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An Ion Exchange Column-Equilibrium Procedure to Measure Biologically Effective Metal Concentrations in Seawater

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AN ION-EXCHANGE COLUMN-EQUILIBRIUM PROCEDURE TO MEASURE BIOLOGICALLY EFFECTIVE METAL CONCENTRATIONS IN SEAWATER

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TABLE OF CONTENTS

		Page
SUMMARY		viii
A.	**INTRODUCTION**	1
1.0	BACKGROUND INFORMATION	2
2.0	THEORY	8
B.	**PHASE I - DEVELOPMENT OF THE ION-EXCHANGE PROCEDURE**	13
1.0	PROCEDURE OVERVIEW	13
2.0	MATERIALS AND METHODS	16
2.1	Reagents and General Procedures	16
2.2	Natural Seawater Collection	19
2.3	Ion-Exchange Analysis	19
2.3.1	Equipment Description	19
2.3.2	Resin Preparation	21
2.3.3	Determination of Metal Concentrations in the Column Eluates	22
2.3.4	Detection Limits	23
3.0	RESULTS AND DISCUSSION	24
3.1	Procedure Development in Artificial Seawater	24
3.1.1	Elution Experiments	24
3.1.2	Equilibration Experiments	29
3.1.3	Sorption Curves	34
3.1.4	Experiments Using EDTA as a Complexing Agent	36
3.1.5	Metal-Metal Interaction	43
3.1.6	Precision of the Ion-Exchange Analysis	45
3.2	Procedure Development in Natural Seawater	49
3.2.1	Flow Rate	49
3.2.2	Method of Resin Equilibration	51
3.2.3	Resin Crosslinkage	52
3.2.4	Resin Batch Experiments	54

TABLE OF CONTENTS (Continued)

		Page
C.	**PHASE II - OYSTER EMBRYO AND ALGAL ASSAY EXPERIMENTS**	59
1.0	INTRODUCTION	59
2.0	MATERIALS AND METHODS	60
2.1	Natural Seawater Preparation	60
2.2	Ion-Exchange Procedure	60
2.3	Oyster Embryo Assays	63
2.3.1	Oyster Conditioning	63
2.3.2	Oyster Embryo Stock Preparation	64
2.3.3	Oyster Embryo Assay Procedure	65
2.3.4	Data Analysis	66
2.4	Algal Assays	66
2.4.1	Nutrient Stock Preparation	66
2.4.2	Algal Stock Preparation	68
2.4.3	Algal Assay Procedure	69
2.4.4	Data Analysis	69
3.0	RESULTS AND DISCUSSION	70
3.1	Oyster Embryo Assays	70
3.1.1	Experimental Design	70
3.1.2	Toxicity Experiments	73
3.1.3	Ion-Exchange Results	81
3.1.4	Comparison of Oyster Assay Ion-Exchange Results	87
3.2	Algal Assays	93
3.2.1	Toxicity Curves	93
3.2.2	Toxicity Experiments	97
3.2.3	Ion-Exchange Results	105
3.2.4	Comparison of Algal Assay and Ion-Exchange Results	110
D.	**RECOMMENDATIONS FOR FUTURE STUDIES**	116
E.	**REFERENCES**	117

APPENDIX I Chelex Preparation
APPENDIX II Atomic Absorption Spectrophotometry Operating Conditions
APPENDIX III Determination of EMC Values
APPENDIX IV Oyster Embryo Assay Results
APPENDIX V Algal Assay Results

LIST OF TABLES

		Page
Table 1	Salt Concentration in SOW 35°/oo	17
Table 2	Composition of SOW Diluted to 25°/oo	18
Table 3	Compounds Tested as Eluents	25
Table 4	Results from an Experiment Using EDTA as the Eluent	27
Table 5	The Effect of EDTA on the Sorption of Metal	41
Table 6	Complexation of Metal with EDTA as Predicted by the Ion-exchange Technique and MINEQL	44
Table 7	Effect of Increasing Pb Concentration on the Sorption of Cd, Cu and Mn	46
Table 8	Effect of Increasing Cd and Mn Concentrations on the Sorption of Cu and Pb	47
Table 9	Precision of the Ion-Exchange Procedure	48
Table 10	The Effect of Flow Rate on the Sorption of Cu	50
Table 11	The Effect of Equilibration Methods on the Sorption of Cu	53
Table 12	The Effect of Resin Crosslinkage on the Sorption of Cu	56
Table 13	Seawater Characteristics	61
Table 14	Nutrient Stock and Final Concentrations	67
Table 15	Results of the Ion-Exchange Analyses for Copper	82
Table 16	Results of the Ion-Exchange Analyses for Cadmium	83
Table 17	Results of the Ion-Exchange Analyses for Zinc	84
Table 18	Results of the Ion-Exchange Analyses for Lead	85

LIST OF TABLES (Continued)

		Page
Table 19	Results of the Ion-Exchange Analyses for Lead	106
Table 20	Results of the Ion-Exchange Analyses for Cadmium	107
Table 21	Results of the Ion-Exchange Analyses for Copper	108
Table 22	Results of the Ion-Exchange Analyses for Zinc	109

LIST OF FIGURES

		Page
Figure 1	Possible Chemical Forms of Heavy Metals in Seawater	4
Figure 2	Ion-Exchange Apparatus	20
Figure 3	Absorbance Readings of 100 nM Cu in Five Concentrations of EDTA	28
Figure 4	Equilibrium Curve for Mn and Pb	30
Figure 5	Equilibrium Curve for Cd	31
Figure 6	Equilibrium Curve for Cu with Sample Volumes to 500 mL	32
Figure 7	Equilibrium Curve for Cu with Sample Volumes to 800 mL	33
Figure 8	Equilibrium Curve for Cu Using Resin in the Hydrogen Form	35
Figure 9	Sorption Curve for Mn	37
Figure 10	Sorption Curve for Cd	38
Figure 11	Sorption Curve for Cu	39
Figure 12	Sorption Curve for Pb	40
Figure 13	Equilibrium Curve for Cu using AG 50W X-4	55
Figure 14	Sorption Curves for Cu using Different Resin Batches	58
Figure 15	Seawater Preparation Scheme	62
Figure 16	Abnormal Development of C. gigas Embryos of Different Spawns Resulting from Exposure to Total Added Cadmium for 48 h.	72
Figure 17	Log-Probit Graph to Determine the LC_{50} of C. gigas Embryos Exposed to Copper for 48 h.	74

LIST OF FIGURES (Continued)

		Page
Figure 18	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus the Total Added Copper Concentration in the Presence of EDTA.	75
Figure 19	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus the Total Added Cadmium Concentration in the Presence of EDTA.	77
Figure 20	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus the Total Added Zinc Concentration in the Presence of EDTA.	79
Figure 21	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus the Total Added Lead Concentration in the Presence of EDTA.	80
Figure 22	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus EMC Values for Zinc.	88
Figure 23	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus EMC Values for Lead.	90
Figure 24	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus EMC Values for Copper.	91
Figure 25	Abnormal Embryonic Development (% Net Risk) of C. gigas Versus EMC Values for Cadmium.	92
Figure 26	Response of T. pseudonana to Copper	94
Figure 27	Response of T. pseudonana to Lead	95
Figure 28	Response of T. pseudonana to Cadmium	96
Figure 29	Response of T. pseudonana to Zinc	98
Figure 30	Cell Yield (% of Control) of T. pseudonana Versus the Total Added Copper Concentration in the Presence of EDTA	99

LIST OF FIGURES (Continued)

		Page
Figure 31	Cell Yield (% of Control) of T. pseudonana Versus the Total Added Lead Concentration in the Presence of EDTA	101
Figure 32	Cell Yield (% of Control) of T. pseudonana Versus the Total Added Cadmium Concentration in the Presence of EDTA	102
Figure 33	Cell Yield (% of Control) of T. pseudonana Versus the Total Added Zinc Concentration in the Presence of EDTA	104
Figure 34	Cell Yield (% of Control) of T. pseudonana Versus EMC Values for Copper	111
Figure 35	Cell Yield (% of Control) of T. pseudonana Versus EMC Values for Lead	112
Figure 36	Cell Yield (% of Control) of T. pseudonana Versus EMC Values for Zinc	113
Figure 37	Cell Yield (% of Control) of T. pseudonana Versus EMC Values for Cadmium	115

SUMMARY

An ion-exchange column-equilibrium procedure capable of measuring biologically active heavy metal concentrations in seawater was developed and tested with Cd, Cu, Pb and Zn. The procedure was based on the assumption that the amount of heavy metal sorbed by a cation-exchange resin of the sulfonic type was related to the free metal cation concentrations in solution. A new term, the Effective Metal Concentration (EMC), was derived from the ion-exchange procedure and was equated to the truly dissolved inorganic metal concentration in a sample. It was the EMC value that was believed to be proportional to the biological active metal fraction in solution.

A series of experiments was conducted to determine the optimal operating conditions for the ion-exchange procedure. The sorption of metals by the resin was affected by a number of factors including flow rate of solution, method of resin prepartion, resin crosslinkage, and batch of resin used. Although variability in the sorption characteristics of the resin was observed between experiments, variability within an experiment was minimal.

Oyster embryo (Crassostrea gigas) and algal (Thalassiosira pseudonana) assays were conducted to generate toxicity data to compare with EMC values determined by the ion-exchange procedure. Experiments of a 6 x 4 factorial design using six metal concentrations and four EDTA concentrations per assay were performed. EDTA was used to vary the free metal ion concentration of a sample (thus its toxicity) while maintaining the total metal concentration constant.

A poor relationship was found between toxicity and total added metal concentrations for Cd, Cu, Pb and Zn when EDTA was added to the samples. In contrast, when the same toxicity data was plotted as a function of EMC, a much

stronger relationship was observed for Cu, Pb and Zn, indicating a proportionality between EMC and the toxic metal fraction. This was evident in both the oyster embryo and algal assay experiments. The EMC values for Cd, however, did not show such a strong relationship to toxicity in either the oyster or algal assays. Although not conclusive, adsorption of the Cd-EDTA complex by the resin appeared to be occurring. This resulted in EMC being an overestimation of the true toxic metal fraction.

A. INTRODUCTION

Envirocon Pacific Ltd. was retained on February 4, 1985 by the Environmental Protection Service and the National Research Council to conduct a research program to develop and test an analytical technique to estimate biologically active heavy metal concentrations in seawater. The proposed technique used an ion-exchange resin and was based on the assumption that the amount of heavy metal sorbed by a strongly acidic cation-exchange resin was related to the free metal ion concentration in solution. The application of this approach to seawater was first described by Zorkin (1983) for copper.

The research program was divided into two Phases. Phase I involved modifying the single-element ion-exchange procedure of Zorkin (1983) to provide a method suitable for multi-element analysis. The procedure was first tested in artificial seawater with cadmium, copper, lead manganese and zinc. Cadmium, copper, lead and zinc were chosen because of their environmental importance and toxic nature, while manganese was chosen because it is known to affect copper toxicity (Sunda and Huntsman, 1983).

The primary objective of Phase II was to demonstrate the biological significance of the ion-exchange measurement. Oyster embryo (Crassostrea gigas) and algal (Thalassiosira pseudonana) assay procedures were employed to provide toxicity data that could be compared to results of the ion-exchange analyses.

The following report provides a description of the ion-exchange procedure and the experiments conducted during the development and testing of this procedure.

1.0 BACKGROUND INFORMATION

Although heavy metals are required in trace quantities for the growth and sustenance of marine organisms, at elevated levels, metals such as Cu, Pb and Zn are toxic. The toxicity of a metal is governed not by its total concentration in solution but rather its chemical form. Of all the metal species possible in seawater, it appears that, for a number of metals, the free hydrated metal ion is the most biologically active.

A strong relationship between free metal ion activity* and toxicity has been demonstrated for a number of organisms, including bacteria (e.g. Sunda and Gillespie, 1979; Sunda and Ferguson, 1983), algae (e.g. Sunda and Guillard, 1976; Anderson and Morel, 1978; Rueter and McCarthy, 1979; Guy and Kean, 1980), zooplankton (e.g. Andrew et al., 1977; Young et al., 1979) and fish (e.g. Waiwood and Beamish, 1978; Howarth and Sprague, 1978; Chakoumakos et al., 1979). Canterford and Canterford (1980) studied the influence of Zn, Cd, Pb, Hg, Ag and Tl on algal growth and found that metal toxicity was correlated to the free metal ion concentration and not the total metal concentration. A number of studies using model ligands such as EDTA or NTA to control metal speciation have shown that the concentration of the free metal ion rather than the total concentration of the metal best relates to the toxic response in algae (Sunda and Guillard, 1976; Anderson and Morel, 1978; Allen et al., 1980). Cadmium toxicity to grass shrimp was found to be influenced by the free Cd ion concentration (Sunda et al., 1978), while the accumulation of Cu and Zn by adult oysters was found to be reduced in the presence of NTA and EDTA (Zamuda and Sunda, 1979; Harrison, 1982), implying that the dissolved inorganic fraction controlled the accumulation rate.

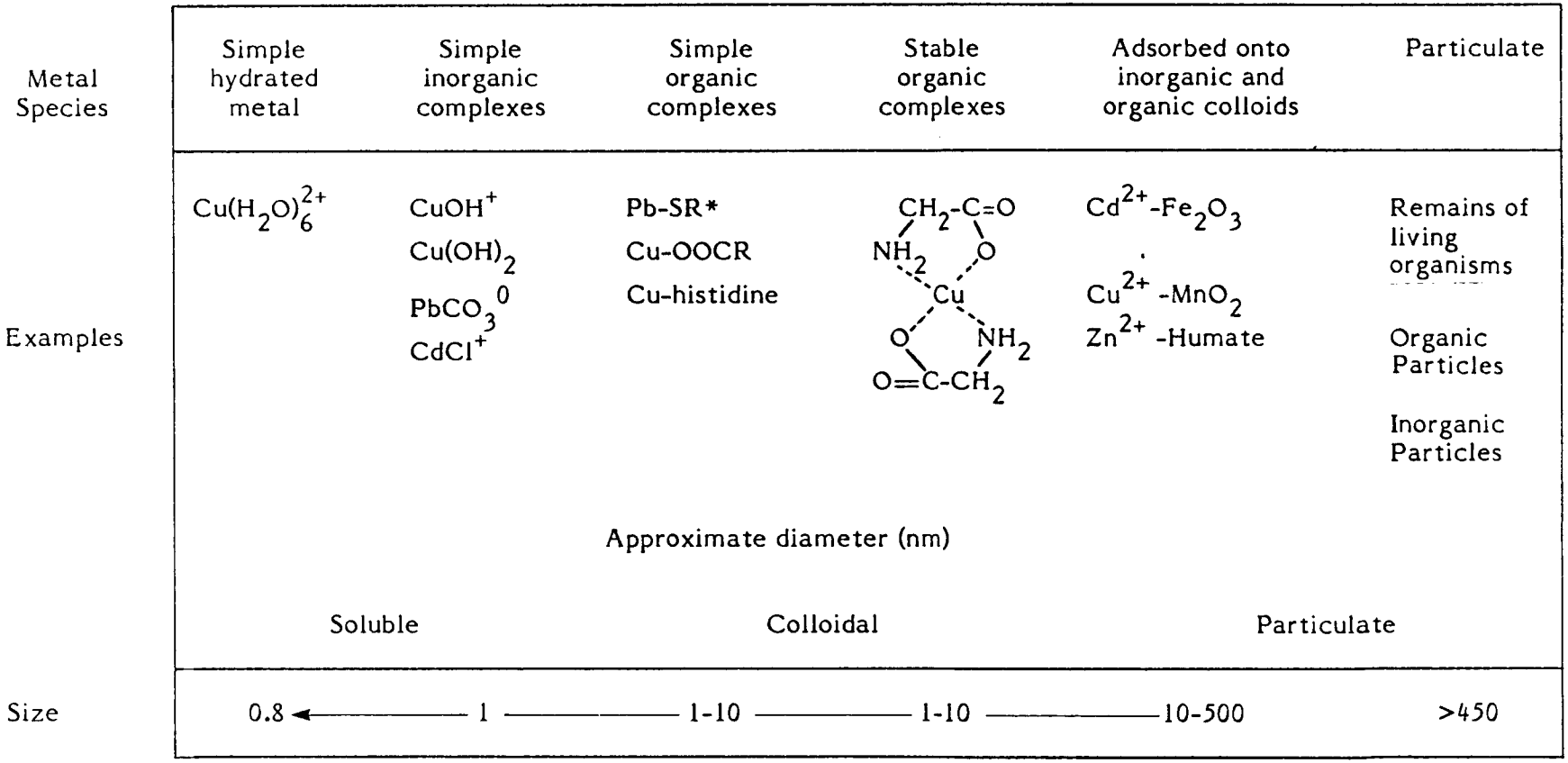
* activity is concentration corrected for the ionic strength of the medium.

Although the free metal ion appears to be the most biologically active metal form its measurement is extremely difficult. This is due to the low concentration levels encountered in natural sea waters and the complex chemistry of seawater. Before discussing the methods presently available for studying metal speciation, a brief overview of heavy metal speciation in seawater will be given.

The chemical forms of heavy metals in seawater can be broadly categorized into particulate, colloidal and dissolved species. Particulate metals are retained by a filter with a 0.45 um pore size while colloidal and dissolved species pass through. Particulate metals usually consist of metal bound to or incorporated into matter such as remains of living organisms. Colloidal metals generally consist of metals adsorbed onto matter such as clay minerals and Fe and Mn oxides. The dissolved fraction is subdivided into 1) simple hydrated ions, 2) metal ions complexed with inorganic anions such as OH^- and CO_3^{2-} and 3) metal ions complexed by organic ligands such as humic and fulvic acids (Figure 1). Since considerable evidence indicates that it is the dissolved inorganic metal fraction which affects aquatic organisms, the dissolved forms of the biologically important metals such as Cd, Pb, Cu and Zn are of particular interest.

