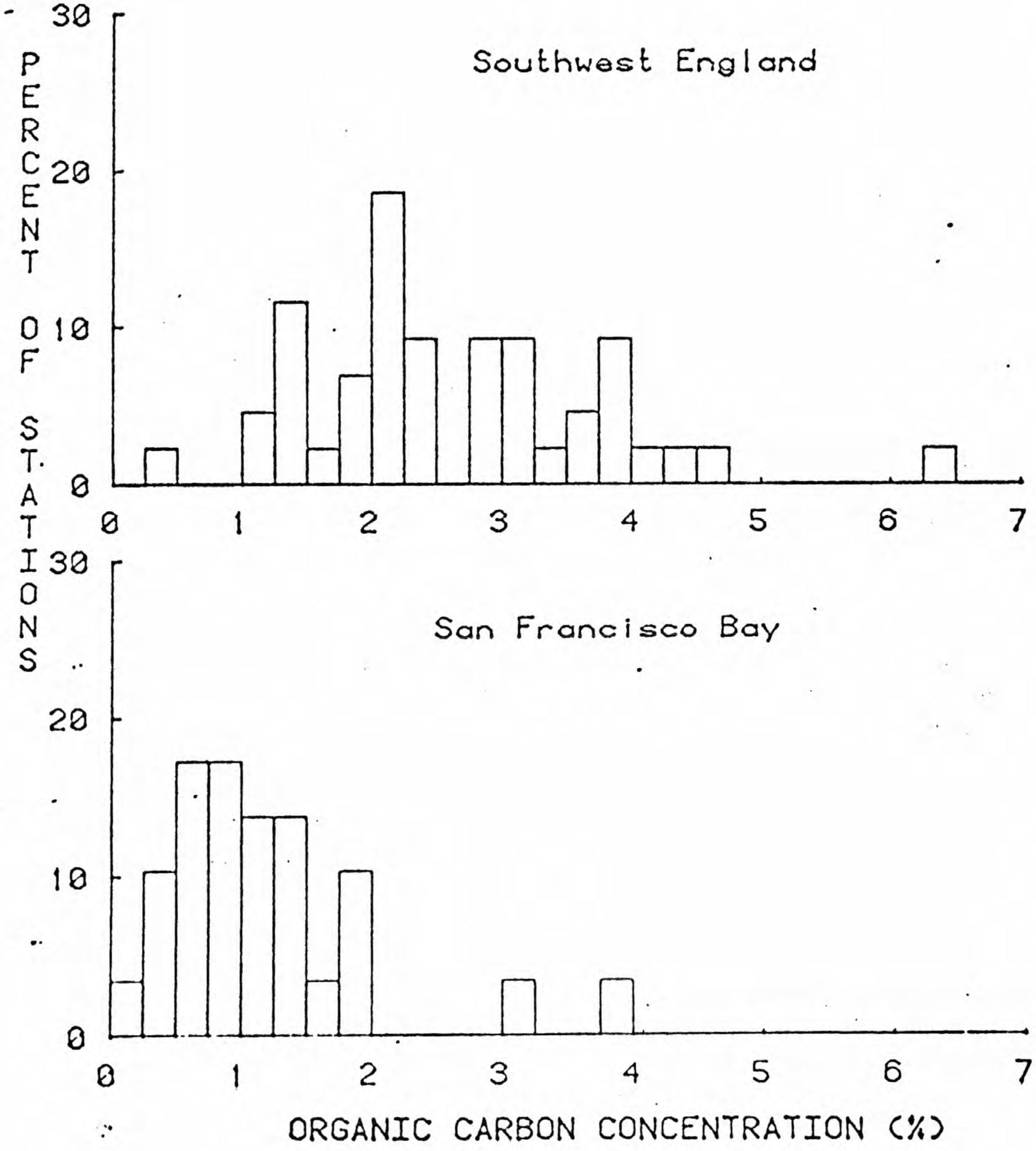

Southwest England
San Francisco Bay
PERCENT OF STATIONS
30
20
10
0
0
1
2
3
4
5
6
7
ORGANIC CARBON CONCENTRATION (%)

Fig. 8-1

(2d)

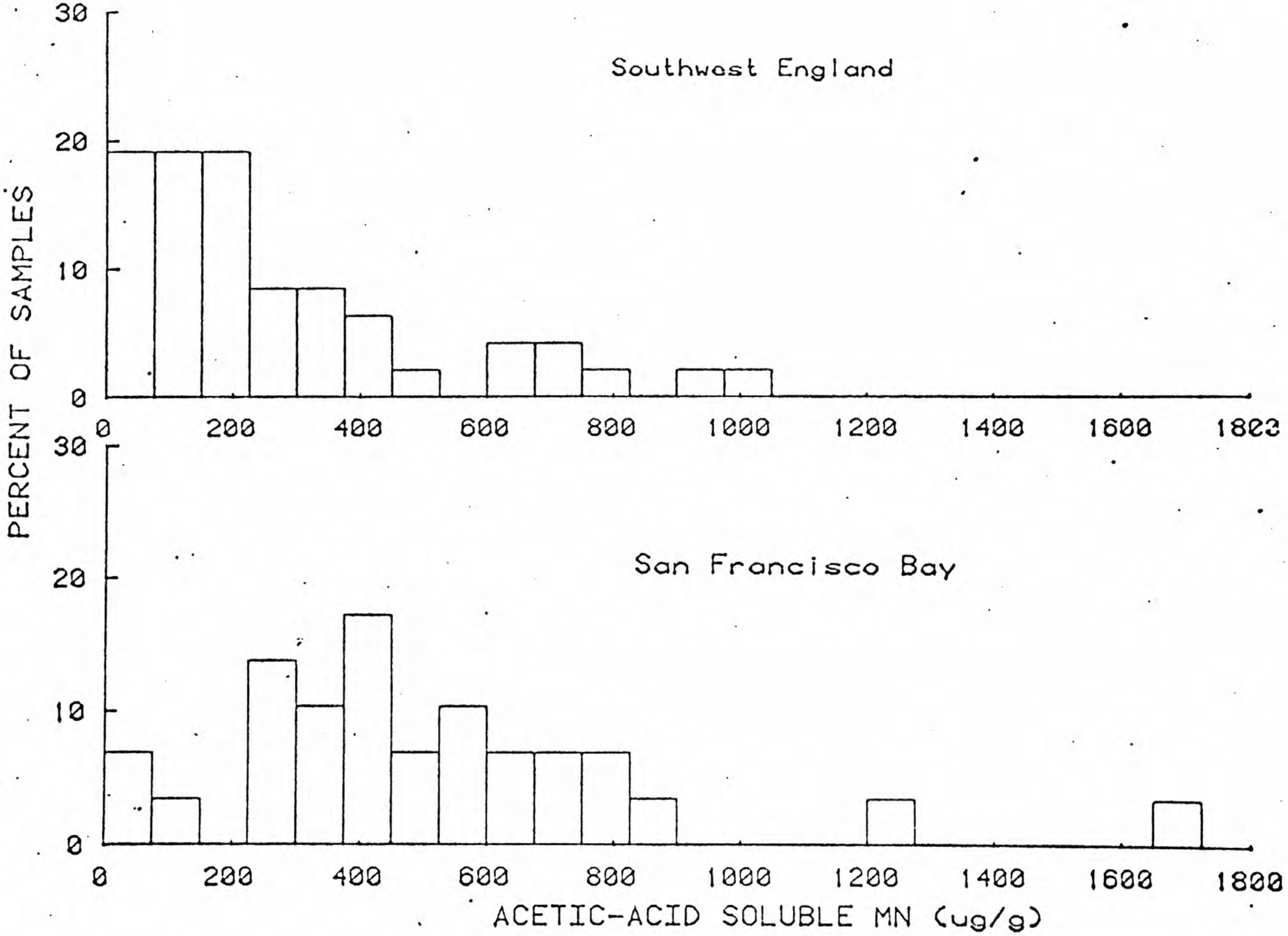

Southwest England
San Francisco Bay
PERCENT OF SAMPLES
30
20
10
0
0
200
400
600
800
1000
1200
1400
1600
1800
ACETIC-ACID SOLUBLE MN (ug/g)

Fig. 8-2

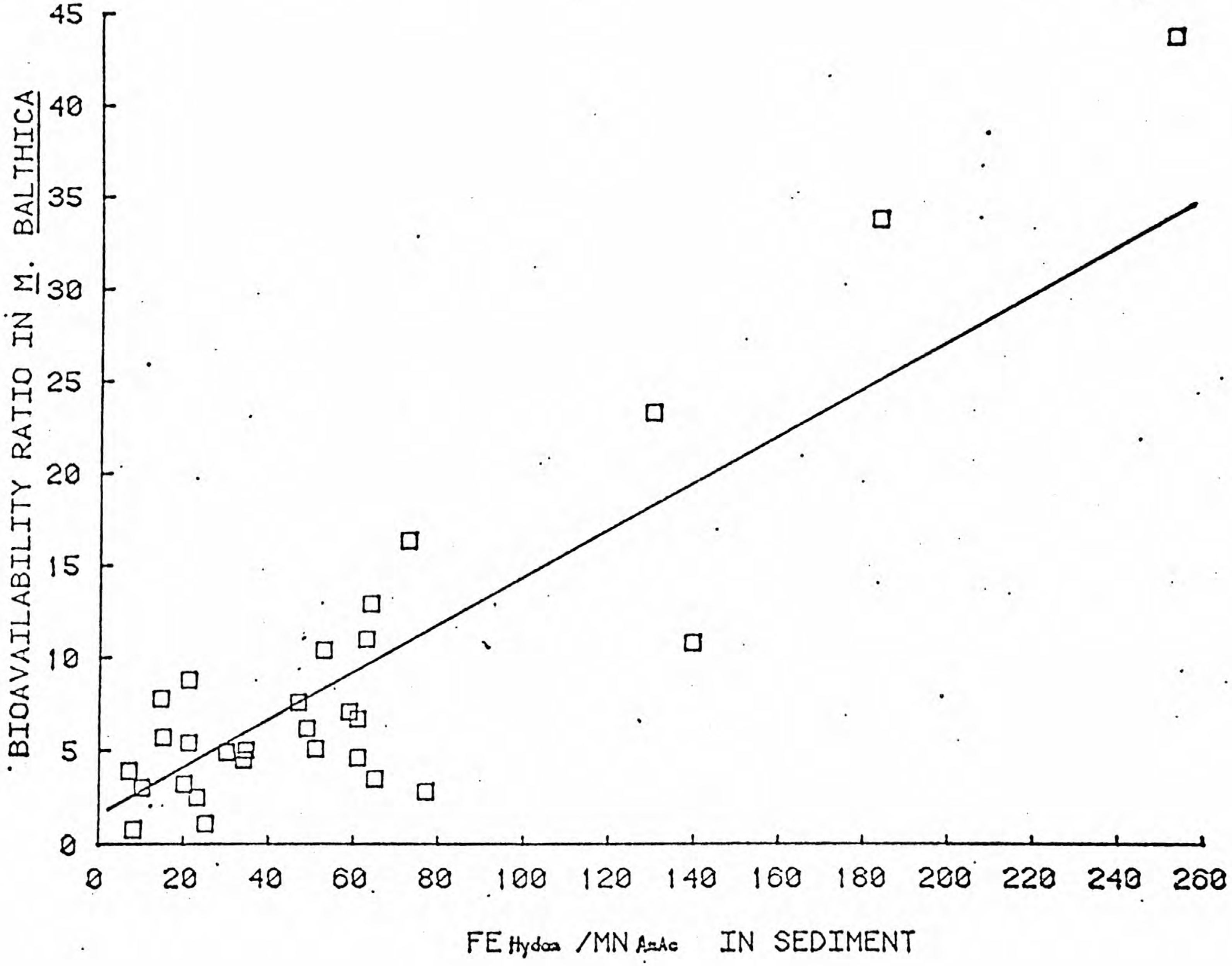

BIOAVAILABILITY RATIO IN M. BALTHICA
45
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
FE$_{Hydox}$ /MN$_{AmAc}$ IN SEDIMENT

Fig. 8-3

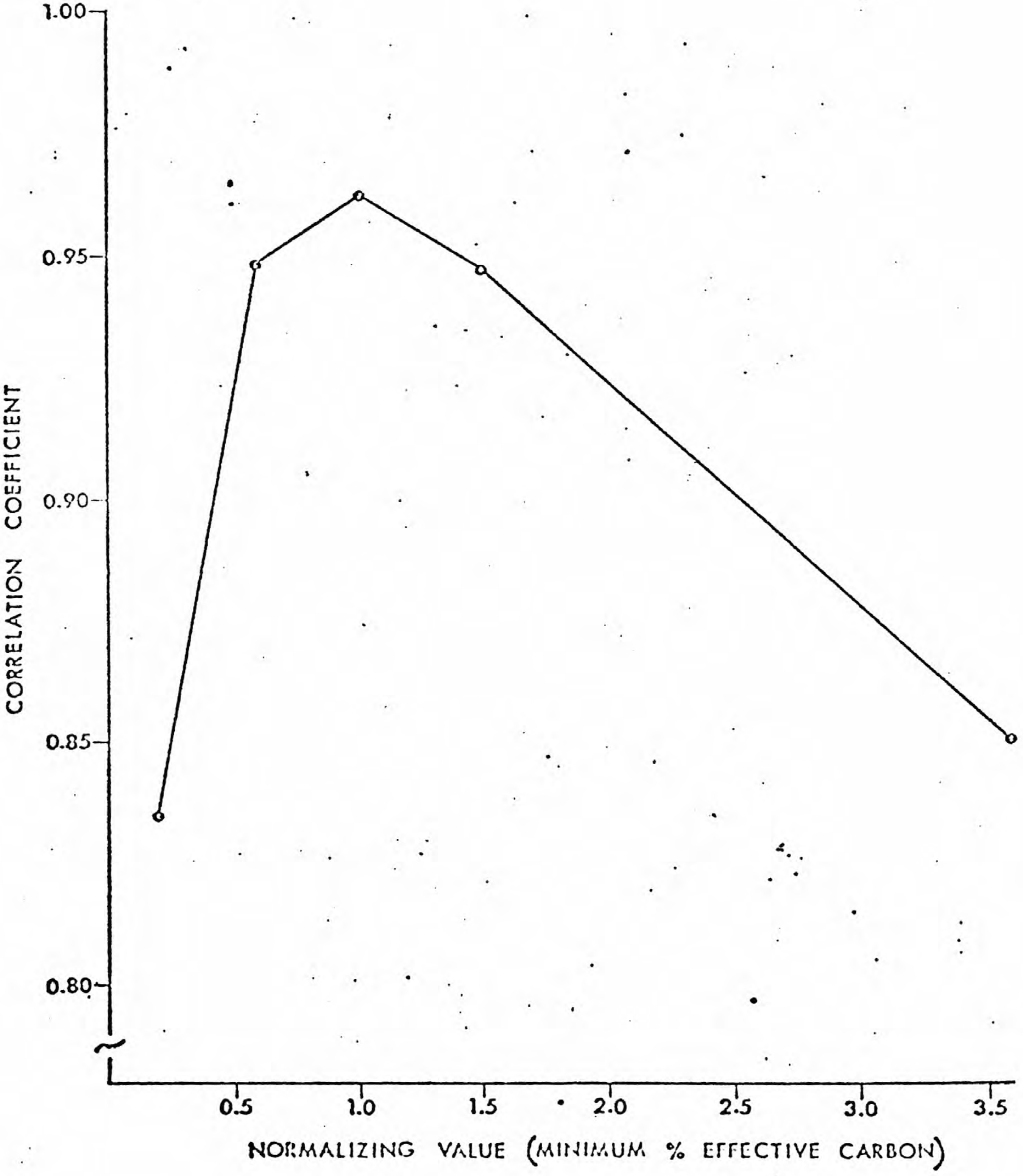

1.00
0.95
0.90
0.85
0.80
CORRELATION COEFFICIENT
0.5
1.0
1.5
2.0
2.5
3.0
3.5
NORMALIZING VALUE (MINIMUM % EFFECTIVE CARBON)

Fig. 8-4

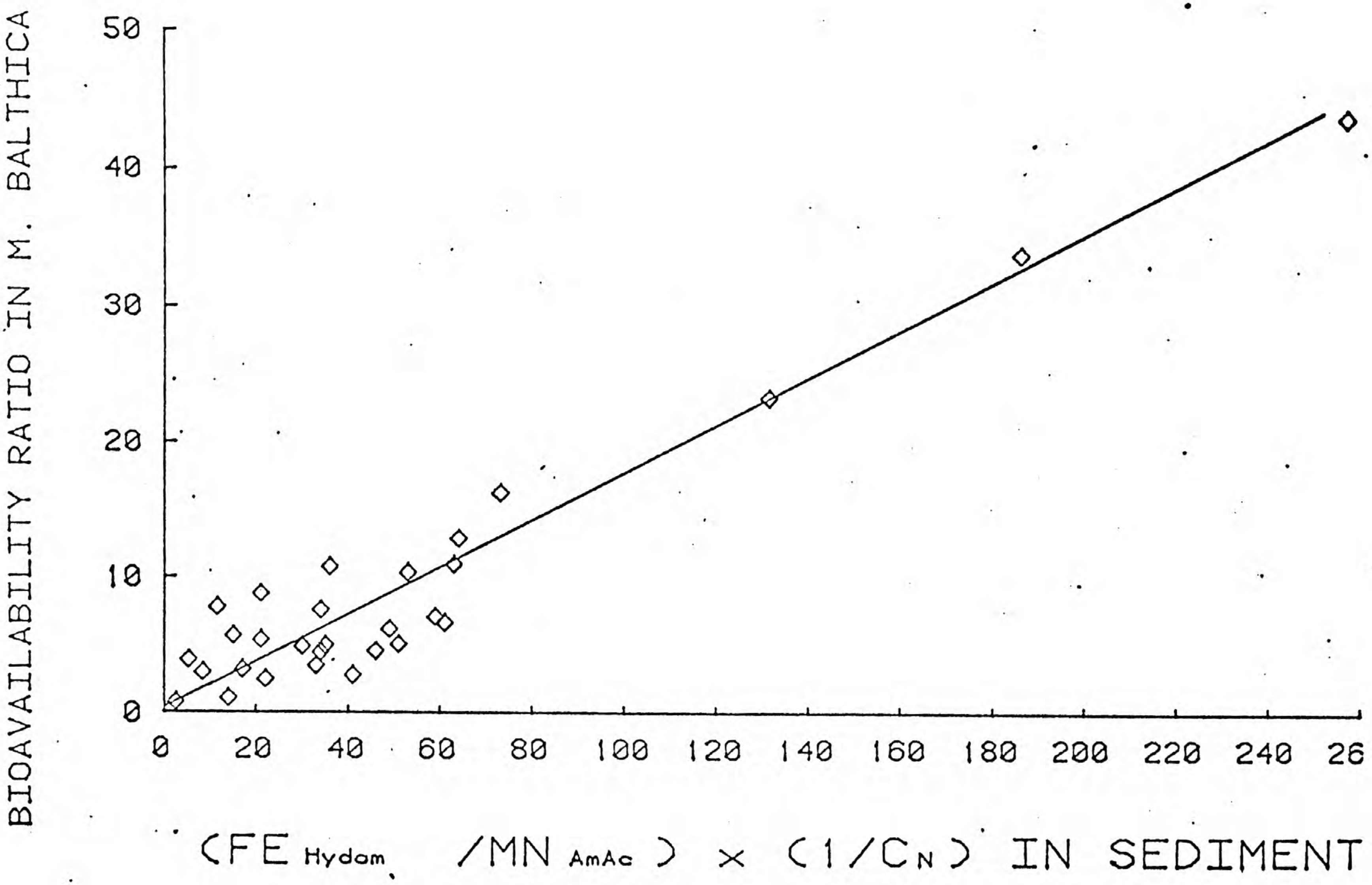
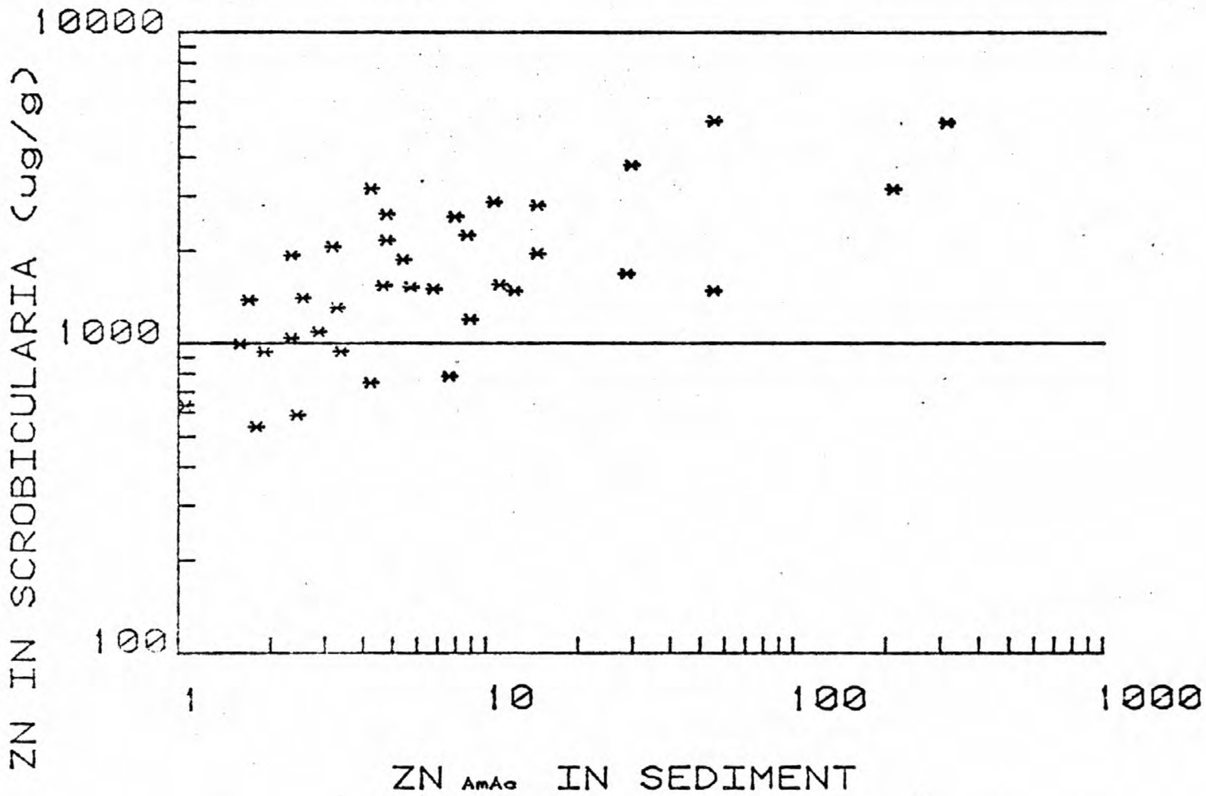
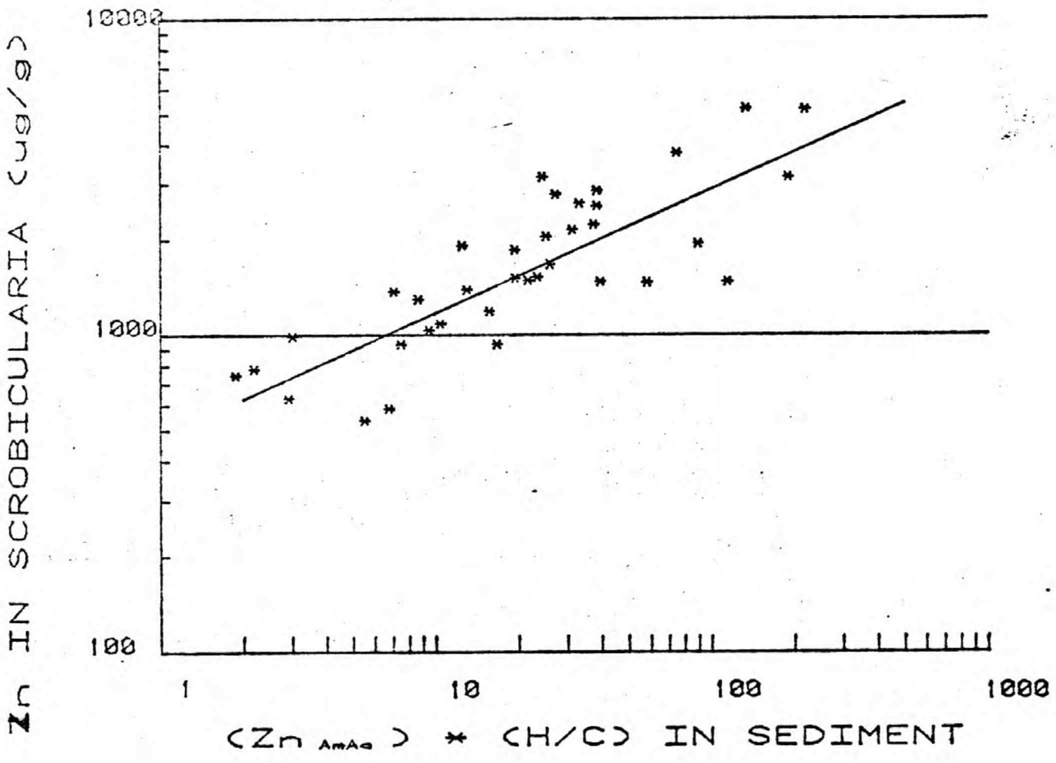

BIOAVAILABILITY RATIO IN M. BALTHICA
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
200
220
240
26
(FE Hydom /MN AmAc) × (1/C N) IN SEDIMENT

Fig. 8-5



10000
1000
100
1
10
100
1000
ZN IN SCROBICULARIA (ug/g)
ZN AmAc IN SEDIMENT



10000
1000
100
1
10
100
1000
Zn IN SCROBICULARIA (ug/g)
(Zn AmAc) * (H/C) IN SEDIMENT

Fig. 8-5a

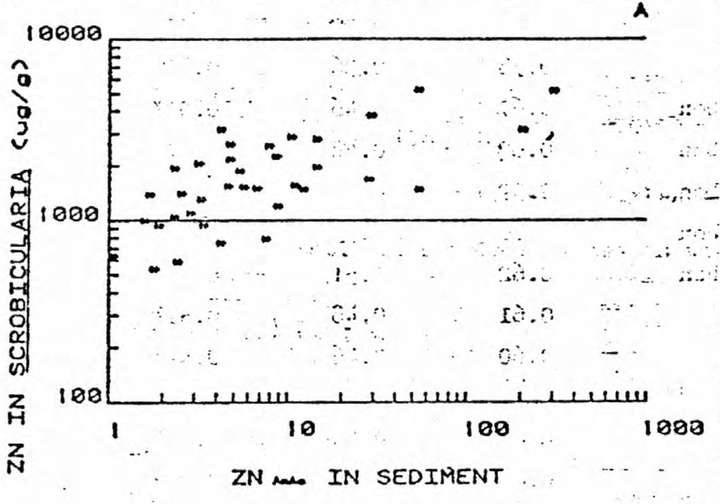

A
10000
1000
100
1
10
100
1000
ZN IN SCROBICULARIA (ug/g)
ZN IN SEDIMENT

Fig. 8-5b.

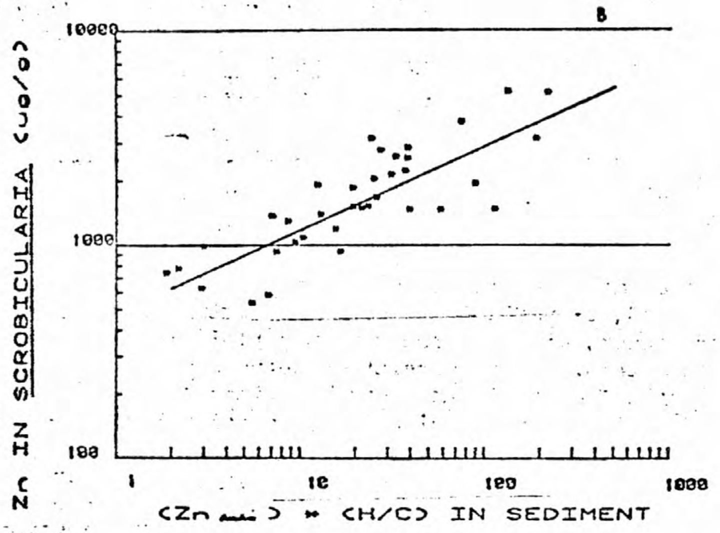
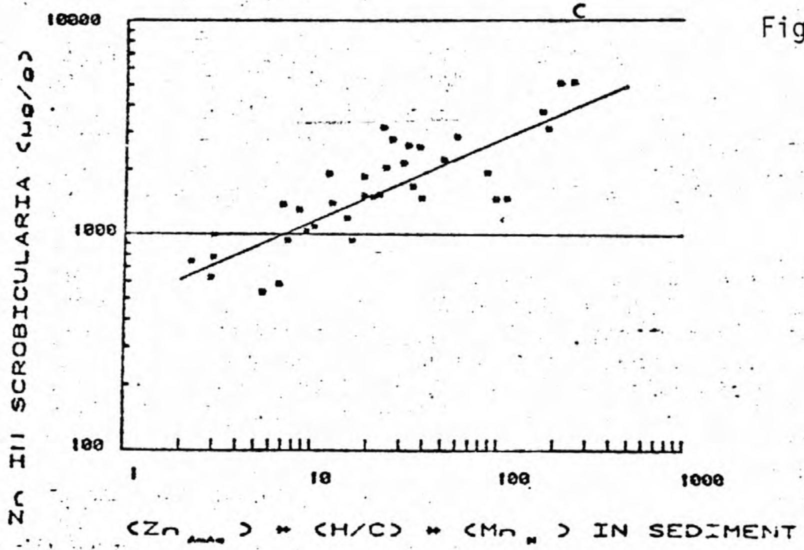


Fig. 8-5c.



Chapter 9

S.N. Luoma and G.W. Bryan

Controls on the Availability of Ag, Cd, Co, Fe and Mn to Estuarine Bivalves (Scrobicularia plana) and Polychaetes (Nereis diversicolor)

The controls on trace metal concentrations in natural populations of aquatic organisms have not been adequately resolved. Uptake of metals from both food and solution is concentration dependent under controlled laboratory conditions, but, in nature, uptake is not simply a function of exposure level. In fact, metal concentrations (or metal effects) in aquatic organisms in natural systems do not closely follow total metal concentration in either food or solution in many instances (Bryan and Hummerstone, 1977; Luoma and Bryan, 1978, 1979; Sunda and Guillard, 1976). Physiocochemical factors explain many of these deviations. The effect of solute Cu on phytoplankton is a function of the activity of the free Cu ion rather than total Cu in solution (Sunda and Guillard, 1976; Anderson and Morel, 1978). The uptake of metals from sediments by deposit feeders differs dramatically with the nature of the sediment to which the metal is bound (Luoma and Jenne, 1976; 1977). Concentrations of Pb and Zn in natural populations of deposit feeding bivalves are largely explained by a combination of metal concentrations in sediments plus the modifying effects of physicochemical variables which affect the form of the bound metal (Luoma and Bryan, 1978; 1979).

Variables other than physicochemical conditions may also affect metal availability to organisms. Concentrations of one metal may inhibit (Bryan, 1969), or enhance (Heisinger, et al, 1979) the uptake of another. Physiological processes such as homeostatic regulation, the inherent "permeability" or the organism to a metal, and cyclic physiological changes may all play a role in determining the concentration of metal observed at any one time in tissues. Likewise, the ecological and behavioral characteristics of the organism may greatly affect exposure and uptake of both bound and solute metals.

Realistic predictions of metal impacts in aquatic environments will require quantitative understanding of the relative importance in metal uptake of physical, chemical, physiological and ecological processes. In this chapter we employ a statistical approach to test the contributions of several of these processes to concentrations of Ag, Cd, Co, Fe and Mn in natural populations of two deposit feeding species -- the bivalve Scrobicularia plana and the polychaete Nereis diversicolor. Similar studies with Cu, Pb and Zn have appeared elsewhere (Luoma and Bryan, 1978, 1979, in prep.).

Trace metal concentrations in Scrobicularia and Nereis relative to sediments and water have been described previously within specific estuaries (Bryan and Hummerstone, 1971, 1973a,b, 1977, 1978; Bryan and Uysal, 1978). Here we statistically compare metal concentrations in the animals with the physiocochemical characteristics of their environment across a number of estuaries and a broad range of conditions (Luoma and Bryan, 1978, 1979; in press, a,b). Not all the parameters important in controlling a process in specific instances are made evident in a broad comparison. However, broad statistical comparisons may be useful in pointing to general phenomena controlling uptake (many of which are beyond the focus of single estuary studies) and in identifying specific questions or profitable lines of research which might be used in future studies.

Approach

Spatial differences in the metal concentrations of the deposit feeders were statistically compared with differences in the chemistry of the sediments using multiple regression. Samples were collected from 37 stations in 19 estuaries of widely varying characteristics (Luoma and Bryan, 1978, 1979; in press a,b). Sediments were treated with 6 extractants to assess the role of operationally defined fractions of metals and binding substrates in controlling metal bioavailability (the action of the extractants was described by Luoma and Bryan, in press a,b). The effect on uptake of total metal concentrations in sediments was tested by comparing metal concentrations extracted by a hot nitric acid digest of the sediments with metal concentrations in organisms. To test if some extractable metal phase corresponded to the biologically available fraction of metals in the sediments, metal concentrations in the animals were regressed against metal concentrations extracted from sediments by acid (1<u>N</u> HCl; 25 percent acetic acid; acid ammonium oxalate), an alkaline extractant (1<u>N</u> ammonia) and a neutral extractant with exchanging cations (1<u>N</u> ammonium acetate).

To assess the influence on uptake of the physicochemical partitioning of sediment-bound metals, concentrations of substrates (oxides of Fe and Mn total organic materials, humic substances, carbonates) which bind metals in oxidized estuarine sediments were included as independent variables in multiple regression calculations. This approach was discussed in detail by Luoma and Bryan (1979). Briefly, it is assumed that the partitioning of a metal is controlled by equilibrium processes. The forces driving partitioning are: (1) the strength of bonding between the metal and each substrate and (2) the relative abundances of the different substrates. Realistic data for (1) are not available, thus mathematical models of partitioning in sediments which contain mixtures of substrates have not been developed. However concentrations of the trace metal-reactive fraction of substrates in sediments may be defined using chemical extractants (Luoma and Bryan, in press a). Differences in the concentrations of these substrate fractions among sediments result in differences in metal partitioning (Luoma and Bryan, in press b). Correlations between metal concentrations in the sediment and metal concentrations in organisms would be expected where differences in substrate concentrations result in a shift in metal partitioning to forms of higher or lower bioavailavailability to the organism.

To test the influence of competitive interactions among metals on uptake, the concentrations of metals extracted from sediments by HCl were entered with the substrate variables into multiple regression calculations. Regression for each metal were conducted with and without concentrations of potentially interacting metals. Metal interactions within tissues were assessed by regressing metal concentrations within organisms against one another.

The influence of uptake from solution was determined from inclusion of metal concentrations in the seaweed *Fucus vesiculosus* with the above variables in multiple regression calculations. Concentrations of many metals in *Fucus* follow metal concentrations in solution within estuaries (Bryan and Hummerstone, 1971, 1977; Bryan and Uysal, 1978) and seaweed will integrate the rapid fluctuations that may occur in some solute metal levels. The use of *Fucus* as an indicator of biologically available solute metal assumes the same form(s) of the solute metal is available to *Fucus*, *Scrobicularia* and *Nereis*. Concentrations of Ag and Fe in *Fucus* were not employed as indicators of solute metal availability because of recent indications that a part of the burden of Ag and Fe in *Fucus* may be stripped from particulates (Chapter 10).

METHODS AND MATERIALS

Sediments were collected from the surface oxidized layer of the intertidal zone, sieved through 100μm mesh and extracted within 24 hours of collection. Extraction techniques and sample handling were described in Chapter 4.

Animals were purged for one week in clean 50 percent seawater (Scrobicularia) or acid washed sand (Nereis) before wet ashing in hot nitric acid (Bryan and Uysal, 1978). Analyses of Ag, Cd, Co, Cu, Fe and Mn were conducted by atomic absorption, using background correction in the Ag and Cd analyses. Criterion discussed by Luoma and Bryan (1978) were used in calculating mean metal concentrations in Scrobicularia.

All combinations of extractable metals in extractable substrates were initially screened using multiple regression to find the extractable phases of both metal and substrate which best explained the variance in metal concentrations in the animals (Table 9-1). Substrates included Fe in all extractants, Mn in all extractants, humic substances extracted by ammonia, total organic carbon and inorganic carbon. The screening calculations were conducted using the program RLSEP from the University of California computerized statistical package (a stepwise multiple regression with a backward glance). The extractable phases of Fe, Mn and metal which yielded the strongest correlations in the screening calculations were used in stepwise multiple regression calculations (with a backward glance) with a statistical software package written by Tektronix Corp. The Tektronix routine allowed breakdown of the contribution to the total variance of each independent variable, and a high degree of flexibility in determining the effects of the order in which variables were entered into the equations. Calculations were run with both log

transformed and untransformed dependent and independent variables, but tranformations seldom made a substantial difference in the outcome of the calculations. Calculations using metal levels in *Fucus* to test the effect of uptake from solution were run separately, since *Fucus* was not found at 5 stations where *Scrobicularia* was present and 8 stations where *Nereis* was present. We will present only the multiple regression results which explained the highest proportion of variance in metal concentrations of *Scrobicularia* and *Nereis* using statistically significant variables.

RESULTS

Concentrations of Ag, Cd, Co, Fe and Mn found in *Scrobicularia* and *Nereis* are shown in Table 9-2. Concentrations of each metal differed among stations by at least an order of magnitude (Table 9-3). The degree of variability was substantially different from metal to metal and among species. Concentrations of Ag, Co and Pb were more variable in animal tissues than in sediments, suggesting organisms responded with sensitivity to small changes in concentrations of these metals. The organisms were much less sensitive to changes in concentrations of other metals (especially Zn). The sensitivity of *Scrobicularia* to changes in sediment metal content differed from the sensitivity of *Nereis*. As a result, metal concentrations in the bivalves correlated only weakly with those in the polychaete. (Correlation coefficients (r) were: Cd=0.62*, Co=0.51*, Mn=0.32; Ag=0.25; Fe=0.24 and Cu=0.13, where *=P 0.01).

Physicochemical Controls on Availability

Cadmium. Cadmium was extracted from sediments only with HCl, due to generally low total concentrations. HCl-Cd in the sediment correlated significantly with Cd concentrations in the animals, explaining 29 percent of the Cd variance in *Scrobicularia*, and 16 percent of the variance in *Nereis* (Fig. The relatively strong correlation between Cd concentrations in *Scrobicularia* and *Nereis* appeared to result from similar physicochemical controls on uptake. Multiple regression calculations indicated concentrations of three substrates in the sediments explained 23 percent of the Cd variance in both animals (Table 9-4). Higher concentrations of Cd were observed in both species where Mn concentrations in sediments were elevated (oxalate-Mn yielded the best correlation of the 6 extractants). Low concentrations of Cd were observed in sediments with high concentrations of either total organic carbon or carbonate. The effects of Mn and TOC on Cd concentrations in the animals are consistent with our assumptions about the influence of substrate concentration on Cd partitioning and, subsequently on the bioavailability of Cd. Several explanations are possible for the negative effects of carbonates, however:

1) Carbonate concentrations may directly affect Cd partitioning to carbonates.

2) Carbonate concentrations may affect Cd partitioning to Mn oxides. Manganese carbonates become more abundant, at the expenses of Mn oxides, in high carbonate sediments (Luoma and Bryan, in prep. b). Thus high carbonate concentrations will reduce the partitioning of Cd to the Mn oxide substrate and reduce the bioavailability of Cd, if our statistical indications that Mn-bound Cd is of high bioavailability are correct. or 3) The negative effect of carbonates may indirectly indicate a negative effect of high salinities on uptake. Carbonate concentrations are highest in the most seaward reaches of estuaries and, within many estuaries, generally correlate negatively with salinity. The largest residuals to the best fit multiple regression equation tended to be stations at the extremes of the salinity range of Scrobicularia and Nereis, lending some credence to this hypothesis.

Concentrations of Cd in solution also significantly contribute to Cd concentrations in Nereis and Scrobicularia (Table 9-4). Concentrations of Cd in seaweed explain 15-16 percent of the Cd variance in both animals. Both Mn-oxalate and TOC were slightly insignficant variables among the 32 stations where both Scrobicularia and Fucus were collected, and the proportion of the variance explained by carbonates was greater than that observed among all 37 Scrobicularia stations. The smaller data set omitted 3 stations where Cd concentrations in Scrobicularia were very high (mouth of Plym; 2 stations in the

Gannel), which undoubtedly influenced the fit of the variables.

The slopes in the multiple regression equations (Table 4) indicated a remarkably parallel response of *Nereis* and *Scrobicularia* to changes in sediment chemistry and solute Cd (except for the greater sensitivity of *Nereis* to changes in concentrations of Mn). The intercept coefficient (a) differed substantially between the two species, however, suggesting the higher concentrations of Cd observed in *Scrobicularia* than in *Nereis* (Table 1) resulted from processes (perhaps physiological) other than those we have considered here.

Cobalt. Concentrations of Co in *Scrobicularia* and *Nereis* were largely controlled by Co concentrations in the sediments (Fig. 2). Concentrations of Co in *Nereis* followed concentrations in the sediments directly over the entire data range. Concentrations of Co in *Scrobicularia* correlated closely with concentrations in sediments among higher values (Co-sediment 1μg/g) and poorly among lower values. The latter suggested some physiological regulation of Co by *Scrobicularia* at low concentration of this metal. The fraction of Co extracted from sediment by HCl or acetic acid followed bioavailability substantially better than did total Co and slightly better than the fraction of Co extracted by other acids (Table 6).

Concentrations of humic substances (positive effect) and total organic carbon (negative effect) signficantly affected Co uptake by *Scrobicularia*, explaining 16-21 percent of the variance (Table 6). Among the stations where *Fucus* and *Scrobucularia* were collected solute Co also appeared to contribute to Co concentrations in the bivalve (explaining 7 percent of the variance) but the overall fit of the equation was poorer than among all stations. As with Cd, high concentrations of Co were found in *Scrobicularia* at several of the stations where *Fucus* was absent, substantially affecting the regression calculation with the smaller data set.

Among all stations, only Co-HCl in sediments significantly contributed to Co concentrations in *Nereis* (Table 9-5). Among the stations where *Fucus* occurred, Co concentrations in solute explained 4 percent of the variance in the polychaete and competitive inhibition of Co uptake by HCl-soluble Ag was indicated.

The significant correlation between Co concentrations in *Scrobicularia* and *Nereis* probably reflected the strong dependence of uptake in both species upon acid extractable concentrations of Co in sediments, and the similar responses of both to solute concentrations of Co (i.e. similar slopes for the *Fucus* variable). At most stations Co concentrations in Scrobicularia exceeded those in *Nereis* (Table 9-2). The differences were generally greatest near the heads of estuaries, where high concentrations of humic substances enhanced Co uptake by *Scrobicularia* and/or in estuaries where high concentrations of Ag in the sediments inhibited Co uptake by Nereis (Fal, Tamar, Gannel, East Looe). Concentrations of Co in *Nereis* exceeded those in *Scrobicularia* only in the Bristol Channel (where uptake by *Scrobicularia* was inhibited by the combination of low humic substances and high organic carbon), in the lower Fowey and in the Hayle.

Iron. The fractions of Fe extracted by HCl and by ammonia yielded the strongest correlations with Fe concentrations in *Scrobicularia* (Fig. 9-3). Both correlations were strongly influenced by the high values observed in the Tamar estuary. Among the middle range of Fe concentrations in sediment there was little relationship among estuaries between sediment-Fe and Fe in *Scrobicularia*.

Multiple regression calculations indicated the organically-complexed Fe extracted by ammonia was the most important variable controlling Fe concentrations in the bivalve (Table 9-7). The strong correlation of untransformed ammonia-Fe with *Scrobicularia*-Fe resulted partly from the emphasis in linear regression on high values such as those from the Tamar, and partly from the similar trends within estuaries (declining concentrations from the head-to-mouth) observed for both Fe-ammonia and Fe-Scrobicularia. The trends within estuaries may be a coincidence, if the high Fe concentrations in Scrobicularia from landward reaches of estuaries are the result of a large upstream solute contribution to uptake (as suggested from tissue distributions by Bryan and Uysal, 1978). The general correlation of ammonia-Fe and Scrobicularia-Fe among estuaries suggests control of Fe uptake by the fraction of Fe complexed with humic substances in sediments is worth further investigation, however.

A small fraction of the amorphous Fe in sediments also appeared to be available to Scrobicularia, as did Fe that was partitioned to Mn oxides (Table 9-7).

Physicochemical variables explained only 26 percent of the variance in Fe concentrations in Nereis. Uptake of Fe was slightly enhanced where TOC concentrations were high and followed a weak function of ammonia-soluble Fe. The small degree of variation among stations (4.1 fold - Table 9-2) and poor correlation with physicochemical variables suggest physiological regulation may play an important role in determining Fe levels in Nereis (as suggested by Bryan and Hummerstone, 1977).

Manganese. Concentrations of Mn in Scrobicularia were controlled by acid-extractable concentrations of Mn in sediments (Fig. 9-4) and the partitioning of Mn between humic substances and total organic materials. The fraction of Mn extracted from sediments with HCl correlated slightly better with Mn concentrations in Scrobicularia than did other extractable fractions in simple regressions (Table 9-6), and provided the best fit among the Mn fractions in multiple regression calculations (Table 9-8). Similar to Co and Zn, the availability of Mn to Scrobicularia was inhibited by high concentrations of organic materials and enhanced by the association of Mn with humic substances. Similar results were observed when ammonia-Mn was employed in the multiple regression as when humic substance concentrations extracted by ammonia were employed as a variable (Table 9-8).

The factors controlling the availability of Mn to Nereis were similar to those controlling availability to Scrobicularia. However, Mn concentrations in Nereis were more sensitive to changes in the concentration of humate-bound Mn (Fig. 9-4) and less sensitive to changes in HCl-Mn than were those in Scrobicularia. The effects of these differences were most evident in the Gannel estuary, where high concentrations of HCl-Mn accompanied by low concentrations of ammonia-Mn resulted in Mn levels in Scrobicularia which exceeded those in Nereis by up to 20 fold (much more so than in other estuaries Table 9-2). Concentrations of total organic materials had a positive effect on Mn availability to Nereis (in contrast to the negative effect observed for Scrobicularia) and carbonates had a slight negative effect (perhaps indicating Mn oxide, not Mn carbonate was the available fraction of HCl-Mn).

No evidence was found for significant Mn uptake from solution by either Nereis or Scrobicularia among all stations. However, uptake of solute Mn by Scrobicularia at stations in the extreme landward reaches of some estuaries (Bryan and Uysal, 1977) may not have been statistically detectable within this broad data range.

Silver. Concentrations of Ag in Scrobicularia correlated weakly with concentrations of Ag extracted from sediment by HCl but showed no significant correlation with total Ag or ammonia-Ag in sediments (Table 9-6, Fig 9-5). Concentrations of Cu in sediments significantly inhibited Ag uptake by Scrobicularia (Fig. 9-6). Together these two variables explained 77 percent of the variance in Ag-Scrobicularia (Table 9-6). Interestingly, the strong control of HCl-Ag on Ag concentrations in Scrobicularia was not highly evident until the inhibitory effects of HCl-Cu in sediment were considered (i.e. HCl-Ag was always the second variable of choice in the multiple regression calculation). The interaction of these two variables was most evident in the Fal and Hayle estuaries where, despite high concentrations of Ag in sediments, low concentrations of Ag were observed in Scrobicularia due to the extreme Cu enrichment of the sediments.

Significant but less substantial variables, affecting the availability of Ag to Scrobicularia, were TOC (positive) and humic substances, which had a negative effect. The form of Mn extracted by ammonium acetate also had a negative effect on the availability of Ag. Luoma and Jenne (1976) showed that much of the Ag associated with pure Mn oxides is extracted by ammonium acetate. Ammonium acetate may extract a form of Mn highly reactive in binding Ag in sediments and this form of Ag may have a low bioavailability.

Concentrations of HCl-Ag were only marginally better than total Ag concentrations as an indicator of Ag availability to Nereis (Table 9-9). Concentrations of Cu in the sediment had no detectable effect on Ag uptake by the polychaete. The physicochemical controls on Ag availability to Nereis differed from the controls for Scrobicularia only in the inclusion of total Mn and the substitution of oxalate-Fe for total organic carbon in the Nereis calculations. Extractable Fe and TOC are themselves strongly correlated in these sediments (Luoma and Bryan, in press a). Thus they may reflect a similar process affecting Ag uptake by both Scrobicularia and Nereis.

Concentrations of Ag in Scrobicularia were generally higher than those in Nereis, except in estuaries where Cu enrichment in sediments inhibited the availability of the metal to the bivalve (e.g., Fal, Hayle, upper Fowey). The regression data could not explain the extreme Ag contamination observed in Scrobicularia (but not Nereis) in the East Looe estuary (see also Bryan and Hummerstone, 1977).

DISCUSSION

Ultimately, simplified regression models of the type we have presented here may be useful in providing first order predictions of the bioavailability of sediment-bound trace metals in natural systems. The specific models we have presented need to be further tested, and amended, by studies in other systems with other species. Although we have considered a broad range of estuarine environments, the chemical characteristics of the estuaries of south and west England generally differ from larger, morphologically more complex estuarine systems; and these differences may influence the controls on bioavailability (e.g. Luoma and Bryan, 1979).

Many of the factors controlling metal uptake by _Scrobicularia_ were similar to those controlling uptake by _Nereis_. However, the two species often responded with a different sensitivity to the same variable (e.g. Mn-ammonia and Mn-HCl); and in a few instances, a variable affected metal uptake in one species but not the other (e.g. Ag inhibition of Co uptake by _Nereis_; Cu inhibition of Ag uptake by _Scrobicularia_). At this point, sufficient differences appear to exist between species to prevent the development of a single quantative model describing bioavailability of any metal to all deposit feeders. However, the two species were sufficiently similar to differentiate the type of estuarine situations where a general enhancement or inhibition of bioavailability might be expected. The latter capability could be useful in predicting the impact of proposed metal discharges in one system relative to another, or in explaining results observed in different natural systems.

In this study, statistical correlations reflect similarities in the spatial patterns of variables as seen across a wide range of data from a number of estuaries. The strength of correlation of variables reflecting the chemistry of sediments with concentrations of Ag, Cd, Co, Fe and Mn in Scrobicularia and Nereis was remarkable, considering the myriad of variables that could interact over the range of conditions that were sampled. From 70-80 percent of the variation in Ag, Mn, Co, Zn (Luoma and Bryan, 1978) and Pb (Luoma and Bryan, 1978) concentrations in the deposit feeders followed differences in sediment chemistry. Over 60 percent of the variance in Fe concentrations of Scrobicularia and Cd concentrations in Scrobicularia and Nereis was explained by the variables we considered. Only Fe and Zn in Nereis did not correlate with sedimentary variables, consistent with other indications of physiological regulations of these metals in the polychaete. Although statistical correlations do not prove cause and effect, the data strongly indicate that the biological availability of sediment-bound Ag, Cd, Co, Fe, Mn, Pb, and Zn dominates uptake by Nereis and Scrobicularia.

Several specific variables appeared to control bioavailability from sediments:

(1) Concentrations of metals extracted from sediment. The extractable concentration of metal in sediment was the single most important variable correlating with concentrations of Ag, Cd, Co, Fe and Mn in Nereis and Scrobicularia. In general metal concentrations extracted by weak acids more closely followed bioavailability than did total metal concentrations in sediments; and HCl-extractable forms were better indicators of bioavailability than were forms extracted by other acids. Concentrations of Fe and Mn extracted by ammonia and concentrations of Zn extracted by ammonium acetate (Luoma and Bryan, 1979) also followed the availability of these metals.

Extractants were also useful in identifying the phases of different substrates which most influenced partitioning (and thus availability) of the different metals. Among the extractants tested, similar methods were optimally useful in defining bioavailability as were suggested for optimally assessing metal partitioning in sediments (Luoma and Bryan, in press, b): HCl, ammonia (or NaOH), acid ammonium oxalate and ammonium acetate. These extractants provide both direct indications of the biologically available fractions, and data concerning substrate concentrations which may be used to predict the modifying effect of physicochemical variables on the concentration dependence of uptake.

Effect of metal partitioning. A significant proportion of the spatial variability in metal concentrations in the animals was explained by concentrations of Fe, Mn, total organic materials and/or humic substances (Fig. 9-7). suggesting differences in metal partitioning to these substrates resulted in differences in metal bioavailability. Substrate effects in the regression differed among metals, as expected from differences in metal chemistry. Significant correlations between metals were observed in the tissues of the animals in instances where physiocochemical controls on availability were highly similar.

In several instances, the substrates which statistically correlated with the bioavailability of a metal were different from the substrates which dominated the partitioning of that metal in the sediments (as determined by Luoma and Bryan, in press, a). For instance, the fractions of Fe bound to Mn oxides and humic substances, and the fraction of Mn associated with humic materials in oxidized sediments are small relative to the bulk of Fe and Mn occurring as hydrous oxides. Because the availability of oxidic Mn and Fe is low, however, the quantitiatively minor forms played a major role in controlling uptake. Luoma and Jenne (1977) suggested that partitioning of a trace metal solely to substrates which bond that metal most strongly in sediments would minimize the bioavailability of the metal, because many differences in availability among metal-substrates associations are directly related to differences in bonding strength. However, if even a small fraction of metal is partitioned to a substrate from which the metal is highly available, that form of the metal could play a major role in determining availability. Moreover, since minor substrates have low bonding strengths for metal, partitioning (and thus bioavailability) may be highly sensitive to differences in minor substrate concentration. Thus changes in bioavailability may often be directly linked (and thus closely correlated) to changes in concentrations of substrates which are quantitatively of minor importance in partitioning. For example, data with Scrobicularia and Nereis suggest humic substances are quantitatively minor substrates in the partitioning of Mn, Fe, Co and Zn, but humic substances have a major effect upon the bioavailability of these metals. Similarly, statistical evidence suggests Mn oxides are of minor importance in the partitioning of Cd and Ag in sediments (Luoma and Bryan, in press b), but play a major role in Cd and Ag availability to animals.

Intermetal interactions. The Ag-Cu interactions suggested statistically from our data have not been reported previously in the literature. The inhibition of Ag uptake by sediment-bound Cu was highly important in explaining Ag burdens in Scrobicularia. Only after the Cu inhibition was considered was it obvious that Ag uptake by the bivalve was concentration dependent. Moreover, indications of a physiological enhancement of Cu uptake by Ag in bivalve tissues (Luoma, et al, unpublished data; Luoma and Bryan, in prep. a) coupled with the inhibition of Ag uptake by Cu in sediments helps explain the very poor correlation between Cu concentrations in sediments and bivalve tissues over wide data ranges.

Uptake from solution. Concentrations of metals in solution appeared to be a minor source of all metals except Cd and perhaps Co (within the limits of our use of Fucus as an inidcator of solute uptake). The indication of an important solute component in Cd uptake is consistent with high Cd concentrations observed in organisms from the Bristol Channel, where substantial quantities of Cd in solution have been reported. The contribution of solute sources could be more important if the correlations with sedimentary variables reflected sediment-controlled release of metals to solution rather than direct uptake from sediments (Luoma and Jenne, 1977). This seems unlikely, however, in these estuaries, where the residence times of water at the sediment-water interface are low.

Salinity. Despite laboratory evidence that low salinities may facilitate uptake of some trace elements in estuaries (Wolfe, 1970), our statistical evidence indicated metal burdens of most metals in Scrobicularia and Nereis could largely be explained without invoking salinity effects. Samples were collected from throughout the salinity range of both species. Strong salinity effects could have been evident as residuals at stations with salinity extremes. Such residuals were observed in calculations with Zn and Cd (although the effect was not consistent among all estuaries for either metal) but not for other metals. Concentrations of humic substances were inversely related to salinity within these estuaries. However, humic substances concentrations varied considerably among estuaries at stations with similar salinities. Thus the positive effect of humic substance concentrations on the availability of Fe, Co and Zn to Scrobicularia, and Mn to both Scrobicularia and Nereis, more likely indicated an enhanced availability of these metals when complexed to humic substances than a salinity-associated effect on availability.

Nereis vs sedimentary characteristics. Only variables which were significant in "Final Runs" I and II are reported.

Independent Variables

Runs	Variables	Extractant
I Screening Run I	(1) Target Metal	One run for each: Total, HCl acid ammonium oxalate, ammonium acetate and ammonia (where detectable)
	(2) Fe, Mn	Each variable (1) was run with all combinations of Fe-total, HCl, acid ammonium oxalate, ammonium, and Mn-total ammonium acetate, HCl, acid ammonium oxalate
	(3) TOC, humic substances, CO_3	
II Screening Run II	(1) Non-target metals	HCl
	(2) All variables as in Screening run I	
III Final Run I		
A. Log transform y	(1) Target Metal	Extractants which gave highest R_2 in screening run
B. No transformation of y	(2) Fe, Mn	Extractants which gave highest R_2 in screening run
	(3) TOC, humic substances, CO^3	
	(4) Any metal which was significant in II	
IV Final Run II		
A. Log transform y	(1) Same as Final Run I	
B. No transformation of y	(2) Target metal in *Fucus*	

Table 9-2 Metal concentrations in µg/g dry weight observed in the plana and the bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor*

		Ag		Cd		CO		Fe		Mn	
Estuary	Station	Scrobic.	Nereis	Scrobic.	Nereis	Scrobic.	Nereis	Scrobic.	Nereis	Scrobic.	Nerei
Plym	head	0.27	1.22	32.4	3.1	10.3	3.5	1124	355	20	8.1
	mouth	0.86	0.31	7.60	0.31	15.6	1.8	709	492	18	10.0
Erme	1	0.67	0.26	1.14	0.20	10.1	2.9	983	425	48	11.3
	2	1.12	0.55	1.23	0.40	13.1	4.3	872	385	53	10.1
West Looe	head	3.87	1.02	1.94	0.40	16.7	6.6	1260	399	71	11.8
	mouth	5.77	1.10	0.90	0.20	5.9	3.5	605	524	54	11.6
East Looe	head	122.0	n.c.	3.10	n.c.	20.3	n.c.	1551	n.c.	66	n.c.
	2	65.5	1.91	1.30	0.12	12.4	2.04	2140	212	49	5.5
	3	45.5	1.94	1.39	0.15	10.5	2.90	1892	432	59	9.1
	mouth	4.1	1.16	0.57	0.25	7.3	2.2	1353	596	42	9.6
Fal	1	0.27	3.05	3.72	0.81	22.5	4.5	2060	378	12.8	12.3
	2	0.26	6.23	2.25	0.97	9.2	7.5	1658	268	20.6	14.1
Poole Harbor		16.2	0.53	15.00	0.24	6.6	5.6	1672	528	5	10.0
Beaulieu	head	5.03	0.32	2.37	0.33	41.7	13.8	2592	471	51	16.5
Hamble		0.36	0.17	1.01	0.27	9.4	4.8	1727	348	38	9.9
Itchen		1.82	0.14	1.03	0.10	5.3	1.9	1093	513	27	13.0
Test		0.56	0.26	0.99	0.18	14.3	7.2	1535	465	29	11.2
Gannel	head	1.94	3.93	14.30	1.45	11.3	13.4	1963	371	252	13.9
	2	0.68	1.94	7.22	0.37	55	7.0	3028	372	197	15.5
	mouth	1.12	3.66	1.53	0.40	23	11.2	1259	431	74	10.9
Camel		0.34	0.60	0.60	0.54	8.0	6.3	2286	561	21	
Hayle		0.63	8.01	1.50	0.17	6.8	8.4	1042	597	19	
Fowey	head	n.c.	0.74	n.c.	0.67	n.c.	9.3	n.c.	533	n.c.	23.5
	2	0.40	0.71	2.69	0.21	15.0	5.6	1323	551	21	13.3
	3	0.28	0.74	2.37	0.39	8.2	4.4	1311	454	30	11.7
	4	0.58	0.62	1.67	0.24	10.2	4.2	1498	453	34	10.7
	5	0.35	0.47	1.08	0.29	4.2	4.6	800	408	24	9.4
	mouth	0.18	0.38	0.64	0.20	2.7	3.3	1179	456	33	13.5
Torridge		1.20	0.50	2.12	0.42	13.3	6.3	1426	457	100	16.6
Bristol Channel	1	8.70	2.64	2.83	0.51	4.8	5.8	1373	425	72	23.5
	2	1.18	2.40	4.50	0.79	9.4	10.4	948	553	60	25.1
Tamar	head	0.30	0.82	2.30	0.43	17.3	9.6	10,614	698	103	52.1
	2	0.32	0.76	2.72	0.54	21.1	10.8	13,256	703	117	32.3
	3	0.39	0.87	2.97	0.27	29.2	10.2	5185	637	57	17
	4	0.25	0.97	3.60	0.65	17.1	8.3	1453	562	39	12.6
	mouth	0.23	1.20	1.61	0.38	10.8	4.3	1395	448	33	18.4
Tavy		0.34	2.64	5.52	1.26	29.2	24.9	1289	292	30	10.0

Table 9-3 Variation in concentrations of Ag, Cd, Co, Cu, Pb, Zn, Fe and Mn among stations in 19 estuaries of south and west England. *Data from Luoma and Bryan, 1979

	Sediment		*Scrobicularia*		*Nereis*	
	Range of total concentration (µg/g)	Magnitude of variation	Range of concentration (µg/g)	Magnitude of variation	Range of concentration (µg/g)	Magnitude of variation (x)
Ag	0.1 - 1.55	15.5x	0.18 - 122	678x	0.17 - 8.0	47.1
Cd	0.1 - 10.8	108x	0.6 - 32.4	54.0x	0.17 - 3.10	18.2
Co	3 - 36	12x	3 - 55	18.3x	1.8 - 24.9	13.8
Cu	17 - 3052	179.5x	17 - 372	21.9x	12 - 1410	116
Fe	10,686 - 48,383	4.5x	605 - 13,256	21.9	212 - 871	4.1
Mn	138 - 1314	9.5x	5 - 252	49.4	5.5 - 52	9.5
Pb	41 - 1602	39.1x	14 - 1016	72.6x	0.6 - 685	1142
Zn*	95 - 3544	37.3x	558 - 4922	8.8x	141 - 467	3.3

Table 9-4 Multiple regression describing physicochemical variables correlating significantly with Cd concentrations in the bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor*. Only variables and transformations found significant ($t<2.04$) by stepwise multiple regression (with a backward glance) are presented. All regressions are significant at $p<0.001$

Sedimentary Variables	a	slope	Percent Variance explained	t-statistic per variable	f-statistic for regression	R^2 for regression
			log - *Scrobicularia* - all data			
log Cd - HCl	.60	(+)0.63	29.0	5.14		
Mn-oxalate		(+)0.00084	9.0	3.31		
Total organic Carbon		(-)0.114	9.0	-2.42		
log carbonate		(-)0.128	5.0	-1.91	8.7	0.521
			log - *Scrobicularia* - seaweed data			
log Cd - HCl	0.135	0.435	30.9	4.86		
Cd - *Fucus*		0.070	16.0	4.75		
log carbonate		(-)0.232	20.5	(-)4.25	19.8	0.671
			log - *Nereis* - seaweed data			
Cd - *Fucus*	-1.09	0.056	15.0	3.39		
log Cd - HCl		0.463	15.7	3.36		
log carbonate		-0.134	9.0	-2.23		
log Mn-oxalate		0.410	5.0	2.50		
TOC		-0.107	9.0	-2.20	5.8	0.537

Table 9-5 Multiple regression describing physicochemical variables correlating with Co concentrations in the bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor* Only variables and transformations found significant (t>2.02) by stepwise multiple regression (with a backward glance) are presented. All regressions are signficant at $p<0.001$

Variable	a	slope	Percent Variance explained	t-statistic per variable	F-statistic for equation	R^2 for equation
Log - Co - *Scrobicularia* - all data (n=36)						
Co-HCl	0.92	0.051	47.2	8.0		
Humic Substances		0.024	4.9	4.4		
TOC		(-)0.149	16.6	-4.2	24.2	0.69
Co - *Scrobicularia* - all data (n=36)						
Co-HCl	9.67	3.26	57.5	9.3		
log TOC		-51.34	9.0	-4.4		
Humic Substances		0.82	6.9	3.0	30.6	0.74
Log - Co - *Scrobicularia* - *Fucus* data (n=32)						
Co-HCl	0.58	0.051	29.6	5.1		
Humic Substances		0.32	10.4	3.0		
log TOC		(-)0.54	6.9	-2.3		
Co-*Fucus*		0.016	7.3	2.1	8.0	0.54
Log - Co - *Nereis* - all data (n=42)						
log Co-HCl	0.28	0.58	66.0	8.0	34.0	0.66
Log - Co - *Nereis* - *Fucus* data (n=33)						
log Co-HCl	0.46	0.70	66.0	8.5		
Ag-HCl		(-)0.19	5.1	-2.6		
Co-*Fucus*		0.017	4.0	2.7	21.4	0.75

Table 9-6 Correlation coefficients (R) comparing correlation of various extractable fractions of trace metals with metal concentrations in Scrobicularia and Nereis. All data were transformed to logarithmns.

	Total	HCl	Acetic acid	Acid ammonium oxalate	Ammonia	Ammonium acetate
			Scrobicularia			
Co	0.49	0.57	0.57	0.55	nd	nd
Fe	0.28	0.44	0.40	0.29	0.46	nd
Mn	0.67	0.75	0.70	0.63	0.30	0.46
Ag	0.04	0.33	nd	nd	0.30	nd
			Nereis			
Co	0.68	0.81	0.77	0.78	nd	nd
Fe	0.25	0.37	0.23	0.46	0.20	nd
Mn	0.62	0.62	0.60	0.62	0.57	0.54
Ag	0.46	0.50	nd	nd	0.50	nd

Table 9-7 Multiple regression describing physicochemical variables correlating with Fe concentrations in the bivalve Scrobicularia plana and the polychaete Nereis diversicolor. Only variables and tran formations found significant ($t>2.04$) by stepwise multiple regression (with a backward glance) are presented. All regressions are significant at $p<0.001$

Variable	Extract	a	Slope	percent variance explained	t variable	F equation	R^2 equation
			Log Fe - *Scrobicularia* - all data				
Fe	ammonia	2.82	0.0022	40.0	4.25		
Fe	HCl		2.3×10^{-5}	12.9	2.87		
Mn	oxalate		4.24×10^{-4}	8.2	2.60	16.8	0.61
			Fe - *Scrobicularia* - all data				
Fe	ammonia	-1117	24.20	50.9	5.3		
Mn	oxalate		3.06	6.6	2.1		
Fe	HCl		0.16	5.3	2.1	18.0	0.63
			Log *Nereis* - all data				
TOC		2.54	0.038	21.4			
Fe	ammonia		0.00025	4.7		6.72	0.26

Table 9-8 Multiple regression describing physicochemical variables correlating with Mn concentrations in the bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor* Only variables and transformations found significant (t>2.04) by stepwise multiple regre sion (with a backward glance) are presented. All regressions are significant at $p<0.001$

Sedimentary variable	a	slope	percent variance explained	t	F regression	R^2 regressi
			Log Mn - *Scrobicularia* (all data)			
Mn - HCl	1.33	0.0012	59.1	10.6		
Log Humic Substance		0.32	20.4*	3.6		
Log Total Organic Carbon		(-)0.65		3.2	30.1	0.795
			Mn - *Scrobicularia* (all data)			
Mn - HCl	9.5	0.19	67.2	13.1		
log TOC		(-)118.3	10.7	-6.0		
Log HA		40.2	6.8	3.8	60.7	0.847
Mn - HCl	44.7	0.16	67.2	10.0		
log TOC		(-)96.7	8.0	-4.4		
log Mn-ammonia		13.7	4.1	1.5	42.1	0.793
			Log Mn - *Nereis* (all data)			
Mn - ammonia	0.94	0.051	38.3	2.7		
Mn - HCl		0.00027	12.8	2.6		
log CO_3		(-)0.067	5.3	-2.5		
log TOC		0.37	8.7	3.4	14.2	0.69

* One value for HA and TOC means variable entered second caused largest increase in R^2 combination of two variables was most important.

Table 9-9 Multiple regression describing physicochemical variations correlating with Ag concentrations in the bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor*. Only variables and transformations found significant (t>2.05) by stepwise multiple regression (with a backward glance) are presented. All regressions are significant at p<0.0001

variable	extractant	a	slope	percent variance explained	t variable	F	R^2
						regression	
			Log Ag - *Scrobicularia* (all data)				
Log Cu	HCl	3.69	(-)1.30	28.4	-10.3		
Log Ag	HCl		1.83	47.8	7.9		
Log Mn	ammonium acetate		(-)0.43	2.0	-2.6		
Humic Substance	ammonia		(-)0.029	3.7	-2.9		
TOC			0.23	2.0	3.2	32.0	0.84
			Log Ag - *Nereis* (all data)				
Log Ag	HCl	1.79	0.54	40.9	4.7		
Log Mn	Total		1.17	14.2	4.9		
Fe	oxalate		2.84	8.8	3.6		
Log Humic Substance	ammonia		-0.42	5.2	-3.7		
Log Mn	ammomium acetate		-0.43	5.6	-2.8	21.2	0.75

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Fig. 9-1 Correlation of Cd extracted from sediment by HCl with Cd concentrations in the bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor*. $r = 0.54$, $p<0.001$ for *Scrobicularia*. $r = 0.40$, $p<0.02$ for *Nereis*.