In seawater, Cd is thought to be present in the dissolved form mostly as the chlorocomplexes ($CdCl_2$, $CdCl^+$) (Sunda, 1984), while the other inorganic species, including the free Cd^{2+} ion constitute only about 3% of the dissolved fraction (Nurnberg, 1983). In coastal seawater, Cd was reported to be neither associated with colloidal compounds nor significantly bound to organo-metallic compounds (Hasle and Abdullah, 1981; Sunda, 1984).

Dissolved Pb is primarily found in seawater as carbonate complexes (e.g. $PbCO_3$). The species $PbOH^+$ and $PbCl_2$ are also present though with the free hydrated metal ion, contribution is less than 2% of the dissolved metal



*R = Organic molecules

FIGURE 1. Possible Chemical Forms of Heavy Metals in Seawater

concentration (Nurnberg, 1983). Hasle and Abdullah (1981) found little or no association of Pb with colloids, but found extensive association of Pb with organic matter in coastal seawater.

The predominant dissolved inorganic forms of Cu are thought to be $Cu(OH)_2$ and $CuCO_3$ (Florence and Batley, 1980). The association of Cu with organic matter and colloids in coastal seawater has been found to be highly variable, though up to 74% of dissolved Cu has been reported to be associated with high molecular weight organic complexes (Hasle and Abdullah, 1981).

Dissolved inorganic Zn seems to exist mainly as hydrated Zn^{+2} ions and chlorocomplexes (Florence and Batley, 1980; Sunda, 1984). There is some confusion about dissolved Zn concentrations and speciation, however, primarily due to problems associated with Zn contamination.

Although numerous methods are available to study metal speciation, most measure a class of metal species based on some physical or chemical property and few are species specific, particularly for the free metal ion. Generally, two approaches have been taken. Metal speciation can be estimated by theoretical computer models or by direct chemical analysis of water samples. Computer models have only limited application in natural seawater because the nature and concentration of naturally occurring complexing agents are generally unknown.

The speciation of a metal estimated through chemical analysis is generally by direct measurement or after some pretreatment step. Although many instrumental techniques are capable of measuring dissolved metal levels directly (e.g. neutron activation, atomic absorption, x-ray fluorescence), such methods give only total metal concentrations and provide no information on particular forms of metal. Ion-selective electrodes (ISE) and anodic stripping voltammetry (ASV), by contrast, are techniques whose response is dependent on the metal's

speciation. Ion-selective electrodes can measure the activity of the free metal ion without affecting the solution equilibria, but their use is limited because of the low sensitivity at metal concentrations found in natural seawater. Anodic stripping voltammetry has been used to estimate metal speciation in seawater (e.g. Nurnberg and Valenta, 1983) because of its high sensitivity and its ability to measure the ASV-labile (free metal ion plus the metal complexes which will dissociate at the Hg interface) species of metals such as Cd, Cu, Pb and Zn in one analysis. However, surface-active compounds in natural seawater samples (e.g. humic and fulvic acids) may adsorb onto the Hg film and interfere with the analysis. Moreover, the ASV-labile fraction cannot be directly related to the free metal ion concentration as complexes having a low stability with the metal dissociate at the electrode surface and thus contribute to the analytical signal.

A number of chemical or physical separation techniques prior to analysis of the sample by analytical methods such as ASV or atomic absorption spectroscopy have been developed. Molecular size separation is often used to divide the dissolved metal fraction into the molecular and colloidal species. Ultrafiltration using membranes with pore diameters ranging from 1 to 15 nm (Hoffman et al., 1981), and dialysis membranes have been used to separate ionic species and soluble complexes from colloidal species (Benes, 1980; Hart and Davies, 1977, 1981). However, filtration and dialysis membranes are often contaminated with trace metals and organic matter and must be rigourously cleaned prior to use. Other disadvantages include the adsorption of some charged metal species onto the membrane and the dissociation of metal complexes at the membrane surface. Less used separation procedures based on molecular size include centrifugation (Benes and Steinnes, 1975), ion exclusion chromatography (Steinberg, 1980) and reverse-phase liquid chromatography (Mills and Quinn, 1981).

Separation methods based on the charge of the metal species have also been used. Chelating resins such as Chelex-100 strongly bind ionic metal while large

molecules and colloids are excluded and not retained on the column. Batley and Florence (1976) and Batley and Gardner (1978) defined seven classes of metal species depending on their ability to adsorb onto Chelex-100 before and after the oxidation of organic matter. However, Chelex-100 dissociates metal-ligand complexes present in natural seawater and thus would overestimate the amount of free metal ion in solution. It is more often used to pre-concentrate metals for total metal analysis than for measurement of specific metal species.

Thiol resins, which contain sulfhydryl functional groups, have been stated to estimate biologically active metal concentrations (Florence, 1982). However, these resins are found to bind metals similar to Chelex-100 and thus were not suitable for application to the measurement of free metal ion concentrations.

Strongly acidic cation (Shuman and Dempsey, 1977) and strongly basic anion (Koide et al., 1984) exchange resins have also been used to study metal speciation. These resins do not demonstrate such a strong binding for metals as Chelex-100 or thiol resins and generally adsorb only ionic metal forms. As a result, they are less likely to dissociate metal-ligand complexes to the extent of chelating resins. Cation-exchange resins have been used to measure free metal ion activities in complex media such as milk (Christianson et al, 1954; Pearce and Creamer, 1974), and to estimate free Ni ion concentrations in sewage samples (Cantwell et al., 1982).

It is the strongly acidic cation exchange resins that are believed to provide the best opportunity to determine free metal ion concentrations in seawater.

2.0 THEORY

The ion-exchange procedure is based on the assumption that the amount of metal sorbed to a strongly acidic cation-exchange resin can be related to the free metal ion concentration in solution. The following section describes the theoretical basis for this assumption. Although copper is used in this discussion, it is representative of a number of divalent heavy metals including cadmium, lead and zinc.

When a cation-exchange resin is placed in contact with a solution containing a divalent transition metal ion such as Cu^{2+}, an exchange reaction occurs that, assuming that the resin is initially loaded with Na^+ ions, can be represented by the equation

$$Cu^{2+} + 2\,\overline{Na}^{+} \rightleftharpoons 2\,Na^{+} + \overline{Cu}^{2+}$$

where the overbar indicates ions bound in the resin phase. Applying the law of mass action to this reaction,

$$K_{Cu,Na} = \frac{\{Na^+\}^2\ \{\overline{Cu}^{2+}\}}{\{\overline{Na}^{+}\}^2\ \{Cu^{2+}\}} = \frac{[Na^+]^2\ [\overline{Cu}^{2+}]}{[\overline{Na}^{+}]^2\ [Cu^{2+}]} \cdot \frac{f_{Cu}\ \gamma_{Na}^2}{f_{Na}^2\ \gamma_{Cu}} \qquad (1)$$

where the curly and square brackets represent, respectively, activities and concentrations, and f and γ are the activitiy coefficients of the ions in, respectively, the resin and solution phases. All applications considered here assume that the resin used is of the strongly acidic type and it achieves equilibrium with the solution phase.

Seawater essentially has a constant relative composition, that is the concentration of each of the major consituents varies with the sample salinity, the latter being a quantity representative of the total amount of dissolved solids

in g/kg solution. In seawater having an average salinity of 35 g/kg, the summed concentration of the major cations is about 0.6 eq/kg whereas that of trace metals such as Cu^{2+} is less than 1 μeq/kg. Hence, after equilibrating a resin with seawater as indicated above, the number of exchange sites occupied by major seawater cations greatly exceeds that occupied by trace metal cations, and any exchange of Cu^{2+} for major seawater cations due to a change in dissolved Cu concentration will not significantly alter the bulk composition of the resin phase. As a result, the activity coefficients of the ions in the resin phase will remain constant as long as the sample salinity, which controls the concentration of major ions, and the pH, which controls the concentration of complex forming bases such as hydroxides and carbonate, remain constant. Rewriting equation (1) in the form

$$\lambda_o = [\overline{Cu^{2+}}] \; / \; [Cu^{2+}] = K_{Cu,Na} \cdot \frac{[Na^+]^2}{[Na^+]^2} \cdot \frac{f_{Na}^2 \; \gamma_{Cu}}{f_{Cu} \; \gamma_{Na}^2} \qquad (2)$$

and noting that equivalent expressions can be written for the exchange of Cu^{2+} with the other major cations K^+, Mg^{2+} and Ca^{2+}, it is evident that λ_o, the distribution coefficient of the free Cu^{2+} ions, will have a constant value for a given pH and salinity.

In seawater, Cu^{2+} occurs not only as free ions but also in various complexes. These include positively charged (e.g. $CuCl^+$, $Cu(OH)^+$), neutral (e.g., $CuCl_2^0$, $CuCO_3^0$), and negatively charged (e.g. $Cu(CO_3)_2^{2-}$) species. Since a cation exchange resin sorbs all cationic species, the total concentration of Cu in the resin phase will be

$$[\overline{Cu}] = [\overline{Cu^{2+}}] + \sum^{i} \sum^{n(+)} [\overline{CuL_{i,n}}] \qquad (3)$$

where $[CuL_{i,n}]$ represents the concentration of complexes formed between Cu^{2+} and n molecules of the ith ligand and $\sum^{n(+)}$ is used to indicate that the summation

includes only values of n resulting in positively charged complexes. Substituting in terms of the distribution coefficients and solution concentrations of the various species, equation (3) becomes

$$\begin{aligned} \lambda\,[Cu] &= \lambda_o\,[Cu^{2+}] + \sum^{i} \sum^{n(+)} \lambda_{i,n}[CuL_{i,n}] \\ &= \left\{ \lambda_o + \sum^{i} \sum^{n(+)} \lambda_{i,n}\,\beta_{i,n}\,[L_i]^n \right\} [Cu^{2+}] \end{aligned} \tag{4}$$

where λ is an overall distribution coefficient for all (cationic) forms of Cu, $[Cu]$ is the total dissolved concentration, and $\beta_{i,n}$ the stability constant of the dissolved complexes. Thus, on substituting for $[Cu]$ using the relationship

$$[Cu] = \left\{ 1 + \sum^{i} \sum^{n} \beta_{i,n}[L_i]^n \right\} [Cu^{2+}] \tag{5}$$

and solving for λ, it is evident that

$$\lambda = \frac{\lambda_o + \sum^{i} \sum^{n(+)} \lambda_{i,n}\,\beta_{i,n}\,[L_i]^n}{1 + \sum^{i} \sum^{n} \beta_{i,n}[L_i]^n} \tag{6}$$

The distribution of Cu between the resin and solution phases will therefore be determined by the pH and salinity of the seawater and by the ligand composition.

In artificial organic-ligand-free seawater, the concentration of inorganic ligands is fixed by pH and salinity. Calculations using the MINEQL computer program (Westall et al., 1976) indicate that the only cationic Cu complexes present in significant amounts in artificial seawater are the monochloro and monohydroxo species, suggesting that these will also occur in the resin phase.

In natural seawaters, a part of the dissolved Cu may be complexed by organic ligands or be bound to colloidal matter. Assuming that none of these forms of Cu bear a cationic charge and thus the cationic metal concentration is directly proportional to the inorganic metal concentration, the amount of Cu sorbed by the resin will be determined solely by the inorganic Cu fraction present in true solution. Thus the amount of inorganic Cu, Cu_{inorg}, can be calculated from the relationship

$$[Cu_{inorg}] = \frac{[\overline{Cu}]_x}{\lambda_{inorg}} \tag{7}$$

where $[Cu]_x$ is the experimentally determined sorbed Cu value for the natural seawater sample and λ_{inorg} is the value of the distribution coefficient of Cu derived from measurements on artificial seawater of the same pH and salinity.

The activity of the Cu^{2+} ion in seawater samples is related to Cu_{inorg} through the relationship

$$\{Cu\} = \gamma_{Cu} \alpha [Cu_{inorg}] \tag{8}$$

where α is the fraction of Cu_{inorg} present as free ions. The value of γ can be estimated from relationships such as the Davies equation (Davies, 1962) while α can be calculated using computer models such as MINEQL. Hence, ion-exchange measurements can, in principle, be used to estimate free metal ion concentrations and activities in natural seawater solutions.

The values of α and γ derived using existing models are at best only crude estimates; consequently, the accuracy of the activities based on their use is rather doubtful. Cu_{inorg}, which is closely related to the activity, is by contrast experimentally well defined, suggesting its use as an alternative parameter for assessing the toxicity of Cu to organisms in natural seawater samples. Since

Cu_{inorg} values will differentiate Cu bound to organic ligands and colloidal matter from that present in inorganic species in true solution, they provide a measure of the biologically effective metal concentration (EMC) of the solution. The EMC value (i.e. Cu_{inorg}) of a sample should be particularly useful in environmental studies.

B. PHASE I - DEVELOPMENT OF THE ION-EXCHANGE PROCEDURE

1.0 Procedure Overview

In the ion-exchange procedure the following conditions must be met: 1) the cation-exchange resin used must not display a high specificity for transition metal cations; 2) the resin must achieve equilibrium with the test solution; and 3) the resin must be used in a column form so that the resin is exposed to unperturbed sample.

A resin that has a similar relative ion selectivity for both transition metal cations and alkaline earth metals is the sulfonic acid type. Such resins are commercially produced and are available in reagent grade quality. Consequently, a sulfonic acid resin was chosen for the present study.

The ion-exchange procedure is divided into four steps:

1) Equilibration of a cation-exchange resin with a sample;
2) Removal of metals sorbed to the resin (elution);
3) Quantification of metal in the column eluate; and
4) Comparison of the amount of metal sorbed from standard solutions to that sorbed from a sample (calibration).

Resin equilibration is achieved by passing solution through resin in a column form until the effluent metal concentration is the same as the influent concentration. The resin is used in a column form so that the final volume of sample passing through the resin is essentially unperturbed; i.e. there is no further net change in the resin's or solution's metal concentration.

After equilibration, metals must be removed from the resin for quantification. There are different strategies that can be taken to achieve complete metal recovery. One procedure is to use a strong acid such as HNO_3. As hydrogen ions

compete with the metal ions for sorption sites, the relatively high hydrogen ion concentration in solution will cause complete displacement of metal ions. A second approach is to use a strong complexing agent such as EDTA. As the complexing agent has a much stronger affinity for metals than does the resin's sorption sites, the metals will tend to form complexes with the ligand.

Once the metals are removed from the resin, their concentration in solution is then determined. A number of methods are available for measuring total metal concentrations in aqueous solutions. These include inductively coupled argon plasma spectrophotometry, x-ray fluorescence, anodic stripping voltammetry, and atomic absorption spectrophotometry. Because of the low metal concentration and composition of sample expected to be analyzed, atomic absorption spectrophotometry was believed to be the best alternative for total metal determinations.

The final step in the analysis is to calibrate the resin to known metal quantities. This involves passing seawater metal standards through resin-columns similar to those used for samples and determining the concentration of metal sorbed from these standards. (The metal standards must match the pH and salinity of the samples undergoing analysis.) The amount of metal sorbed from the standards is then compared to the amount of metal sorbed from the sample to derive a value termed the Effective Metal Concentration (EMC). The EMC value is defined as follows:

$$EMC^{*} = \frac{\text{Conc. of Sorbed Metal From Sample}}{\text{Conc. of Sorbed Metal from Standard Seawater Solution}} \times \text{Conc. of Seawater Standard}$$

* will have the same units as the seawater standard.

2886

At present, the seawater metal standards used to calibrate the resin are defined in terms of the inorganic metal concentrations in solution. Because of this, an EMC value is equated to the true dissolved inorganic metal concentration in a sample. (This is valid as long as the truely dissolved inorganic composition of the standards and samples match.) Although it would be preferable to define metal standards in terms of free metal ion concentrations and thus have EMC so equated, there are presently no reliable methods to determine this quantity in seawater.

The metal standards used in the present study have been prepared in artificial seawater because it has a well defined composition and is free of organic complexing agents. However, natural seawater could be substituted for artificial seawater provided it is properly pretreated to remove organics and to reduce background metal concentrations.

The development of steps 1-3 are described in Phase I. The calibration procedures (step 4) were developed and applied in Phase II.

2886

2.0 MATERIAL AND METHODS

2.1 Reagents and General Procedures

All chemicals were reagent grade. Trace metal stocks (Cd, Cu, Mn, Pb, and Zn) were prepared from high purity metals and were made up in 1% HNO_3. Stock solutions were prepared at a concentration of 10^{-2}M. Working standards were made by serial dilution of the stock.

Standard Ocean Water (SOW) was prepared according to the recipe of Morel et al., (1979), in 20 L batches. Seawater salts (Table 1), excluding $MgCl_2$, were added to 18 L of glass-distilled water (GDW) in a 20-L polycarbonate carboy and bubbled with acid cleaned (1N H_2SO_4), filtered (0.45 um Nuclepore) air until the mixture was completely dissolved. Magnesium chloride was then added to the salt solution which was brought up to 20 L with GDW ($MgCl_2$ was dried for 2 days at 70°C before use because of its hygroscopic nature). The medium was bubbled with air overnight to allow equilibration and to adjust the pH to 8.0 $\pm$ 0.05. After equilibration, the solution was passed through an ion-exchange resin (Chelex-100, 100-200 mesh) to reduce the level of trace metal contaminants and stored in 12-L polycarbonate carboys until use. The preparation of Chelex-100 resin is described in Appendix I. For the experiments in Phase I, SOW (35°/oo) was diluted with GDW to 25°/oo and adjusted to pH 8.0 $\pm$ 0.05 by bubbling with air. The composition of diluted SOW is given in Table 2.