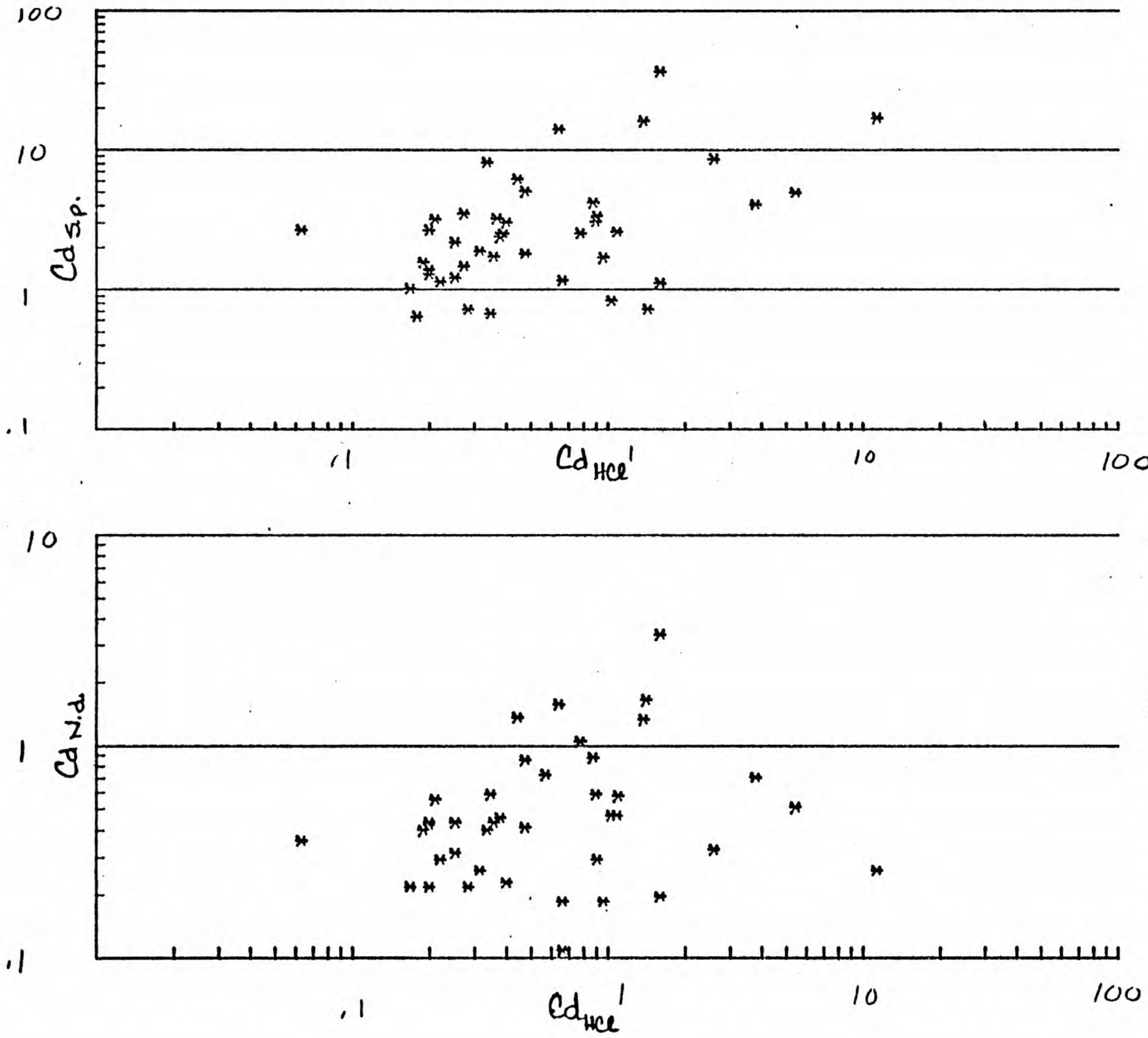
Fig. 9-2 Correlation of Co extracted from sediment by HCl with concentrations of Co in *Scrobicularia plana* and *Nereis diversicolor*. $r = 0.57$, $p<0.001$ for *Scrobicularia*. $r = 0.81$, $p<0.001$ for Nereis.

Fig. 9-3 Correlation of Fe concentrations extracted from sediment by ammonia with Fe communications in *Scrobicularia plana* and *Nereis diversicolor*. $r = 0.46$, $p<0.005$ for *Scrobicularia*. $r = 0.20$, $p>0.10$ for *Nereis*.

Fig. 9-4 Correlation of Mn concentrations extracted from sediment by HCl with Mn concentrations in *Scrobicularia plana* and Mn extracted by ammonia with Mn in *Nereis diversicolor*. $r = 0.75$ $p<0.001$ for *Scrobicularia*; $r = 0.57$, $p<0.001$ for *Nerei*

Fig. 9-5 Correlation of Ag concentrations extracted from sediment by HCl with Ag concentrations in *Scrobicularia plana* and *Nereis diversicolor*. $r = 0.33$, $p<0.05$ for *Scrobicularia*; $r = 0.50$, $p<0.005$ for *Nereis*.

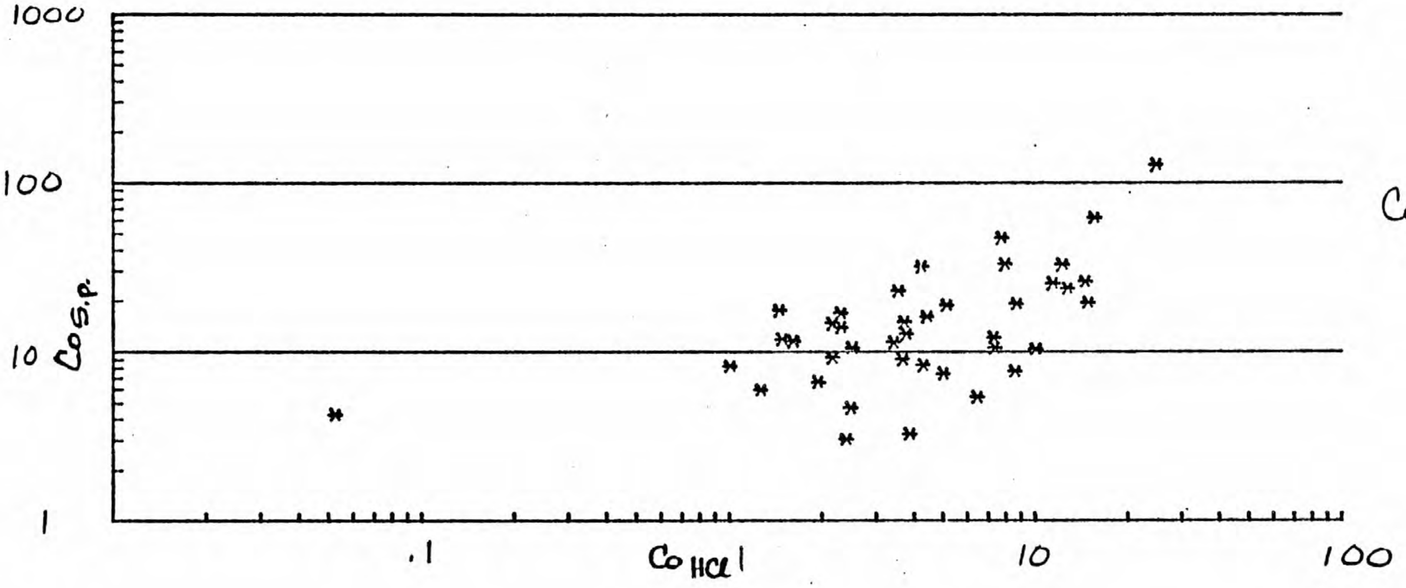
Fig. 9-1



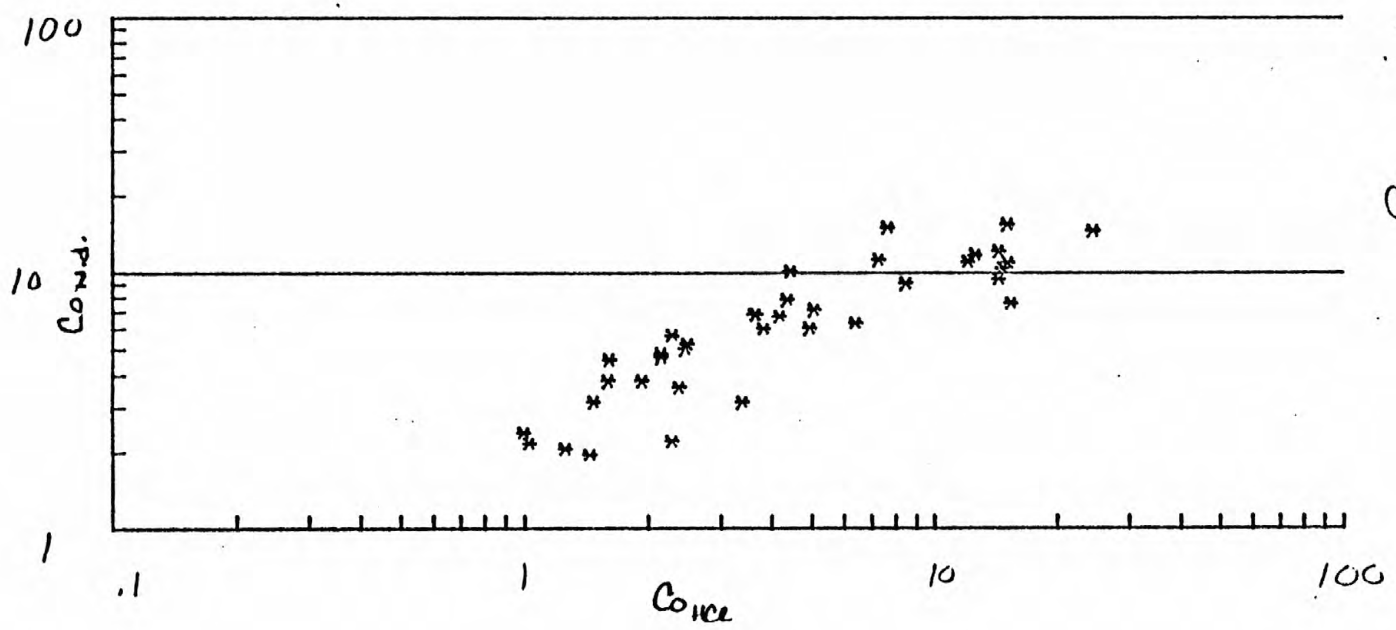
$\mathrm{Cd}_{\mathrm{HCl}}$ vs.
$\mathrm{Cd}_{\underline{\text{Scrobicularia plan}}}$

$\mathrm{Cd}_{\mathrm{HCl}}$ vs.
$\mathrm{Cd}_{\underline{\text{Nereis diversicolor}}}$

Fig. 9-2

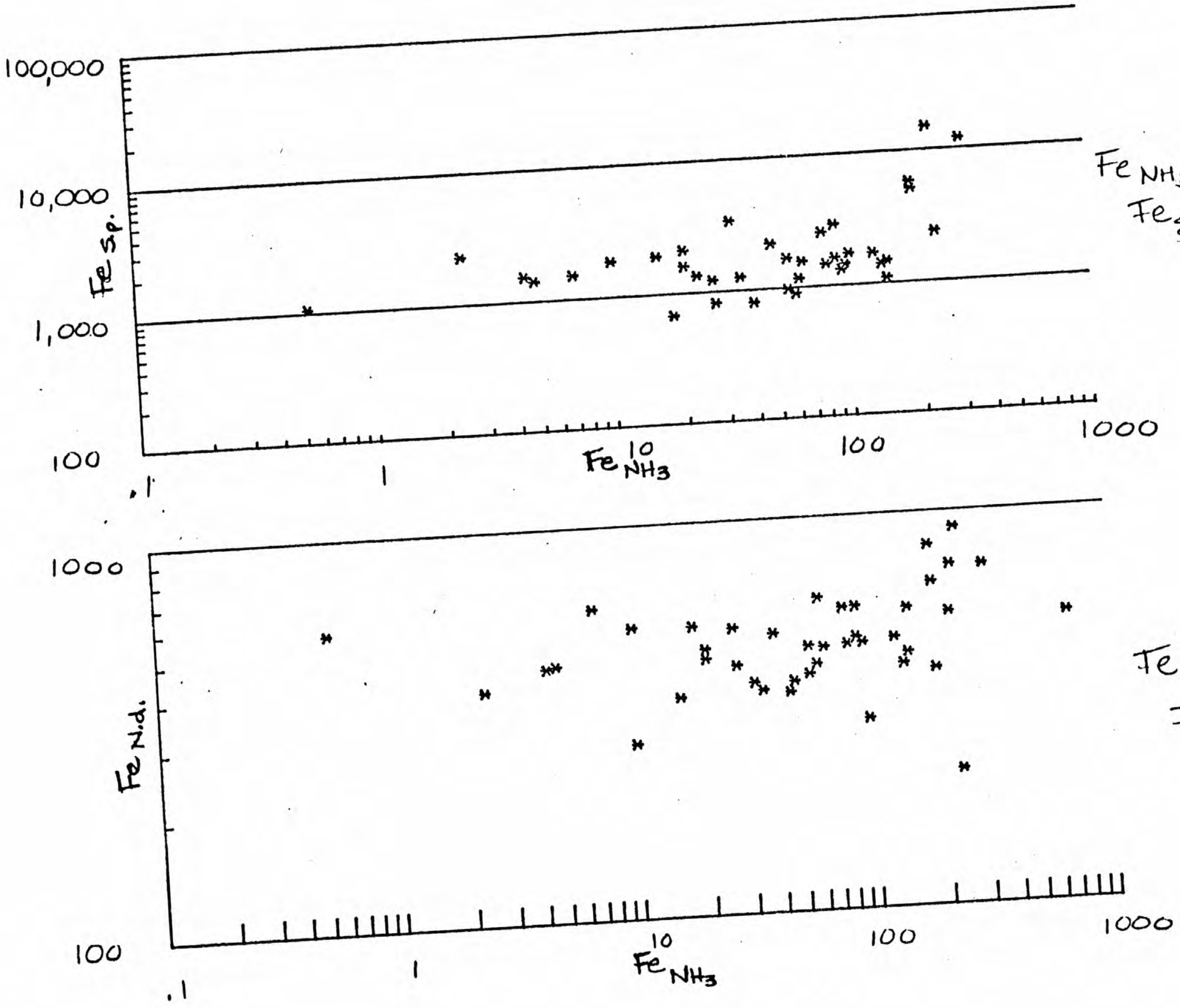


Co HCl vs. Co *Scrobicularia plana*



Co HCl vs. Co *Nereis diversicolor*

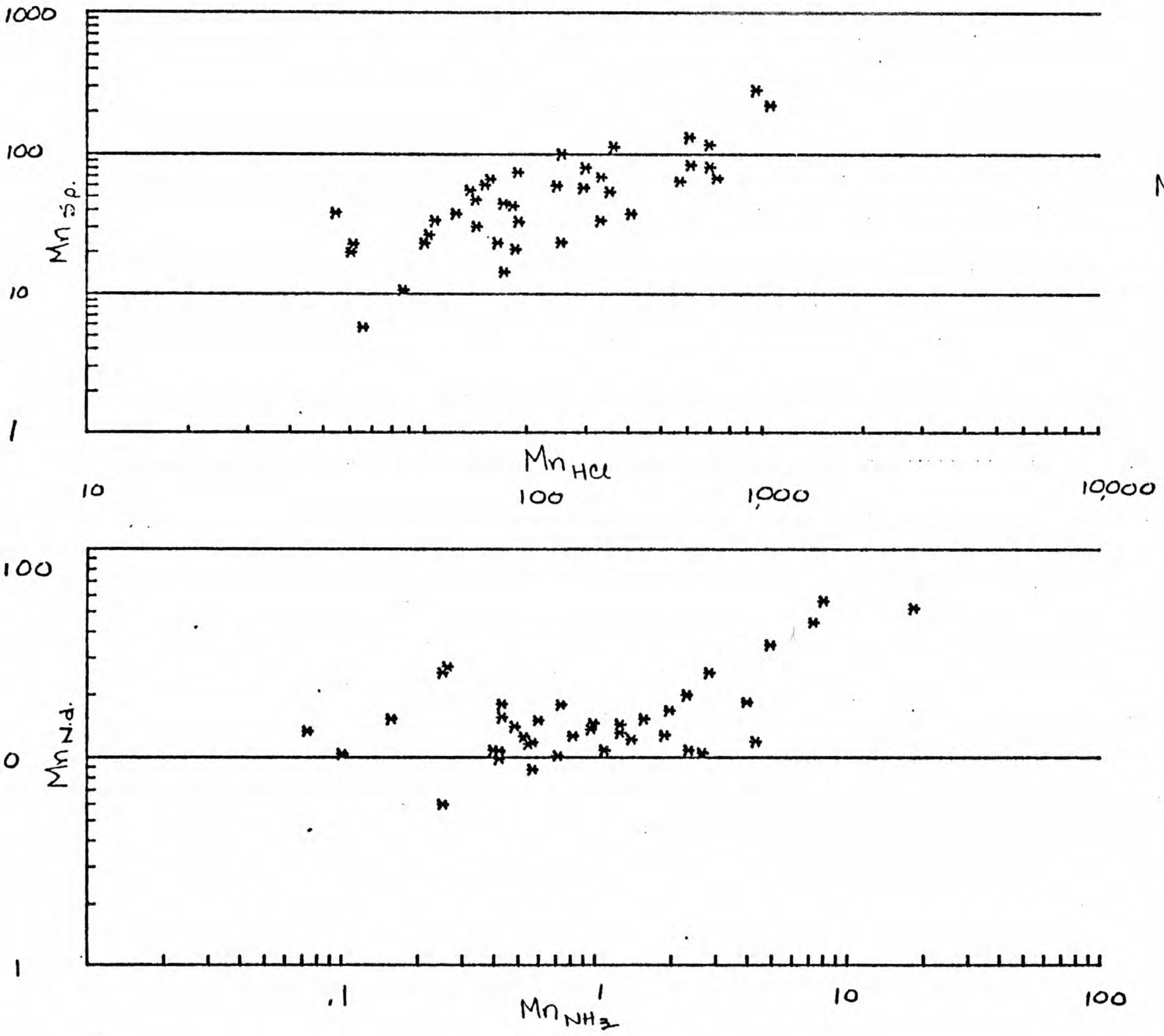
Fig. 9-3



Fe_{NH_3} vs.
$Fe_{\text{Scrobicularia plana}}$

Fe_{NH_3} vs.
$Fe_{\text{Nereis diversicolor}}$

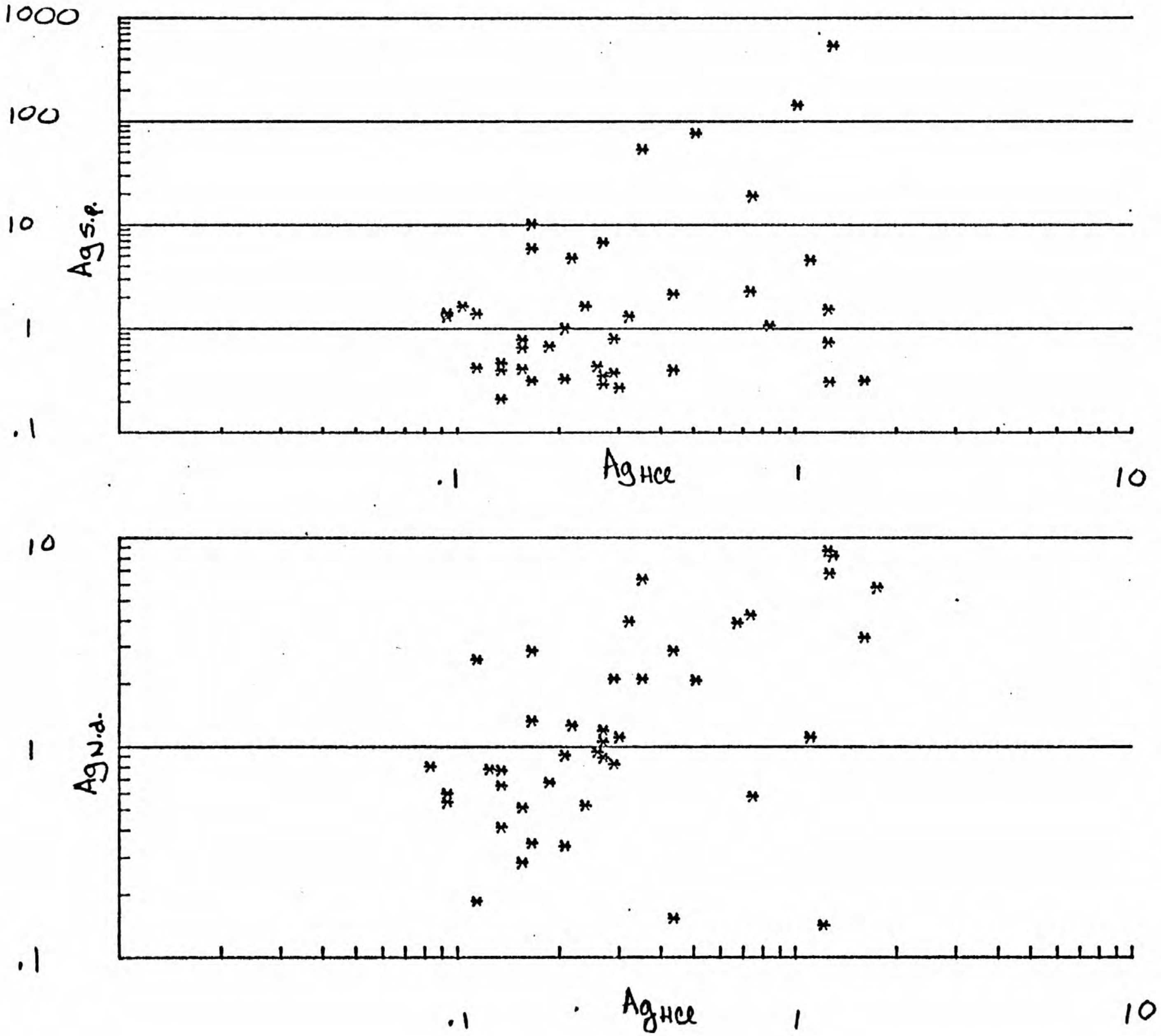
Fig. 9-4



Mn_{HCl} vs.
Mn Scrobicularia plana

Mn_{NH_3} vs.
Mn Nereis diversicolor

Fig. 9-5



Ag_{HCl} vs. Ag *Scrobicularia plana*

Ag_{HCl} vs. Ag *Nereis diversicolor*

Chapter 10

Controls on Cu Concentrations in Estuarine Bivalves (*Scrobicularia plana*), polychaetes (*Nereis diversicolor* and Seaweed (*Fucus vesiculosus*)

S.N. Luoma and G.W. Bryan

INTRODUCTION

Multiple regression calculations indicate that the biological availability of Ag, Cd, Co, Fe, Mn, Pb and Zn in deposit feeding bivalves (*Scrobicularia plana*) and polychaetes (*Nereis diversicolor*) from the estuaries of south and west England are controlled by the concentrations and the chemical forms of these metals in surficial, oxidized sediments (Luoma and Bryan, 1978, 1979, in prep. a). In this paper similar regression models are calculated to assess controls on Cu availability to *Scrobicularia*, *Nereis* and the seaweed *Fucus vesiculosus*. Concentrations of Cu in *Nereis* and (surprisingly) *Fucus* correlate very strongly with total-Cu in sediments. However, Cu in *Scrobicularia* appears to be controlled largely by physiological interactions with Ag and possibly, Pb. Copper concentrations and form in sediments play less of a role in controlling burdens in *Scrobicularia* than with most metals. However, anomalous stations are reported where the availability of Cu to *Scrobicularia* is greatly enhanced in response to a combination of anoxia and undefined variables.

Methods and Approach

Sediments and organisms were collected from 37 stations in 19 estuaries of broadly varying characteristics. The methods and statistical approach for sediments, *Scrobicularia* and *Nereis* have been described in previous chapters. Concentrations of Cu in *Fucus* were determined as described by Bryan and Hummerstone (1973).

RESULTS

Scrobicularia

Concentrations of Cu in Scrobicularia differed by 34 fold among stations (Table 10-1). In general, concentrations were higher toward the head of most estuaries than toward the mouth, in rough correspondence with intra-estuarine trends in the concentration of Cu in sediments (as reported elsewhere-Bryan and Hummerstone 1977; Bryan and Uysal, 1978). However, among estuaries no correspondence between Cu concentrations in sediments and concentrations in Scrobicularia was observed (Fig. 10-1).

A multiple regression assessment of factors affecting the bioavailability of Cu (described by Luoma and Bryan, 1979; in press) indicated 26 percent of the variance in the Cu concentrations of Scrobicularia was explained by the equation

$$\text{Log Cu - Scrobicularia} = (0.47 \log \text{HCl-Ag}) - (0.72\ \text{TOC}) - (0.12) \qquad (1)$$

where HCl-Ag is HCl soluble Ag and TOC is total organic carbon.

Uptake of Cu was apparently weakly enhanced where Ag concentrations in sediments were high and inhibited at high concentrations of organic carbon. The cross-correlated variables oxalate-Fe and total organic carbon (Luoma and Bryan, in press b) were interchangeable in the equation.

Inspection of the data in Table 10-1 and Fig. 10-1 indicated an anomalous mid-estuarine enhancement of the availability of Cu to Scrobicularia contributed to the poor correlation between the animals and the sedimentary variables. Populations of Scrobicularia with unexpectedly high concentrations of Cu were found in 6 (and possibly 7) estuaries sampled from head to mouth (Table 10-1, 2). In each estuary the Cu concentrations in bivalves from the station of enhanced availability fell outside the declining head to mouth gradient observed at other stations. The enhanced Cu levels in Scrobicularia did not coincide with any visible local sources of Cu or with elevated concentrations of Cu in either sediments, polychaetes, seaweed or interstital water. Differences in the extractability of Cu from sediments were not evident at stations where availability was enhanced; nor were any unusual differences in particle size or concentrations of organic carbon, carbonates, humic substances, or extractable Fe and Mn. An unexplained enhancement of Cu availability to Scrobicularia was also observed in an earlier study of the Looe estuary (Bryan and Hummerstone, 1977) and has been observed in the Erme estuary (Bryan unpublished data).

The magnitude of the mid-estuarine enhancements in Cu concentrations of Scrobicularia differed among estuaries (Table 10-2). The differences in magnitude were not related to Cu concentrations in the sediments. In general the largest enhancement was observed in estuaries with low concentrations of amorphous Fe and total organic carbon in sediments (consistent with the indication in equation 1 that one or both of these variables affect Cu availability to Scrobicularia). A negligible enhancement of Cu availability was observed in the Tamar an estuary with very high sedimentary concentrations of amorphous Fe; and the largest enhancement occurred in the Looe, where Fe concentrations were lowest. While differences in Fe concentrations did appear to explain the differing degrees of enhancement among estuaries, such differences were not related to the occurrence of the enhancement within any estuary.

In general the anomalous Cu peaks in Scrobicularia occurred within each estuary in an area of sediment deposit, i.e. in a small bay or on a mudflat in the protected lee of a point of land. The sediments in these areas were generally of a high water content and noticably more anaerobic than surrounding stations. The correlation of enhanced Cu availability to Scrobicularia with poorly drained, anaerobic sediments was tested in two mudflats in the Looe estuary.

In the east Looe Scrobicularia were collected from the soft anoxic sediments in the middle of the small bay where enhancement had been observed in previous collections. A second station was sampled approximately 50 meters directly offshore from the bay station, on the edge of the river channel in slightly sandier and obviously more well-drained sediments. For comparison, samples were also collected high on the shore and low on the shore in the Torridge estuary. The effect of shore height on Cu concentrations in Scrobicularia from the Torridge was similar to that reported by Bryan and Hummerstone for the Tamar (Table 10-3). At the East Looe Station, however, collections 50 m apart from sediments of different water content (and apparently different degrees of anoxia), resulted in a dramatic difference in the Cu concentration of the bivalves (Table 10-3).

Further evidence of the association of enhanced Cu availability with undefined aspects of anoxia was obtained in the West Looe estuary. The color of the shell of Scrobicularia often varies among and within mudflats. Animals from mudflats where subsurface sediments are more anoxic tend to have blacker shells. After a week in oxidized seawater the black portions of the shells turn orange, suggesting the blackness of the shell is a result of the adsorption of Fe sulfide on to the shell surface. Scrobicularia collected from the West Looe station where enhanced Cu availability had previously been observed were divided into 3 groups based upon the color of their shells. The concentrations of Cu in the soft tissues of the individuals with the blackest shells was dramatically higher than concentrations in individuals collected from less anoxic microhabitats within the same mudflat (Table 10-4). Animals from all 3 groups were of similar size, and the differences in Cu concentrations among groups was much greater than the variability normally observed among *Scrobicularia* from a single mudflat.

Consistent with these findings, Luoma and Cain (in press) observed a dramatic increase in the Cu concentrations of the bivalve *Macoma balthica* in San Francisco Bay when a mudflat in the bay became anoxic due to a macro algal bloom (Table 10-5). During the bloom, *Macoma* came to the surface of the sediment probably to avoid the anoxic conditions, and Cu concentrations were 3-4 times those observed at the same time of year under less anoxic conditions on that mudflat.

Not all anoxic conditions enhance the availability of Cu to *Scrobicularia*. For instance, the Tamar estuary often has highly anaerobic sediments, but Cu concentrations in *Scrobicularia* from the Tamar are always low (especially relative to the Cu-contaminated sediments). Moreover, no area of substantially enhanced Cu availability to *Scrobicularia* has been found in the Tamar. Thus some combination of undefined variables (including some influence of Fe or TOC) accompanying anoxia appears to be mobilize Cu to a form of especially high biological availability. The significance of these observations is that a very small anthropogenic Cu input into areas of enhanced Cu availability (e.g. the middle reaches of the Looe estuary) may have much greater biological effects than a much larger Cu discharge to an estuary such as the Tamar where Cu availability to *Scrobicularia* is generally low.

A physiological association of Cu with Ag in the tissues of *Scrobicularia* also appeared to affect Cu burdens in the bivalve. Concentrations of Cu in *Scrobicularia* correlated significantly ($p<0.01$) with concentrations of both metals (for Pb $r=0.64$; for Ag $r=0.47$). Uptake of both Pb and Cu by *Scrobicularia* is inhibited by Fe in sediments (Luoma and Bryan, 1978), although the effect on Pb uptake is much more obvious than the effect on Cu. Similarities in the physicochemical controls in the bioavailability of Cu and Pb may contribute to the correlation of these two metals in *Scrobicularia*. However, the possibility of a physiological association between Pb and Cu is also indicated by the occurrence of "granules" containing both metals within the tissues of some invertebrates (Brown, 1977).

No similarities in the physicochemical control of Cu and Ag uptake are detectable. In fact, uptake of Ag by *Scrobicularia* is stimulated at high concentrations of organic carbon (opposite to Cu) and inhibited by Cu in sediments (Luoma and Bryan, in prep.). Thus a physiological coupling of Cu and Ag uptake seemed the best explanation of the correlation between the two metals. Such a coupling was consistent with the occurrence of HCl-Ag in sediments as a significant variable in equation 1 since Ag uptake by *Scrobicularia* is a strong function of HCl-Ag in sediments (Luoma and Bryan, in prep.). Moreover, the estuaries where the enhancement of Cu uptake by anoxia was greatest also were estuaries with high concentrations of biologically available Ag (Table 10-2).

To statistically test the hypothesized coupling of Cu and Ag in *Scrobicularia* Ag-*Scrobicularia* was included as an independent variable in multiple regression calculations for Cu-*Scrobicularia*. In this calculation 38 percent of the variance in Cu concentrations of *Scrobicularia* was explained by the equation

$$\log \text{Cu-}\textit{Scrobicularia} = (0.17 \log \text{Ag-}\textit{Scrobicularia} + (0.0012\ \text{Cu-Ammonia}) \\ (0.38 \log \text{TOC}) + 1.83 \qquad (2)$$

Ag-*Scrobicularia* explained 21 percent of the total variance, ammonia-Cu 11 percent and TOC 5.5 percent. Ammonia-Cu was only a slightly better choice than was HCl-Cu or total Cu (all three are highly inter-correlated) as the extractant for Cu concentrations in sediments. The stations where anoxia enhanced Cu availability the most substantially (one station in each of the East Looe, West Looe and Gannel) did not fit the regression (as expected) and were not included in the calculations.

Inspection of the relationship between Cu and Ag in *Scrobicularia* (Fig. 10-2) indicated that the stations with low concentrations of Cu relative to Ag were all stations with low ratios of Cu/Fe in sediment (i.e. low Cu availability) and/or very high Ag concentrations in *Scrobicularia*. If the presence of Ag in bivalve tissues stimulates the uptake of Cu stoichimetrically, one would expect a reduced Ag /Cu ratio at stations where the environment of *Scrobicularia* provides insufficient Cu to fill the "demand" created by Ag (i.e. where Ag levels are very high and/or Cu availability is very low). The interaction of Ag demand in tissues and Cu availability from sediments might be expressed quantitatively by the equation

B-Cu = (Ag-*Scrobicularia*) x (Fe-oxalate/Cu-ammonia) (3)

where Fe ox/Cu-amm = 1/(Cu-am/Fe-ox). When B-Cu exceeds some value, Cu concentrations in *Scrobicularia* will not keep up with the demand created by the presence of Ag and negative residuals in the Cu-Ag regression will result. Below some value of B-Cu, Cu and Ag should correlate strongly in *Scrobicularia*.

This hypothesis was tested using the statistical filtering technique of Luoma and Bryan (in press b.). Multiple regression of Cu-*Scrobicularia* vs sedimentary variables in Ag-*Scrobicularia* were calculated using data with values of B-Cu below a progressively lower threshold. Table 10-6 indicates that progressively larger F-values and higher values of R^2 were observed as higher values of B-Cu were dropped from the regression calculation. Among the 19 stations where B-Cu<125 the regression explained 76.3 percent of the variance in the Cu concentrations of *Scrobicularia*, with Ag-*Scrobicularia* and Cu sediment being the dominant variables.