Only plastic laboratory ware was used in this study as greater amounts of metal adsorb to glass than to plastic. All plasticware was initially soaked in 6N HNO_3 for a day, soaked in 1N HCl for a number of days and finally rinsed thoroughly with glass distilled water (GDW). For subsequent uses, the plasticware was rinsed with 1N HNO_3 and rinsed 3 times with GDW.

TABLE 1
Salt Concentrations in SOW (35°/oo)[a]

Substance	Weight (g)	Volume (L)	Final Concentration (M)
NaCl	490.6	20	4.20×10^{-1}
$CaCl_2 \cdot 2H_2O$	30.8	20	1.05×10^{-2}
KBr	2.0	20	8.40×10^{-4}
NaF	0.06	20	7.14×10^{-5}
KCl	14.0	20	9.39×10^{-3}
H_3BO_3	0.6	20	4.85×10^{-4}
Na_2SO_4	81.8	20	2.88×10^{-2}
$NaHCO_3$	4.0	20	2.38×10^{-3}
$SrCl_2\ 6H_2O$	0.34	20	6.38×10^{-5}
$MgCl_2\ 6H_2O$[b]	222.0	20	5.46×10^{-2}

[a]Bubbled with air to pH 8.0 $\pm$ 0.05
[b]Added after other salts.

2886

TABLE 2
Composition of SOW diluted to 25°/oo[a]

Substance	Analytical Concentration (M)	Computed Major[b] Species (%)
Chloride	4.00×10^{-1}	Cl^- (100)
Sodium	3.43×10^{-1}	Na^+ (99) $NaSO_4^-$ (1)
Sulphate	2.06×10^{-2}	SO_4^{2-} (51) $MgSO_4$ (24) $NaSO_4$ (20) $CaSO_4$ (4)
Magnesium	3.89×10^{-2}	Mg^{2+} (87) $MgSO_4$ (13)
Calcium	7.50×10^{-3}	Ca^{2+} (89) $CaSO_4$ (10)
Potassium	7.36×10^{-3}	K^+ (97) K_2SO_4 (3)
Carbonate[c]	1.70×10^{-3}	HCO_3^- (72) $MgCO_3$ (14)
Bromide	6.00×10^{-4}	Br^- (100)
Strontium	4.56×10^{-5}	Sr^{2+} (100)
Borate	3.46×10^{-4}	H_3BO_3 $B(OH)_4^-$
Fluoride	5.10×10^{-5}	F^- (66) MgF^+ (33)

[a]pH adjusted to pH of sample.

[b]Calculated by the chemical equilibrium program MINEQL for pH 8.0.

[c]HCO_3^- and CO_3^{2-}

2886

2.2 Natural Seawater Collection and Preparation

Natural seawater was collected from the West Vancouver Laboratory (of the Department of Fisheries and Oceans) seawater system, which is collected by pipe from a depth of approximately 20 m, and transported to the University of British Columbia in two 45 gal polypropylene barrels. The barrels were cleaned with 2N HNO_3 and 3N HCL prior to use. Within four hours of collection, the seawater was passed through a 293 mm Whatman #1 paper filter (nominal pore size of 11 um) to remove zooplankton and large particulate matter. The initial 2-3 L of filtrate was discarded. A Masterflex peristaltic pump (industrial model) fitted with acid washed silicone tubing was used in all filtration procedures. The barrels were covered with black plastic to reduce algal growth during storage.

For each batch of seawater, pH, salinity, alkalinity, and dissolved metal measurements were made. The pH was measured with a Corning (model 130) pH meter equipped with a Corning semi-micro combination electrode. Salinity was determined by an Endeco (Type 102) refractometer ($\pm 0.5^{o}/oo$). Total alkalinity was determined using the method described in Strickland and Parsons (1972). Dissolved metal concentrations were determined by Analytical Services Laboratory, Vancouver, B.C. The samples were analyzed after filtration (0.45 um) using the APDC-MIBK method in conjunction with atomic absorption spectrometry.

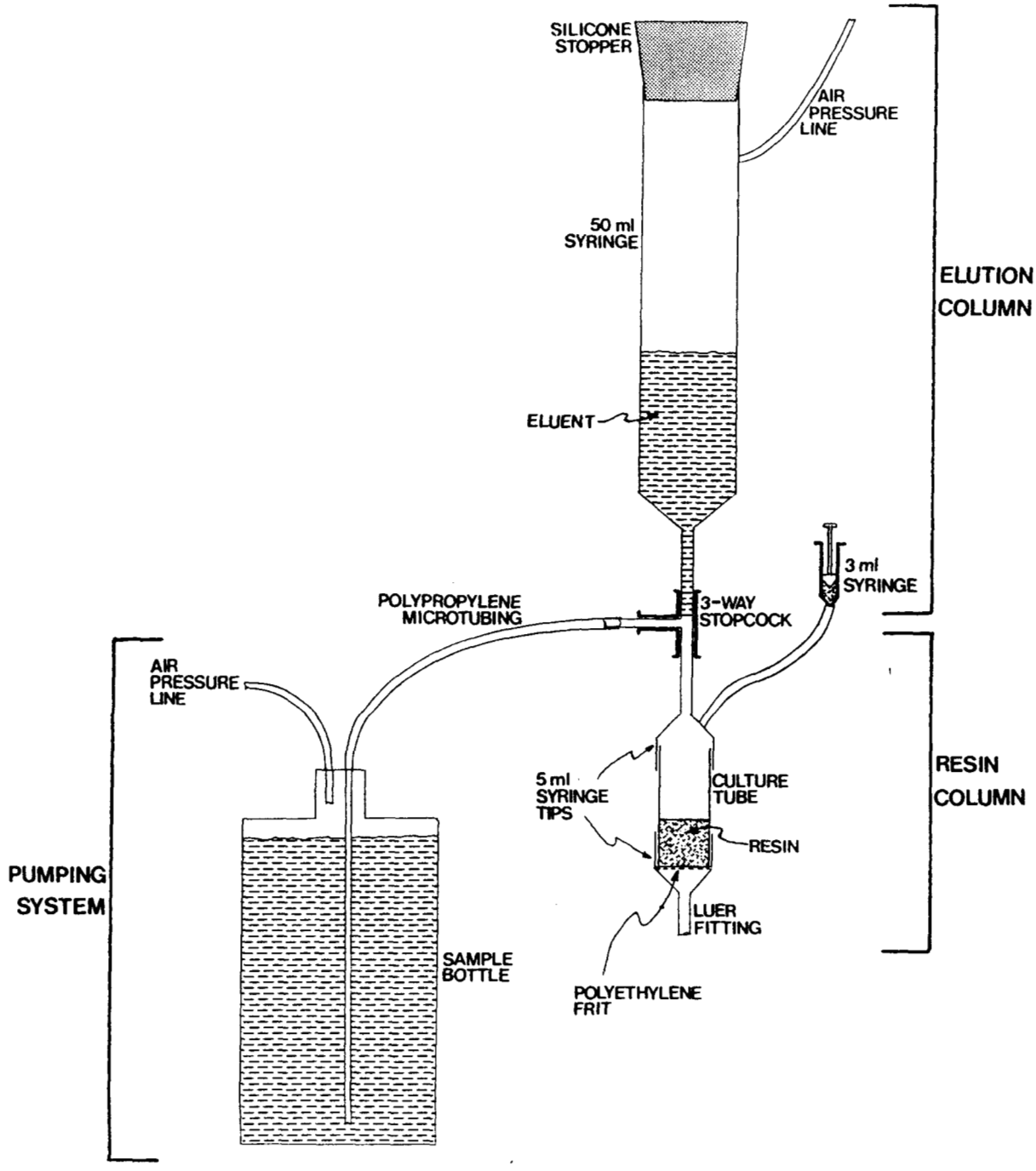
2.3 Ion-Exchange Analysis

2.3.1 Equipment Description

The apparatus used in the ion-exchange procedure was comprised of 1) a pumping system, 2) a resin-column, and 3) an elution-column (Figure 2). The pumping system consisted of a 500 mL polypropylene bottle connected to a filtered

FIGURE 2

ION-EXCHANGE APPARATUS



(0.45 um) air pressure source. Air pressure was used to control the flow rate of solution through the resin-column. A pressure of approximately 1 psi produced a flow rate of approximately 5 mL/min. The bottle was connected to a resin-column by 0.062" I.D. polypropylene microtubing.

Resin and elution columns were constructed from 12 x 75 mm polypropylene culture tubes, 5 mL and 50 mL Plastipak* polypropylene syringes, Pharmaseal* plastic three way stopcocks, polyethylene frits (35 um), and Luer* miniature plastic fittings. Resin-columns were constructed by cutting the sealed end off culture tubes and fitting the tips of 5 mL syringes over each end. To provide a bed support for the resin, polyethylene frit was sandwiched between a syringe tip and one end of the culture tube. Teflon tape was used to provide a tight fit between the culture tube and syringe tip. To provide a method of filling the column with fluid to any desired level, a 3 mL syringe was attached to the column via plastic microtubing.

The elution column consisted of a 50 mL syringe fitted with a silicon stopper and connected to an air pressure source (e.g., an aquarium pump). A 3-way stopcock was attached to the Luer fitting of the syringe to provide a method of flow control and a connection to the resin-column.

Twelve resin and elution columns were fitted into a plexiglass sampling box. Three sampling boxes were constructed to enable a set of 36 columns to be run at one time, if required.

2.3.2 Resin Preparation

Bio-Rad* AG 50W (200-400 mesh, hydrogen form) analytical grade cation-exchange resin was used as the ion-exchanger. The resin is composed of sulphonic acid groups attached to a styrene divinylbenzene polymer lattice (R-

* Registered Trademark

2886

SO_3^-). Batches of resin were initially prepared by rinsing with GDW, settling and decanting three times with GDW to remove fines, and finally, drying at 70°C for two days. The resin was stored in a dessicator until use.

Resin-columns were prepared by slurry loading 0.75 g of resin into a column and eluting with 20 mL of 3N HCl to remove trace metal contaminants. The resin was rinsed with GDW and equilibrated with the major seawater cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) by passing 50 mL of diluted SOW (29°/oo, pH 8.0) through the resin.

The columns were regenerated after use. This involved stirring the resin with a glass rod to remove any air bubbles, then sequentially passing through the resin 20 mL of GDW, 20 mL of 3N HCl (to remove trace metal contaminants and to convert other resin to the protonated form), 20 mL of GDW and finally, 50 mL of diluted SOW.

2.3.3 Determination of Metal Concentrations in the Column Eluates

A Perkin-Elmer Model 560 atomic absorption spectrophotometer equipped with a graphite furnace was used to determine the metal concentrations in the samples. Pyrolytically coated graphite tubes were used for all analyses. The operating conditions for each metal are presented in Appendix II.

Metal-EDTA standards were used to determine metal concentrations in samples generated in the ion-exchange analysis. Concentrations of unknowns were calculated from calibration curve equations where equations were derived by computerized regression analysis of data obtained from analyses of the standards. Reagent blanks were run for all analyses and were subtracted from standard and sample readings. The approach taken to derive metal concentrations in samples from the ion-exchange analysis is given in detail in Appendix III and will not be repeated here.

2886

2.3.4 Detection Limits

The limit of detection (LOD) is defined as the lowest concentration level that can be determined to be statistically different from a blank (ACS Committee, 1983). The LODs of the ion-exchange procedure for the various metals were calculated based on the following formula for small sample sizes (n 20):

$$\frac{t_{0.001(1),\ n-1}\ S_B}{m}$$

where t is the appropriate Student's t value for n-1 degrees of freedom, S_B is the standard deviation of replicates of samples which give readings slightly above the detection limit and m is the slope of the calibration curve. The slope is used to convert the absorbance readings of the AAS analysis to concentration units.

The Limit of Quantification (LOQ) is the level above which quantitative results may be obtained with a specified degree of confidence (ACS Committee, 1983). LOQ was calculated as follows:

$$LOQ = \frac{10\ S_B}{m}$$

where S_B and m are the same as given above.

2886

3.0 RESULTS AND DISCUSSION

3.1 Procedure Development in Artificial Seawater

A number of experiments were performed to determine the optimal conditions and analytical steps required to apply the ion-exchange procedure to the measurement of Cd, Cu, Pb, Mn and Zn in seawater. Experiments were first conducted in artificial seawater to eliminate potential interferences associated with organic and colloidal matter present in natural seawater. The following section describes these experiments.

3.1.1 Elution Experiments

As the metals sorbed to the resin must be removed before they can be quantified, the first set of experiments were designed to determine a method to allow complete removal of sorbed metals. An eluent was desired that could quantitatively remove Cd, Cu, Mn, Pb and Zn from the resin with the least volume possible (maximize sensitivity) while still being a suitable background matrix for graphite furnace atomic absorption spectrophotometry (GFAAS).

In a preliminary study using Cu and Mn in natural seawater (Zorkin, unpubl.), a variety of compounds were tested as eluents (Table 3). Quantitative elution using an eluent volume of less than 40 mL was possible with sulphuric, hydrochloric and nitric acids and with the complexing agent EDTA and NTA. EDTA, however, was the only compound to undergo further testing.

The volume of EDTA required to completely remove Cd, Cu, Pb, Mn and Zn from the resin was then determined for different EDTA concentrations. Twelve resin-columns were loaded with metals by passing 500 mL of diluted SOW ($25^{o}/oo$) containing a concentration of 100 nM of Cd, Cu, Mn, Pb and Zn (11.2, 6.4, 5.5,

TABLE 3
Compounds Tested as Eluents

ACIDS	Formula	Eluent Concentration
Acetic	CH_3COOH	3N
Hydrochloric	HCl	3N
Nitric	HNO_3	3N
Sulphuric	H_2SO_4	3N
COMPLEXING AGENTS	**Acronym**	
Ammonium pyrrolidine-N-carbodithioate	APDC	10^{-2} M
Cyclohexanediaminetetraacetic acid	CDTA	10^{-2} M
Ethylenediaminetetraacetic acid	EDTA	10^{-2} M
Nitrilotriacetic acid	NTA	10^{-2} M

20.7 and 6.4 µg/L, respectively) through the columns. After resin equilibration, the columns were eluted with 25 mL of EDTA (pH 4.7) in 5 mL aliquots and at concentrations ranging from 10-50 mM in 10 mM increments. Duplicate columns were run for each EDTA concentration.

Quantitative elution was achieved for Cd, Mn and Pb with 10 mL of EDTA at all EDTA concentrations tested (Table 4). Copper, on the other hand, required 20 mL of EDTA for complete elution but, as with the other metals, increasing the concentration of EDTA did not reduce the volume required to acheive quantitative elution.

The results for Cu also differed from the other metals in that its concentration in the eluates* appeared to decrease as the concentration of EDTA increased (Table 4). However, as all resin-columns were loaded with the same amount of Cu, the concentration of Cu in the eluates should have been similar.

After further experimentation, the discrepancy in the Cu levels was found to be due to an EDTA effect in the GFAAS analysis. Figure 3 shows the results of an experiment where 100 nM of Cu in the presence of five different concentrations of EDTA was analyzed by GFAAS. As the concentration of EDTA in solution increased, the absorbance readings for Cu decreased. Since the absorbance readings of the metal standards used to quantify the metal concentrations in the eluates were made up in only one concentration of EDTA (30 mM), the concentration of Cu in eluates having an EDTA concentration lower or higher than that of the standards would thus be over or underestimated. This demonstrated the importance of exactly matching the EDTA concentration of the standards with the EDTA concentration of the eluates.

* An eluate is the eluent after passing through the resin-column.

TABLE 4
Results from an Experiment Using EDTA as the Eluent.
Values are Metal Concentrations (nmol) in Each 5 ml of Eluent Volume.

Metal	Eluent Volume	EDTA[a] Concentration (mM) 10	20	30	40	50
Mn	1st 5 ml	2.39[2]	2.59	2.66	2.74	2.72
	2nd 5 ml	0.10	N.D.[b]	N.D.	N.D.	N.D.
Pb	1st 5 ml	5.07	5.08	4.65	4.59	4.87
	2nd 5 ml	0.14	0.42	0.78	0.79	0.34
	3rd 5 ml	N.D.	N.D.	N.D.	N.D.	N.D.
Cd	1st 5 ml	0.441	0.439	0.422	0.429	0.377
	2nd 5 ml	N.D.	N.D.	0.007	0.010	N.D.
	3rd 5 ml	N.D.	N.D.	N.D.	N.D.	N.D.
Cu	1st 5 ml	10.27	10.46	6.97	6.87	6.47
	2nd 5 ml	2.04	2.45	1.03	2.41	1.90
	3rd 5 ml	0.32	0.41	0.35	0.36	0.29
	4th 5 ml	0.20	0.18	0.17	0.19	0.12
	5th 5 ml	N.D.	N.D.	N.D.	N.D.	N.D.
Zn	1st 10 ml		0.175[c]			
	2nd 10 ml		0.005			
	3rd 10 ml		0.010			

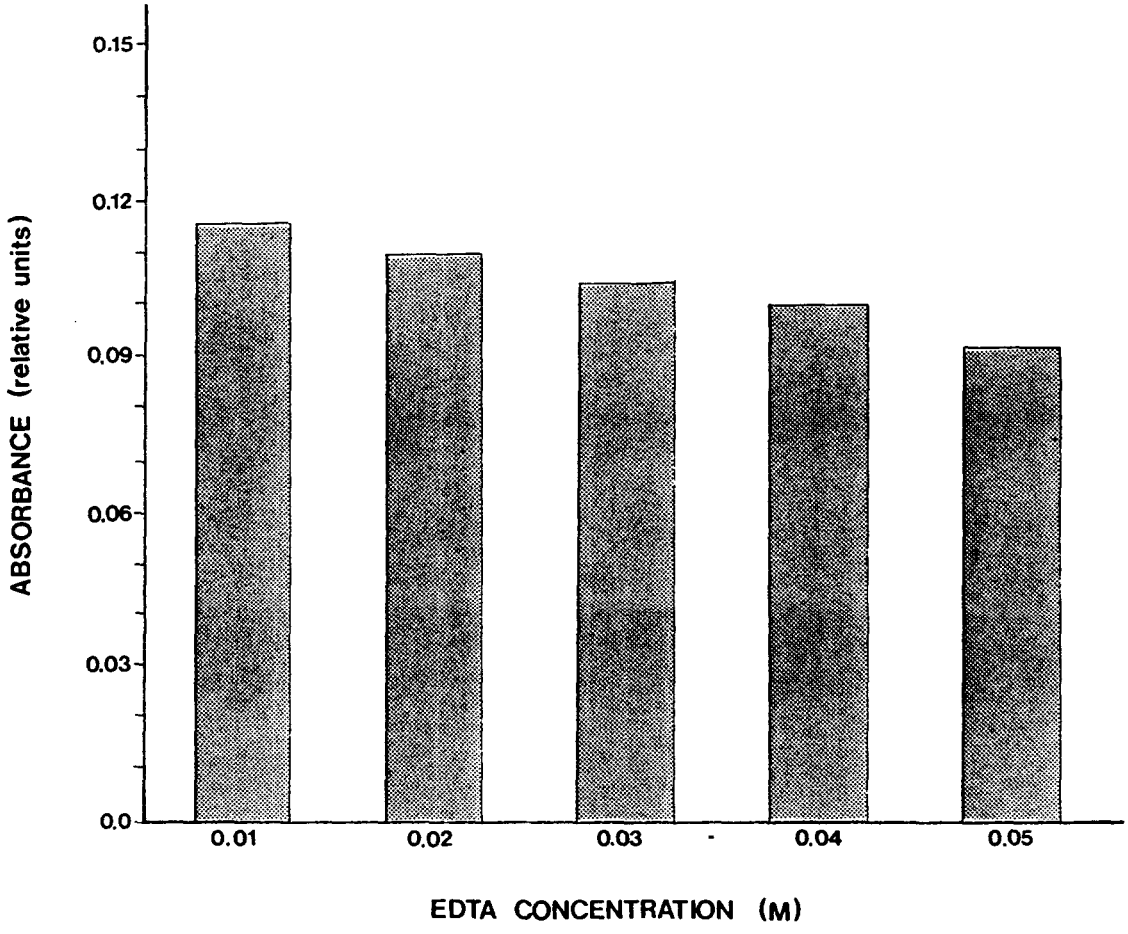
[a]EDTA: pH 4.7

[b]Limit of Detection: Mn - 0.04 nmol/5 ml
Pb - 0.10 nmol/5 ml
Cd - 0.004 nmol/5 ml
Cu - 0.08 nmol/5 ml

[c]Arbitrary Units: Problems were encountered with the Zn standards and with the overall Zn analysis. Because of this, only the absorbance readings are reported.

FIGURE 3

ABSORBANCE READING OF 100nM Cu IN FIVE CONCENTRATIONS OF EDTA



Zinc also appeared to be completely removed within 10 mL of EDTA. However, because of numerous problems encountered with the measurement of Zn by GFAAS such as contamination from the graphite tubes and non-linearity of response, the reliability of the data was in question. Methods other than GFAAS seemed to be more appropriate and were later applied to the measurement of Zn in the column eluates.

Based on the elution experiments, an EDTA concentration of 10 mM and a volume of 20 mL was adopted as standard elution conditions for subsequent analyses. However, to provide a longer contact time between the eluent and the resin, the eluent was passed through the resin in 10 mL aliquots to allow for a five minute pause between each aliquot.

3.1.2 Equilibration Experiments

As discussed in Section B.1.0, the resin and solution phases must achieve equilibrium before any metals sorbed to the resin can be quantified. Equilibrium is achieved when the heavy metal concentrations in the resin does not change with any further exposure to the sample. To determine the volume of sample required to bring 0.75 g of resin (AG 50W X-8) into equilibrium with Cu, Cd, Mn and Pb, various volumes of diluted SOW ($25^{o}/oo$) containing 100 nM of each metal was passed through a set of 12 columns. The volume of diluted SOW ranged from 0 to 500 mL at 50 mL intervals. After resin equilibration, the columns were eluted with EDTA and the metal concentrations in the eluates measured. The equilibrium curves for the metals are presented in Figures 4-6.

Cadmium and Mn achieved equilibrium with the least volume requiring only 50 mL of solution. Lead achieved equilibrium within 150 mL, while Cu required at least 450 mL. As the equilibrium curve for Cu was suspect because of the large concentration increase in sorbed metal between 400 and 450 mL of solution (Figure 6), another experiment was conducted using sample volumes up to 800 mL. In this experiment, the concentration of sorbed Cu remained constant after 450 mL of sample (Figure 7).

FIGURE 4

EQUILIBRIUM CURVE FOR Mn AND Pb

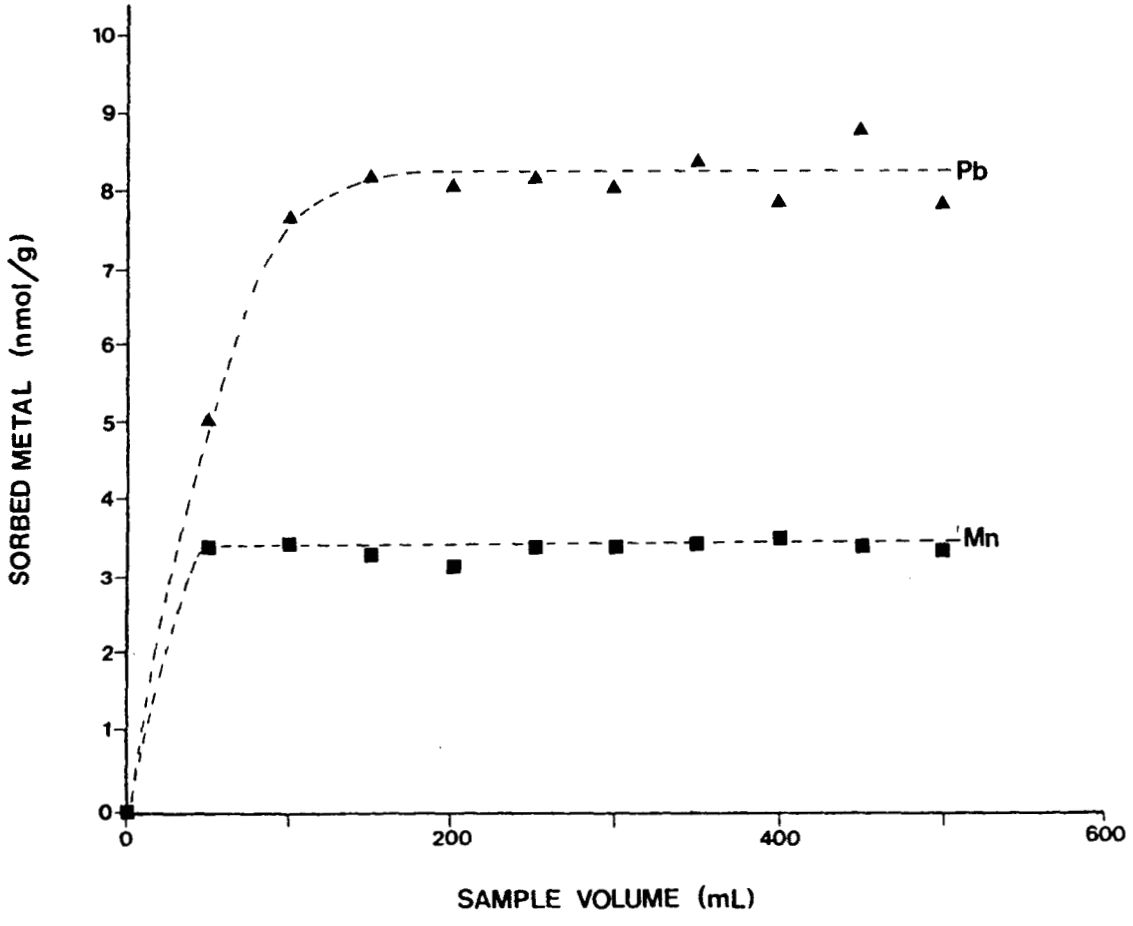


FIGURE 5

EQUILIBRIUM CURVE FOR Cd

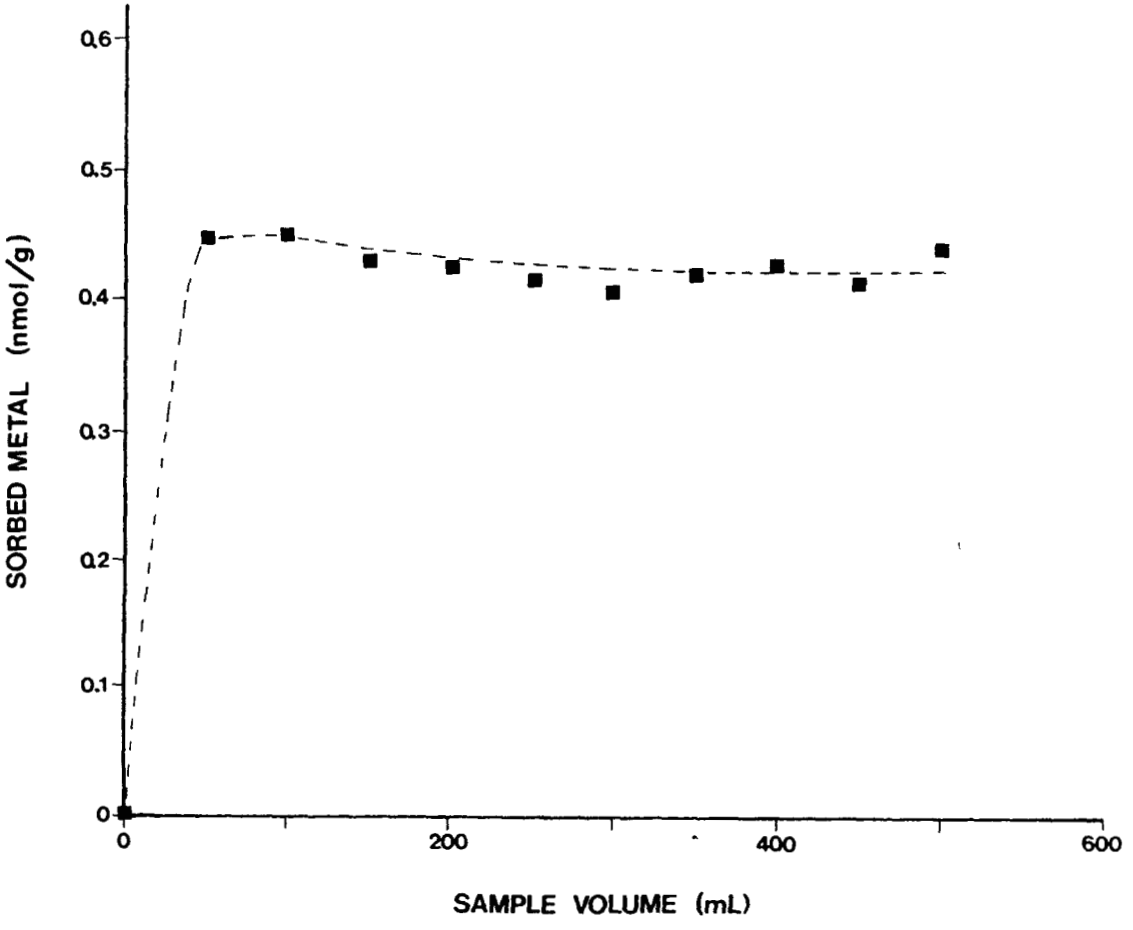


FIGURE 6

EQUILIBRIUM CURVE FOR Cu WITH A SAMPLE VOLUME TO 500 mL

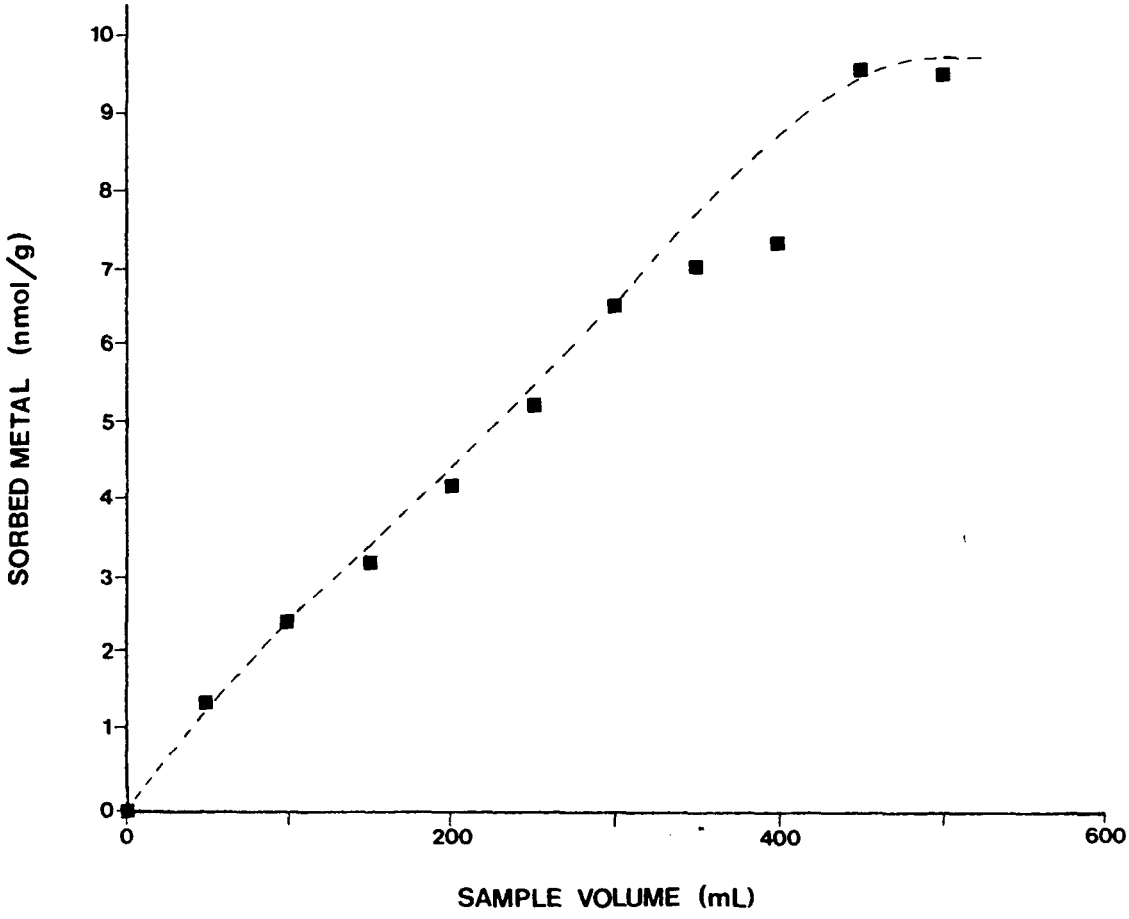
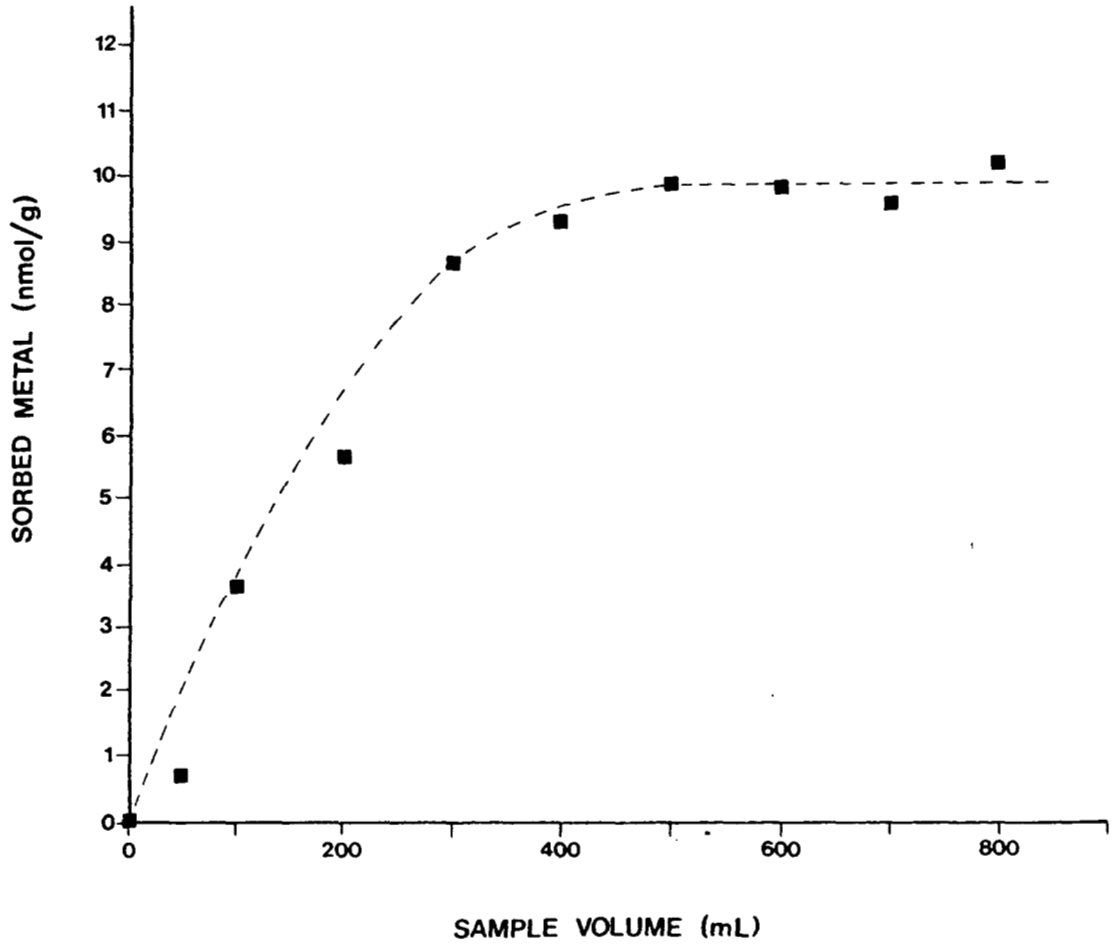


FIGURE 7

EQUILIBRIUM CURVE FOR Cu WITH A SAMPLE VOLUME TO 800 mL



A further experiment was conducted to determine if the equilibrium volume for Cu could be reduced so that less sample would be required for the analysis. An experiment was run similar to the one described above except that instead of using resin pre-equilibrated with SOW, resin in the hydrogen form was used. It was thought that loading Cu onto the resin at the same time as the major seawater cations may result in equilibration at a lesser sample volume. However, this was not the case. Copper did not acheive equilibrium even after 700 mL of solution and the equilibrium of the metal appeared to be quite erratic (Figure 8). It was evident that using resin in the hydrogen form was not suitable for this application. Consequently, a sample volume of 500 mL using resin pre-equilibrated with SOW was adopted as standard procedure for the ion-exchange procedure.