A similar multiple regression calculation was conducted for the stations where S-Cu>125 to test the reverse of the hypothesis; i.e. at high B-Cu, the concentrations of Cu in *Scrobicularia* are controlled by the availability of Cu. As hypothesized Ag-*Scrobicularia* was not a significant variable in this calculation, Cu levels in *Scrobicularia* being a positive function of Cu levels in sediments and a negative function of amorphous Fe and concentrations of organic materials.

The suggestion of a physiological coupling between Cu and Ag in *Scrobicularia* tissues also helps explain why Cu concentrations in *Scrobicularia* correlate so poorly with Cu concentrations in sediments in simple regressions. Uptake of Ag by *Scrobicularia* is inhibited by Cu in sediments (Luoma and Bryan, in prep.). Thus, where Cu concentrations in sediments are lowest, Ag concentrations in *Scrobicularia* are elevated, resulting in an enhancement of Cu uptake at low sedimentary Cu, and a skewing of the relationship between Cu in sediments and Cu in the bivalve.

Fucus. Concentrations of Cu in Fucus correlated almost perfectly with total-Cu in sediments (Fig 10-3). This correlation was not the result of contamination with particulates since correlations with other metals are much weaker than with Cu (Table 10-7). Concentrations of Cu in Fucus follow concentrations of Cu in solution within estuaries (Bryan and Hummerstone); and Fucus has commonly been used as an indicator of biologically available solute Cu. The data in Fig. suggest, however, that either (1) solute concentrations of Cu in estuaries are generally controlled by total-Cu in sediments or (2) the differences in Cu burdens of Fucus from different estuaries reflect Cu stripped from sedimentary particulates (i.e. a large portion of the Cu burdens in Fucus is derived from particulate bound Cu).

Suggestion (1) seems unlikely. Luoma and Jenne (1977) showed that sediment-water distribution vary widely among different forms of sediment-bound metals. Luoma and Bryan (in press b.) showed that Cu was partitioned among several forms in the estuaries of southwest England. Thus, if sediments do control solute concentrations of Cu in these estuaries, those controls should not be a function of total-Cu in sediments.

At low tide Fucus lies directly on the sediment surface, contacting bottom sediments and particulates which adhere to the plant. If the strength of Cu complexation to algal proteins exceeds the strength of Cu bonding to all other substrates in the sediments then Cu would be stripped from all types of bonding sites in sediments and a correlation of Total-Cu with Fucus-Cu might be expected.

The correlation of sedimentary concentrations of other metals with concentrations in Fucus are weaker than with Cu (Table 10-7). However, the strength of Cu complexation by algal polyphenols (Ragan, et al 1979) greatly exceeds that of other metals. Furthermore, the strength of the correlation between sediments and *Fucus* for the different metals follows the same order as the strength of complexation by the algal proteins (Table 10-7), suggesting that the stronger a metal is complexed by the algae the larger the proportion of the body burden of that metal that is stripped from sediments.

Nereis

Concentrations of Cu in *Nereis*, like those in *Fucus* were strongly correlated with total Cu concentrations in sediment (r=0.85; R^2= 0.72). Extractable forms of Cu showed similar or weaker correlations (HCl-Cu, r=0.86; oxalate-Cu, r=0.59; acetic acid-Cu r=0.75; ammonia-Cu, r=0.79). An additional 15 percent of the variance (R^2=0.87) in *Nereis*-Cu was explained by the multiple regression equation

$$\log \text{Nereis-Cu} = (0.81 \log \text{total-Cu}) + (1.00 \log \text{total-Mn}) - (0.30 \log \text{HCl-Pb}) - 2.07. \qquad (4)$$

A small enhancement of Cu availability was apparent where total Mn concentrations in sediments were high (i.e. where Cu was partitioned to Mn oxides or Mn carbonates) and some inhibition of Cu uptake by Pb in sediments was suggested.

DISCUSSION

Statistical correlations over a wide data range indicate very different factors control concentrations of Cu in the deposit feeders Nereis diversicolor and Scrobicularia plana. Concentrations of Nereis generally follow total Cu in sediments, with some minor influence from the form of Cu and the presence of a competing metal (Pb). At least three factors interact to produce Cu burdens observed in Scrobicularia: (1) An enhancement of Cu availability by a combination of anoxia, low concentrations of Fe and other undefined variables. While this enhancement was most evident at several stations within estuaries, it may also have affected differences observed among stations in different estuaries. (2) an apparent physiological coupling of Cu and Ag uptake. The presence of Ag in Scrobicularia tissues enhanced the uptake of Cu to the extent that Cu was available in the environment. (3) the concentration and form of Cu in sediments. The extraction data weakly indicated that forms of Cu associated with humic substances (extracted by ammonia) may be more available than other Cu forms in sediments. The association of Cu with Fe oxides and/or bulk organic material appeared to reduce Cu availability. The availability of Cu controlled Cu concentrations Scrobicularia only where there was insufficient Cu available to meet the physiological "demand" created by Ag. Where sufficient Cu was available, Ag levels in the bivalve controlled Cu concentrations. Little Cu uptake occurred beyond that related to Ag. Even in estuaries such as the Fal, where Cu was 0.3 percent of the sediment weight, the positive residuals in the relationship of Cu-Scrobicularia vs Ag-Scrobicularia were small, suggesting the quantity of Cu taken-up without an Ag association was small. Preliminary laboratory experiments have demonstrated a similar dependence of Cu uptake on Ag tissue burdens in the bivalve Macoma balthica (Cain and Luoma, unpublished data). Neither study has indicated the physiological basis of the Cu-Ag association.

The strong association of Cu burdens in Fucus with total-Cu in sediments prevented using Fucus as in indicator of the contribution of solute Cu to burdens in Scrobicularia. A large proportion of the variance in Scrobicularia-Cu was unexplained by our analysis. Bryan and Uysal, (1978) suggested from tissue distributions that solute sources might contribute significantly to Cu uptake by the bivalve.

The correlation of sediment-Cu and Fucus-Cu also raised questions about the proper interpretation of metal concentrations in algal tissues, and the use of benthic algae as indicators of solute concentrations of metals in systems with widely differing concentrations of sediment-bound metals. If further investigation indicates sediments do contribute a significant proportion of Cu, Pb, Ag and Fe to body burdens in benthic algae, then residuals of correlation between sediments and algae might be better indicators of differences in biologically available solute concentrations of metals than are absolute concentrations of these metals in algal tissues.

Table 10-1 Concentrations of Cu in *Scrobicularia*, *Nereis*, *Fucus* and sediments from 19 estuaries in south and west England. Precise locations of stations are in Luoma and Bryan (1978).

Estuary	Station	Cu		Fucus	Sediment (Tota
		Scrobic.	Nereis		
Plym	head	26	32	37	67
	mouth	116	33	NC	81
Erme	1	95	30	10	30
	2	98	39	7	17
West Looe	head	128	29	27	64
	mouth	342	55	20	43
East Looe	head	150	NC	9	30
	2	88	16	11	37
	3	372	38	13	36
	mouth	75	44	12	37
Fal	1	92	832	804	3052
	2	98	932	1612	2814
Poole Harbor		29	13	NC	98
Beaulieu	head	31	18	10	24
Hamble		47	17	20	46
Itchen		148	18	19	131
Test		17	12	7	42
Gannel	head	79	67	NC	176
	2	62	86	NC	109
	mouth	230	257	33	230
Camel		27	32	27	88
Hayle		50	947	50	1170
Fowey	Head	NC	104	NC	130
	2	38	96	38	142
	3	46	78	33	120
	4	30	39	23	122
	5	23	31	21	116
	mouth	21	44	17	120
Torridge		11	20	11	26
Bristol Channel	1	23	61	23	29
	2	13	77	13	42
Tamar	Head	141	236	141	410
	2	93	192	93	354
	3	61	168	61	408
	4	28	157	51	361
	mouth	26	133	51	282
Tavy		55	204	77	314

Table 10-2 Concentrations of Cu and Ag in *Scrobicularia* and concentrations of Cu and Fe in sediments at stations in 7 estuaries where the availability of Cu was enhanced compared to the same variables at stations landward and seaward of enhancement.

Estuary	Landward of enhancement (μg/g)				Enhancement of Availability (μg/g)				Seaward of peak (μg/g)			
	Cu-*Scrobic.*	Cu-Sediment	Fe-Oxalate	Ag-*Scrobic.*	Cu-*Scrobic.*	Cu-Sediment	Fe-Oxalate	Ag-*Scrobic.*	Cu-*Scrobic.*	Cu-Sediment	Fe-Oxalate	Ag-*Scrobic.*
East Looe	88	37	2376	65.5	267	36	2188	29.2	44	37	2030	1.2
West Looe	101	64	3638	3.9	274	43	2450	5.8				
Gannel	86	109	4360	1.9	168	107	4273	0.9				
Fowey	25	120	2499	0.3	72	122	2732	0.5	28	116	2436	0.3
Elorn	21	61	8336	0.7	59	54	10,369	1.3	30	64	7802	0.9
Tamar[a]	48	410	10,255	0.3	67	354	10,359	0.2	44	408	11,995	0.40
Itchen												

[a] It is questionable whether this "peak" is significant.

Table 10-3 Variation in copper concentrations of *Scrobicularia plana* within two mudflats. Numbers in parentheses represent number of individuals analyzed.

	East Looe Estuary		Torridge Estuary	
	Sediment conditions	[Cu] *S. plana* (μg/g dry wt)	Sediment conditions	[Cu] *S. plana*
Adjacent to channel	well drained; *weak* reducing conditions in subsurface sediments	92±35 (17)	well drained; *weak* reducing conditions in subsurface	24±0.1 (8)
Upper Flat	high water content; *strong* reducing conditions in subsurface sediments.	235 (6)	high water content; *weak* reducing conditions in subsurface	34±7 (13)

Table 10-4 Variations in the copper concentrations of S. plana of different shell color from mudflat in the West Looe estuary with an apparent enhancement of Cu availability. Numbers in parentheses are number of individuals analyzed.

Shell color	Concentration of Cu in soft tissues (μg/g)	Frequency of ripe gonads
White with Black	127 (5)	5/5
Black with White	225 (13)	12/13
Black	602 (5)	0/5

Table 10-5 The concentration of Cu in *Macoma balthica* from a San Francisco Bay mudflat subjected to a period of severe anaerobiosis.

Date	Sediment condition	Concentration of Cu in *Macoma balthica* (µg/g)
Jun. 25, 1975	normal	78±20 (8)
Aug. 11, 1975	*Polysiphonia* bloom. Severe anoxia	436±142 (24)
Oct. 31, 1975	Sandier bar with surviving population of clams (rest of mudflat anoxic)	378±124 (14)
Aug. 25, 1976	Sandier bar one year after bloom	141±86 (17)

Table 10-6 Correlation of log Cu-*Scrobicularia* with sedimentary variables by stepwise multiple regression within data sets from which stations with values of B-Cu were excluded as indicated.

B-Cu=(Ag-*Scrobicularia*) x (Cu-ammonia/Fe-oxalate) Eq. 2

a and b are regression coefficients.

B-Cu	Variable	a	b	t statistic per variable	f statistic for regression	R^2 for regression	n
all data*	log Ag-Scrobicularia	1.83	0.17	3.3			
	Cu-ammonia		0.0012	2.4			
	log TOC		-0.38	-1.6	6.0	0.3	33
<600	log Ag-*Scrobicularia*		0.60	4.7			
	log Cu-ammonia	1.59	0.30	3.5	8.8	0.54	28
<200	log Ag-Scrobicularia						
	log Cu-ammonia	1.54	0.63	4.3			
			0.35	3.8	9.9	0.62	23
<125	log Ag-Scrobicularia	1.94	1.32	6.0			
	log Cu-ammonia		0.0031	4.0			
	Fe-oxalate		-0.033	-2.5	11.1	0.77	19
>125	log Fe-lxalate	2.45	-0.74	2.4			
	Cu-ammonia		1.81	1.8			
	TOC		-0.092	-1.8	5.0	0.54	17

*Stations with exceptional enhancement of Cu availability (East Looe, West Looe and Gannel) not included in these calculations.

TABLE 10-7 A COMPARISON OF THE CORRELATION BETWEEN METAL CONCENTRATIONS IN *FUCUS* AND TOTAL METAL IN SEDIMENTS, WITH THE SELECTIVITY COEFFICIENT (REGAN, ET AL, 1979) DESCRIBING THE STRENGTH OF METAL COMPLEXATION WITH POLYPHENOLS FROM *FUCUS*.

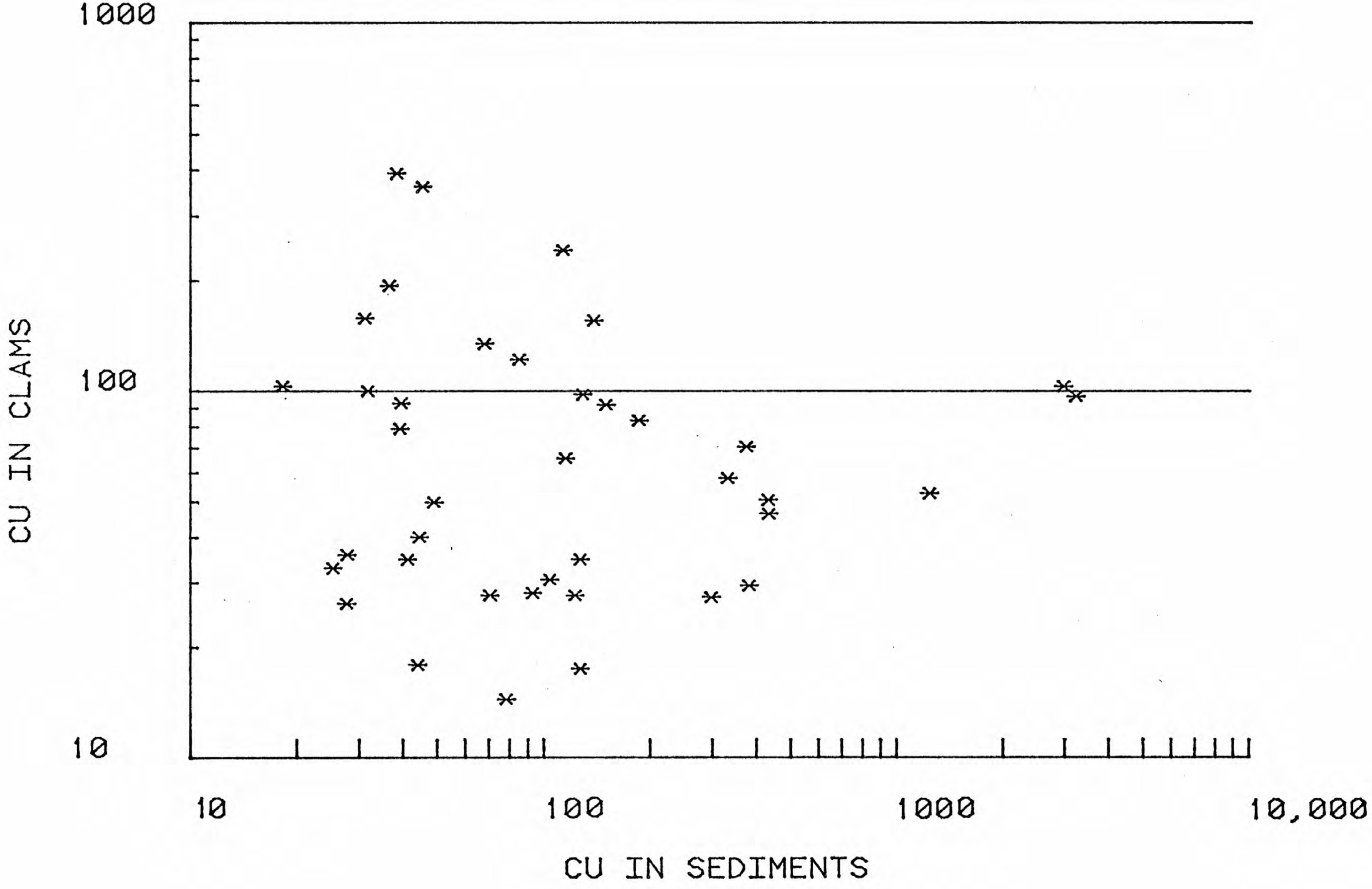
METAL	CORRELATION WITH TOTAL METAL IN SEDIMENT		SELECTIVITY COEFFICIENT FOR CHELATION WITH *FUCUS* POLYPHENOLS[a]
	R	P	
CU	0.98*	$P<0.001$	106
PB	0.67*	$P<0.001$	81.3
ZN	0.42*	$P<0.01$	5.5
AG	0.30	$P<0.10$	N.M.
FE	0.26	$P>0.10$	N.M.
CO	0.10	$P>0.10$	3.6
CD	0.004	$P>0.10$	2.7
MN	0.001	$P>0.10$	1.0

a=RAGAN, ET AL (1979)

NM=NOT MEASURED

List of Figures

Fig. 10-1



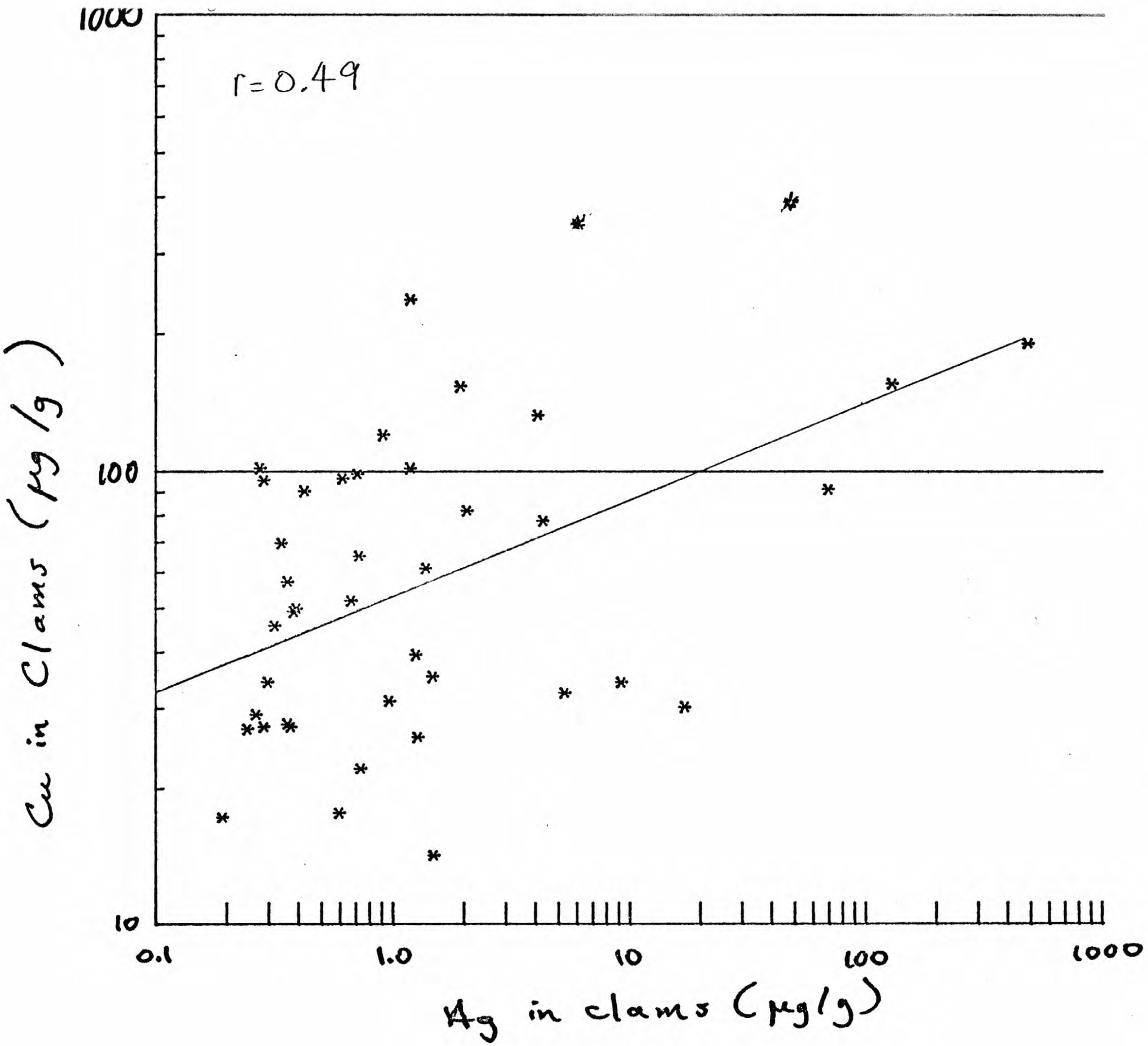
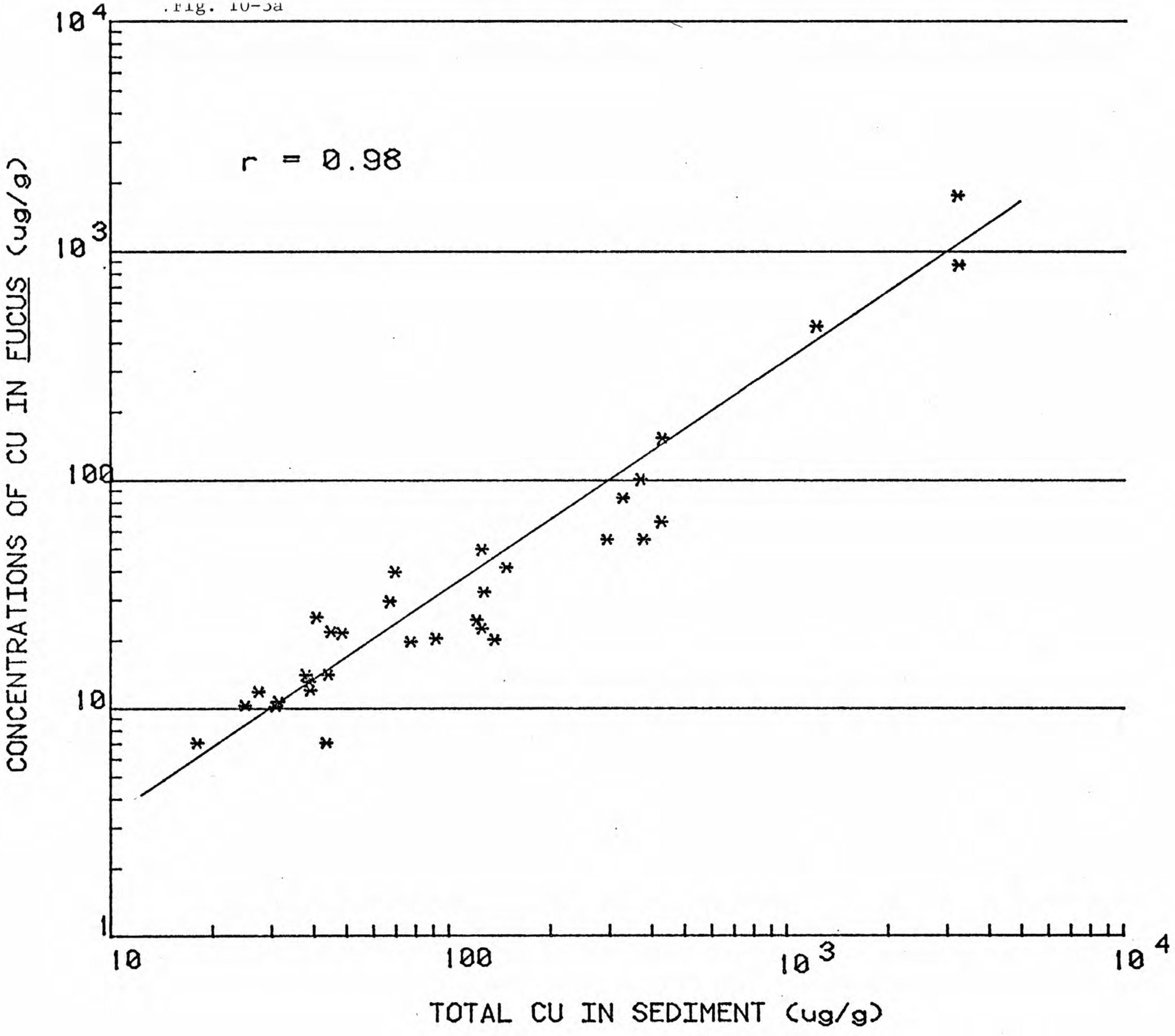

r=0.49
1000
100
10
0.1
1.0
10
100
1000
Cu in Clams (µg/g)
Ag in clams (µg/g)

Fig. 10-3a



10^4
10^3
100
10
1
r = 0.98
CONCENTRATIONS OF CU IN FUCUS (ug/g)
10
100
10^3
10^4
TOTAL CU IN SEDIMENT (ug/g)

Fig. 10-3b

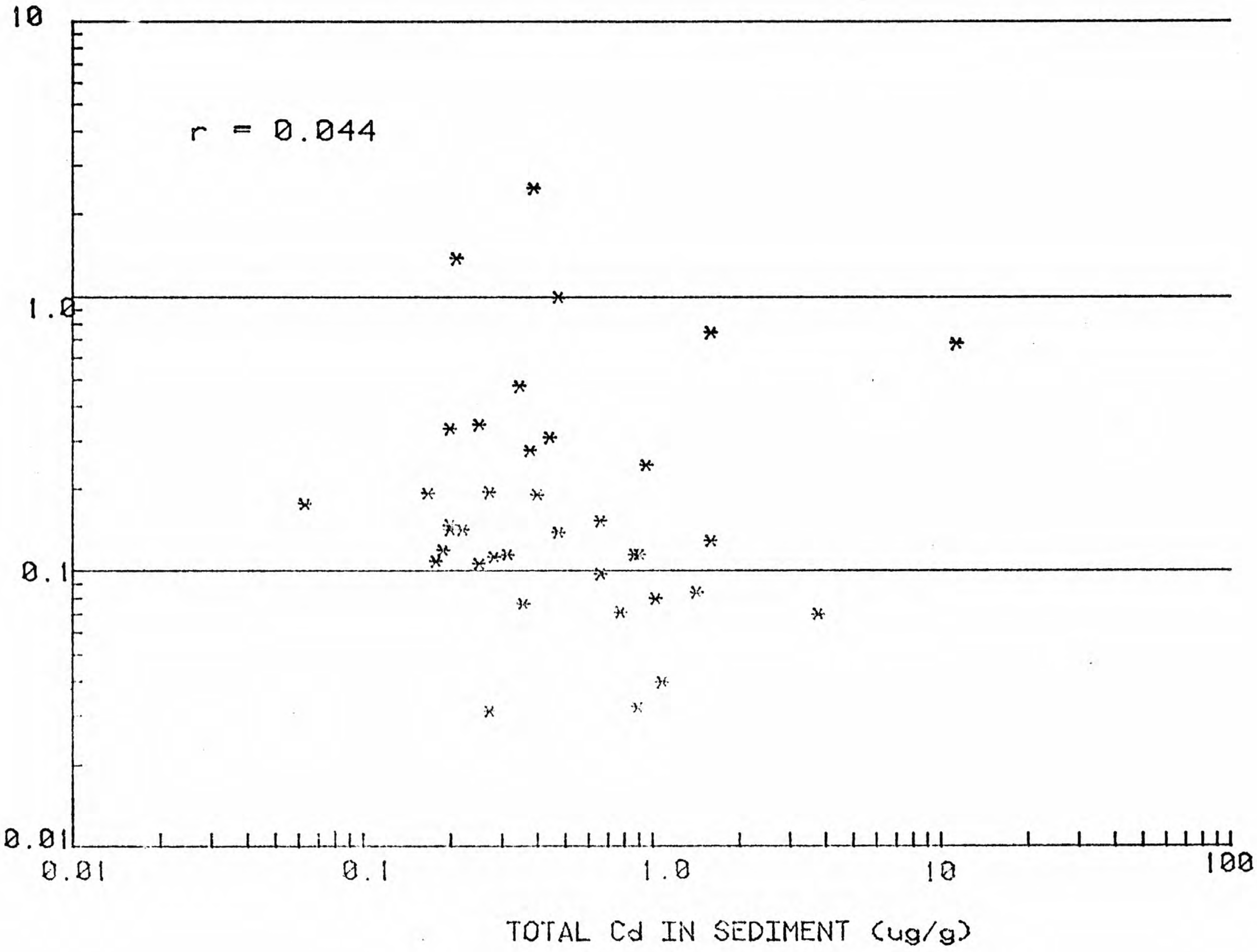

r = 0.044
CONCENTRATION OF Cd IN FUCUS (ug/g)
10
1.0
0.1
0.01
0.01
0.1
1.0
10
100
TOTAL Cd IN SEDIMENT (ug/g)

Fig. 10-3c

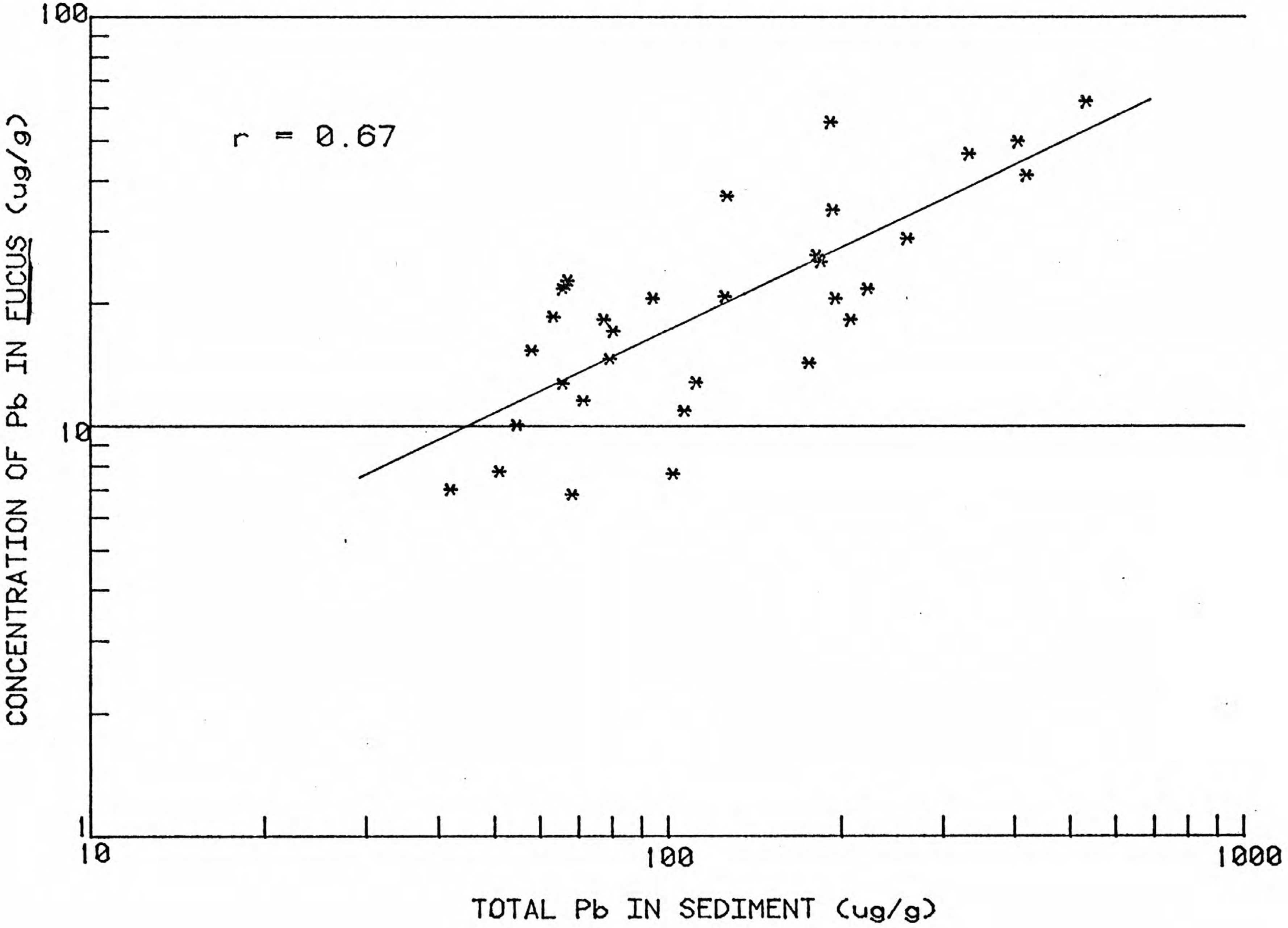
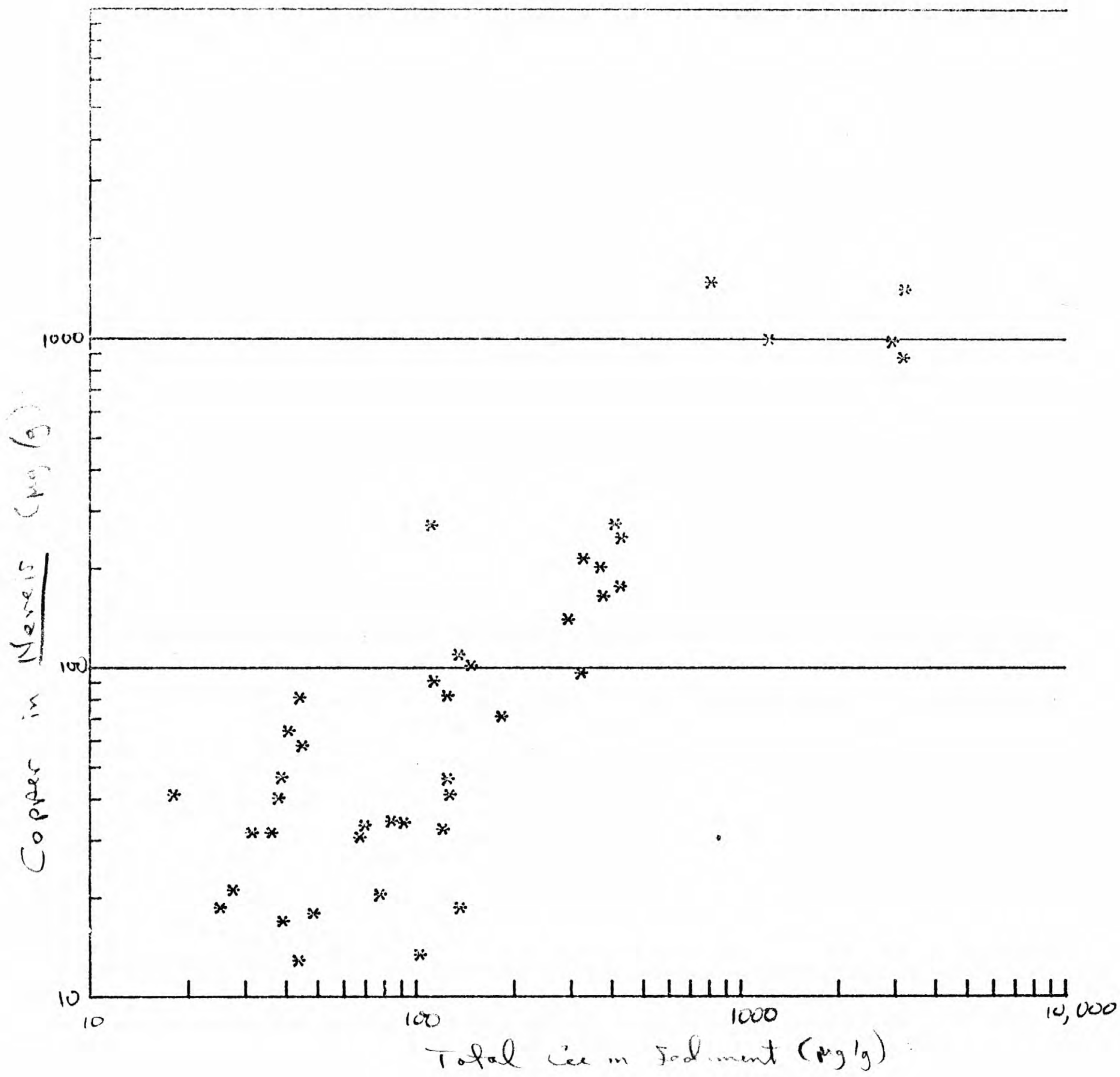

100
10
1
r = 0.67
CONCENTRATION OF Pb IN FUCUS (ug/g)
10
100
1000
TOTAL Pb IN SEDIMENT (ug/g)

Fig. 10-4


1000
100
10
10
100
1000
10,000
Copper in Nereis (µg/g)
Total Cu in Sediment (µg/g)

Chapter 11 Concentrations of Metals in Sediments and Shellfish in San Francisco Bay

W.L. Bradford and S.N. Luoma

INTRODUCTION

The purpose of this chapter is to review the data available in the literature on heavy-metal concentrations in sediment and shellfish in San Francisco Bay and to introduce our results within the perspective of work done in the past on the Bay. A portion of this chapter was written with Dr. Wesley Bradford of the U.S.G.S.

Area Description

San Francisco Bay (fig. 11-1), consists of two arms. The north arm is the drowned mouth of the Sacramento and San Joaquin Rivers that drain the Central Valley of California. The south arm is a tectonically built estuary that was created and is maintained, in part, by local land subsidence (Pritchard, 1967).

The major freshwater inflow is into the North Bay from the Sacramento and San Joaquin Rivers. Freshwater inflows into the south bay are from wastewater treatment plants and, taken together, are very small compared to the tidal prism. During the winter rainy season, high flows commonly create a salinity stratified estuary throughout the northern arm. Occasional floods push lenses of freshwater into the South Bay, causing rapid renewal of water. During the dry season, salinity stratification commonly occurs only in the neighborhood of Carquinez Strait. The south bay becomes well mixed and the water renewal rate is very slow (Bradford and Iwatsubo, 1979).

The patterns of flow out of the Sacramento-San Joaquin delta are so complex that an accurate direct measurement of discharge has never been made. But discharge is known to exceed 2800 m^3/s regularly during high flows and to be as low as 140 m^3/s during low flows.

The Sacramento River is also the major source of suspended sediments entering the bay, with daily transport ranging from 281 to 24,200 tonnes passing Sacramento. Annual loads passing Sacramento since 1956 have ranged from 0.201×10^6 tonnes in 1977 to 5.11×10^6 tonnes in 1965. Several sources account for some loading, mostly during the winter spring rainy season. Conomos and Peterson (1977) estimated a mean annual sediment input into the bay of about 4.2×10^6 tonnes resulting in a uniform deposition rate of about 35 mg/cm^2 considering all sources of suspended solids.

San Francisco Bay is the center of one of the world's major metropolitan areas. For much of the last 100 years, domestic and industrial wastes and drainage from the urban areas flowed untreated into the bay.

Since World War II--and especially in the past 10 years--with the passage of State and Federal water quality legislation, efforts to upgrade treatment of domestic and industrial discharges have reduced raw waste loading considerably. Storm runoff has been partly controlled by retention basins and combined sewer systems. Controls on the sources of heavy metals to sewage systems are being exercised and are gaining in effectiveness (Teng Chung Wu, Regional Water Quality Control Board, San Francisco Bay Region, oral commun., 1979).

Large shallows and marshes dominate portions of north and south bays. These shoals currently support large populations of the snail, *Nassarius obsoletus*, the eastern softshell clam *Mya arenaria*, the Japanese little-necked clam *Tapes japonica*, and the two smaller bivalves *Macoma balthica* and *Macoma nasuta*. The olympic oyster *Ostrea lurida* is present in small numbers. The mussel *Mytilus edulis*, which is of commercial importance and a popular shellfish in Europe, is abundant in the bay, as is the mussel *Ischadium demissus* (Nichols, 1973; Girvin et al., 1975). Some of these species may be marketable, and most are generally numerous enough to be considered at least a sport fishery (W. Dahlstrom, California Fish and Game, oral commun. 1979). The eastern oyster, *Crassostrea virginica* was once grown comercially from juveniles to marketable size in the Bay, but this fishery collapsed in the early 1900's due to undefined water quality problems.

In this chapter we report concentrations of heavy metals observed in the common mulluscs from San Francisco Bay to provide background for our studies of Cu, Zn and Ag availability to *Macoma balthica* in the Bay.

HEAVY-METAL CONCENTRATIONS

Shellfish

From the standpoint of the public health, there are few reference points against which to judge heavy-metal concentrations in shellfish. The U.S. Food and Drug Administration (FDA) has set a limit on the concentration of only Hg at 0.5 mg/kg wet weight of meat. At the 7th National Shellfish Sanitation Workshop sponsored by the FDA in 1971, the Task Force on chemistry summarized the data on 6 heavy metals in *Mya arenaria* on the east coast (Anon., 1973) and set alert limits (table 11-1) for wet weight concentrations based on the summary data. The alert limits do not imply imminent danger to the public health, but are an attempt to set concentrations above which further investigation and, perhaps, consideration from the public health viewpoint may be warranted.

Girvin et al. (1975) analyzed *M. arenaria* collected at several locations around San Francisco Bay where commercial or sport harvesting is possible. The Zn concentration at Tara Hills, and the Cd and Hg concentrations at Tara Hills, and the Hg concentrations at Foster City are equal to the alert limits. Elsewhere, heavy-metal concentrations were well below the alert limits (fig. 11-2).

One may also assess the effects of anthropogenic heavy-metal discharges by comparing metal concentrations in shellfish from San Francisco Bay with concentrations in the same species living in more pristine environments. Tomales Bay, a small embayment a few kilometers north of San Francisco, receives only small and insignificant discharges of wastewaters or urban runoff. Mean concentrations of metals in the soft tissues of mussels (*Mytilus edulis*) from Tomales Bay compared with the mean of metal concentrations observed in *M. edulis* from 28 stations in San Francisco Bay (table 11-2) indicate significant differences for Ag, As, Fe, Hg, Mn, Ni, Pb and Zn ($p<0.05$; 2-tailed Student-t test, Sokal and Rohlf, 1969). For all metals but Ag, mean concentrations were higher in San Francisco Bay. Differences in Ag concentrations may be due to differences in analytical techniques and should not be considered meaningful.

Metal concentrations in organisms from different stations in an estuary as complex as San Francisco Bay are not, in reality, a single population of values. Comparisons of metal concentrations in several shellfish species from within and outside the bay provide a better indication of spatial variability and sites of anthropogenic metal discharge (fig. 11-2). Concentrations of Pb and Zn are higher in mussels from Albany Hill, Bayview Park, Coyote Point, and Redwood Creek than in mussels from Tomales Bay. Redwood Creek mussels also appeared to be very contaminated with Cd, Cu, and Hg when compared to Tomales Bay mussels.

In the southernmost reach of the south bay near the Palo Alto Yacht Harbor, high concentrations of Ag (23-28 mg/kg) and Cu (36-37 mg/kg) occur in the mussel *Modiolus modiolus* (Luoma, unpublished data). Although data from different species of mussels may not be directly comparable, it is noteworthy that the Ag values from *M. modiolus* are at least 5 times higher than any reported for *M. edulis* by "mussel-watch" (Goldberg et al., 1978) or in Risebrough et al.'s (1977) summary of observations from throughout the world, and the Cu concentrations in *M. modiolus* are about equal to the highest concentrations reported in those surveys.

Concentrations of Ag, Cu and, in some cases, Zn, appear higher in tissues of the clam *Macoma nasuta* taken from San Francisco Bay than those taken from Bodega Bay, another pristine coastal embayment north of San Francisco (table 11-3). Also, Ag, Cu, and Zn concentrations in tissues of the commercially exploitable clam *Tapes japonica* taken from San Francisco Bay are higher than in those taken from Princeton Harbor located on the coast south of San Francisco (table 11-4). In the latter comparison, concentrations are particularly high in samples from Redwood Creek. Girvin et al. (1975) also reported high heavy-metal concentration in the hepatopancreas of the oyster *C. gigas* from Redwood Creek over those from Tomales Bay. Concentrations were higher by 100-fold for Ag, 31-fold for Cu, 22-fold for Zn, 17-fold for Hg, and 7.5-fold for Cd.

Luoma and Cain (1979), found that Ag concentrations in soft tissues of the clam Macoma balthica were very high (up to 170 mg/kg) in samples from near the Palo Alto Yacht Harbor and decreased in samples collected progressively seaward. No Ag source could be distinguished in Redwood Creek or elsewhere. Apparently Ag concentrations in M. balthica collected throughout the south bay were affected by a single source in Palo Alto.

Concentrations of Ag, Cd, and Cu in the common snail Nassarius obsoletus (table 11-5) were also very high in samples from the Palo Alto Yacht Harbor compared to samples from elsewhere in the bay, again suggesting the presence of an important source of these metals at Palo Alto. Zinc concentrations were generally uniform throughout the bay except in the sample near Pinole Point, suggesting a Zn source nearby.

The data on heavy-metal concentrations in San Francisco Bay shellfish illustrate both the complexity and the value of using biota as indicators of contamination. Tissue concentrations obviously vary widely among species found in the same locality. Moreover, data from different species may lead to different conclusions about sites of contamination if interpreted too narrowly. For example, Cu and, in the case of mussels, Ag contamination in the south bay are not always pronounced from analyses of Mya arenaria, Mytilus edulis, or Tapes japonica, whereas data from other species, especially oysters, show contamination clearly.

Comparing data from all species collected in San Francisco Bay against data from their counterparts from pristine coastal embayments nearby suggests: (1) generally higher concentrations of biologically-available Ag, Cd, and Cu are present on the western shoal of the south bay near Palo Alto, and to a certain extent, baywide, (2) the Redwood Creek area is substantially contaminated with Ag, Cd, Cu, Pb, and Zn and (3) sites of Pb contamination are found throughout, especially at Albany Hill, Bayview Park and Coyote Point.

Salinity effects (Risebrough et al., 1977) are an unlikely explanation for most such differences, since salinities throughout all but the northernmost reaches of the bay approach those of the marine waters in the summer and fall. Moreover, Luoma and Cain (1979) observed no relationship between temporal fluctuations of Ag and Cu in M. balthica and fluctuations of salinity in the south bay. Physical-chemical differences (concentrations of solute ligands, particulate organic carbon, humic substances or distributions of sedimentary particle size) between the estuarine environment of the bay and the marine-dominated environments of the coastal embayments used in the comparisons may have contributed to the differences in biotic heavy-metal concentrations.

Since there are no FDA standards on heavy-metal concentrations in shellfish for human consumption, a conclusive assessment of the risk to public health from eating bay oysters and mussels cannot be made. However, heavy-metal concentrations might be of some concern in a few instances particularly in oysters such as C. gigas reared in the south bay near Redwood Creek and Palo Alto. The two bivalves most likely to be exploited, M. arenaria and T. japonica, are not especially efficient at concentrating most metals. Even concentrations in M. arenaria from Redwood Creek exceed the East Coast alert limits only for Cd and Hg, and then only by about 30 percent and 42 percent respectively. By contrast, oysters accumulate heavy metals in their tissues to a much greater extent.

The biological effects of the contamination are difficult to assess. In view of the abundance of T. japonica and M. arenaria (Girvin et al., 1975), it seems unlikely that they are under significant baywide stress. C. gigas planted on stakes or trays grow well except in Redwood Creek where evidence of stress has been seen (W. Dahlstrom, oral commun., 1979). Near the Palo Alto Yacht Harbor periodic die-offs of M. balthica have been observed (Luoma and Cain, 1979; Luoma, unpublished data) coincident with annual maximum concentrations of Ag and Cu. At most other stations where heavy-metal contamination is evident, the ecology of the benthic community is too poorly understood to detect effects if they were occurring.

Sediments

Mean concentrations of 12 heavy metals in sediments from 9 areas of particular interest are listed in table 11-6. The median range of metal concentrations measured elsewhere in the bay are shown in table 11-7. Bay sediments are higher in Ag, As, Cd, Cr, Ni, Pb, Se, and Zn relative to average shale (Krauskopf, 1967) (figs. 11-3 and 11-4)suggest some general anthroprogenic sources. The discrepancies between average shale and bay sediments are especially great for Ag and Cd. It is possible that shale values may not represent local background composition for these metals. Data from the southern California coast (SCCWRP, 1973) suggest background Ag concentrations in sediments are near 1.0 mg/kg. However, analytical difficulties are common with both Ag and Cd, due to matrix interferences and the low concentrations of these metals in sediments.

Viewed from the perspective of other commercial waterways, such as Los Angeles and Baltimore Harbors (fig. 11-5), sediments in San Francisco Bay are generally low in heavy metals. Only Cr and Ni show consistently higher median concentrations, while Mn is higher at a few specific sites.

Concentrations in sediments of at least some heavy metals differ substantially among stations within the bay. Local metal discharge may explain some of these differences. However, many differences may simply reflect differences in the physical characteristics, such as particle size, distributions and the concentrations of sedimentary sinks with high adsorption capacities, such as humic material and Fe oxides.

To determine the extent of physical-chemical influence on metal variations in bay sediments during the summer of 1977, we measured concentrations of Cu, Zn, amorphic Fe, organic carbon, and particle size (determined as percent particles less than 14 mm diameter) on oxidized surface sediments at 13 stations (Fig. 1) (Methods were the same as in Chapter IV). The highest concentrations of the heavy metals (Cu and Zn) were found in areas of sluggish circulation--the southernmost reach of the south bay, Redwood Creek, San Leandro Bay, the northern shoal of San Pablo Bay, and near China Camp. The lowest concentrations of Cu and Zn occurred near Carquinez Strait and on the eastern shoal of the south Bay where wind-generated sediment resuspension is intense.

Among the 13 stations, Cu and Zn concentrations were linearly correlated with percent of particles less than 14 µm (P) ($p < 0.01$ in both cases), with oxalate extractable Fe ($p < 0.02$ in both cases), and with organic carbon concentrations (p 0.01) in both cases). Organic carbon correlated significantly ($p < 0.01$) with particle size, but extractable Fe did not. The correlation between organic carbon (C) and particle size (P) fit an exponential model almost perfectly ($R^2 = 0.97$)

$$C = ae^{bP}. \tag{1}$$

On the other hand, correlations of both Cu and Zn with particle size best fit a linear model

$$M = a + b \cdot P \tag{2}$$

suggesting that particle size, not organic carbon, was the controlling variable in the correlation with metal concentration, M.

Since grain size, correlated with organic carbon, only one can be used in a multiple regression model. In this case, P was chosen. However, oxalate-extractable Fe was not correlated with P and so qualifies as an independent variable.

Multiple linear regression of Zn against P and Fe accounted for 73 percent ($R^2 = 0.73$) of the variance in sediment Zn concentrations. If one anomalous value from San Leandro Bay was excluded, the R^2 value rose to 0.89. The regression equation excluding the one value is

$$Zn = 35.2 + 3.39\ P + 2.15\ Fe. \tag{3}$$

The residuals of the relationship (table 11-8) indicate a point source contribution accounting for about 133 mg/kg Zn to the sediments in San Leandro Bay and about 34 mg/kg Zn to the sediments in North San Pablo Bay. At Carquinez Strait, the Zn concentration is lower than predicted

Elsewhere the model predicts concentrations to within 15 percent.

Variations in Cu concentrations among stations could also be largely explained ($R^2 = 0.895$) by particle size distributions and concentrations of extractable Fe. The multiple regression best fit the equation

$$Cu = 0.11 + 2.17\ P + 1.16\ Fe. \tag{4}$$

The residuals (table 11-8) suggest significant sources in Palo Alto Yacht Harbor, and west Pinole Point, and lack of sources at Carquinez Strait.

CONCLUSIONS

The low-to-moderate heavy-metal concentrations found in San Francisco Bay sediments, compared to those found in other commercial waterways, belie the high concentrations in shellfish at some locations, particularly Redwood Creek and the Palo Alto Yacht Harbor. Although the discharge of Cu into the bay is not large compared to that in many estuaries, the physical-chemical characteristics of some stations appear to enhance the bioavailability of Cu, making local organisms susceptible to contamination. Thus a small discharge of a metal such as Cu, while not greatly affecting concentration in sediments, may be the biological equivalent of a much greater discharge in an estuary with different physicochemical characteristics. Determination of the factors enhancing the susceptibility of bay organisms to contamination by some metals is an especially important research need. Prceeding chapters discussed a statistical approach to this problem employed in a spatially intensive study in south and west England. In the following chapters we present initial results from a temporally intensive study of factors affecting the bioavailability of Cu, Zn and Ag in San Francisco Bay.

Chapter 11 SUMMARY

Concentrations of 11 heavy metals--Ag, As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn--are, generally, higher in the soft tissues of clams, mussels, and oysters from San Francisco Bay than in comparable organisms from pristine coastal embayments nearby. Concentrations of Ag, Cd, Cu, Hg, and Pb in clams and mussels at Redwood Creek and Palo Alto in the south bay are particularly elevated over the pristine-environment organisms. Heavy-metal concentrations in oysters (which are more efficient than clams or mussles at accumulating heavy metals) grown and harvested in the bay south of Foster City may be high enough to be a public health concern. However, except for Hg, there are no U.S. Food and Drug Administration concentration limits on shellfish heavy-metal concentrations.

Concentrations of heavy metals in bay sediments generally exceed the averages for shale, but compared to concentrations in sediments from other commercial waterways--Baltimore and Los Angeles Harbor for example--bay sediments may be judged low to moderate. There is poor correlation between heavy-metal concentrations in sediments and in shellfish.

The variance in Cu and Zn concentrations in oxidized sediments is largely (90 and 89 percent) accounted for by oxalate-extractable Fe and percentage of particles less than 14 μm in diameters. In the South Bay, Zn from wastewater was found to move from solution to suspended solids and then to bottom sediments. The mechanism may involve coprecipitation with Fe oxides around resuspended sediments.

Table 11-1 --Heavy metal concentration alert limits for the eastern soft-shelled clam *Mya arenaria* (mg/kg dry weight). Converted to dry weight basis using wet/dry weight ratio of 5.92 (Girvin et al., 1975)

Cd	Cr	Cu	Hg	Pb	Zn
3.0	29.6	148	1.2	29.6	178

Table 11-2--Mean heavy-metal concentrations (mg/kg dry weight) in *Mytilus edulis* from San Francisco Bay (Risebrough et. al., 1977) and from Tomales Bay (Anderlini et. al., 1975 a,b). Parentheses enclose one standard deviation. Numbers of analyses are 28 and 20 respectively

	Ag	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
Tomales Bay	1.3	5.5	6.4	<1	7.5	73	0.26	6.3	1.2	0.9	5.4	82
	(0.5)	(0.9)	(1.1)		(1.1)	(5)	(0.01)	(1.7)	(0.5)	(0.3)	(0.5)	(11)
San Francisco Bay	0.43	7.1	5.3	--	8.0	287	0.38	29.6	2.2	3.3	4.5	170
	(0.33)	(1.5)	(3.0)		(2.3)	(312)	(0.19)	(30.5)	(1.2)	(4.9)	(2.5)	(58)
	*	*				*	*	*	*	*		*

* Probability of accepting the Null Hypothesis using a 2-tailed Student-t test <.05.

Table 11-3 --Concentrations of Ag, Cu, and Zn in soft tissues of the clam *Macoma nasuta* from three stations in San Francisco Bay, and one station in Bodega Bay. (Numbers of replicates 5-20)

Station	Date	Ag	Cu	Zn
		in mg/kg dry weight		
Bodega Bay	7/76	F0.90	20	200
San Francisco Bay				
Foster City	8/75	5.1	34	270
Foster City	1/77	12	25	80
Bayview Park	11/77	3.8	27	110
Bayview Park	2/77	3.7	44	200
Albany Hill	8/76	7.7	55	250

Table 11-4 --Concentrations of Ag, Cu, and Zn in soft tissues of the clam *Tapes japonica* from San Francisco Bay and Princeton Harbor. (Numbers of replicates 5-20)

Station	Date	Ag	Cu	Zn
		in mg/kg dry weight		
Princeton Harbor	1/77	0.70	5	41
San Francisco Bay				
Bayview Park	1/78	6.7	10	80
Bayview Park	3/76	6.6	11	100
Coyote Point	8/75	2.5	15	92
Foster City	3/76	8.4	8	59
Foster City	7/76	11	14	53
Redwood Creek	1/76	16	29	190
Redwood Creek	7/76	65	33	41

Table 11-5.--Concentrations of Ag, Cu, Zn, and Cd in soft tissues of the snail *Nassarius obsoletus* from San Francisco Bay. Transect samples taken at 0.5 km intervals between Palo Alto Yacht Harbor (T1) and the Palo Alto sewage outfall (T5). (Number of replicates always >20)

Station	Date	Ag	Cd	Cu	Zn
			in mg/kg dry weight		
Palo Alto	7/78	320	12	4130	750
East Dumbarton Bridge	5/78	150	3.6	1990	530
Redwood Creek	7/78	150	7.0	1770	640
Bayview Park	5/78	47	4.4	2690	530
San Leandro Bay	6/78	35	6.6	4390	760
China Camp	6/78	50	6.2	2400	830
East Pinole Point	6/78	49	8.6	3030	1350
North San Pablo Bay	6/78	22	2.6	1960	590
Transect at Palo Alto Yacht Harbor					
T1	4/78	310	12	2570	680
T2	4/78	260	9.3	2450	500
T3	4/78	190	11	2210	630
T4	4/78	210	9.8	2200	510

Table 10-6 --Summary of heavy metal concentrations in sediments from nine areas in San Francisco Bay. Data from Baltimore Harbor, Los Angeles Harbor and shale added for comparison
[Mean $\bar{x}$, standard deviation s and number of observation n. Concentrations in mg/kg except Fe (g/kg) dry weight)

Sites		Ag	As	Cd	Cr	Cu	Fe (percent)	Hg	Mn	Ni	Pb	Se	Zn	References
Mare	$\bar{x}$	2.2	11.9	1.49		70	73	0.38	970	93	38	2.4	149	1,3,5,7,8
Island	s	.5	.8	.80		19	--	.18	---	5	13	1.3	30	
Strait	n	6	6	22		19	3	16	3	6	19	6	15	
Carquinez	$\bar{x}$	2.0	11.6	1.52		71		.32		84	42	1.9	134	1,5,7,8
Strait	s	.6	2.7	.46		23		.18		9	10	.4	23	
	n	7	7	18		17		14		7	17	7	13	
Alcatraz-	$\bar{x}$	1.2	7.9	1.11	201	38	33	.24	439	115	34	1.9	105	2,8
Angel Island	s	.4	1.0	.43	17	11	3	.09	60	23	8	.6	17	
Spoil Area	n	12	12	17	12	17	12	15	12	12	17	12	15	
Oakland	$\bar{x}$		10	1.66	310	98	53	.87	516	189	79	2	203	2,3,7,8
Inner	s		--	.67	---	44	11	.70	108	---	24	-	57	
Harbor	n		2	8	2	11	6	10	6	2	11	2	9	
Oakland	$\bar{x}$	3.1	11	1.00	285	72	43	.62	520	150	61	1.7	177	2,3,5
Outer	s	1.8	1	.67	49	9	5	.14	55	34	17	.3	34	
Harbor	n	4	4	9	4	6	7	7	7	4	7	4	7	
Hunters	$\bar{x}$			1.24		55		.33			46		146	5,7,8
Point	s			.25		11		----			4		---	
	n			7		5		3			5		3	
Foster	$\bar{x}$	0.1		.92		38	36	.30	444		29		109	4,5,6,7,8
City	s	---		.19		9	--	.08	---		4		18	
	n	1		6		7	2	5	2		7		5	
Redwood	$\bar{x}$			.94		43	52	.36	578		38		145	3,5,6,7,8
Creek	s			.41		13	12	.22	188		24		62	
	n			7		8	4	6	4		9		7	
South Bay	x	1.8		.78		50	61	.25	654		29		167	5,6,7
Appendage	s	---		.17		7	6	.29	129		3		38	
	n	1		11		4	4	11	4		4		4	
Baltimore Harbor				1.3	174	167		.7	340	40	158		440	11
Los Angeles Harbor			27.9	7.3	144	255	45	1.43	513	73	214		504	10
Shale		0.1	6.6	0.3	100	57	47	0.4	850	95	20	0.6	80	9

References: 1) Anderlini et. al. (1975a); 2) Anderlini et. al. (1975b); 3) Serne and Mercer (1975); 4) Girvin et. al. (1975); 5) Moyer and Budinger (1974); 6) Bradford (1976)*; 7) McCulloch et. al. (1971); 8) Liu et. al. (1975); 9) Krauskopf (1966); 10) Chen and Wang (1974); 11) Villa and Johnson (1974).

* Bradford's 1976 data obtained by partial extraction are normalized to totals by multiplying by the ratio of total metal to extractible metal from Serne and Mercer (1975). Samples analysed with depth in a core are averaged to obtain one value for the core.

Table 11-7.--Summary of heavy-metal data sites other than those in table 6 .

[in mg/kg except Fe in g/kg]

Element	Median	Range	n
Ag			
As	8.1	7.5-8.4	3
Cd	1.24	0.50-3.31	35
Cr	290	264-464	4
Cu	72	29-130	8
Fe	45	17-56	8
Hg	.32	0.04-1.05	15
Mn	480	226-728	8
Ni	103	63-133	4
Pb	52	9-174	8
Zn	155	73-233	8

References

Serne and Mercer (1975)
Girvin et al. (1975)
Moyer and Budinger (1974)
Bradford (1976)
McCulloch et al. (1971)

Table 11-8--Concentrations of Cu and Zn, (mg/kg dry wt.) observed at 13 stations in San Francisco Bay, as compared to concentrations predicted from multiple regressions of metal concentrations against particle size plus extractable concentrations of Fe (eq 3 and 4). The residuals of the multiple regression are also shown. Data collected in summer 1977. (Station locations shown as dots in fig. 1.)

Station	Zn			Cu		
	Observed	Estimated	Residual	Observed	Estimated	Residual
1. Carquinez Strait	65	95	-30	20	35	-15
2. East Pinole Point	117	109	+8	50	45	+5
3. North San Pablo Bay	176	172	+4	80	83	+3
4. West Pinole Point	140	106	+34	53	43	+10
5. China Camp	138	139	-1	73	64	+9
6. Albany Hill						
7. San Leandro Bay	318	185	+133	100	92	+8
8. Bayview Park				42	51	-9
9. San Leandro Creek	62	64	+2	23	17	+6
10. San Francisco Airport						
11. Foster City	79	90	-11	27	33	-6
12. Redwood Creek	194	194	0	88	99	-11
13. Redwood Creek	182	192	-10	102	99	+3
14. East Dumbarton Bridge	120	115	+5	39	50	-11
15. Palo Alto Yacht Harbor	112	108	+4	57	45	+12

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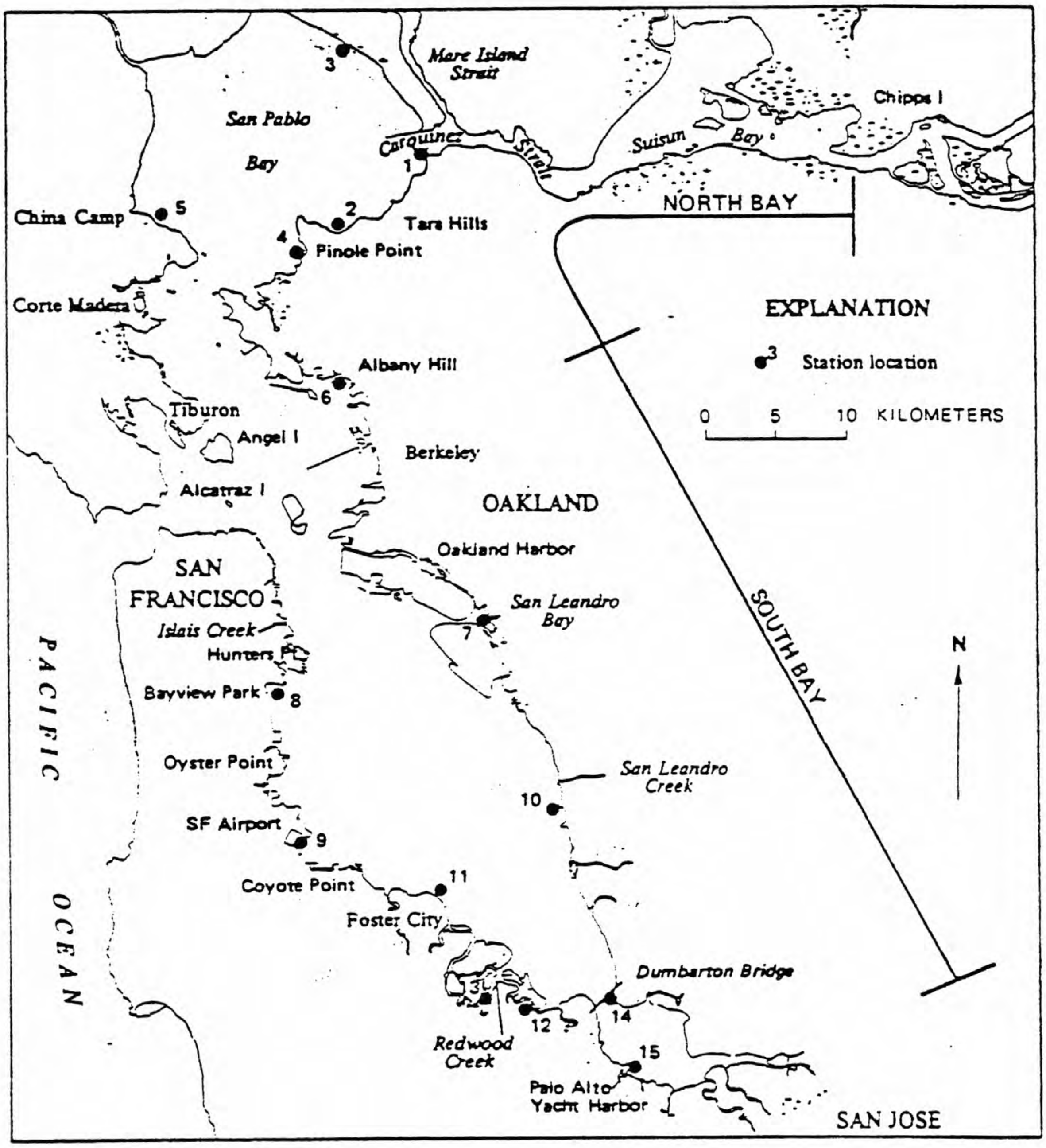
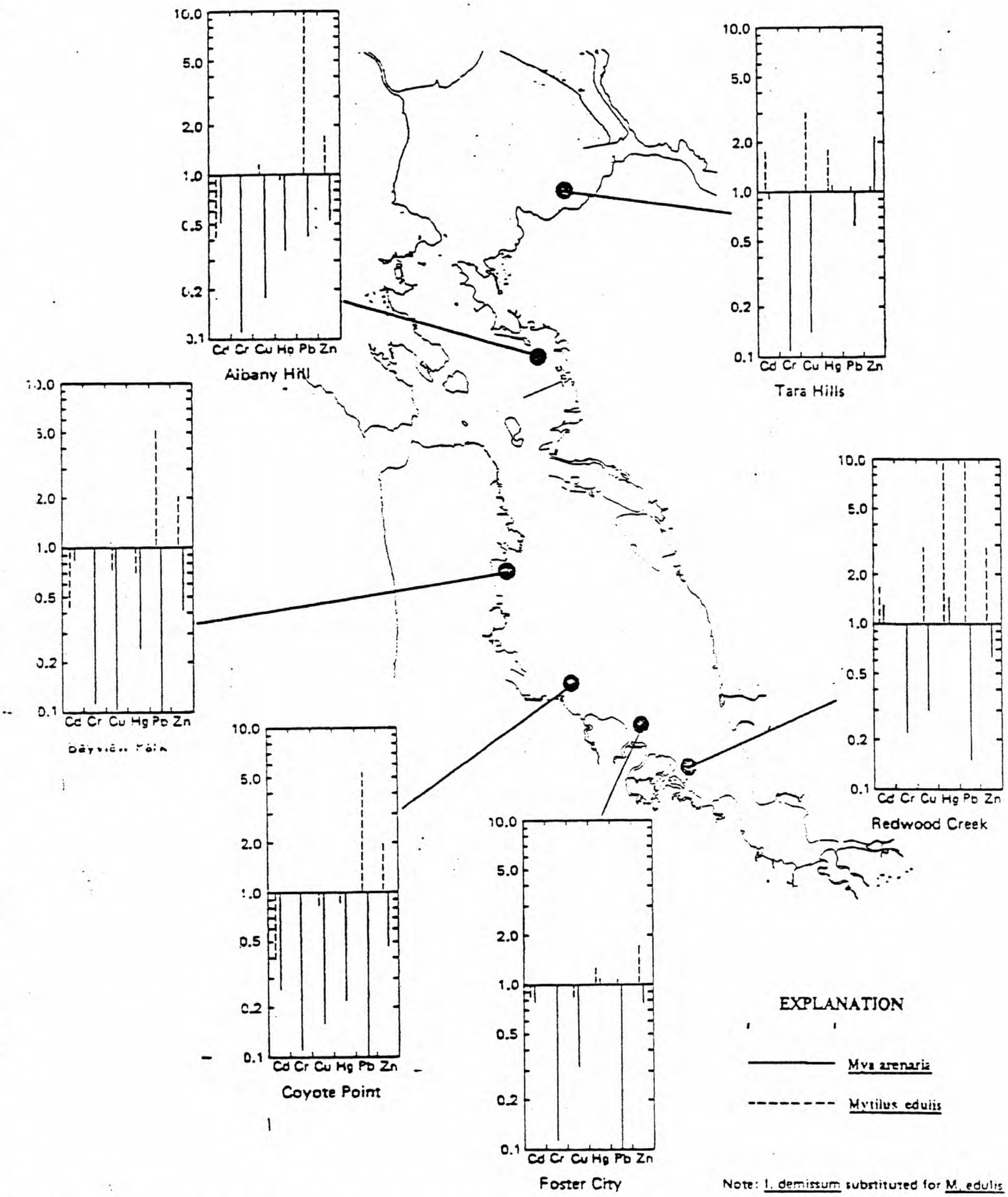


Figure 11-1 --San Francisco Bay and local points of interest in this report.