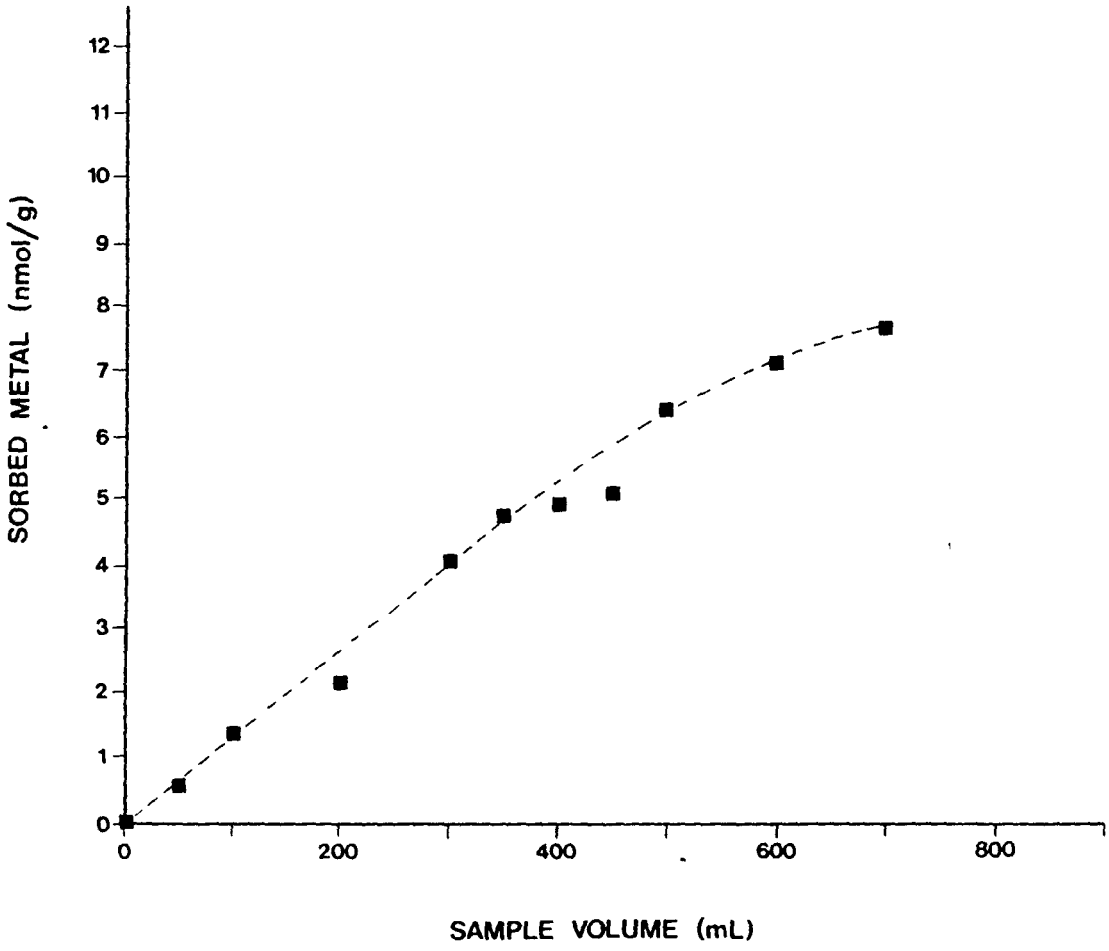
The relative selectivity of the resin for the four trace metals can also be obtained from the concentration data generated in these experiments. The order of the resin's selectivity under the operational conditions described above was Cu > Pb > Mn > Cd.

3.1.3 Sorption Curves

To determine the relationship between the concentrations of sorbed metal and solution metal, the amount of metal sorbed to the resin was determined as a function of the total dissolved inorganic metal concentration in diluted SOW. Cadmium, Cu, Mn and Pb were studied over the concentration range of 0-800 nM. Metals were added to 500 mL aliquots of diluted SOW to give a final concentration of 0, 25, 50, 100, 200, 400 or 800 nM. This concentration range was chosen because it was the approximate concentration range expected to be used in the bioassays. The samples were passed through a set of resin columns, the columns eluted, and the metal concentrations in the eluates measured. (The metal concentrations in the eluates were converted to nmol/g of resin values following the procedures used in Appendix III.)

FIGURE 8

EQUILIBRIUM CURVE FOR Cu USING RESIN IN THE HYDROGEN FORM



For Mn, the relationship between the sorbed and solution metal concentration was linear over the entire concentration range (correlation coefficient r=0.9999; Figure 9). The relationship for Cd and Cu was linear to 400 nM (r=0.9999 and 0.9995, respectively), but demonstrated some curvature from 400 to 800 nM (Figures 10 and 11). Lead, on the other hand, showed curvature over the entire concentration range (Figure 12) with the curve best fitted to a second degree polynominal. The coefficients of the regression equation for Pb are given in Figure 12.

Because of the linear response for Cd, Cu and Mn over the concentration range of 0-400 nM, calibration of the resin within this concentration range would require only a limited number of standard solutions; in theory, only one standard would be required. The response for Pb, however, changed with an increase in the metal's concentration. As such, the calibration of the resin to Pb will require a number of standards that bracket the concentration of the samples undergoing analysis.

3.1.4 Experiments Using EDTA as a Complexing Agent

To verify that only cationic metal species were sorbed to the resin and sorption of anionic metal species was negligible, a series of experiments was performed using the organic complexing agent EDTA. EDTA was chosen because it forms anionic species with the metals under study.

Experiments were conducted using diluted SOW containing 100 nM of Cd, Cu, Pb and Mn that had EDTA added at concentrations ranging from 25 to 2000 nM. The results are reported as the concentration of metal sorbed per g of resin for each metal-ligand combination. For all the metals tested, increasing the EDTA concentration in solution resulted in the concentration of sorbed metal to decrease (Table 5). This indicated that the ion-exchange procedure could detect

FIGURE 9

SORPTION CURVE FOR Mn

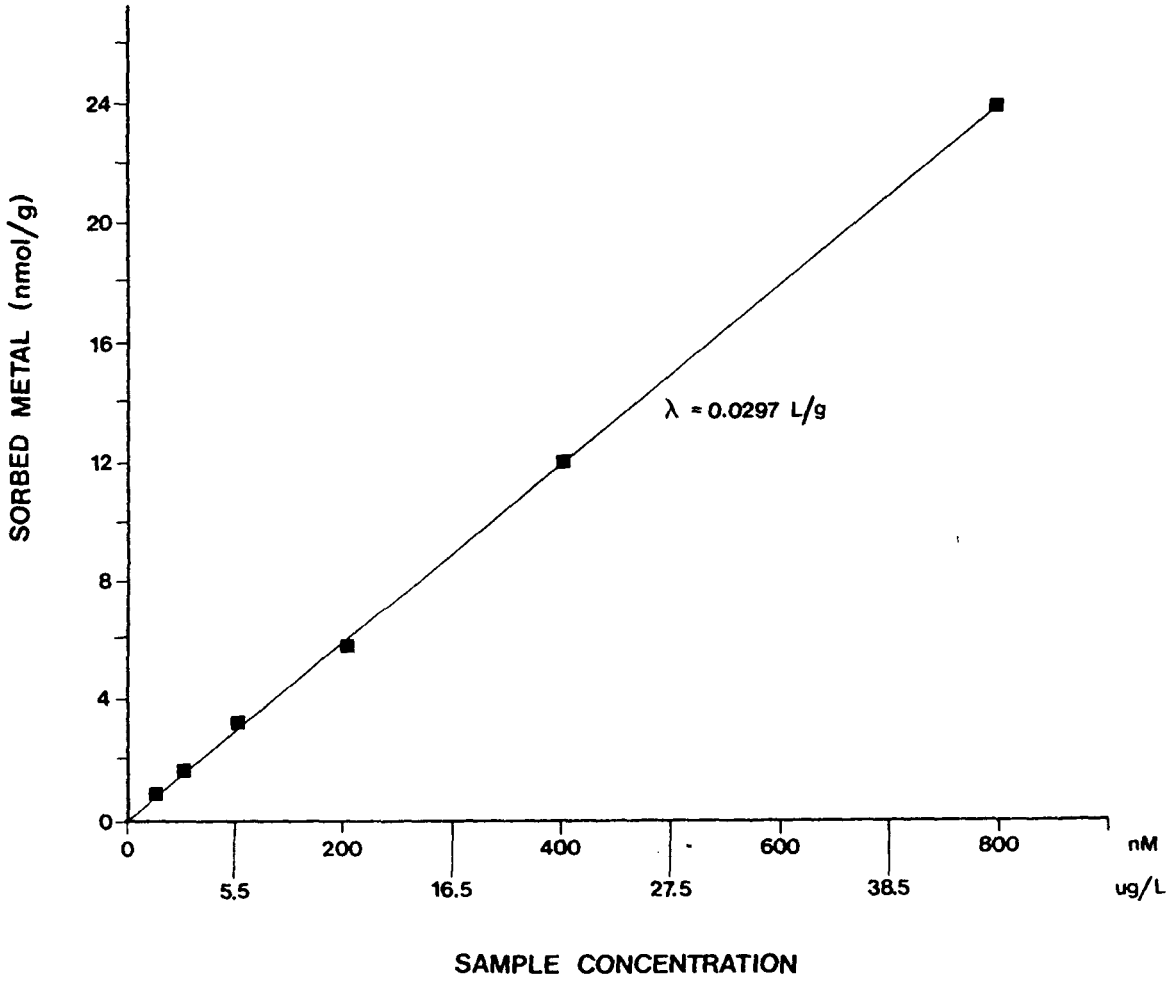


FIGURE 10

SORPTION CURVE FOR Cd

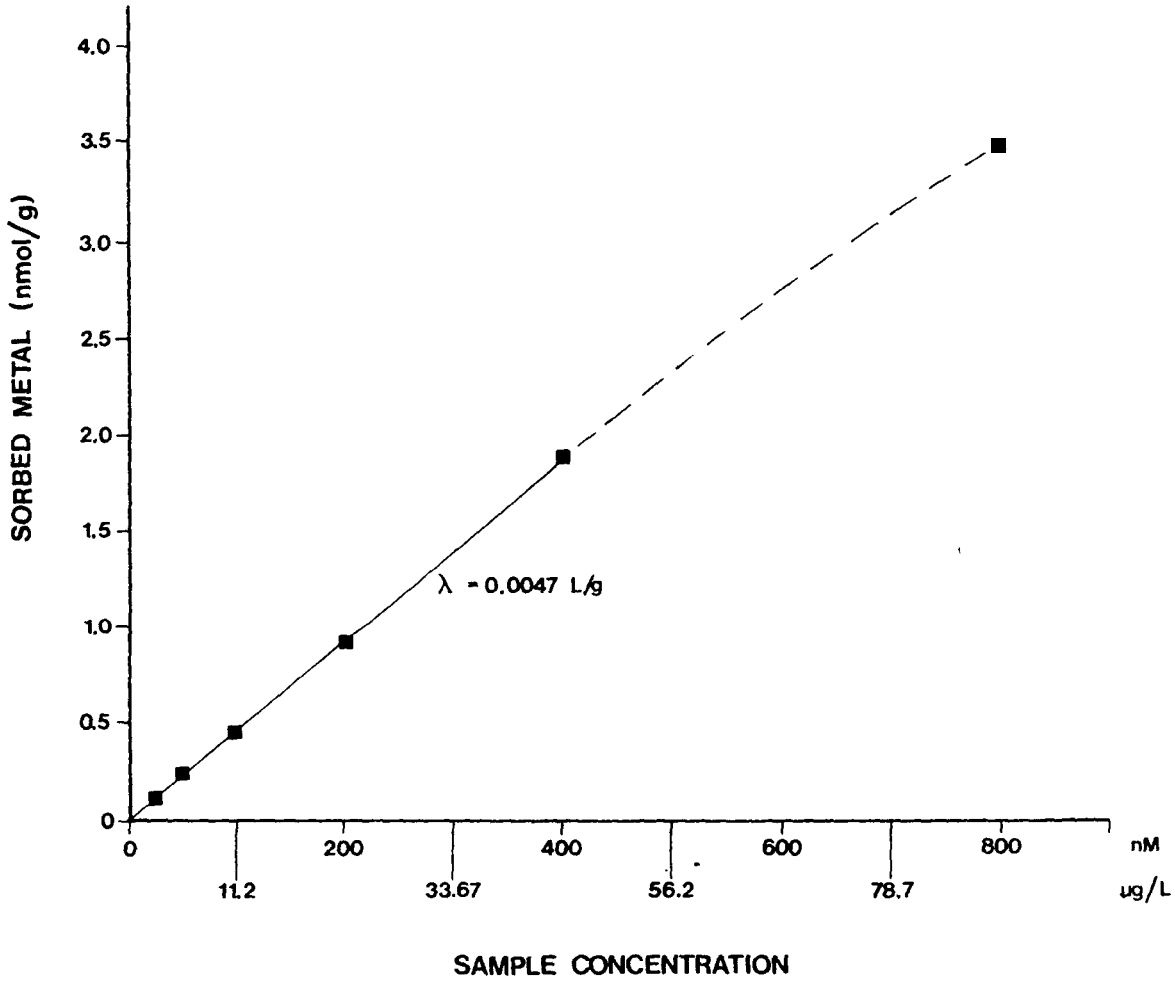


FIGURE 11

SORPTION CURVE FOR Cu

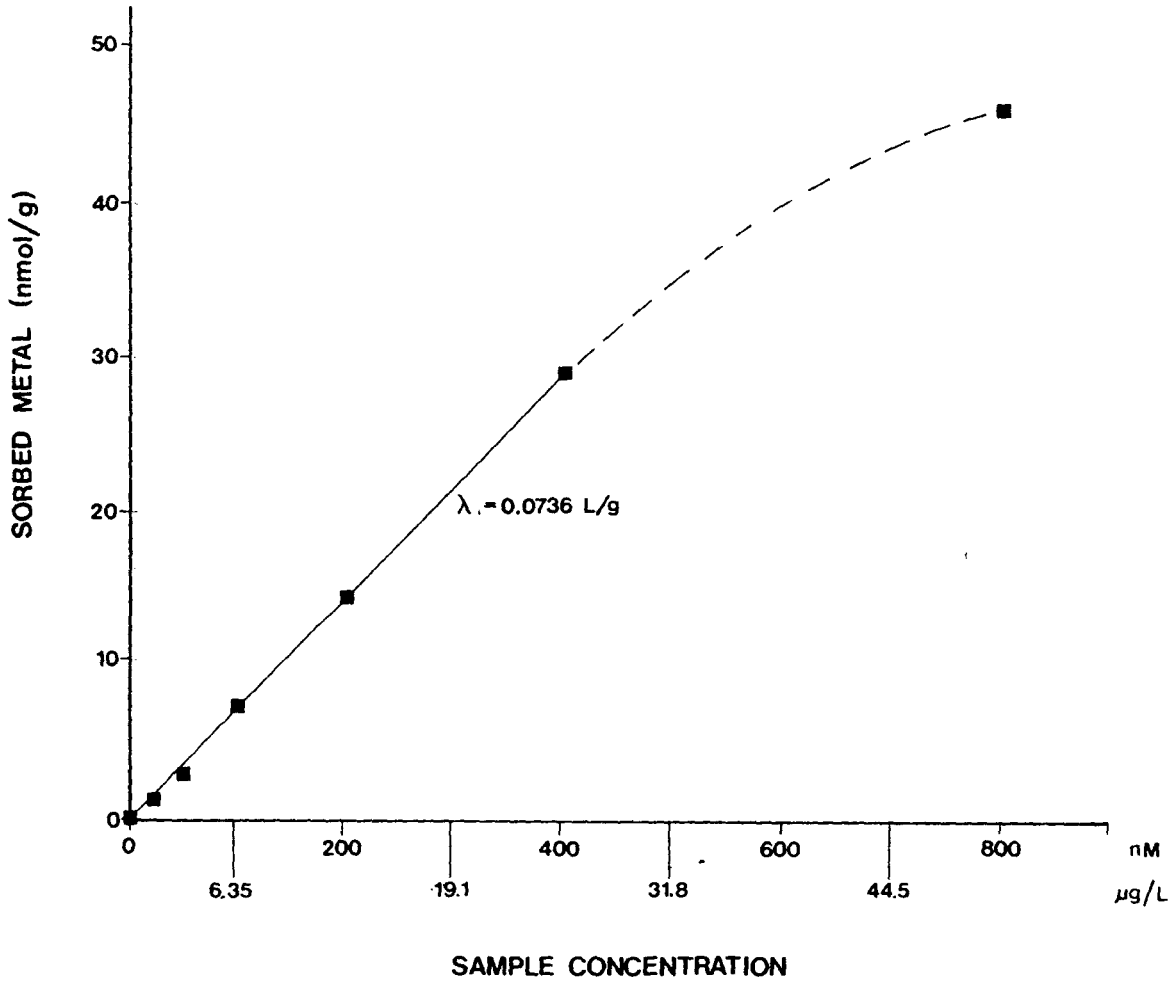


FIGURE 12

SORPTION CURVE FOR Pb

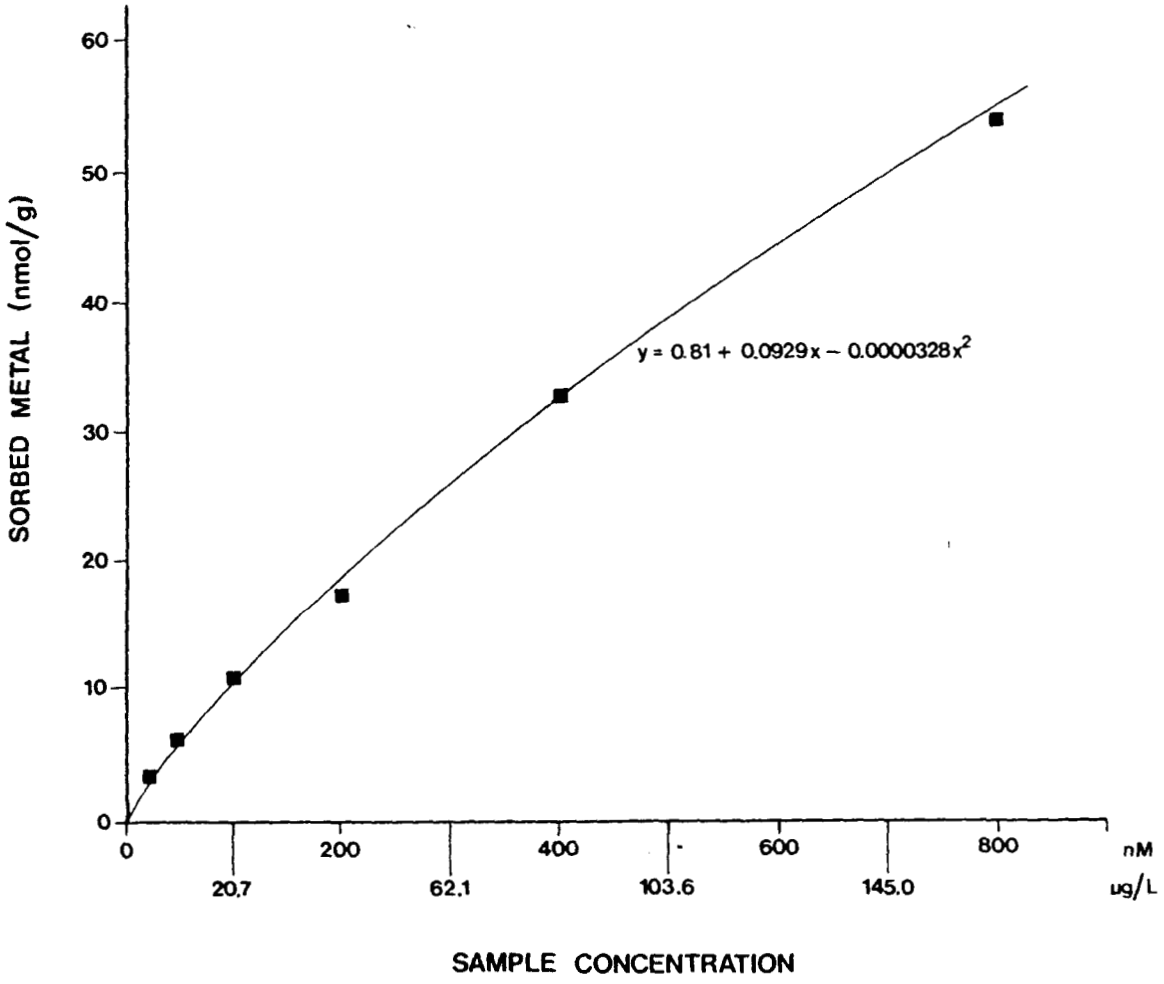


TABLE 5
The Effect of EDTA on the Sorption of Metal.
Values are Sorbed Metal Concentrations (nmol/g)

SERIES[a]	EDTA Conc. Added (nM)	Metal			
		Cu	Pb	Mn	Cd
A	0	6.05	8.12	3.19	0.429
	25	5.86	7.48	3.24	0.440
	50	5.21	6.79	3.18	0.420
	100	4.35	5.50	3.15	0.418
	200	1.80	2.58	3.11	0.400
	400	0.37	0.81	3.10	0.329
B	0	12.11	7.91	3.57	0.459
	500	0.56	0.35[b]	3.50	0.306
	750	0.27	N.D.[b]	3.40	0.227
	1000	N.D.	N.D.	3.25	0.205
	1500	N.D.	N.D.	3.26	0.174
	2000	N.D.	N.D.	2.96	0.163

[a]Metals added to solution was 100 nM.

[b]Non-Detectable: Limit of Detection: Cu - 0.23 nmol/g
Pb - 0.35 nmol/g
Mn - 0.25 nmol/g
Cd - 0.030 nmol/g

2886

the metal's complexation with EDTA and that the metal-EDTA complex did not appear to be sorbed to the resin.

The ability of EDTA to reduce the concentration of sorbed metal was related to the stability of the metal-EDTA complex. Copper and Pb, with the highest formation constants for EDTA (log K^*_1 = 18.07 and 17.97, respectively), showed a 70% reduction in sorbed metal at a 1:2 metal-EDTA concentration ratio. Manganese (log K^*_1 = 13.31), on the other hand, had only a 17% reduction in the sorbed metal concentration at the 1:20 metal-EDTA concentration ratio.

The percentage of metal bound to EDTA could also be estimated from the sorbed metal concentration data. The % bound metal values were calculated as follows:

$$\% \text{ bound metal} = 100 - \frac{\text{Concentration of metal sorbed from a solution with EDTA}}{\text{Concentration of metal sorbed from a similar solution with no EDTA}} \times 100$$

The percentage of metal bound to EDTA for each metal-EDTA concentration combination was also estimated by the computer model MINEQL (Westall et al., 1976). The chemical model calculates the concentration of metal species in solution when the total metal concentration and the stability constants for all metal complexes are given. However, as most stability constants are derived in solutions unlike that of seawater and are determined at a different ionic strength, the extrapolation of these stability constants to a seawater matrix and the concurrent ionic strength corrections needed seriously compromise the accuracy of the computer model. With these limitations in mind, the trends in the measured and calculated % bound metal values are discussed.

* Ionic strength of 0.55

2886

The amount of metal complexed with EDTA as estimated by the ion-exchange technique was less than that predicted by the computer model for all the metal-EDTA concentration combinations (Table 6). This was consistent with the work of Zorkin (1983) who found that the computer model overestimated the complexation of Cu by EDTA in SOW of 35°/oo. However, considering the limitations of the computer model, reasonable agreement was obtained between the trends predicted by the model and the ion-exchange results suggesting that the resin was related to the inorganic metal fraction and sorption of the negatively charged metal-EDTA species was negligible.

3.1.5 Metal-Metal Interaction

In theory, varying the concentration of a trace metal cation in the resin phase by varying its solution concentration should not affect the concentration of any other heavy metal cation sorbed to the resin. Previous work, however, has indicated that changes in the Fe content of a sample may affect the sorption of Cu (Zorkin, 1983). To determine if metal-metal interactions was occurring with the metals under study, a set of experiments was conducted where the sorbed metal concentration of one metal was varied while monitoring the concentration of other sorbed metals.

In the first experiment, Cd, Cu and Mn were added to 6 L of diluted SOW (25°/oo) to give a final concentration for 50 nM of each metal. The solution was divided into 12-500 mL aliquots to which Pb was added at a concentration of 25, 50, 100, 200, 400 or 800 nM. Each Pb concentration was run in duplicate. The samples were then passed through a set of columns, eluted, and the concentration of metals measured in the eluates. A second experiment was conducted following the same procedure as above except that both Cd and Mn concentrations were increased while the concentrations of Cu and Pb were kept constant at 50 nM.

TABLE 6
Complexation of Metal with EDTA as Predicted By the Ion-Exchange Technique and MINEQL

EDTA Conc. (nM)	% of Metal Complexed with EDTA							
	Cu		Pb		Mn		Cd	
	Meas.[a]	Calc.[b]	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.
0	0	0	0	0	0	0	0	0
25	3	21	8	2	0	0	0	0
50	14	41	16	5	0	0	2	0
100	28	71	32	15	1	0	3	3
200	70	93	68	48	2	0	7	14
400	94	99	90	89	3	0	23	60
500	95	99	95	94	2	2	33	75
750	98	99	99	98	5	4	50	89
1000	99	99	99	99	9	6	55	93
1500	99	99	99	99	9	11	64	96
2000	99	99	99	99	17	15	93	97

[a]measured by ion-exchange technique:

$$\% \text{ complexed} = 100 - \frac{\text{concentration of metal sorbed from a solution with EDTA}}{\text{concentration of metal sorbed from a similar solution with no EDTA}} \times 100$$

[b]Calculated by MINEQL; pH 8.0, Ionic strength 0.55.

2886

In both experiments, increasing the concentration of a metal in the resin phase had no significant effect on the concentration of any of the other metals in the resin phase (Tables 7 and 8). Consequently, there should be no problems in using the ion-exchange procedure in situations where the concentration of one or more metals far exceeds the concentration of another.

3.1.6 Precision of the Ion-Exchange Analysis

The precision of the metal sorption measurements was determined by pumping diluted SOW containing 50 nM of Cd, Cu, Mn and Pb through a set of columns and analysing the eluates by GFAAS. The coefficient of variation of the observations was highest for Cu at 4.1% and was 2.8% for the other metals (Table 9). As a component of the variability in the analysis is associated with the GFAAS procedure used to quantify the metals in the eluates, the precision of the sorption of metal onto the resin was considered adequate.

However, the amount of Cu and Pb sorbed from the same metal concentrations in solution did vary from one experiment to the next. This was unexpected as the resin should sorb approximately the same amount of metal for each experiment. The change in sorption is thought to be due to some change in the operating conditions between runs such as flow rates, resin preparation, etc. However, this does not present a problem as the standard solutions to calibrate the resin are always run in each experiment.

Sorption variability also affects the Limit of Detection (LOD) reported for each analysis. As LOD is dependent on the slope of the calibration curve, a change in slope, which is the amount of metal sorbed per unit concentration, changes the LOD of the analysis. This is why the LODs have slightly different values for each experiment conducted in Phase I.

2886

TABLE 7
Effect of Increasing Pb Concentration on the Sorption of Cd, Cu and Mn. Values are Means (One Standard Deviation) of Sorbed Metal Concentrations (nmol/g) for Duplicates.

Pb Conc. in Solution (nM)	Pb	Cd[a]	Cu[a]	Mn[a]
25	3.05 (0.09)[b]	0.230 (0.007)[b]	2.33 (0.07)[b]	1.83 (0.06)[b]
50	5.94 (0.13)	0.226 (0.006)	2.40 (0.03)	1.90 (0.02)
100	10.84 (0.03)	0.229 (0.004)	2.29 (0.02)	1.86 (0.00)
200	17.29 (0.48)	0.228 (-)	2.18 (-)	1.90 (-)
400	32.37 (0.32)	0.222 (0.004)	1.82 (0.16)	1.92 (0.04)
800	54.16 (-)	0.225 (0.016)	2.15 (0.08)	1.96 (0.04)

[a]Metal added at a concentration of 50 nM to the sample.

[b]Limit of Detection: Pb - 0.32 nmol/g
Cd - 0.019 nmol/g
Cu - 0.33 nmol/g
Mn - 0.18 nmol/g

TABLE 8
Effect of Increasing Cd and Mn Concentrations on the Sorption of Cu and Pb. Values are Means (One Standard Deviation) of Sorbed Metal Concentrations (nmol/g) for Duplicates.

Cd and Mn Conc. in Solution (nM)	Cd	Mn	Cu[a]	Pb[a]
25	0.104 (0.003)	0.82 (0.02)	4.40 (0.03)	4.90 (0.09)
50	0.228 (0.004)	1.61 (0.06)	4.35 (0.10)	4.89 (0.03)
100	0.441 (0.0)	3.23 (0.02)	4.28 (0.14)	4.94 (0.22)
200	0.907 (0.014)	5.71 (0.22)	4.16 (0.24)	4.94 (-)
400	1.877 (0.081)	11.93 (0.53)	4.53 (0.21)	5.16 (0.14)
800	3.473 (-)	23.82 (-)	4.52 (-)	4.98 (-)

[a]Metal added at a concentration of 50 nM to the sample.

Limit of Detection: Pb - 0.21 nmol/g
Cd - 0.014 nmol/g
Cu - 0.28 nmol/g
Mn - 0.10 nmol/g

2886

TABLE 9
Precision of the Ion-Exchange Procedure.

Metal	$\bar{x}$	SD	CV(%)	No. of Columns[a]
Cd	0.227[b]	0.007	2.9	11
Cu	4.36	0.18	4.1	11
Mn	1.89	0.052	2.8	11
Pb	4.97	0.14	2.8	10

[a] SOW containing 50 nM was passed through a set of columns

[b] nmol/g of sorbed metal

3.2 Procedure Development in Natural Seawater

The following section describes a number of experiments conducted in natural seawater. Unlike the previous experimentation, which was conducted prior to the oyster and algal assays, these experiments were conducted at various times throughout the project. Thus, the modifications made to the ion-exchange procedure described in this section may not have been included in all analyses conducted in oyster or algal assays.

3.2.1 Flow Rate

As a known volume of solution is required to pass through the column to achieve resin equilibration, the length of time needed for this step is dependent on the solution's flow rate. Since an increase in flow rate would result in a decrease in the analysis time, an experiment was conducted to determine the fastest flow rate that could be used without compromising the analytical results.

Six litres of filtered (0.45 um), natural seawater was spiked with Cu to give a final concentration of 0.10 uM and left for 12 hours to equilibrate. Twelve columns were prepared and 500 mL aliquots of seawater were passed through the columns at flow rates of 0.7 ± 0.2, 4.0 ± 0.2 or 6.0 ± 0.2 mL/min. Four columns were used for each flow rate. After equilibration, the columns were eluted with EDTA and the eluates analyzed for Cu by GFAAS.

Table 10 shows the results of the flow rate experiment. The columns having a flow rate of 0.7 mL/min were found to have the least amount of Cu sorbed to the resin. This was unexpected as flow rate should not affect the amount of sorbed metal unless very high flow rates are used.

TABLE 10

**The Effect of Flow Rate on the Sorption of Copper.
Means, Standard Deviations and Analysis of Variance of Sorbed Cu Concentrations**

Flow Rate (ml/min)[a]	0.7	4.0	6.0
Number of replicates	4	4	4
Treatment Mean (nmol/g)	1.77	2.18	2.07
Standard Deviation	0.06	0.24	0.13

Analysis of Variance Table

H_o = No difference due to flow rate.

Source of Variation	Degrees of Freedom	Mean Square	F Ratio	Results of Test
Total	11			
Treatment	2	0.184	6.89	$P<0.05$ Reject H_o
Error	9	0.027		

[a] Resin: AG 50W X-8 (#27947)

2886

One explanation for a decrease in metal sorption at the lower flow rate is that a portion of the resin was not fully exposed to the sample and thus not completely equilibrated with the sample. This could occur if there was channeling of the sample through the resin (i.e. the solution passes through only a portion of the total cross sectional area of the column). Increasing the flow rate could possibly decrease the channeling effect. Further experimentation was conducted to test this hypothesis.

3.2.2 Methods of Resin Equilibration

An experiment was designed to test different methods for equilibrating the resin with the test solution. Six litres of seawater similar to that described above (0.10 μM Cu) was prepared and 500 mL aliquots of sample were passed through a set of 12 columns. The columns were manipulated as follows:

1. Three columns were used in the normal manner; that is, there was no column or resin manipulation, the sample was simply pumped through the resin.

2. Three columns were rotated 120^{o} after each 50 mL of solution had passed through. As the delivery tube for the sample is to one side of the column, rotating the column would ensure that channeling of the sample down the side of the delivery tube was not occurring.

3. The resin in three columns was stirred with a glass rod after each 100 mL of sample.

4. The sample in three of the columns was pumped up through the bottom of the column. This method resulted in the column completely filling with solution and the resin continually being circulated within the

column by the flow action of solution. Both ends of the columns were sealed by a polyethylene frit to prevent loss of resin.

Table 11 shows the results of the column manipulation experiment. As compared to no column manipulation, rotating the columns or circulating the resin within the columns resulted in a higher sorbed Cu concentration. This suggested that these manipulations allowed more resin to come into equilibrium with the test solution and that some channeling of the sample could be occurring in the normal column procedure. Stirring of the resin after every 100 mL of solution, however, was found to reduce the amount Cu sorbed to the resin. There is no apparent explanation for this result except that the physical agititation of the resin may have caused release of Cu in some manner. Further experimentation would be required to determine the reasons for a decrease in sorbed metal due to stirring.