Note: I. demissum substituted for M. edulis in Tara Hills samples.

Figure 11-2 --Ratios of concentrations of 6 heavy metals in *Mya arenaria* to the alert limit concentrations (data from 5) and in *Mytilus edulis* relative to concentrations in *M. edulis* from Tomales Bay (data from 2).

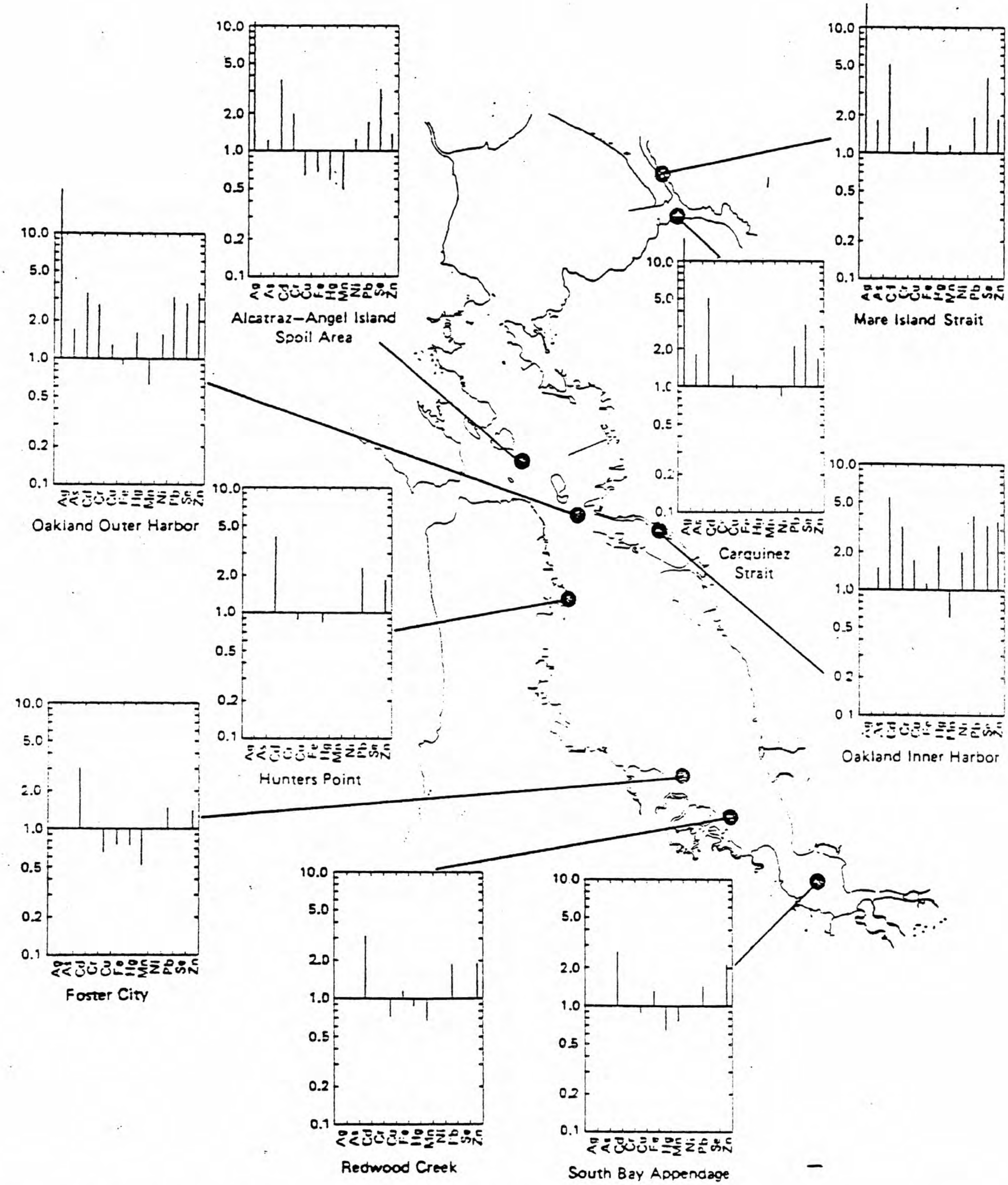


Figure 11-3 --Ratios of the concentrations of 12 heavy metals in San Francisco Bay sediments to concentrations in average shale.

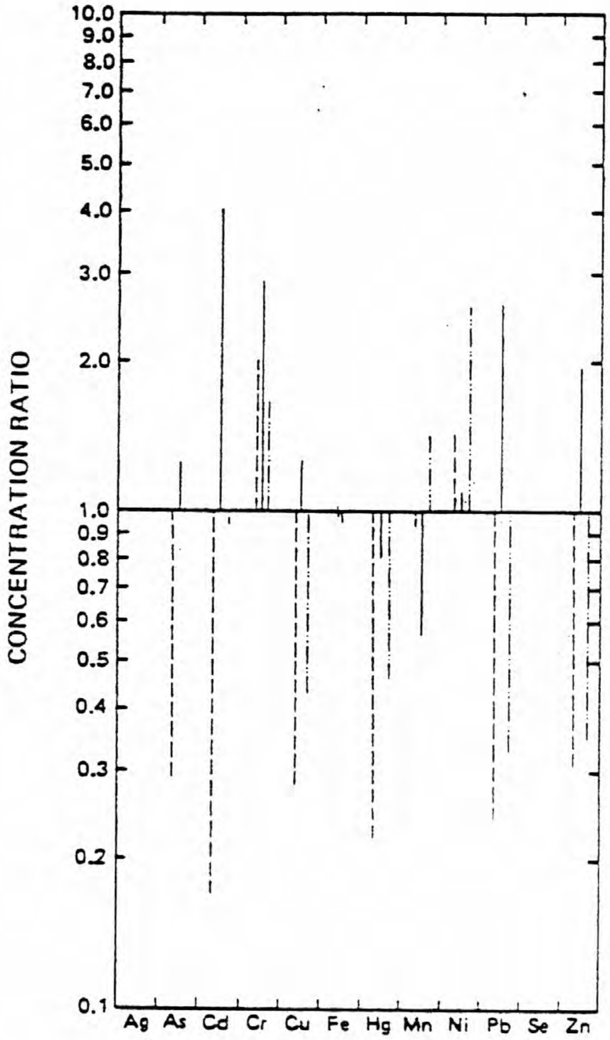


Figure 11-4 Ratios of median heavy metals concentrations in bay sediments (Table 11-6) to concentrations in average shale (——), in sediments from Los Angeles Harbor (---), and in sediments from Baltimore Harbor (-··-··).

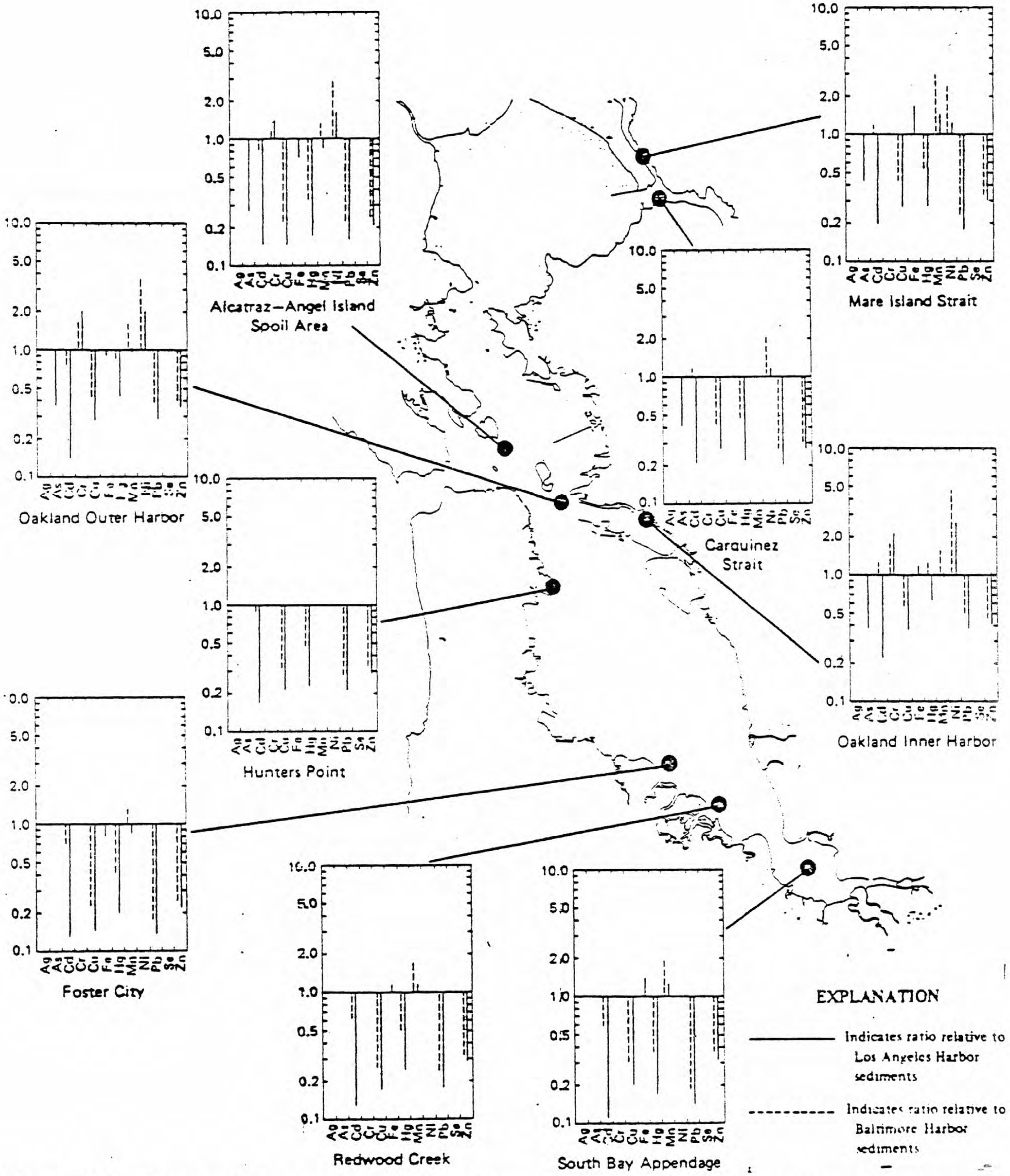


Figure 11-5 --Ratios of concentrations of 12 heavy metals in San Francisco Bay sediments to concentrations in sediments from Los Angeles Harbor and from Baltimore Harbor.

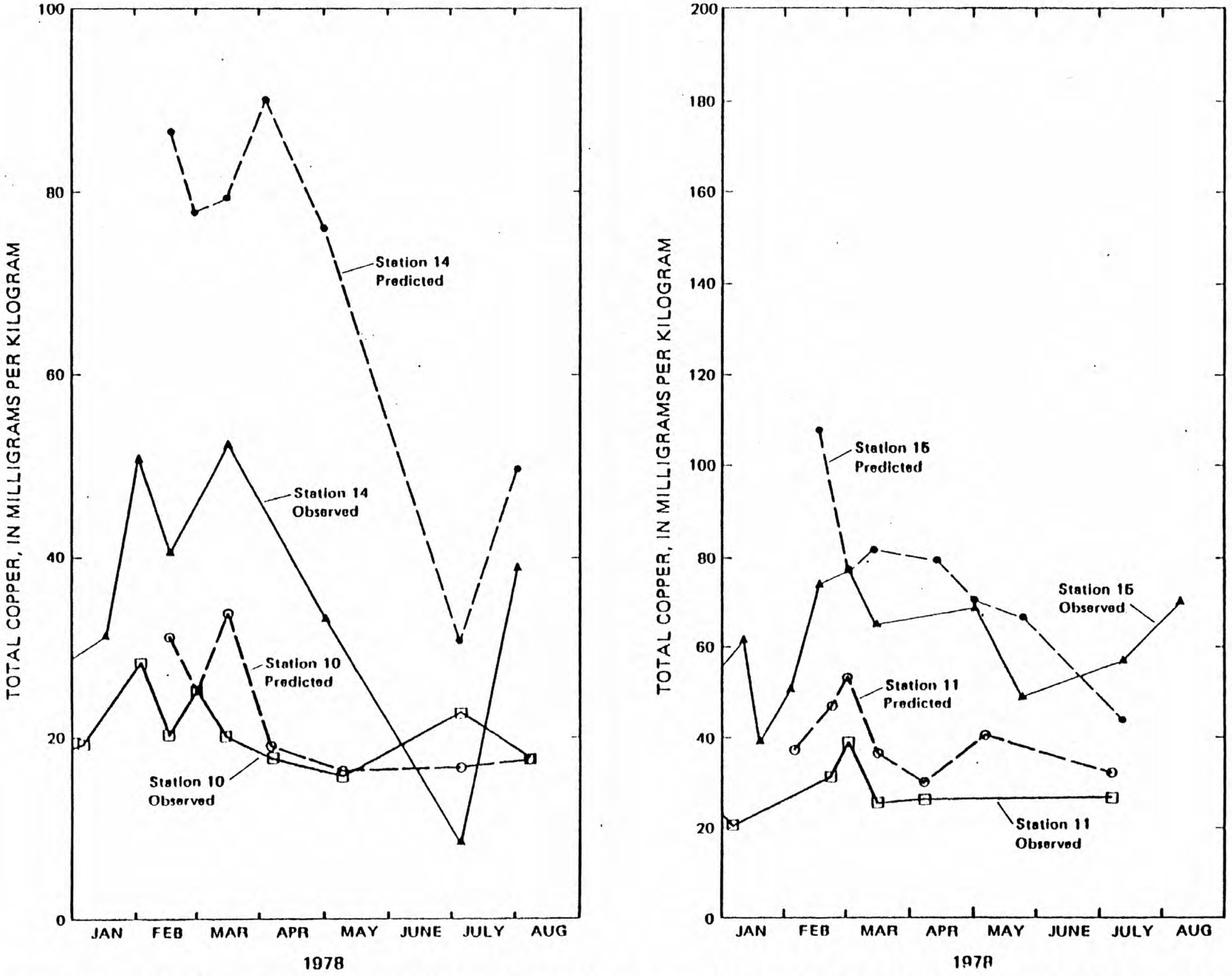


Fig. 11-6 Variations with time in the concentration of total Cu observed at stations 10, 11, 14 and 15 in south San Francisco Ba between January and September 1978. Dashed lines are variations predicted from fluctuations in amorphic Fe and particle size.

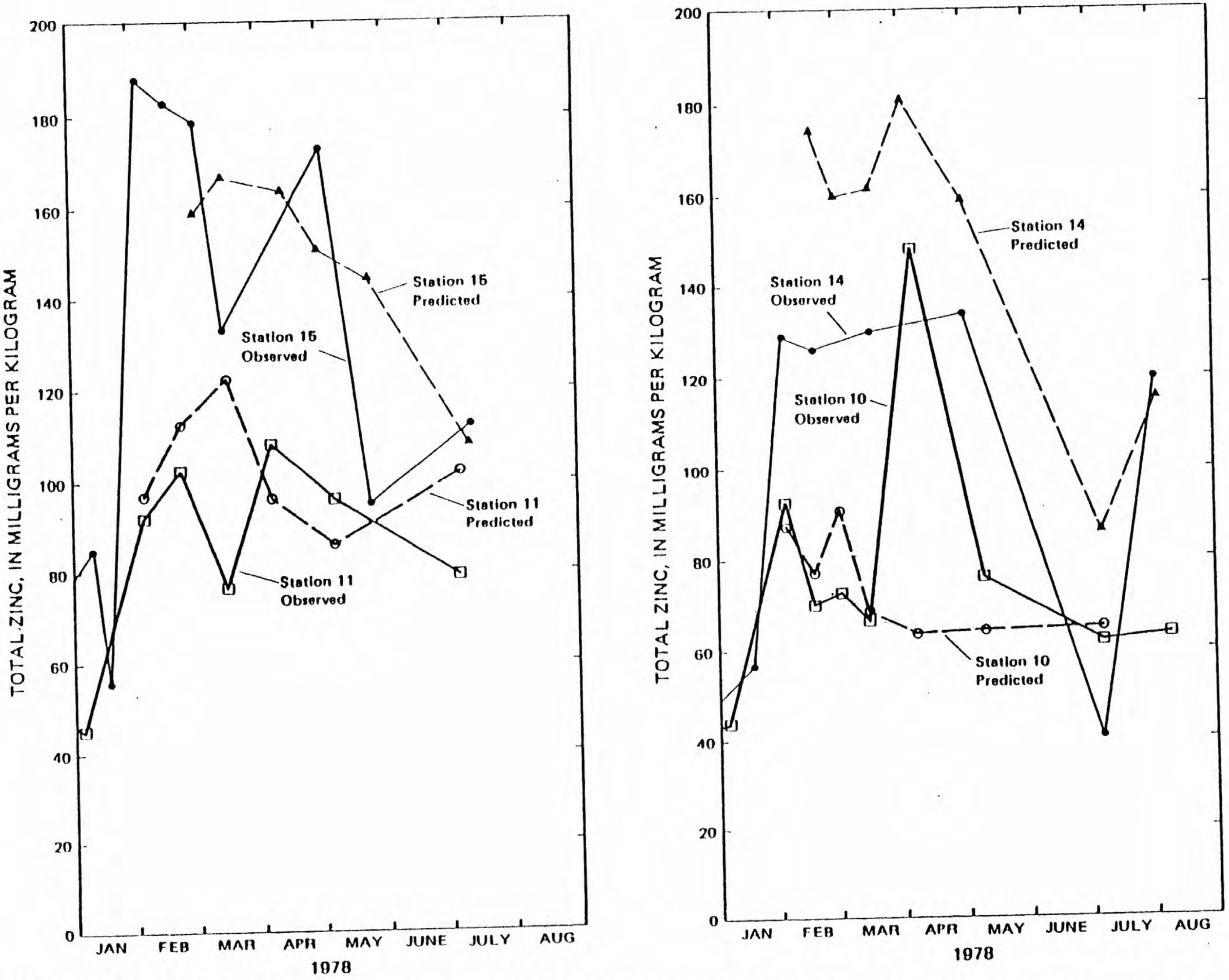


Figure 11-7 Variation with time in the concentration of total Zn observed at stations 10, 11, 14 and 15 in south San Francisco Bay between January and September 1978. Dashed lines are variations predicted from fluctuations in amorphic Fe and particle size.

Percentage of total heavy metal in sample

Treatment condition	Cd	Cu	Hg	Fe	Mn	Pb	Zn
Centrifuged							
Strongly exchanging (ammonium acetate)					◦[1]		
Weakly reducing (hydroxylamine)				◦	○[1]		(1)
Strongly oxidizing (hydrogen peroxide)	●	•	•	○	○	•	•
Strongly reducing (citrate—dithionate)		◦[1]		•	◦	(1)	◦
Total dissolution (HNO_3, HF, HCl_4)	◦	●	●	●	●	●	●

EXPLANATION

blank	<1 percent
◦	1—4.9 percent
○	5—24 percent
•	25—49 percent
●	>50 percent
1	The Mare Island Strait samples, which showed evidence of being better aerated than the other samples, were the exception. See details in text.

Figure 11-8.--General pattern in release of heavy metals in the semiselective extraction procdure (data from 3).

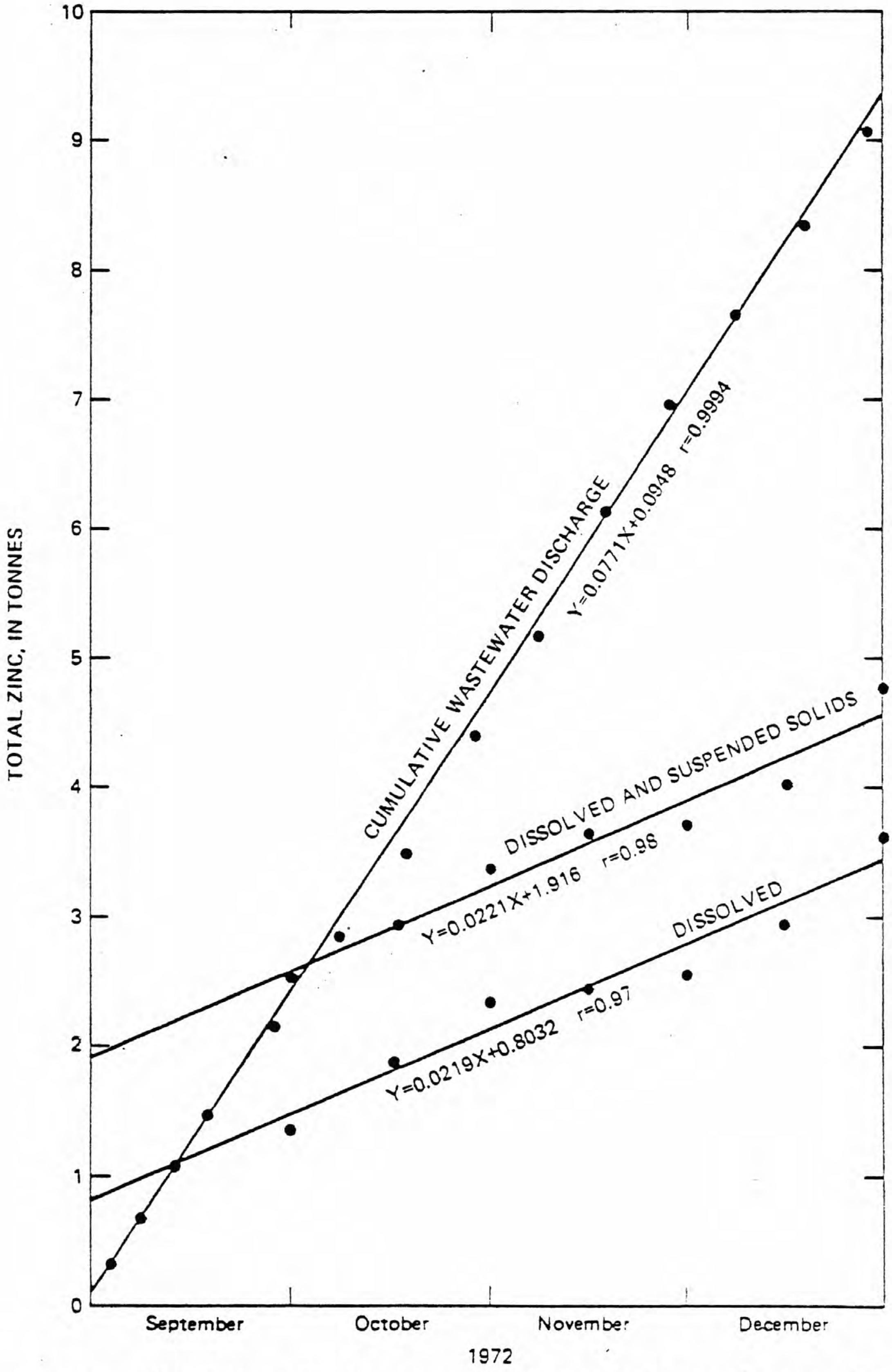


Figure 11-9--Cumulative discharge of zinc in wastewater and the total mass of zinc in the aqueous and combined aqueous and suspended-solid phases.

Chapter 12

Fluctuations of Copper, Zinc and Silver in Clams from Southern San Francisco Bay as Related to Freshwater Discharge

S.N. Luoma and D.J. Cain

Trace metal contamination is often associated with the type of industrial/ urban development which surrounds San Francisco Bay. South San Francisco Bay may be especially vulnerable to trace contaminant effects because the residence times of South Bay waters are long during most of the year. Mcculloch et al. (1970), Imberger et al. (1977) and conomos (1979) suggest that South Bay is well flushed only during periods of high freshwater discharge from the Sacramento-San Joaquin Delta. Girvin et al (1975) suggest that pollutants may accumualte in South Bay waters, sediments, and biota during periods of restricted flushing.

The discharge of freshwater into South Bay may affect the concentration of trace metals available to organisms in several ways:

(1) River, stream and sewer discharge may carry elevated concentrations of solute and particulate-bound metals into the estuary during the rainy season. Urban storm runoff is characterized by high concentrations of many trace metals (Pitt and Amy 1973).

(2) Terrigenous (land-derived) sediments are carried into the estuary primarily during the winter (Conomos and Peterson 1977). The physicochemical characteristics of the terrigenous sediments (especially those in urban runoff) may differ from the characteristics of the estuarine sediments. These differences may affect both the partitioning of metals between particulates and solute

and the ability of organisms which ingest particulates to accumulate metals (Luoma and Jenne 1977; Luoma 1977a). (3) The facilitation of flushing by freshwater discharge into South Bay may reduce concentrations of solute trace metals which accumulated in the water and/or change the chemistry of the sediments (again, affecting the availability of the metals to organisms), and (4) decreases in salinity of ambient waters, associated with freshwater influx, may directly affect metal uptake by organisms (Phillips 1977a).

Any or all of the above affects should be reflected in temporal changes in the metal concentrations of organisms in South Bay. Temporal variability in the metal concentrations in bivalves (Anderlini et al. 1975) and seston (Flegal 1977) were reported in North San Francisco Bay, but the causes of the variation were not discussed. Careful analyses of the causes of such variability have been useful in studying biological, physical and chemical influences on metal cycling in other estuaries (Luoma 1977a; Frazier 1975). In this Chapter we present data from South San Francisco Bay on temporal changes in the concentrations of silver (Ag), copper (Cu) and zinc (Zn) in the soft tissues of the tellenid clam Macoma balthica. The data extend from March 1975, to February 1978, and include a two year period of severe drought, which significantly affected physical and chemical processes relevant to San Francisco Bay. We show that increases in freshwater discharge enhance the biologically available concentrations of all three metals in South Bay, but also appear to modulate the removal of Cu and Ag from the estuary.

Macoma balthica was chosen for study because it is a deposit feeder (ingests sediment and associated organic matter for food), it concentrates a number of metals to a greater extent than the two other clams (Tapes japonica and Mya arenaria) common in South Bay (Luoma, unpublished data), and it is widespread on intertidal mudflats throughout the Bay (see also Nichols 1979, and Carlton 1979). Silver, Cu and Zn were chosen because all are potentially toxic to estuarine organisms and are potentially important contaminants in South Bay.

Methods and Materials

Clams and sediments were collected at eight intertidal stations and one subtidal station in South Bay (Fig. 12-1). Our discussion will focus primarily on two of these stations (1 and 5) which have been sampled periodically since early 1975. Sediments were scraped from the surface oxidized layer, sieved through 250 μm polyethylene mesh and extracted within 24 h of collection with either hydroxylamine hydrochloride in 0.01 N nitric acid, 25 % acetic acid, 0.1 N sodium hydroxide or a mixture of concentrated nitric and sulfuric acids (for "total" metal).

The sediment extractions were used to assess the effects of freshwater discharge on sediment chemistry, and to identify periods of terrigenous sediment movement in South Bay. Sodium hydroxide extractions were used to estimate the concentration of humic materials in the sediments. Humic acid concentrations in the extract were measured by absorbance at 480nm. Humic materials originate primarily from bacterial metabolites in terrestrial soils, and occur at higher concentrations when terrigenous input into sediments increases (Luoma and Bryan unpublished data). Hydroxylamine and acetic acid extractions were used to estimate the proportion of freshly precipitated iron in the sediments. In oxidized sediments, Fe occurs as a hydrated oxide primarily associated with the surface of particles (Jenne 1968, 1977). Iron oxides are highly amorphous when they precipitate but gradually become more crystalline with age. The solubility of Fe in hydroxylamine hydrochloride declines more rapidly as iron oxides age (crystallize) than does the solubility of Fe in acetic acid (Luoma, 1974). Thus, the ratio of Fe extracted from sediments by hydroxylamine relative to that extracted by acetic acid is an index of the proportion of freshly formed iron oxide. Freshly formed iron oxide enters the estuary and its tributaries primarily during periods of high runoff (Elder et al 1976; Trefrey and Presley 1976). When terrigenous input of Fe is low, the oxides should increase in crystallinity as the age or mix with the "older" Fe in marine sediments. These seasonal changes should be reflected in the relative extraction of Fe by the two extractants.

Fifteen to 30 clams were collected at each sampling. The animals were kept for 24 h after collection in clean seawater to clear their gut of undigested sediments, Soft tissues were dissected from the shells, weighed, digested in a mixture of concentrated nitric and sulfuric acids (2:1, with the addition of excess HNO_3 where necessary), then evaporated to dryness and reconstituted in 25% HCl. (Reconstituted in HCl was essential to prevent precipitation of Ag in the samples). Analyses were conducted on either samples of pooled animals (in 1975 and early 1976) or on the tissues of individual animals by atomic absorption spectrophotometry with background correction where necessary. In a few instances significant correlations between metal concentration and the weight of individual animals were observed. Metal concentrations calculated for a median-sized animal (100 mg) from the regression equation for the concentration-weight relationship were used instead of mean concentration in all such samples.

River flow data were obtained from calculations of a Sacramento River - San Joaquin Delta Outflow Index (see Arthur and Ball 1979), stream flow data were from the U.S. Geological Survey (U.S.G.S.) gauging station on San Francisquito Creek (Fig. 12-1); and rainfall data were from National Weather Service data for Palo Alto. Estuarine water salinities were determined with a salinometer from shallow pools on the surface of the mudflats.

Results

Physical and Chemical Environment

Physical variables. Delta discharge provides the major source of freshwater influx into the entire San Francisco Bay system (Conomos 1979). San Bruno shoals and the narrows at the Dumbarton Bridge may impede the penetration of this Delta water into the southern reaches of the Bay (Imberger et al, 1977). Thus, local streams and sewers may be a very important source of freshwater in South Bay despite their relatively low rates of discharge. Local stream discharge was highly irregular during our study, closely reflecting the pattern of local rainfall (Fig. 12-2). Discharge from most local streams was negligible during the drought summers of 1976 and 1977. The discharge of the Sacramento River also declined substantially as the drought progressed (Fig. 12-2).

Chemical Variables. Salinities at stations 1 and 5 were lower in the spring and winter of both drought years than in the summer, reflecting some significant seasonal increase in freshwater inflow in South Bay despite the drought (Fig. 12-3, 12-4). Local stream discharge appeared to be a significant source of this freshwater. The minimum salinities observed at station 1 in 1976 (late March), and in 1977 (January, April, November) all followed the largest pulses of discharge from San Francisquito Creek observed in those years. Reduced salinities at station 5 in March 1976, and April 1977 also followed large pulses of stream discharge. The minimum salinity observed at station 5 (5 May 1977), however, was preceded by more than 30 days of relatively low stream discharge, suggesting Delta discharge may have penetrated to station 5 in May 1977. A surprising salinity minimum occurred in late August 1977 at stations throughout the South Bay (1, 3, 5, 6, 7, 8). This followed 90 days of no rainfall and zero discharge from all local streams (USGS Data Report in preparation; Santa Clara Valley Water District unpublished data) and coincided with a period of very low Delta discharge.

The humic acid concentrations measured in 1977-78 indicated little terrigenous input of sediment at station 5 until November (following the second storm of the year, but the first pulse of stream runoff measured at the USGS gauging station) and at station 1 until January (Fig. 12-3, 12-4). The proportion of fresh iron oxide in the sediments at station 5 followed the expected seasonal pattern with minor peaks following the large pulse of stream discharge in April 1977 and the first storm of the fall in September 1977 (Fig. 12-4). The proportion of freshly precipitated iron oxide at station 1 was consistently higher and less variable than at station 5 (Fig. 12-3). This is consistent with the strong influence of a high, relatively constant input of sewage into the southernmost reach (Imberger et al, 1977). Peaks in the proportion of fresh iron oxide in April 1976, and in February and April 1977, following the largest stream discharges of those years, suggested runoff also contributed Fe to the sediments at station 1. The winter maximum and the end of the summer minimum in the proportion of fresh iron oxide occurred earlier at station 5 than at station 1 in both years, as did the beginning of the winter increase in humic acid concentration. At both stations the anomalous salinity minimum in August, 1977 was accompanied by a small increase in the proportion of fresh iron oxide, and the winter increase in humic acid concentrations preceded the increase in fresh iron.

Trace metal concentrations

To facilitate a meaningful perspective, the concentrations of Cu, Ag and Zn observed in South Bay sediments and clams (Table 12-1).

were compared to similar observations from 17 English estuaries described in previous chapters. The tellenid clam Scrobicularia plana, from the English estuaries, is ecologically,morphologically and behaviorally quite similar to M. balthica. Where the two species co-occur in England their Ag, Cu and Zn concentrations are comparable (Bryan and Hummerstone, 1977). Median Ag concentrations in clams from station 1 were nearly 100 times greater than concentrations in clams from more pristine environments (Table 12-1). Median Ag enrichment in both sediments and animals was as great at station 1 in South Bay as in any estuary in the English survey (Fig. 12-5) which included several estuaries with silver mines in their drainage basin. The Ag enrichment appeared to originate from a point source near station 1 on the western shoal, below Dumbarton Bridge, and was rapidly diluted north and east of station 1 (Table 12-1).

The concentrations of Cu and Zn in South Bay sediments were low relative to concentrations observed in metalliferous, or industrially enriched areas (Fig. 12-5; Table 12-1). Concentrations of Zn in M. Balthica were also low for this family of bivalves (Fig. 12-5). In contrast, the concentrations of Cu periodically observed in clams at stations 1 and 4 in South Bay were 30 times background (Luoma, unpublished data) and the highest Cu levels were as high as any observed in the English clams. The high degree of biological Cu enrichment in some parts of South Bay suggested the benthic community of this portion of the estuary was especially vulnerable to Cu input.

Silver and Copper dynamics in *M. balthica*

Between March, 1975 and February, 1978, concentrations of Ag in clams at station 1 varied by over 30 times (from 7 to 220 $\mu g/g^{-1}$) and concentrations of Cu by six times (74 to 440 $\mu g/g^{-1}$, Fig. 12-3). Copper concentrations in the clams varied by five times during the same period at station 5 (Fig. 12-4). Concentrations of Ag were too low at station 5 for assessments of the range of variation to be meaningful. The variation of Cu and Ag concentrations in the animals did not follow the variations of concentrations in the sediments.

The highest concentration of Cu observed in *M. balthica* occurred at station 1 in August 1975 (Fig. 12-3). This peak coincided with a period of severe anoxia on the mudflat (due to an intense bloom of the benthic macro-algae *Polysiphonia sp.*) which destroyed all but a few isolated patches of the benthic infauna (see also Nichols, 1979). Mobilization of biologically available Cu has been observed in other estuaries under similar types of anaerobic conditions (Chapter 10) although the mechanism involved has not been explained.

With the exception of the Cu peak in late 1975, the dynamics of Ag and Cu in *M. balthica* at station 1 followed a similar pattern (Fig. 12-3). The lowest concentrations of both metals were observed in early summer. Concentrations began to increase with the onset of infrequent rainfall in the late summer and fall. Concentrations peaked in the early winter, followed by a relatively rapid decline, which coincided with the period of maximum rainfall. Temporal variations in Cu concentrations in *M. balthica* at station 5 followed a pattern similar to that observed at station 1 (Fig. 12-4).

The autumn build-up of Cu and Ag in the clams was apparently caused by the influx of local runoff into South Bay. In both 1976 and 1977 the onset of metal accumulation at both stations followed early storms in the watershed of South Bay. Moreover, the concentration of Ag and Cu in the animals, between the time of minimum and maximum concentration in each year, was a function of cumulative rainfall during that period of the year (Fig. 12-6). If Ag and Cu enter South Bay primarily in runoff, then cumulative rainfall should be an indirect measure of the sum of the metal discharge into South Bay at a given point during the year. The concentration of Cu and Ag in the clams at a given quantity of rainfall (i.e. quantity of metal discharged) was significantly greater in 1977 than in 1976, and (for Ag) slightly higher in 1976 than in 1975. Freshwater discharge from all sources declined between 1975 and the end of 1977 (Fig. 12-2) suggesting an inverse relationship between the rate of freshwater discharge and the accumulation of Cu and Ag in the clams per unit metal discharge in runoff. To quantify this relationship, a metal discharge index, $\underline{M}_d$, was calculated for each sampling date between the time of minimum and maximum Ag and Cu concentrations in the clams where

$$M_d = (\Sigma R)(1/FW_d)$$

with ΣR = cumulative rainfall (cm) over the stated period and FW_d = the discharge rate of freshwater ($m^3 \cdot s^{-1}$) from a specified source. The concentrations of Ag in the clams from station 1, as observed during the period of accumulation in all three years of the study, fell into a single, highly significant regression with the metal discharge index (Fig. 12-7) when FW_d was determined from Delta discharge 10 days before the sampling date (the 10-day lag was chosen somewhat arbitrarily, but made little difference in the discharge value chosen). The correlation with Ag concentrations in the clams was insignificant when FW_d was determined from San Francisquito Creek discharge within the week prior to sampling, or as the mean discharge rate of the creek for either the 10 or 20 days before the sampling date. Copper concentrations at both station 1 and station 5 also showed highly significant correlations with the metal discharge index when Delta discharge was used for FW_d (Fig. 12-7). There was no significant difference between the slopes of the relationships at the two stations; however, Cu concentrations at station 1 were consistently higher than those at station 5.

Zinc dynamics

The dynamics of Zn in *M. balthica* at stations 1 and 5 differed from the dynamics of Ag and Cu in several ways (Fig. 12-8). (1) The period of low discharge was characterized by declining or stable Zn concentrations in the clams. Zinc accumulation in the animals did not begin until after the period of maximum freshwater discharge, and was not related to cumulative rainfall. (2) The magnitude of the winter peaks in Zn concentrations declined as freshwater discharge declined over the course of the drought. (3) whereas Cu and Ag concentrations in the clams showed no consistent relationship with salinity, temporal fluctuations in Zn concentrations were consistently the inverse of temporal fluctuations in salinity. (4) Zn concentrations in *M. balthica* were more similar at stations 1 and 5 throughout the study period than were Cu and Ag concentrations.

DISCUSSION

Biologically significant points of Ag and Cu enrichment occur in South Bay. The Ag enrichment is largely the result of Ag discharge from a point source on the western shoal of the southernmost reaches of the Bay. Copper enrichment appears to result from undefined physicochemical conditions in the Bay, which make the system especially susceptible to the relatively small discharges of Cu. Zinc does not appear to be a contaminant of great significance but its behavior is of interest in that it differs significantly from Ag and Cu. The concentrations of Cu and Ag observed at station 1 in November 1977, were as high as any ever reported for tellenid clams. Within two months of this peak, adult *M. balthica* essentially disappeared from the mudflat at this station (but not from station 3 where metal concentrations are lower). Proving that trace contaminants are affecting organisms in a natural system is nearly an impossible task (Nichols, 1979; Luoma 1977b). However, the coincident occurrence of high tissue concentrations of two potent toxicants, and the disappearance of a population suggests the Cu and Ag enrichment in South Bay is worth further investigation.

Freshwater discharge rates were very important in determining the degree of biological Ag and Cu contamination at least at stations 1 and 5. The accumulation of Cu and Ag in the clams as rainfall increased in frequency through the summer and fall, and the annual correlation between metal concentrations in the clams and cumulative rainfall, suggested local runoff provided the primary input of the two metals. This was substantiated by the observation by Girvin et al. (unpublished data) of elevated concentrations of Ag in solution at a location offshore from our station 1 (where our data indicate biological contamination is diluted relative to station 1) in September 1976, following a rainstorm. Three days later the high concentrations of Ag had disappeared. In a diel study at the same station they also observed increasing concentrations of Ag in solution with the onset of rainfall.

The rate of freshwater discharge also had an inhibitory effect on biologically available Cu and Ag during the summer and fall period. The year of lowest freshwater discharge was the year when metal accumulation in the clams per unit rainfall was greatest. Moreover, 60-80% of the temporal variance in the summer-fall concentrations of Ag and Cu in the clams was explained by our metal discharge index, which included the inverse of Delta discharge as one term. The correlation with the metal discharge index suggested concentrations of

Cu and Ag available to M. balthica in South Bay between 1975 and 1978 were controlled during the summer-fall period by a dynamic balance between metal input rates from local runoff and a slow rate of metal loss, modulated by freshwater discharge rates. (Of course, the input and loss functions need not represent metal loadings alone, but could also occur through chemical changes in the sediments or water column that enhance or decrease the biological availability of the metals.) The magnitude of the freshwater discharge during this period of relatively long water residence times in South Bay, appeared to be the crucial factor determining the size of the annual peak in Cu and Ag concentrations.

The rapid decline of Cu and Ag concentrations in the clam tissues during winter and spring occurred as significant quantities of freshwater and terrigenous sediment entered South Bay. The onset of the decline in Cu concentrations at station 5 coincided with the minimum salinity observed at that station in 1975-76 and with the onset of declining salinity and increasing humic acid concentrations in the sediments in 1977-78. At station 1 the onset of the 1975-76 decline coincided with a salinity decline and a gradual increase in the proportion of freshly precipitated iron oxide in the sediment. In 1977-78 the decline did not occur until salinities fell below 26 $^{0}/oo$, but coincided with a sharp increase in the humic acid content of the sediments. The 1976-77 decline at station 1 did not coincide with a salinity decline, but the proportion of freshly precipitated iron in the sediment did rise as Ag and Cu levels in the clam began to drop.

There are at least two possible explanations for the decline in Ag and Cu concentrations in the animals. First, at some point in the early winter, the flushing action of freshwater discharge is sufficiently strong to remove biologically available Cu and Ag from South Bay as rapidly as it enters in the runoff. By limiting the residence time of the contaminated water and/or sediment, the exposure of the clams to Ag and Cu is limited. The second possibility is that as the rainy season progresses the chemical and/or physical nature of the terrigenous sediment in the runoff changes (V. Kennedy, pers. comm.) Early runoff should be dominated by sediment from the urban watershed of South Bay. Not only will the initial storms wash urban streets containing several months' accumulation of contaminants, but also the initial rainfall of the year in the less urbanized areas will largely be converted to groundwater, rather than running off, until the soils are saturated. An example of the latter was observed after the first storm of the year in September 1977, when no stream flow was detected in either San Francisquito Creek or Redwood Creek (USGS Data Report, in prep.) at gauging stations upstream from most of the urban area. However, a significant pulse of flow was observed at an urban gauging station in Stevens Creek (Santa Clara Valley Water District unpublished data). Later in the rainy season the urban runoff will carry less sediment, but more importantly, the stream discharge will also carry a heavy load of less contaminated sediment from the more rural parts of the basin. Humic acids should be more concentrated in the rural runoff than in the urban runoff; the close correspondence of declines in Ag and Cu concentrations in 1977-78 with increased humic acid concentrations could reflect a change in the dominant sediment type in the runoff. These hypotheses are, of course, not mutually exclusive since any flushing modulated by freshwater discharge would also be important in removing urban runoff from the estuary.

The metal discharge index represents a first step toward quantifying the degree of Cu and Ag enrichment we might expect in South Bay organisms as freshwater discharge into the estuary is reduced. Unfortunately, the index will not be useful in predicting environmental impact until we understand more about the relative importance in flushing of South Bay by local stream discharge versus Delta discharge. Initial model studies of hydrodynamics in South Bay suggest significant quantities of freshwater from the Delta should not have penetrated beyond San Bruno shoals during the summer or fall in either 1976 or 1977 (Imberger, et al. 1977). If so, local stream discharge is the most likely source modulating the different rates at which biologically available Cu and Ag were removed from South Bay in these two years. The winter decline in concentrations of Cu also clearly followed major pulses of local runoff more closely than Delta discharge at station 5 in March, 1976, and November 1977, and at station 1 in January 1977, (although the chemical nature of the sediment in the runoff may also explain these effects). Several lines of evidence raise questions about the dominance of local stream discharge as cleansing force in South Bay, however: (1) The lowest Ag concentrations ever observed at station 1 followed a period of zero rainfall between 1 May and 20 June 1975. The Ag minimum suggests a negligible input of Ag combined with a rapid flushing of available Ag at this time. Local stream flow declined to relatively low levels by June, 1975; but Delta discharge peaked at a sufficient rate to penetrate San Bruno shoals shortly before the Ag minimum. At least in the spring of 1975, Delta discharge was the most likely force driving the removal of biologically available Ag at station 1. (2) The salinity anomaly in August 1977, was clearly not the result of local runoff. This salinity minimum coincided with a small influx of terrigenous sediment (reflected by an increase in the proportion of freshly precipitated iron) at both stations 1 and 5, but, more importantly, with a sharp reduction in Cu concentrations in clams at station 5. A similar reduction in Cu concentrations at station 8 was observed at this time, but not at stations 1, 3 or 6 (Luoma and Cain, unpublished data). (3) When the metal discharge index was calculated from the inverse of local stream flow, no correlation with the three years of data on Cu and Ag levels in the clams was observed. It is possible that we did not adequately quantify crucial aspects of the highly irregular stream flows in the relationship; but it is also possible that Delta discharge is a more important flushing force on the shoals of the South Bay than predicted from the initial studies of Imberger et al. (1977) in the main channel of the estuary.

The differences between Zn dynamics and the dynamics of Cu and Ag in South Bay demonstrate that all trace metals do not behave similarly in the system. The differences between the metals may reflect their source, or the source of the chemical factors that control their availability. In 1977-78, Zn concentrations in the clams were lower at both stations than in 1975-76, and, like Cu, concentrations at station 1 exceeded those at station 5 approximately twofold. The distribution of Zn and Cu in 1977-78 suggest factors controlling the availability of the metals originated from similar local sources, probably throughout South Bay (since the northward dilution of Ag greatly exceeded that of Cu and Zn). The south-to-north gradient in Cu and Zn concentrations in 1977-78 could reflect differences in discharge rates, dilution by an increasing volume of water toward the north, or more efficient flushing in the northern reaches. The similarity of the slopes of the regressions between Cu in the clams versus M_d at stations 1 and 5 suggests one of the latter two possibilities is most likely.

In contrast to Cu, the south-to-north gradient for Zn was not observed in 1975-76 when Delta discharge was certainly sufficient to flow southward of San Bruno shoals. Zinc concentrations at station 5 exceeded those at station 1 in 1975-76, and peak concentrations at both stations were much greater than in the following years of low freshwater discharge. Although further study is required, it appears that Delta-derived water may control the biological availability of Zn in years of normal rainfall. This hypothesis is consistent with our earlier suggestion that Zn is not discharged in sufficient quantities into South Bay to be a biologically significant contaminant.

Factors such as temperature, salinity (Wolfe 1971; Phillips 1977a), and biological phenomena (tissue growth; seasonal variations in physiology - Frazier 1975; Betzer and Pilson 1975; Phillips 1977b), have also been cited to explain temporal variations in the metal concentrations of benthic organisms within estuaries. In South Bay, temperature was not found to directly affect Zn, Ag or Cu concentrations in _M. balthica_. The temporal pattern of variation in metal concentrations was roughly the inverse of the seasonal temperature cycle. Seasonal contrasts in the relationship with Cu and Ag concentrations also suggested salinity was not a variable to which these metals were directly responding. Concentrations of Cu and Ag in the clam varied inversely with salinity at station 1 in the spring (March, April 1976; April 1977) and positively with salinity at both stations 1 and 5 in the winter. Zinc concentrations in the clam varied inversely with salinity in a consistent manner; thus, the possibility that salinity variations may have had some direct effect on Zn uptake rates by _M. balthica_ cannot be discounted.

The winter decline in Cu and Ag concentrations in the clams coincided with the period of maximum growth of the organism (Nichols, in prep.; Cloern and Nichols, 1978). Two arguments suggest the effects of tissue growth on metal concentrations were minimal, however: (1) Temporal changes in the content (μg) of Ag and Cu in the animals closely followed changes in concentration ($\mu g/g^{-1}$) thus eliminating the possibility that the seasonal differences in metal concentrations were the result of simply a changing tissue mass, rather than metal fluxes; and (2) Sharp changes in the Cu concentrations of _M. balthica_ were not observed during the spring at stations 6 and 8 in 1977 (Luoma and Cain unpublished data). If undefined physiological processes were responsible for the temporal changes observed, those processes were specific to stations 1 and 5 - a highly unlikely prospect.

Our evidence strongly suggests that the interaction of hydrodynamic processes, local weather, and, quite possibly, the chemical characteristics of sediments entering South San Francisco Bay in local runoff, play the major roles in controlling the contamination of organisms south of the San Mateo Bridge with Cu and Ag. The rate of freshwater discharge into the Bay is a primary factor mitigating the effects of those interactions, and appears to control the amplitude of the annual peak in contaminant concentrations.

Summary - Chapter XII

Significant contamination of the tellenid clam Macoma balthica with Cu and Ag was observed at stations in Southern San Francisco Bay. The degree of contamination appeared to be greatly influenced by the discharge of freshwater into South Bay. Local runoff appeared to provide an important source of the contaminants, especially in the summer and fall. Freshwater discharge, either from local sources or from the Sacramento-San Joaquin Delta, also provided the force that flushed biologically available Cu and Ag from South Bay, and the degree of this flushing force appeared to determine the magnitude of annual peak in Cu and Ag concentrations of the clam. A metal discharge index, combining an indirect estimate of annual metal loading (derived from cumulative rainfall) with the inverse of freshwater discharge at the Delta, was found to explain 60-80% of the temporal variance in the Ag and Cu concentrations of M. balthica. The index represents a first step toward quantitatively predicting the effect of any reduction in freshwater discharge into the Bay on Ag and Cu contamination in South Bay. Significant differences between temporal variations in Zn concentrations in the clams and the variations in Cu and Ag concentrations suggest all contaminants do not behave similarly in South Bay.

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Figure 12-4. Salinity, the proportion of freshly precipitated iron oxide in sediments, humic acid concentrations in sediments, and concentration of copper (Cu) in *Macoma balthica* observed at station 5 between August, 1975, and February, 1978.

Figure 12-5. Concentration of Zn, Cu, and Ag in *Macoma balthica* from San Francisco Bay and in *Scrobicularia plana* (0) from southwest England, compared to concentrations of the metals in sediments from the two locations.

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Figure 12-7. Correlation of Ag concentrations at station 1 and Cu concentrations at stations 1 and 5 with the metal discharge index, M_d. Regression statistics: for Ag, $r = 0.91$ and $\log y = 24 + 0.45 \log x$; for Cu at station 1, $r = 0.79$ and $\log y = 69 + 0.26 \log x$; for Cu at station 5, $r = 0.87$ and $\log y = 25 + 0.31 \log x$. All correlations are significant ($p<0.01$).

Figure 12-8. Zinc (Zn) concentrations in *Macoma balthica* at stations 1 and 5 between August, 1975, and February, 1978.

Fig. 12-1

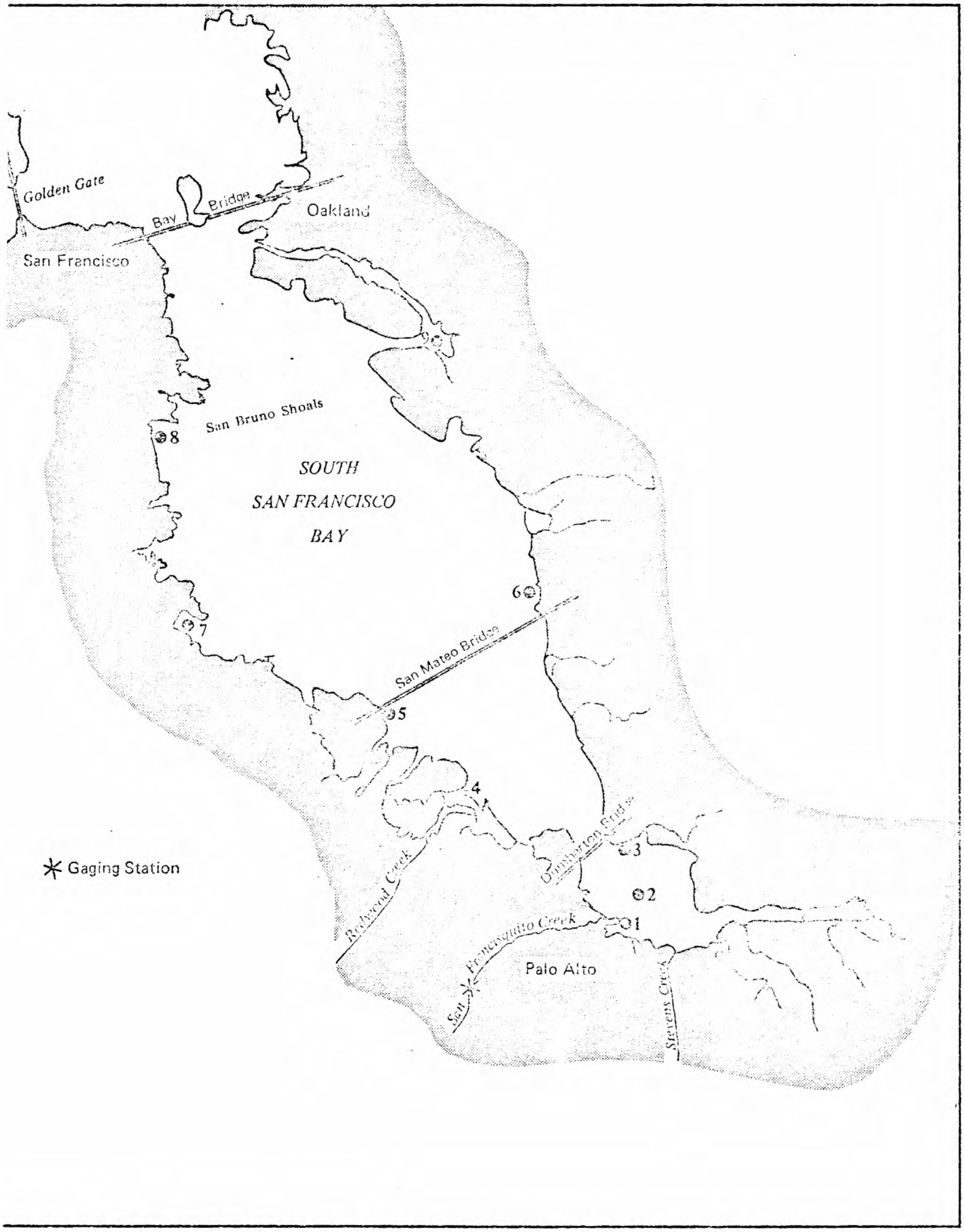

Golden Gate
Bay Bridge
Oakland
San Francisco
9
San Bruno Shoals
8
SOUTH
SAN FRANCISCO
BAY
6
7
San Mateo Bridge
5
4
Dumbarton Bridge
3
2
1
Redwood Creek
San Francisquito Creek
Palo Alto
Stevens Creek
Gaging Station

Fig. 12-2

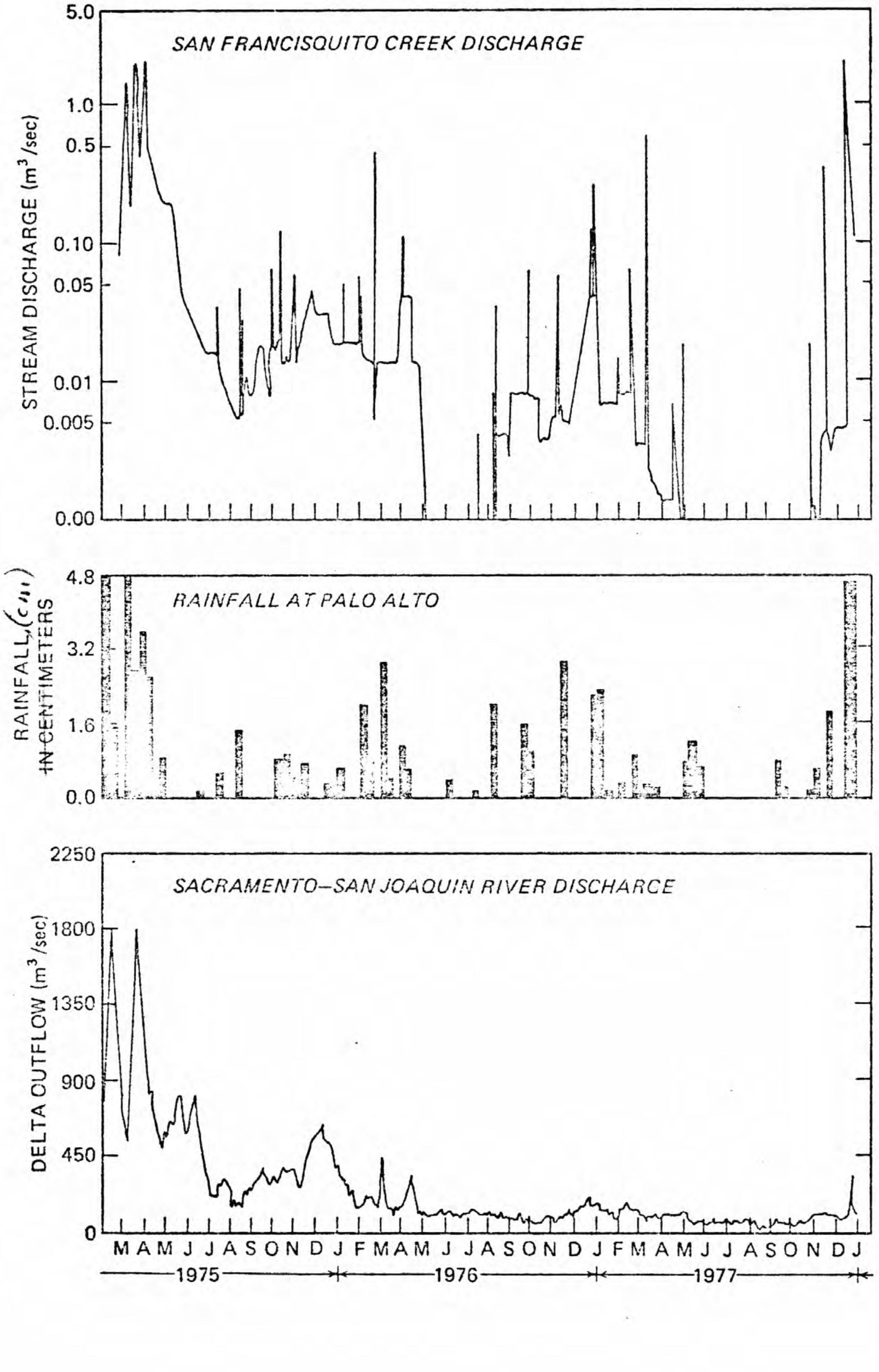

SAN FRANCISQUITO CREEK DISCHARGE
STREAM DISCHARGE (m^3/sec)
5.0
1.0
0.5
0.10
0.05
0.01
0.005
0.00
RAINFALL AT PALO ALTO
RAINFALL (cm) ~~IN CENTIMETERS~~
4.8
3.2
1.6
0.0
SACRAMENTO–SAN JOAQUIN RIVER DISCHARGE
DELTA OUTFLOW (m^3/sec)
2250
1800
1350
900
450
0
M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J
1975
1976
1977

Fig. 12-3

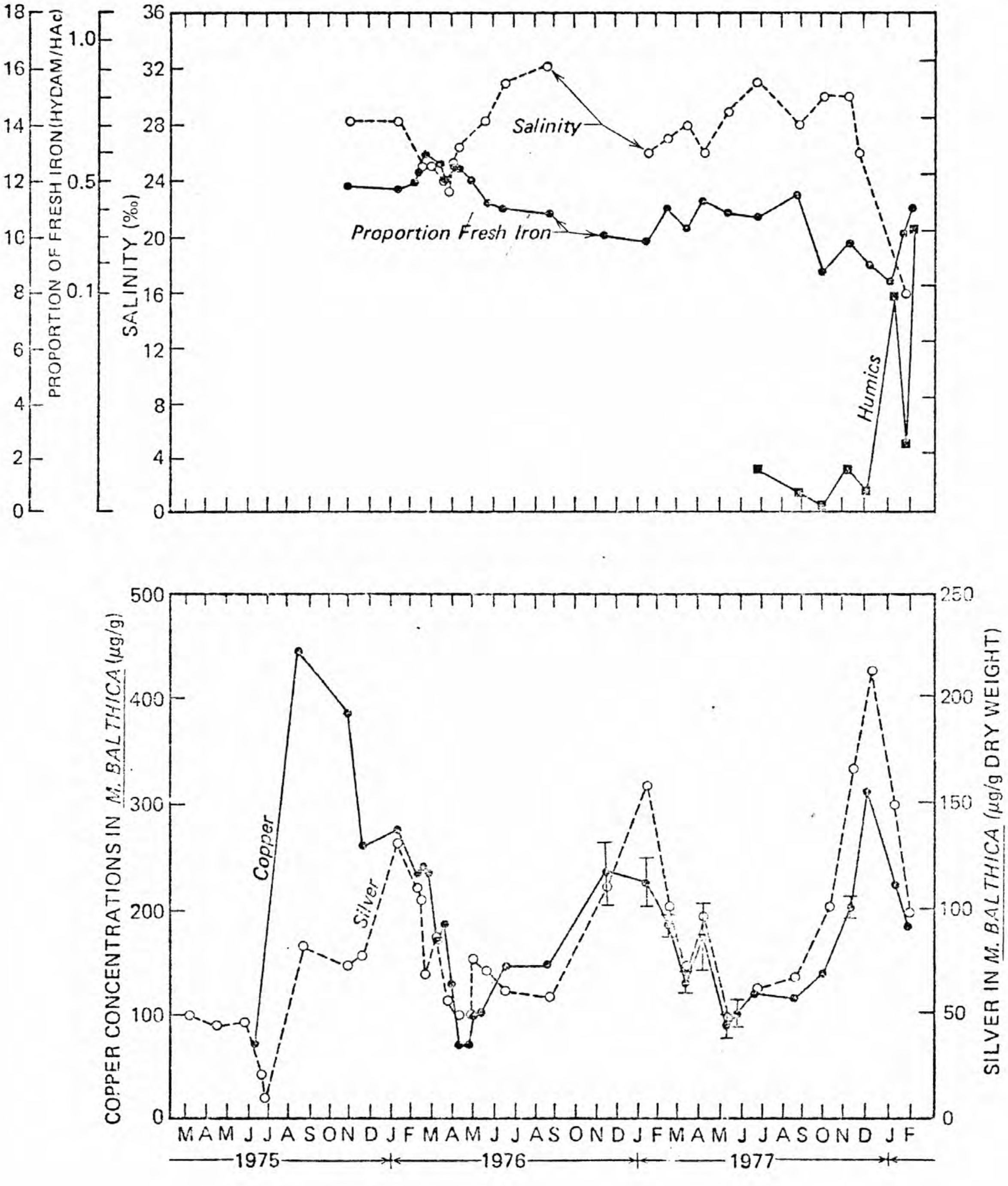

18
16
14
12
10
8
6
4
2
0
PROPORTION OF FRESH IRON (HYDAM/HAc)
1.0
0.5
0.1
SALINITY (‰)
36
32
28
24
20
16
12
8
4
0
Salinity
Proportion Fresh Iron
Humics
500
400
300
200
100
0
COPPER CONCENTRATIONS IN M. BALTHICA (µg/g)
Copper
Silver
250
200
150
100
50
0
SILVER IN M. BALTHICA (µg/g DRY WEIGHT)
M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1975
1976
1977

Fig. 12-4

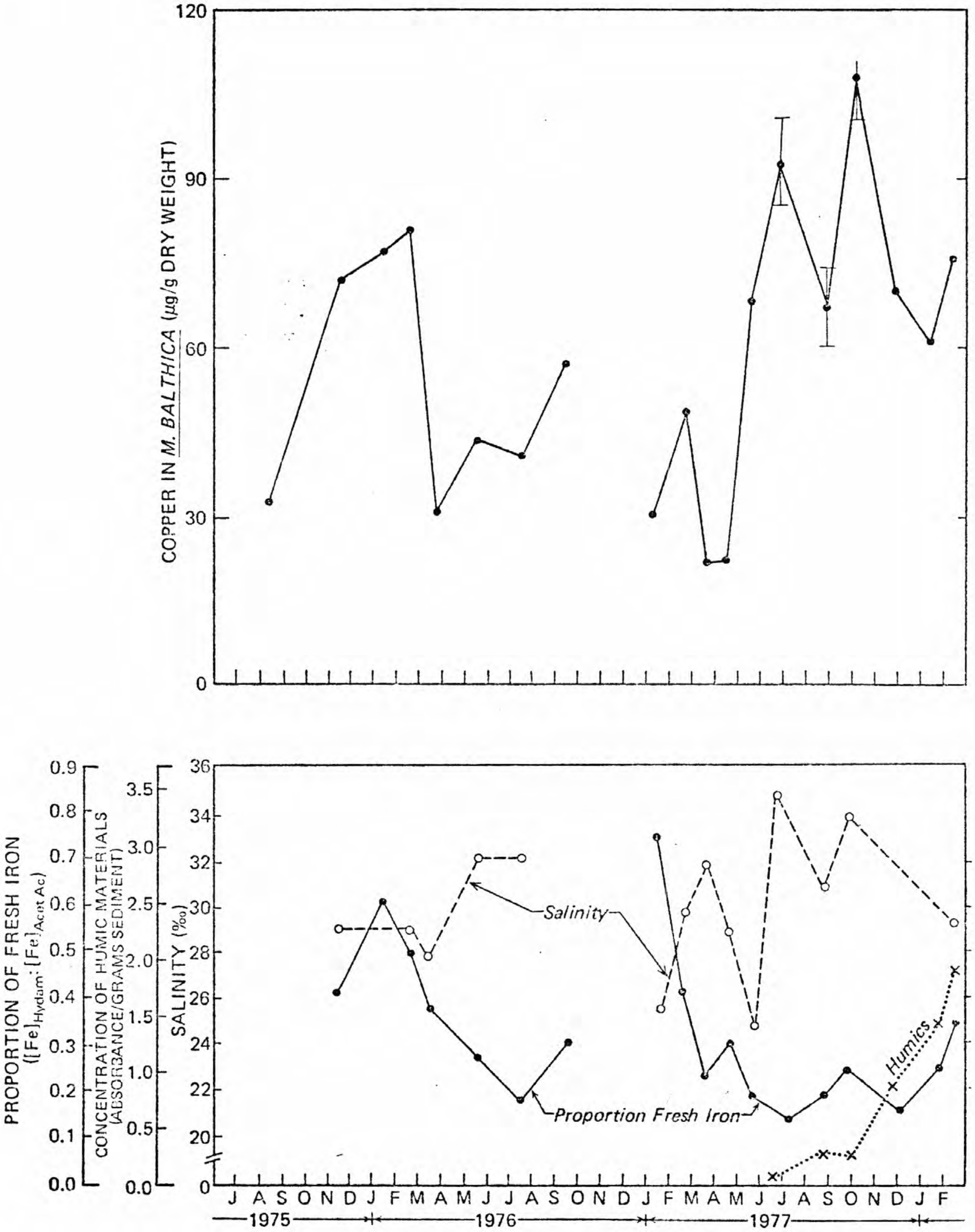

COPPER IN M. BALTHICA (µg/g DRY WEIGHT)
120
90
60
30
0
PROPORTION OF FRESH IRON
([Fe]Hydam:[Fe]Acet Ac)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CONCENTRATION OF HUMIC MATERIALS
(ABSORBANCE/GRAMS SEDIMENT)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SALINITY (‰)
36
34
32
30
28
26
24
22
20
0
Salinity
Proportion Fresh Iron
Humics
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1975
1976
1977