3.2.3 Resin Crosslinkage

Experiments were conducted using different crosslinked resins as it was thought that lower crosslinkage, which gives the resin a more open structure and thus more permeable to higher molecular weight compounds, would resolve the problem of resin equilibration.

The first experiment examined the sorption of Cu to different crosslinked resins: Ag 50W X-8 (X-8 indicates 8% crosslinkage) as compared to Ag 50W X-4 (X-4 indicates 4% crosslinkage). An aliquot (3 L) of filtered natural seawater was spiked with Cu to give a final concentration of 200 nM (0.20 μM) and left for 12 hours to equilibrate. Six columns were prepared with 0.75 g of AG 50W X-8 and six columns with 0.75 g of AG 50W X-4, with aliquots (500 mL) of seawater being passed through each column. After equilibration, the columns were eluted with EDTA and the eluates analyzed for Cu by GFAAS.

TABLE 11

The Effect of Equilibration Methods on the Sorption of Copper. Means, Standard Deviations and Analysis of Variance of Sorbed Cu Concentrations.

	Equilibration Method[a]			
	Normal Procedure	Columns Rotated	Resin Stirred	Resin Circulated
Number of replicates	3	3	3	2
Treatment Mean (nmol/g)	3.40	3.67	2.98	3.86
Standard Deviation	0.07	0.05	0.13	0.16

Analysis of Variance Table

H_o = No difference due to equilibration method.

Source of Variation	Degrees of Freedom	Mean Square	F Ratio	Results of Test
Total	11			
Treatment	3	0.39927	38.6	$P<0.05$ Reject H_o
Error	8	0.01035		

[a] Resin: AG 50W X-8 (#27947)

2886

The effect of crosslinkage on metal sorption is shown in Table 12. Although the same amount of Cu was present in solution, the lower crosslinked resin (AG 50W X-4) sorbed higher levels of Cu. These results suggested that lower crosslinkage allowed more sorption sites to come into equilibrium with the solution and more complete resin equilibrium was obtained.

To further examine the characteristics of AG 50W X-4 in natural seawater, an experiment was conducted to determine the volume of sample required to achieve resin equilibrium. Six litres of filtered natural seawater was spiked with Cu to give a final concentration of 100 nM (0.10 μM) Cu. Twelve columns were prepared with 0.75 g of resin and sample volumes of 200 to 700 mL in 100 mL increments were passed through the columns. Two columns were used for each sample volume.

Figure 13 shows the equilibrium curve for the resin. The resin achieved equilibrium after a sample volume of 400 mL, as little change in the sorbed Cu concentrations was observed at greater volumes. This was the approximate volume for resin equilibrium observed for AG 50W X-8 in artificial seawater. Because of these findings, the resin AG 50W X-8, which was used in the oyster assays, was changed to AG 50W X-4. The new resin was used in the algal assays.

3.2.4 Resin Batch Experiments

Although it appeared that lower crosslinkage affected the sorption characteristics of the resin, further work indicated that other factors may be more important than crosslinkage. This was discovered during the algal assay experiments (see Section D.3.2) where the resin AG 50W X-4 (control #21934) was initially used in the ion-exchange analyses. During these experiments, however, a new bottle of resin (AG 50W X-4; control #30008) was purchased to

FIGURE 13

EQUILIBRIUM CURVE FOR Cu USING AG 50W X-4

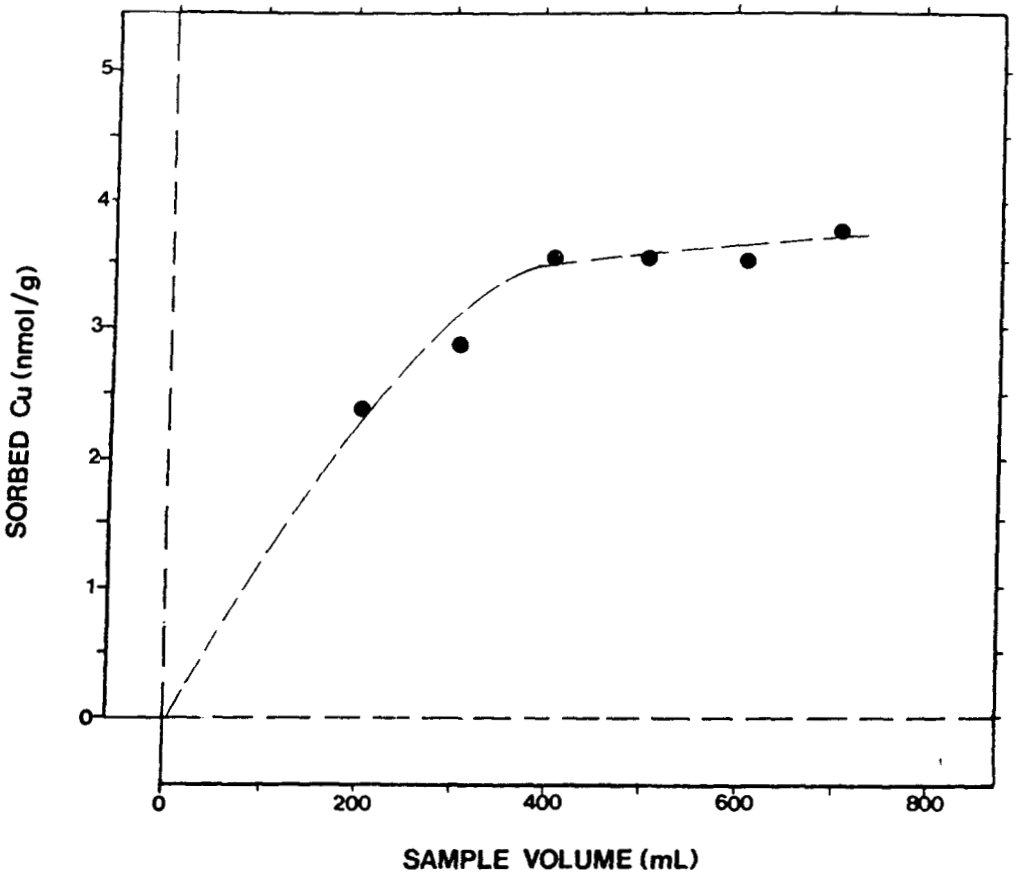


TABLE 12

The Effect of Different Resin Crosslinkage on the Sorption of Cu. Means and Standard Deviations of Sorbed Cu Concentrations (nmol/g).

	Resin	
	AG50W X-4	AG50W X-8
Number of replicates	3	3
Treatment Mean	5.58	4.35
Standard Deviation	0.23	0.49

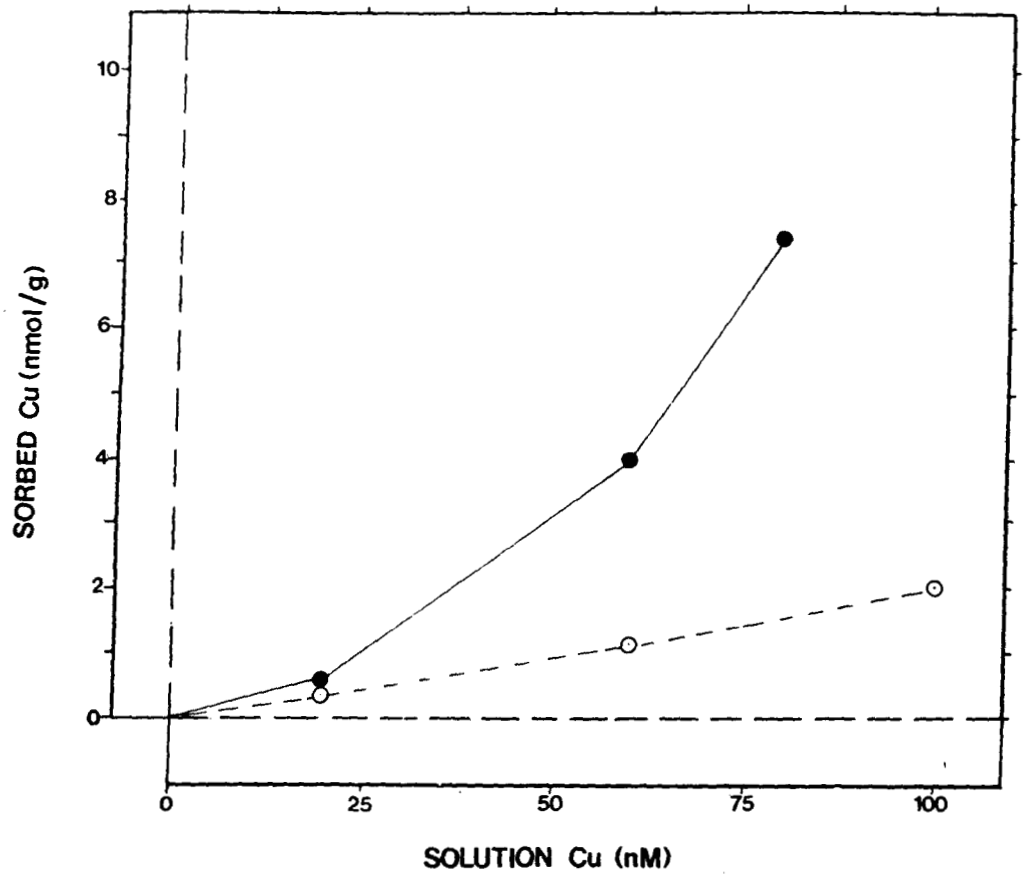
replace the old resin and with its use, it became evident that the sorption characteristics of the two resins were dramatically different. This is illustrated in Figure 14 where seawater standard curves for Cu from two separate algal experiments are presented. Although the new resin had a much lower sensitivity to Cu, its relationship to the metal concentration in solution was linear as compared to the non-linear response obtained with the first batch of resin (control #21934).

A number of factors could explain the difference in sensitivity between these resins. First, although it is possible that different batches of resin could have such different sorption characteristics due to the manufacturing process, it is unlikely due to the quality control measures that are taken by the manufacturer. Another explanation is associated with the age of the resin. With age, these resins undergo chemical degradation which may result in more sites becoming available for sorption. Although the age of the first batch was unknown, it was at least two years older than the newly purchased resin and could account for the difference in the sorption curves.

Obtaining a non-linear standard curve as presented here, poses many problems for the analyst conducting the ion-exchange analysis. It is therefore recommended that for each batch of resin purchased, an initial characterization of the resin be conducted to ensure an appropriate sorption response.

FIGURE 14

SORPTION CURVES FOR Cu USING DIFFERENT RESIN BATCHES



LEGEND

● Experiment A

○ Experiment B

C. PHASE II - OYSTER EMBRYO AND ALGAL ASSAY EXPERIMENTS

1.0 INTRODUCTION

In Phase II, the biological significance of the ion-exchange procedure developed in Phase I was tested by employing oyster embryo and algal assay procedures. These assays were used to generate toxicity data that could be compared to results obtained from ion-exchange analyses conducted on similar samples as those used in the bioassays.

The scope of the work in Phase II was as follows:

1. Determine the optimal experimental design for the toxicity experiments.

2. Determine the concentration ranges of Cd, Cu, Pb and Zn that are toxic to oyster embryos and the diatom Thalassiosira pseudonana.

3. Determine the response of these organisms to toxic metal concentrations in the presence of EDTA.

4. Analyze water samples similar to those used in the bioassays by the ion-exchange procedure and determine an Effective Metal Concentration (EMC) for each sample.

5. Compare the results from the bioassay and ion-exchange analyses to determine the biological significance of the ion-exchange measurement.

2.0 MATERIALS AND METHODS

2.1 Natural Seawater Preparation

Just prior to use, the stored seawater (see Section B.2.2) was filtered a second time through a 142 mm Whatman #1 paper prefilter (cut to size) and a 0.45 um Gelman (GA-6) cellulose triacetate filter. The first two litres of filtrate was discarded. The salinity, pH, alkalinity and dissolved metal concentrations of the various batches of seawater used in the experiments are given in Table 13.

For the oyster assays, three litre aliquots of filtered seawater were put into a set of 4-L narrow-mouthed polyethylene jugs. The aliquots were spiked with the appropriate addition of metal and EDTA, swirled vigorously, and allowed to equilibrate overnight. One litre was used for the column analysis and 1.5 L for the oyster embryo assays.

For the algal experiments, a 10 L aliquot of filtered seawater was placed in a 12-L Nalgene polycarbonate carboy and spiked with nutrients, trace metal and a vitamin mix. Two litre aliquots of the enriched seawater were then put into a set of 4-L polyethylene jugs, and these were spiked with the appropriate addition of metal and EDTA. One litre was used for the column analysis and 750 mL for the algal assays. The seawater preparation scheme for algal and oyster assays is depicted in Figure 15.

2.2 Ion-Exchange Procedure

A set of 28 resin-columns were run at one time. Six columns were used for seawater metal standards (duplicates of three metal concentrations), 21 columns were used for samples and one column for a column blank. Two sets of columns were run per oyster or algal assay.

2886

TABLE 13

Seawater Characteristics

	Batch #4	Batch #5	Batch #6
S °/oo	28.1	28.3[b] ± 0.7	29.1[b] ± 0.8
pH	7.75	7.69[a] ± 0.01	7.76[b] ± 0.03
Alkalinity (meq/L)	2.01	2.00[a] ± 0.02	1.99[b] ± 0.01
Dissolved (0.45 µm) Metals (mg/L):			
	Filter Blank		
Cu	L0.001[c]	L0.001[c,d]	L0.001[c]	L0.001[c,d]
Pb	L0.001[c]	L0.001[c,d]	L0.001[c]	L0.001[c,d]
Cd	L0.0005[c]	0.0006[c,d]	L0.0005[c]	L0.0005[c,d]
Zn	L0.001[c]	0.002[c,d]	0.002[c]	0.002[c,d]
Mn	L0.001[c]	L0.001[c,d]	L0.001[c]	L0.001[c,d]

L = Less than (detection limit)
a = Based on 7 replicates
b = Based on 4 replicates
c = GFAAS analysis after chelation (APDC) and solvent extraction (MIBK)
d = Based on 2 replicates.

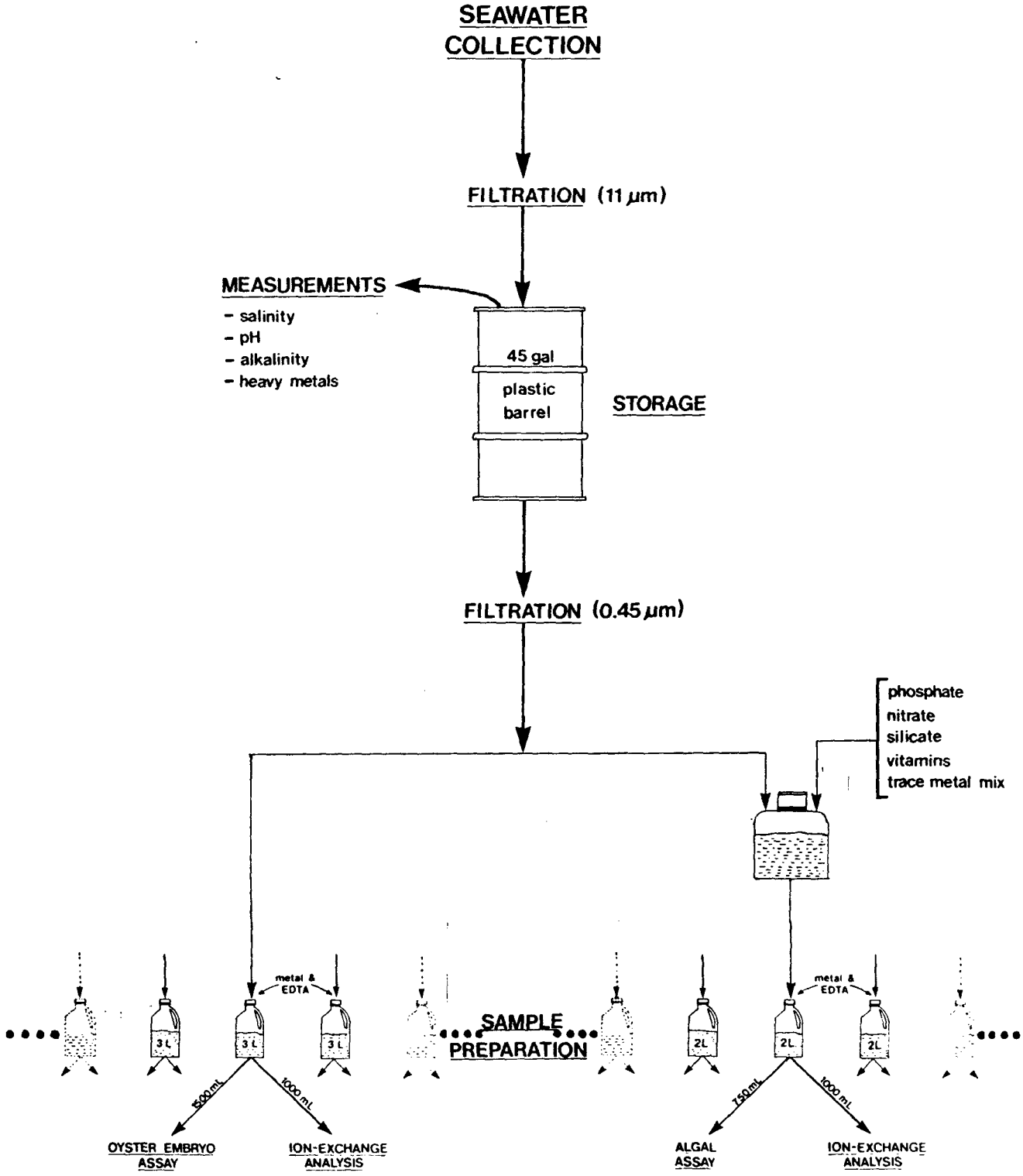


FIGURE 15: SEAWATER PREPARATION SCHEME

Seawater metal standards were made up in SOW diluted with GDW to the same salinity as the samples undergoing analysis; SOW was chosen as the standard medium because it has a well defined and consistent chemical composition. The pH of the standards was adjusted to match the pH of the samples by bubbling with acid cleaned (1N H_2SO_4), filtered (0.4 um Nuclepore) CO_2; this procedure was used instead of acid or base addition so there would be no change in the total alkalinity of the standard. One litre of standard was prepared for each metal concentration by the addition of a metal stock to the diluted SOW.