Fig. 12-5

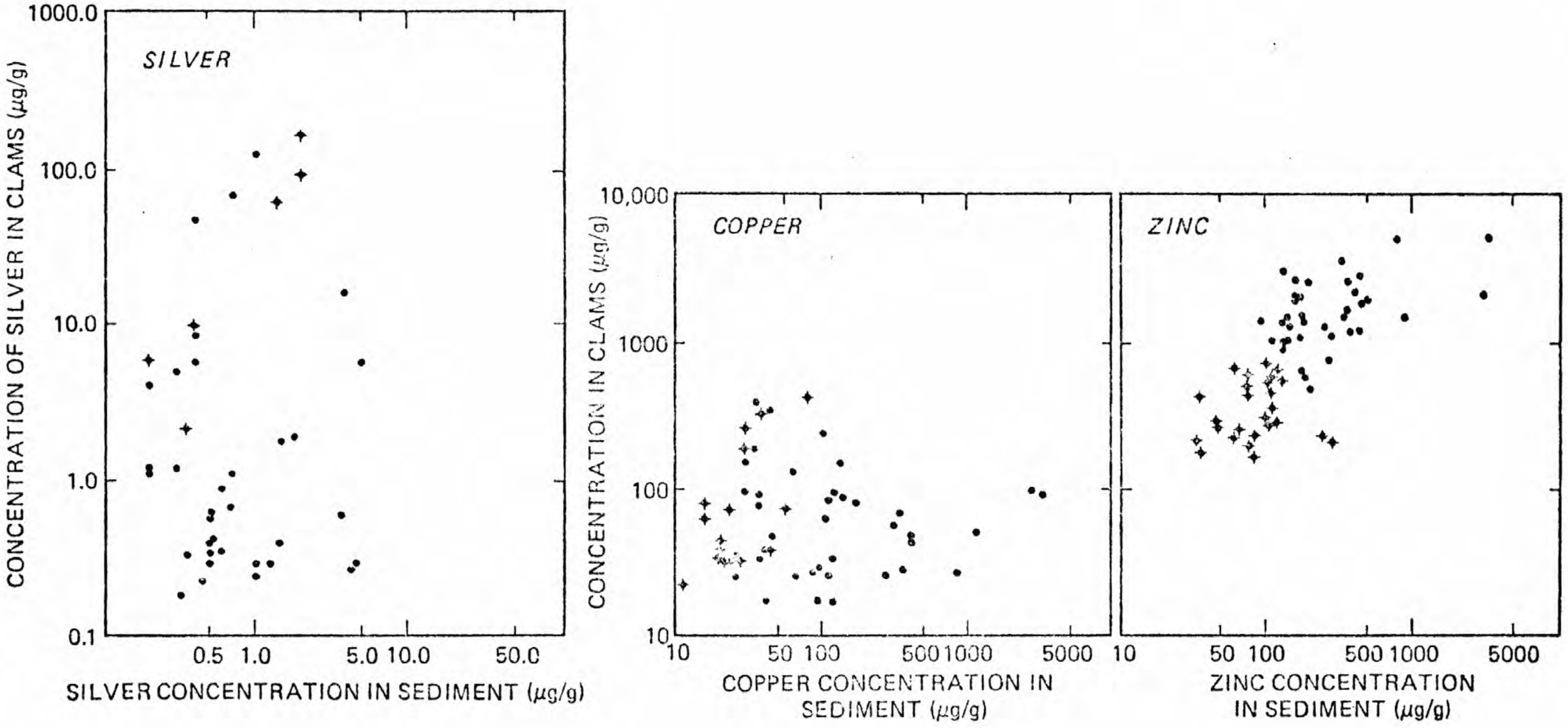
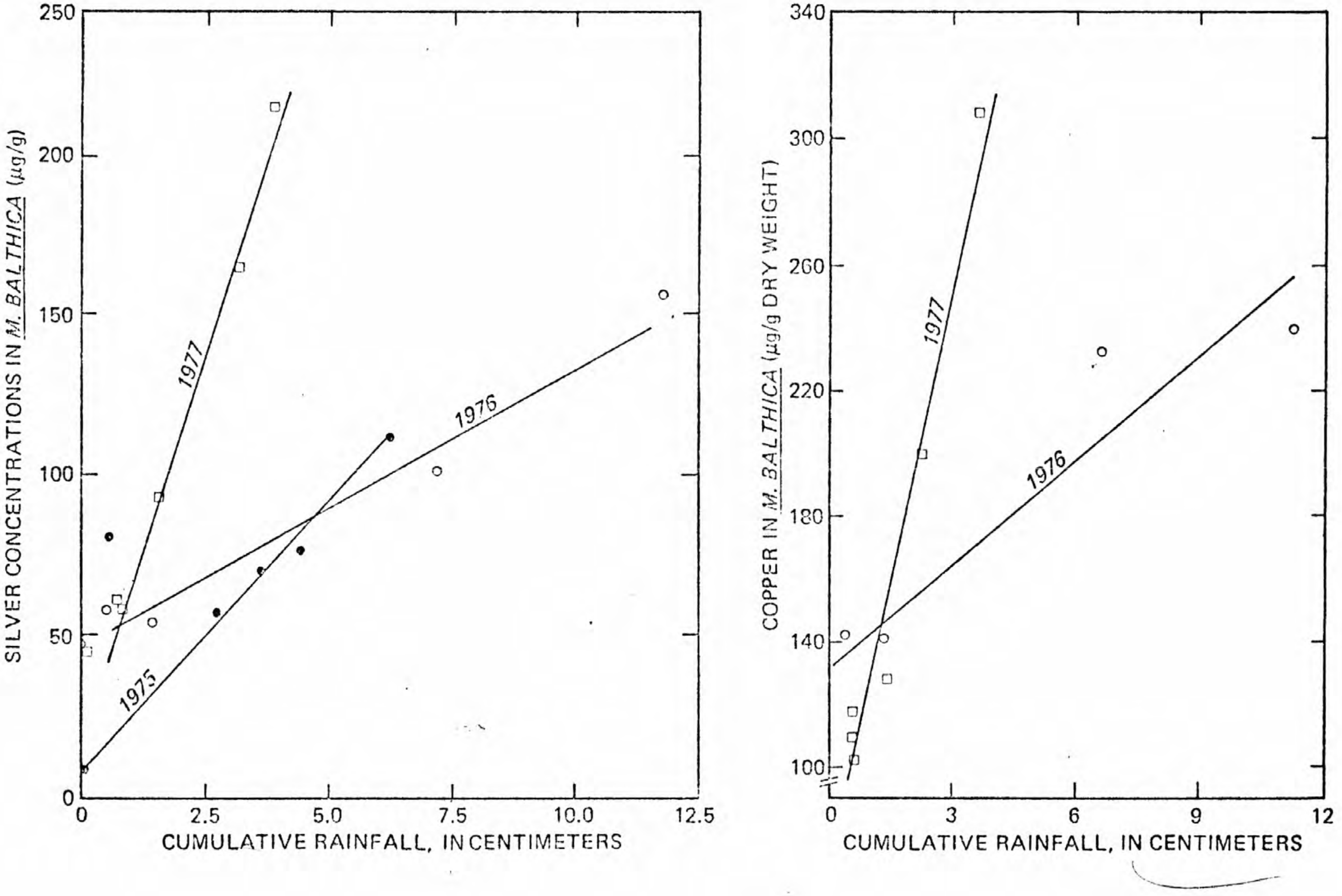

SILVER
1000.0
100.0
10.0
1.0
0.1
CONCENTRATION OF SILVER IN CLAMS (µg/g)
0.5
1.0
5.0
10.0
50.0
SILVER CONCENTRATION IN SEDIMENT (µg/g)
COPPER
10,000
1000
100
10
CONCENTRATION IN CLAMS (µg/g)
10
50
100
500
1000
5000
COPPER CONCENTRATION IN SEDIMENT (µg/g)
ZINC
10
50
100
500
1000
5000
ZINC CONCENTRATION IN SEDIMENT (µg/g)

Fig. 12-6



SILVER CONCENTRATIONS IN M. BALTHICA (µg/g)
250
200
150
100
50
0
0
2.5
5.0
7.5
10.0
12.5
CUMULATIVE RAINFALL, IN CENTIMETERS
1977
1976
1975
COPPER IN M. BALTHICA (µg/g DRY WEIGHT)
340
300
260
220
180
140
100
0
3
6
9
12
CUMULATIVE RAINFALL, IN CENTIMETERS
1977
1976

Fig. 12-7

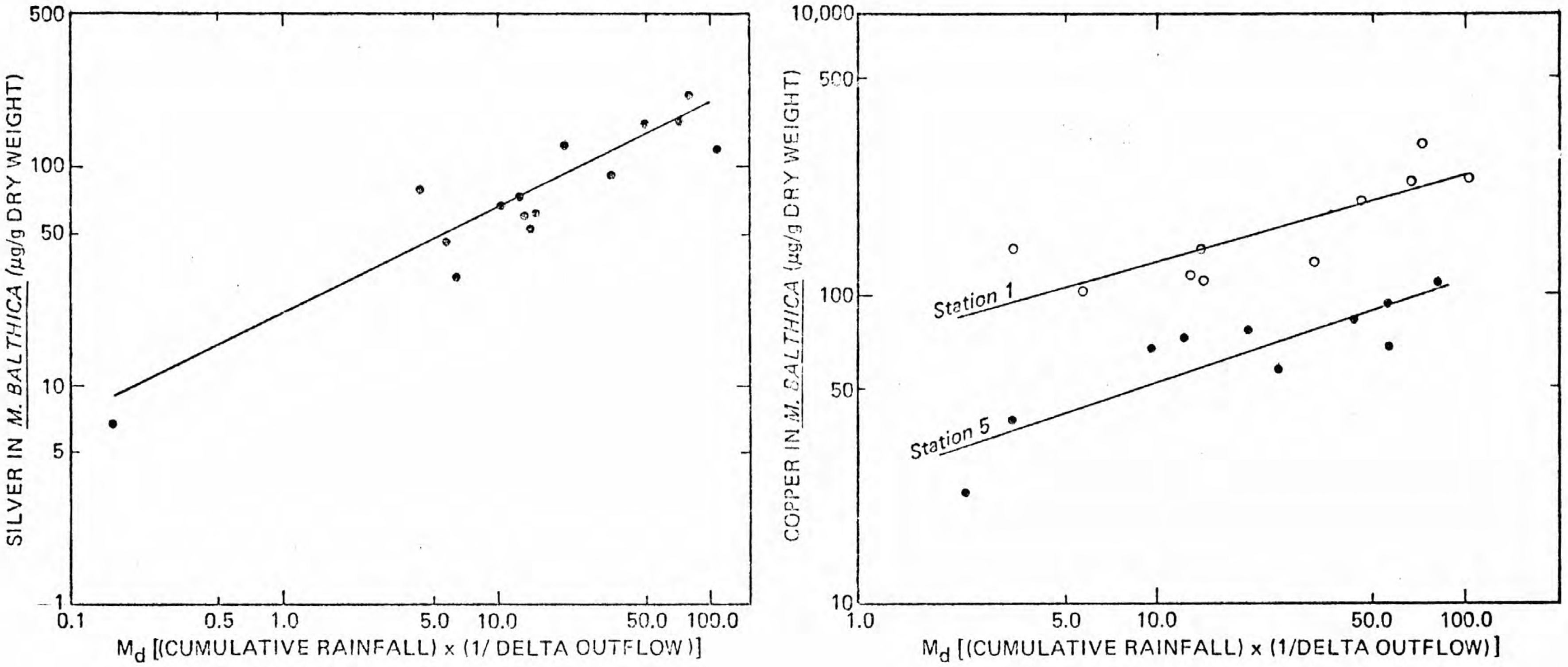
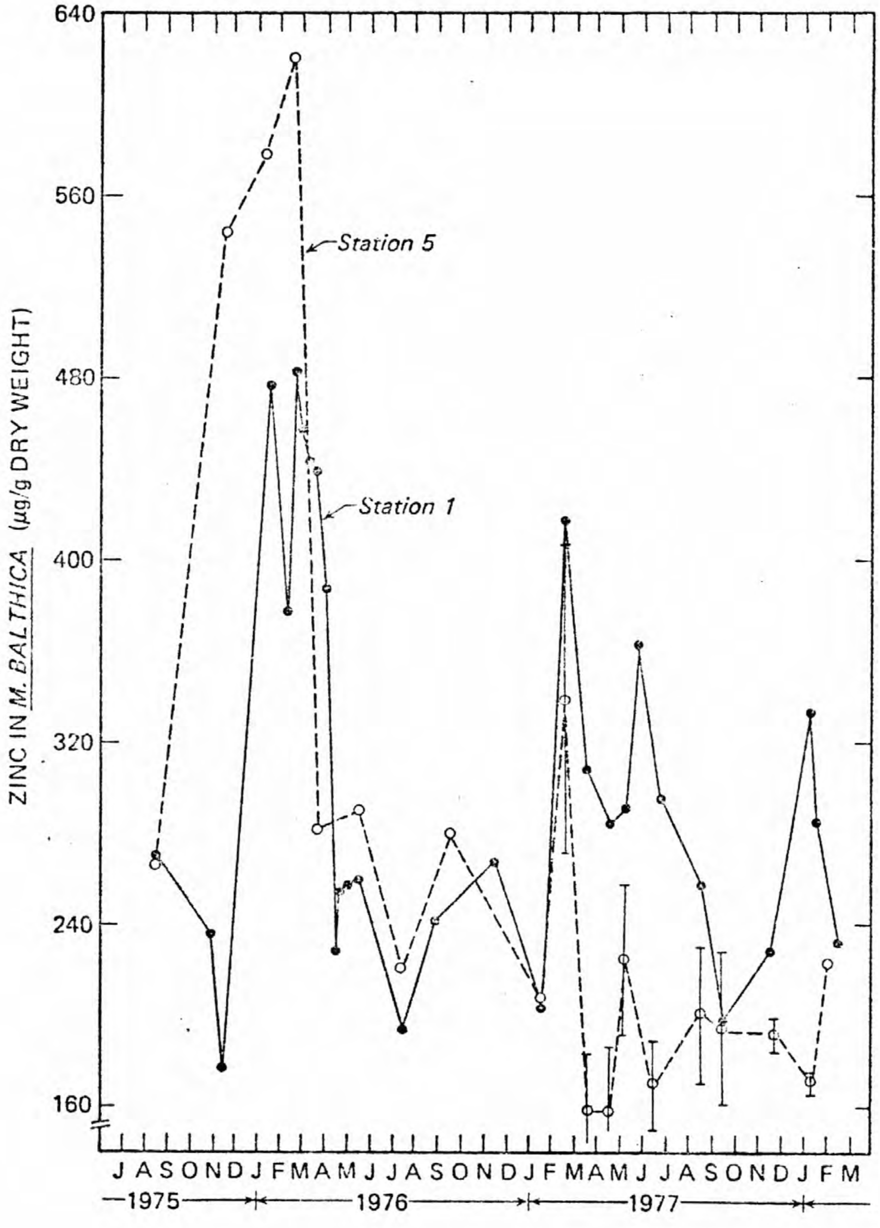

500
100
50
10
5
1
SILVER IN M. BALTHICA (µg/g DRY WEIGHT)
0.1
0.5
1.0
5.0
10.0
50.0
100.0
Md [(CUMULATIVE RAINFALL) x (1/ DELTA OUTFLOW)]
10,000
500
100
50
10
COPPER IN M. BALTHICA (µg/g DRY WEIGHT)
Station 1
Station 5
1.0
5.0
10.0
50.0
100.0
Md [(CUMULATIVE RAINFALL) x (1/DELTA OUTFLOW)]

Fig. 12-8


640
560
480
400
320
240
160
ZINC IN M. BALTHICA (µg/g DRY WEIGHT)
Station 5
Station 1
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M
1975
1976
1977

Chapter 13

Detection of Trace Contaminant Effects: Theory

S.N. Luoma

INTRODUCTION

Two of the most serious gaps in our knowledge of trace contaminant impacts in aquatic environments are (1) the link between the physical-chemical environment and the bioaccumulation of the contaminants and (2) the link between bioaccumulation and toxic effects. The bulk of this report has dealt with the previous problem asking what are the physicochemical and physiological controls on bioaccumulation. In this chapter we propose an indirect method for determining whether accumulated metal has affected a population. In the following chapter we use the method to test for metal effects in San Francisco Bay.

Direct evidence that trace contaminants adversely affect natural populations of aquatic organisms is relatively scarce. Extrapolation of toxicity data to natural systems is limited by the physicochemical complexity of the natural environment and the inherent limitations of toxicity bioassays (Lee 1973). Elevated concentrations of trace contaminants are often observed in ecosystems where the degradation of a biological community is suspected (e.g. Odum et al 1969; Hudson and Sprague 1975). However, proving that trace contaminants cause the observed effects is difficult, as is separation of the relative biological significance of individual contaminants present. In lieu of more direct methods, I propose application of a well-studied toxicological principle as a means of helping determine i) if trace contaminants are affecting organisms in a given situation, and ii) which contaminants are having the more significant effect.

Tolerance to toxicants

The resistance of some insects to both inorganic and organic insecticides is widely documented (Brown 1958; O'Brien 1967). Tolerance to heavy metals has also been studied in terrestrial grasses from polluted areas (McNeilly 1968; Rocovich and West 1975). In both grasses and insects, toxin resistance has similar characteristics: i) resistance results from Darwinian selection for resistant genotypes; it cannot be induced over the lifetime of individuals; ii) physiological mechanisms specific for a single toxicant or group of toxicants are usually involved in resistance, rather than selection for vigorous strains; iii) resistance reduces the overall fitness of the population; when the selective pressure of the toxicant is removed the populations revert, in a few generations, to dominance by intolerant genotypes; and

iv) the degree of tolerance is related to the level of exposure to the toxicant.

Resistance to toxicants has also been reported within aquatic species. Resistance to the insecticide endrin occurs in at least six species of fish (Grant 1976). Resistant strains are found only in areas of known contamination; the level of resistance reflects the degree of contamination; and the mechanism of resistance is specific for insecticides. Resistance to cyclodiene insecticides in populations of mosquito fish also occurs in contaminated areas, but is not present where cyclodienes are not present (Ferguson 1970).

Populations of phytoplankton (*Scenedesmus acutiformis* and *Chlorella fusca*) from metal-contaminated lakes near Sudbury, Ontario, are much more resistant to the toxic effects of copper and nickel than populations of these species from uncontaminated areas (Stokes et al 1973; Stokes 1975). Populations of the polychaete *Nereis diversicolor* from English estuaries contaminated with Cu, Cd, Pb and Zn are more resistant to Cu than are *N. diversicolor* from estuaries less contaminated with the metals (Bryan and Hummerstone 1971; 1973; Bryan 1974). *N. diversicolor* from some, but not all, of the polluted estuaries are also resistant to Zn. No resistance to Cd or Pb is detectable despite high concentrations in sediments. In laboratory studies, resistance to Cu could not be induced within individuals from one generation of *N. diversicolor* collected from an unpolluted estuary.

The range of genotypes expressed in a population from a given location reflects selective pressures against that population. To induce tolerance, a toxicant must be present in biologically available quantities sufficient to limit the reproductive success of a proportion of the individuals in a population (i.e., the non-resistant genotypes). Greater resistance to a toxicant in a population from one location than in a population from a second location is direct evidence the toxicant is exerting selective pressure on the species at the former site. Moreover, resistance to one toxicant, but not to others to which the population is exposed, suggests the former contaminant is the cause of the biological response.

Evidence that a toxicant has exerted selective pressure on one species within a community suggests a strong possibility of toxic effects on other species. Many of the organisms in which resistance is observed (e.g., *Scenedesmus*, *Chlorella*, *Gambusia*) are ecological "opportunists." Such species are characterized by a broader genetic plasticity than species that occupy more specialized niches (Ayla 1968; Grassle and Grassle 1974). The opportunists' ability to survive is enhanced by the variety of genotypes available for colonizing ecologically open space. When a toxicant is introduced into an ecosystem, the probability of an opportunist species developing a resistant population, and surviving, is greater than the probability of a more specialized species exhibiting such resistance. Simplified ecosystems will result when species disappear because they lack contaminant-resistant genotypes. Higher trophic level organisms appear especially susceptible to such effects. Where opportunistic species of fish exhibit resistance to endrin, the number of species in the community is restricted, top carnivores being most commonly absent (Grant 1976). Similarly, predaceous insects are less likely to develop insecticide resistance than are pests (Georghion 1972). The result of insecticide applications is often a simplified insect community, with a reduced capability for internal biological regulation.

Applications

Viewing toxicant resistance as an indicator of selective pressure in an ecosystem may provide a new tool for assessing the causes of simplification in aquatic communities. For example, the absence of toxicant sensitive species in a community may be more convincingly attributed to a toxicant if it has induced resistance in a surviving species. Similarly, the resistance of polychaetes to Cu and occasionally Zn, but never Pb or Cd, in estuaries contaminated by all four metals (Bryan 1974) is a strong suggestion that Cu and Zn are the contaminants of primary concern in these ecosystems. The use of toxicant resistance to relate cause and effect requires comparing toxicity to individuals from a contaminated system with toxicity to individuals of the same species from a less contaminated location. Such comparisons are valid only if individuals of the same life stage are used, and the tests are conducted simultaneously (to avoid temporal effects) under similar physicochemical conditions. Small, random variations in the susceptibility of different populations may make toxicity to highly susceptible genotypes difficult to detect with standard bioassay techniques. However, bioassays can detect general population shifts to resistant genotypes (Ferguson 1970; Bryan 1974; Stokes 1975) and increases in resistance with increasing contaminant concentrations (Grant 1975).

Further study of toxicant resistance in natural systems would enhance the value of its usefulness as a tool in environmental studies. An understanding of the community changes which accompany the resistance of surviving species to specific types of toxicants may facilitate cause and effect analyses, and point to toxicant-sensitive species. Comparisons of the biologically available concentrations at which different contaminants induce the onset of resistance would facilitate a better understanding of the relative toxicity of such contaminants in nature. More knowledge about cross-resistance (the development of resistance to a second toxin by exerting pressure with a first) in aquatic species is also necessary. Some cross-resistance to insecticides has been observed in insects (O'Brien 1967), but cross-resistance to trace metals appears to be rare, at least in plants and polychaetes (McNeill 1968; Bryan 1974).

The use of toxicant resistance to assess contaminant effects has several advantages over direct extrapolation of toxicity data to natural systems. Using in situ population characteristics to indicate effects eliminates the necessity of correlating toxicity and contaminant concentration. While the latter method is preferable where sufficient information is available, attempts at such correlations have been largely unsuccessful due to the physiological and physiochemical complexities of trace contaminant behavior. Similarly, the need to assess such complexities in bioassays is reduced if the tests are used to compare relative susceptibilities rather than absolute levels of toxicity. Direct evidence of toxicant effects may also facilitate natural system studies of the complexities of toxicant behavior.

Determinations of selective pressures on the basis of population characteristics must always be undertaken with caution. However, until contaminant interactions with the physical-chemical-biological environment are better understood, the view of toxicant resistance as a response to such pressure might have an important place in monitoring, studying and interpreting the effects of trace contaminants.

SUMMARY - Chapter 13

If one population of a species is more resistant to a toxicant than are other populations, it is direct evidence that the concentration of the toxicant in the environment of the resistant population is sufficient to elicit biological effects. The presence of a toxicant-resistant population of one species in an ecosystem further suggests that other species may have been affected by the resistance-eliciting substance.

Bioaccumulation and Toxicity to Cu in Macoma balthica: Inter-population Heterogeneity and the Role of Ag.

D.J. Cain and S.L. Luoma

INTRODUCTION

In the previous chapters we demonstrated that Cu concentrations in Scrobicularia plana were statistically associated with concentrations of Ag in the tissues of the bivalve, suggesting a physiological coupling affected uptake of the two metals. We also showed fluctuations of Cu and Ag in Macoma balthica followed closely similar patterns at several stations in San Francisco Bay; also consistent with a physiological couple. The field studies do not prove that a coupling of the metals exists since statistical relationships do not prove cause and effect. In this chapter we begin to test the hypothesis that Ag concentrations in Macoma affect Cu uptake and toxicity.

We have also suggested that enhanced tolerance to a toxin in a population of organisms proves that the toxin is affecting that population. In the process of testing the hypothesis concerning Cu-Ag coupling we compare tolerances to Cu among 3 populations of Macoma in San Francisco Bay, to test for metal effects in the severely contaminated station near Palo Alto.

MATERIAL AND METHODS

Macoma balthica were collected from three intertidal mudflats of San Francisco Bay between February and July 1979. Each sample consisted of approximately 100 individuals representing a range of sizes characteristic of the population.

The clams were held with sediment in 25 0/00 seawater at 12° C for 3-4 days, then depurated for 2 days in clean seawater prior to experimentation. The clams were separated into size classes by shell length, and an equal number of individuals in each size class was distributed among a control and the treatment solutions.

All experiments were run at 12° C in 25 0/00 seawater adjusted to pH 7.9 ± 0.2. Between 15 and 20 animals were immersed in 2 liters of water containing Cu concentrations ranging from 10 μg/l to 1900 μg/l. The latter solution was saturated with Cu. Water was replaced daily in the experimental solutions.

The condition of the clams and the proportion of animals with protruded siphons were recorded once or twice daily. Dead animals were immediately removed from the aquaria. After eleven days of exposure the surviving animals were removed and pooled into 1 mm size classes for removal of soft tissues and analysis, as described in previous chapters.

RESULTS

Concentrations of Cu and Ag in the tissues of Macoma collected from Palo Alto were considerably higher than concentrations in animals from either Hayward or West Pinole (Table 14-1). The Hayward and West Pinole animals contained similar burdens of Cu, but concentrations of Ag at Hayward were approximately 10 fold higher than at West Pinole.

Animals collected from the West Pinole population appeared to be most sensitive to copper toxicity while the Palo Alto animals were least sensitive. (Table 14-2).

Hayward animals were intermediate in their response to the copper.

Uptake by (the final tissue concentration minus concentration of the control) each population is presented in Figure 14-1. Hayward and West Pinole animals were quite similar for all treatments. By comparison, bioaccumulation was greater in the Palo Alto animals at Cu concentrations greater than 50 ppb.

Macoma reacted behaviorally to Cu concentrations of 100 ppb or greater by retracting their siphons and closing their valves in the initial days of the experiment. The animals generally opened after a couple of days, stimulated, probably by the need to respire aerobically. To account for this behavioral response, the bioaccumulation data were normalized by dividing by the average percentage of animals open during the sensitive period. The sensitive period was defined as the time during which less than 50% of the animals in the aquaria were feeding. When the data were normalized bioaccumulation was a more linear function of the concentration of (Fig. 14-2) Cu in solution. The greater bioaccumulation in the Palo Alto animals than in West Pinole and Hayward animals was also more striking.

DISCUSSION

Bioaccumulation rates and tolerance to Cu were highest in the population of *Macoma* from the most highly contaminated area. Susceptibility to Cu poisioning was inversely related to bioaccumulation, since the most tolerant population of animals accumulated the greatest burden of Cu at a given level of exposure. Bioaccumulation rates were similar between West Pinole and Hayward, but animals from West Pinole displayed greater sensitivity to Cu. Cu concentrations in *Macoma* at the two stations were comparable, but the concentration of Ag was roughly an order or magnitude higher at Hayward than at West Pinole. The tolerance to Cu observed at Palo Alto could reflect effects of exposure to either Cu or Ag, since concentrations of both are considerably higher than at Hayward or West Pinole. However, the difference in sensitivity between Hayward and West Pinole follows only the difference in concentrations of Ag in the two populations. Bryan and Hummerstone (1973a) suggested the possibility of co-tolerance between Cu and Ag in the polychaete *Nereis diversicolor*. Our data suggested relatively small exposures to Ag may greatly affect the sensitivity to Cu of different populations of *Macoma*.

Direct tests of the effect of different exposures to Ag on Cu uptake, and of the physiological mechanisms involved in the Cu-Ag interactions are in progress.

Table 14-1. Concentrations of Cu and Ag in *Macoma balthica* from 3 stations in San Francisco Bay

	Cu (μg/g)	Ag (μg/g)
Palo Alto	148±19	57±6
Hayward	33±4	4±0.4
West Pinole	59±9	0.76±0.07

Table 14-2 Percent mortality in three populations of *Macoma balthica*

Cu in seawater	Palo Alto	Hayward	West Pinole
10			
50			36
100		32	31
500		54	100
1000	44	50	100
1900	86		

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Figure 14-1

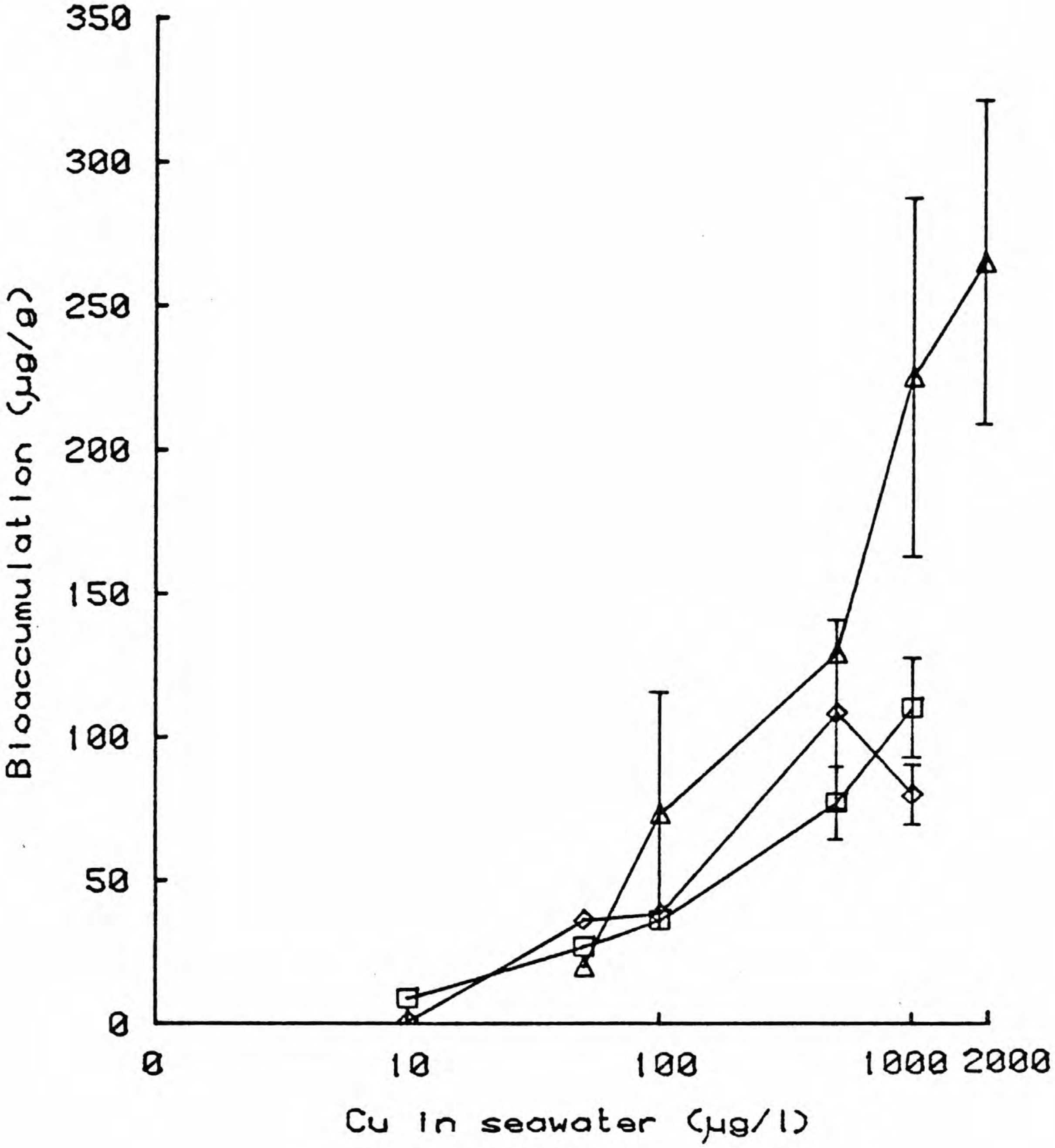
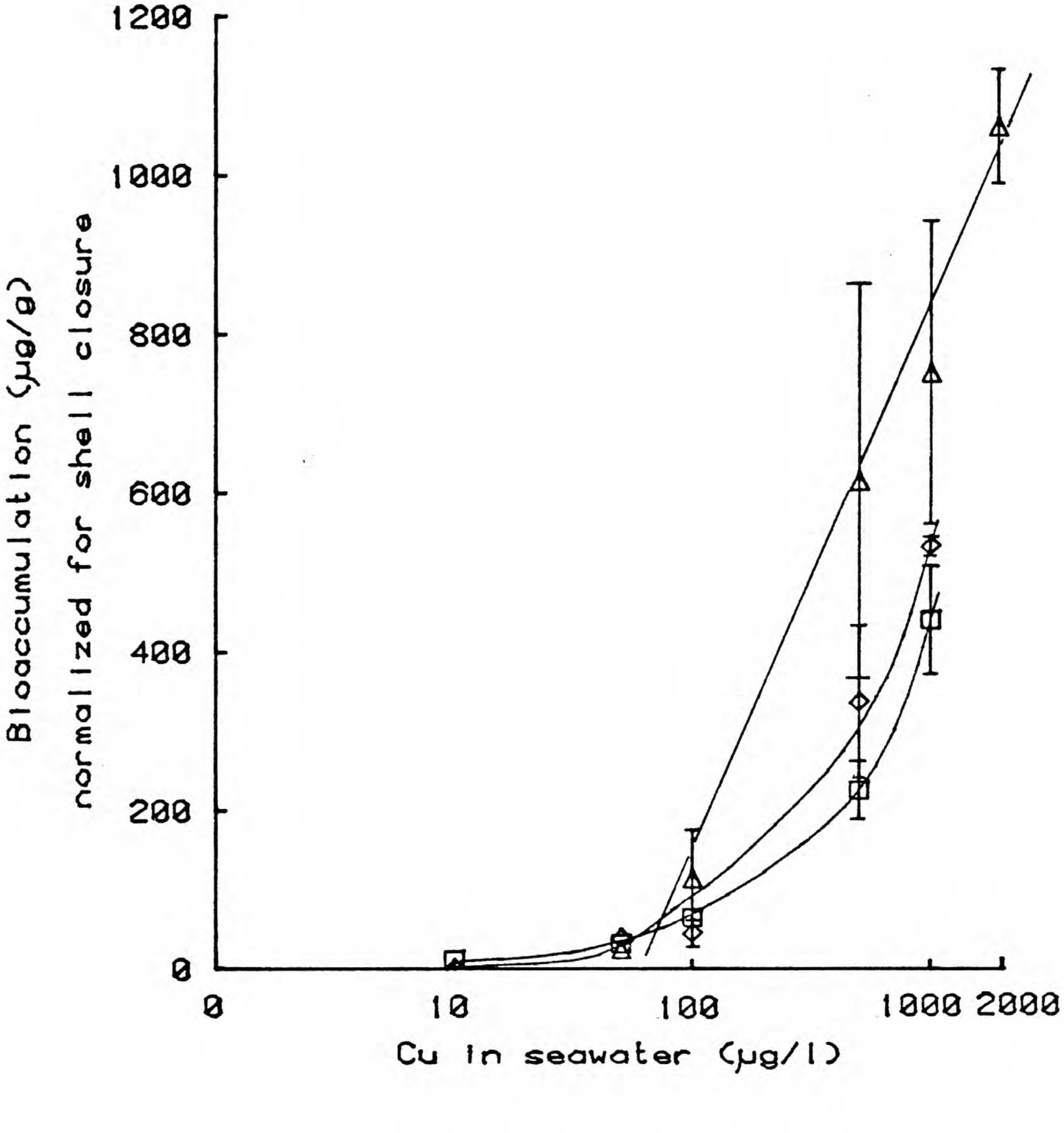

350
300
250
200
150
100
50
0
Bioaccumulation (µg/g)
0
10
100
1000
2000
Cu in seawater (µg/l)

Figure 14-2


1200
1000
800
600
400
200
0
Bioaccumulation (μg/g)
normalized for shell closure
0
10
100
1000
2000
Cu in seawater (μg/l)

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