The methods used to derive EMC values in the ion-exchange procedure are given in detail in Appendix III and will not be repeated here.

2.3 Oyster Embryo Assays

2.3.1 Oyster Conditioning

Oysters (Crassostrea gigas) were purchased from a local wholesaler, generally within two days of their procurement from a commercial oyster grower. Only single oysters measuring greater than 4 inches in length were used. The oysters were scrubbed to remove extraneous debris and placed in a set of five 45-L glass aquaria for conditioning. Twelve to fifteen oysters were placed in each tank depending on their size. Seawater of 27 $\pm$ 1°/oo was dripped into the aquaria at a rate sufficient to completely replace the volume every two days; seawater was obtained from the closed circulating seawater system at U.B.C. Temperature was kept constant at 20 $\pm$ 1°C by an Ebo-Jager automatic aquarium heater or an Hagen radiant aquarium heater with thermostat control. The seawater in the aquaria was filtered by a Hagen Aquaclear combination mechanical/biological filtration system and was aerated by bubbling air through air stones. The aquaria were monitored daily for oyster mortalities and any dead oysters were promptly removed. The aquaria were cleaned every three to four weeks, or more

frequently if there was an accumulation of feces and debris. If an oyster spawning occurred, all oysters in that aquarium were discarded and the aquarium was cleaned.

Oysters were conditioned for various lengths of time to produce sexually mature adults. Oysters collected in the spring generally required 3-4 weeks of conditioning at 20°C while oysters collected in the summer months required no more than one week.

2.3.2 Oyster Embryo Stock Preparation

To prepare an embryo stock, an oyster was opened, removed from its shell and checked for its overall condition. If immature or unhealthy it was discarded. Otherwise a small incision was made in the gonad and a small sample of gametes was taken to determine the viability of eggs or sperm. Sperm viability was determined by motility and eggs were considered ripe when golden brown and pear shaped.

Eggs and sperm were collected by the stripping method. To prepare an egg suspension, the membrane covering the gonad was removed and eggs were gently washed from the gonad into a 2-L polypropylene beaker with a stream of filtered (0.45 um) natural seawater. A final volume of one litre of egg suspension was obtained in this manner. Sperm were removed by making a small incision in the gonad and extracting them with a pipettor. Approximately 0.5 mL of sperm was added to 400 mL of seawater in a 500 mL Erlenmeyer flask. Ten millilitres of the sperm suspension was then added to the egg suspension and the solution stirred to initiate fertilization.

After 30 min, excess sperm, blood cells and body fluids were removed from the fertilized egg suspension by passing it through a Nitex 15 um mesh screen and

backwashing the fertilized eggs from the screen with natural seawater into a one litre polypropylene beaker. The filtration and backwashing step was conducted a number of times to obtain the density of embryos required for the embryo stock. Density was determined using a Coulter Counter (model Z_f). If the readings were less than 6000 eggs/mL, the stock was concentrated further.

2.3.3 Oyster Embryo Assay Procedure

One millilitre of fertilized egg stock (approximately 6,000 fertilized eggs) was added to a set of 500 mL polypropylene beakers containing 250 mL of test solution. All experiments were initiated within 1 hour of fertilization. Triplicate cultures were established for each treatment and six cultures to which no EDTA nor metals were added were used as controls. The beakers were covered in Saran Wrap and kept in a controlled environment room at 20°C for the duration of the assay.

After 48 hours, the assay was terminated by filtering the test solutions through a Nitex screen to retain the oyster larvae. The larvae were backwashed with 7.5 mL of natural seawater into screw-capped glass scintillation vials. To stop further development and preserve the sample, 100 uL of 37% formalin was added to each vial.

For enumeration, each vial was swirled and a sub-sample of approximately 0.5 mL was added to a Sedgewick-Rafter counting cell to obtain counts of greater than 120 larvae. Those embryos that did not develop into D-hinge veliger larvae and those that developed into abnormally shaped D-hinge larvae were scored as abnormal. Two counts were made per sample and the results averaged. Enumeration was performed using a WILD dissecting microscope.

2.3.4 Data Analysis

The basic statistic of interest is the mean percentage of abnormal larvae per treatment. This value is the result of response measurements made on single cultures taken from each of several replicate cultures at a treatment level. However, the resultant abnormality rate is due to the action of two factors; the quality and handling of the oyster embryos, and the treatment itself. As control cultures are used as a measure of the quality and handling of the embryos, Woelke (1972) presents the statistic of percent net risk which would be representative of the effect of the treatment only.

This is defined as:

$$\% \text{ Net Risk} = \frac{\% \text{ treatment abnormal} - \% \text{ control abnormal}}{100 - \% \text{ control abnormal}} \times 100$$

The % net risk statistic is the percentage of abnormal larvae corrected for control abnormals, assuming that the effect of handling and quality is synchronous and independent of the effect of the treatment. Both % abnormality and the % net risk statistic are used in the present report.

2.4 Algal Assays

2.4.1 Nutrient Stock Preparation

Stock solutions of phosphate, nitrate and silicate were prepared separately (Table 14). The pH of all stocks was adjusted to 8.0 and then passed through Chelex-100 to remove trace metal impurities.

Two separate trace metal stocks were prepared, the first consisting of Cu, Mo and Co, and the second of Mn and Zn (Table 14). Aliquots of each were then

TABLE 14

Nutrient Stock and Final Concentrations

	Substance	Stock Concentration (M)	Medium Concentration (M)
Nutrients:	$NaH_2PO_4 \cdot H_2O$	1.00×10^{-2}	1.00×10^{-5}
	$NaNO_3$	1.00×10^{-1}	1.00×10^{-4}
	$Na_2SiO_3 \cdot 9H_2O$	1.25×10^{-2}	1.25×10^{-5}
Trace Metals:	$CuSO_4 \cdot 5H_2O$	9.97×10^{-4}	9.97×10^{-10}
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.50×10^{-3}	1.50×10^{-9}
	$CoCl_2 \cdot 6H_2O$	2.50×10^{-3}	2.50×10^{-9}
	$MnCl_2 \cdot 4H_2O$	2.30×10^{-2}	2.30×10^{-8}
	$ZnSO_4 \cdot 7H_2O$	4.00×10^{-3}	4.00×10^{-9}
	$FeCl_3 \cdot 6H_2O$[a]	4.51×10^{-4}	4.51×10^{-7}
Vitamins:	B_{12}	1.1×10^{0} g/l	5.5×10^{-7} g/l
	Biotin	1.0×10^{-1} g/l	5.0×10^{-7} g/l
	Thiamine HCl	2.0×10^{-1} g/l	1.0×10^{-4} g/l

[a]Added as precipitated ferric hydroxide.

combined to make a trace metal mix. Prior to each algal experiment, an Fe stock was freshly prepared at 0.45 mM and allowed to equilibrate for a few hours to allow the formation of ferric hydroxide. Freshly percipitated ferric hydroxide has been found to be an effective source of Fe to phytoplankton (Wells et al., 1983).

Stocks of B_{12}, biotin and thiamine-HCl were prepared separately, then combined to make a vitamin mix (Table 14).

The prepared stocks were stored in 500 mL polycarbonate Erlenmeyer flasks at 4°C in the dark with the exception of the vitamin mix, which was frozen in 20 mL aliquots in glass scintillation vials. The silicate stock was stored for a maximum of 2 weeks as it is unstable at pH 8.0. All the stocks were allowed to reach room temperature (22°C) before addition to the assay medium. The final concentrations of the nutrients, vitamins and trace metals in the assay medium are given in Table 14.

2.4.2 Algal Stock Preparation

The marine centric diatom Thalassiosira pseudonana (= Cyclotella nana Hustedt) Hasle and Heimdal (WHOI clone 3H) was used as the algal assay organism. It was chosen because of its sensitivity to heavy metals, its uniform size and shape (factors which make it amenable to measurement by electronic particle counting), and its fast growth rate (up to 2.5 doublings/day). The organism varies from 2.5 to 10 um in size and normally appears singly or in pairs in laboratory cultures.

Stock cultures were initially obtained from the Northeast Pacific Culture Collection (University of B.C., Vancouver, B.C.) and maintained in filtered (0.45 um) natural seawater to which nutrients, trace metals and vitamins were added

(Table 14). Inocula for the assays were obtained from exponentially growing stock cultures. Cultures were maintained in exponential growth by transferring approximately 1 mL of existing stock to fresh media every 3 to 4 days.

2.4.3 Algal Assay Procedure

Aliquots (250 mL) of test solution were put into a set of 500 mL polypropylene bottles and stock culture was added to give a final cell concentration of 1000-2000 cells/mL. Triplicate cultures were run for each treatment. Six cultures to which no metal nor EDTA were added served as controls. The organisms were grown in a controlled environment room at 20 $\pm$ 1°C with a light intensity of 95 uEin/m^2/sec under continuous light. The cell concentrations were measured daily at approximately the same time each day using a Coulter Counter (model Z_f) over a period of 5 days. For enumeration, each bottle was swirled and a sub-sample (2.0 mL) taken. Four counts were obtained per sample and the counts averaged.

2.4.4 Data Analysis

The bioassay results were expressed as cell yield taken as a percentage of the control cultures. The cell yield is the total population at the end of the experimental period minus that at the start. Cell yield (% of control) is determined by taking the cell yield of the treatment culture and the cell yield of the control cultures and applying the following formula:

$$\text{Cell yield (\% of control)} = \frac{\text{Cell yield treatment}}{\text{Cell yield control}} \times 100$$

2886

3.0 RESULTS AND DISCUSSION

3.1 Oyster Embryo Assays

3.1.1 Experimental Design

Initially, a 6 x 1 factorial design employing six metal concentrations and one concentration of EDTA was to be used per oyster assay; the overall number of experiments to be conducted for a given metal was therefore determined by the number of EDTA concentrations required to generate sufficient toxicity data for a metal. Using the results from a set of toxicity experiments was believed to be valid as it was assumed that oyster embryos from different parent stocks would respond to toxic metal concentrations in a similar manner (i.e. the same percentage of abnormal larvae would be obtained for a given toxic metal concentration in every experiment). After preliminary investigation, however, it was realized that there was sufficient variability in the sensitivity of oyster embryos from different parent stocks to invalidate a comparison of results between experiments. This is best illustrated with results obtained from an experiment conducted with Cd.

Cadmium was added to natural seawater to give total concentrations of 0.0, 1.0, 2.0, 3.0, 4.0 and 6.0 μM. An embryo stock was prepared, the samples inoculated, and a 48-hour bioassay conducted. The bioassay was then terminated in the normal manner except that, after filtration, the test solutions were re-inoculated with embryos taken from a second spawn and another 48-hour bioassay conducted. The parent oysters used to prepare the second embryo stock had been conditioned in the same tank and for approximately the same length of time as the oysters used for the initial stock.

Figure 16 shows the toxicity curves generated for the experiment. It was evident the relationship between toxicity and the inorganic metal concentration was different for the embryos from each stock. The embryos from the second spawn appeared to be more sensitive to Cd than were the embryos from the first spawn. As all other conditions were kept constant between the bioassays, the difference in the response to Cd was primarily attributed to differences in the sensitivity of the embryos from the different spawns. Such variability was also evident in experiments conducted with the other metals, though not nearly as pronounced as presented here for Cd.

Similar findings with Crassostrea gigas embryos have been reported for Cu. Coglianese and Martin (1981) reported toxicity data that showed considerable variability in the sensitivity of embryos from different spawns. For example, at a Cu concentration of 10 µg/L, the percent normal embryonic developments in three experiments using embryos from different spawns were 3.0, 21.3 and 44.0%. They also found a similar range of values for other Cu concentrations.

The change in embryo sensitivity could be due to a number of factors, which includes conditioning of the parent oysters. Conditioning would affect gonad ripeness and the overall quality of the oyster sperm and eggs (Russell, 1963). However, it was beyond the scope of the present study to determine the factors affecting embryo sensitivity to metals and therefore further experimentation was not conducted.

To eliminate the problem of sensitivity changes, the overall experimental design was modified by increasing the number of samples in a single toxicity experiment fourfold. This would allow a set of metal-ligand concentration combinations analyzed in four separate experiments to be analyzed using a single embryo stock. All toxicity experiments presented below were of a 6 x 4 factorial design that used six metal concentrations and four EDTA concentrations per experiment.

2886

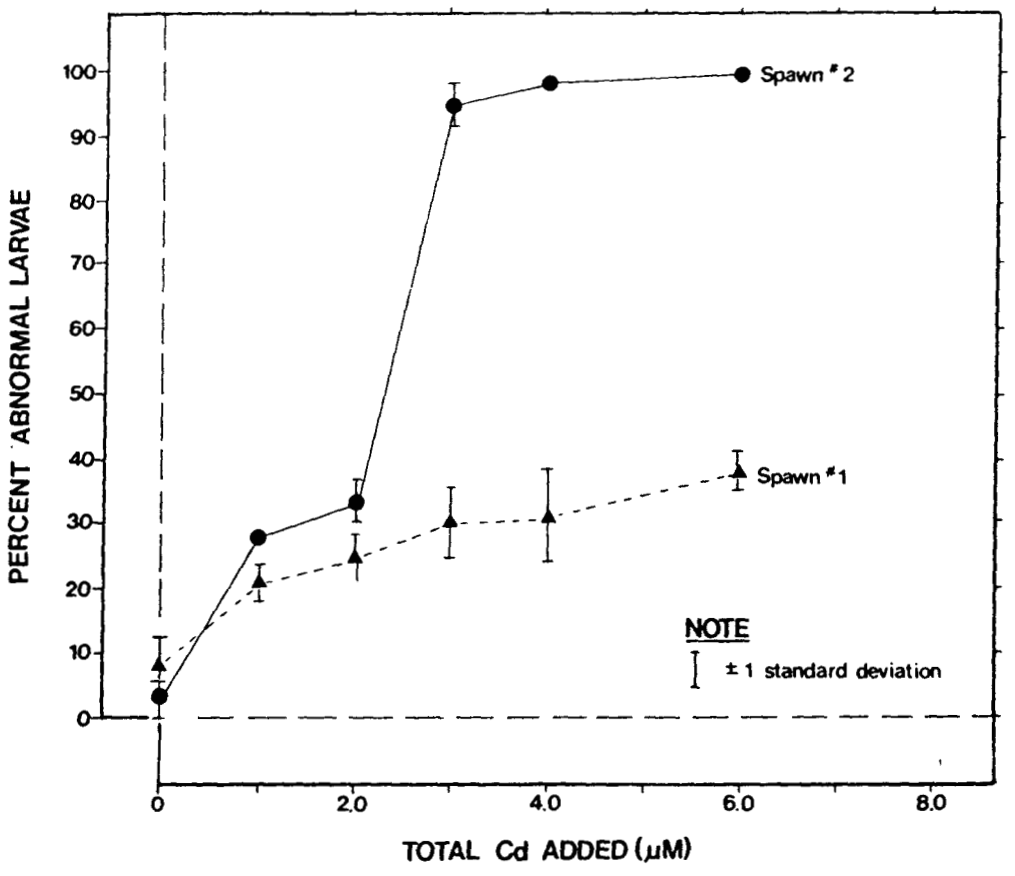


FIGURE 16

ABNORMAL DEVELOPMENT OF C. gigas EMBRYOS OF DIFFERENT SPAWNS RESULTING FROM EXPOSURE TO TOTAL ADDED CADMIUM FOR 48 HOURS

3.1.2 Toxicity Experiments

Metal-EDTA concentration combinations were added to natural seawater and analysed by the oyster embryo assay. The purpose of adding a model ligand such as EDTA was to vary the toxicity of a sample while maintaining the total metal concentration in the sample constant; EDTA varies a metal's toxicity by complexation of the free metal ion, which is the metal species believed to be most toxic. EDTA was chosen as the organic ligand because it has a strong affinity for heavy metals (log K values for Cd, Cu, Pb and Zn are, respectively, 16.5, 18.1, 18.0 and 15.8; corrected for Ionic strength of 0.5) and the ligand would therefore be required in a relatively low concentration to affect metal toxicity.

Copper

Of the four metals studied Cu was found to be the most toxic. The values obtained from a bioassay where no EDTA was added yielded an average LC_{50} of 0.21 µM by log-probit analysis (Figure 17). This is consistent with the work of Harrison et al. (1981) who obtained an LC_{50} of 0.18 µM for Crassostrea gigas embryos using a similar 48-hour static bioassay test. Greater than 80% larval abnormality was found with exposure to 0.3 µM total added Cu.

Figure 18A shows the toxicity curves for the embryos when exposed to Cu in the presence of 0.000, 0.025, 0.050 and 0.100 µM EDTA. The response of the embryos is represented here as the % net risk statistic which is the percentage of abnormal larvae in a treatment corrected for control abnormals. The similarity in the four curves indicated that the amount of EDTA added was not sufficient to reduce Cu toxicity, except at the 0.20 µM Cu level where the addition of EDTA reduced net risk from 29% to approximately 10%.

FIGURE 17

LOG-PROBIT GRAPH TO DETERMINE LC_{50} OF C. gigas EMBRYOS EXPOSED TO COPPER FOR 48 HOURS

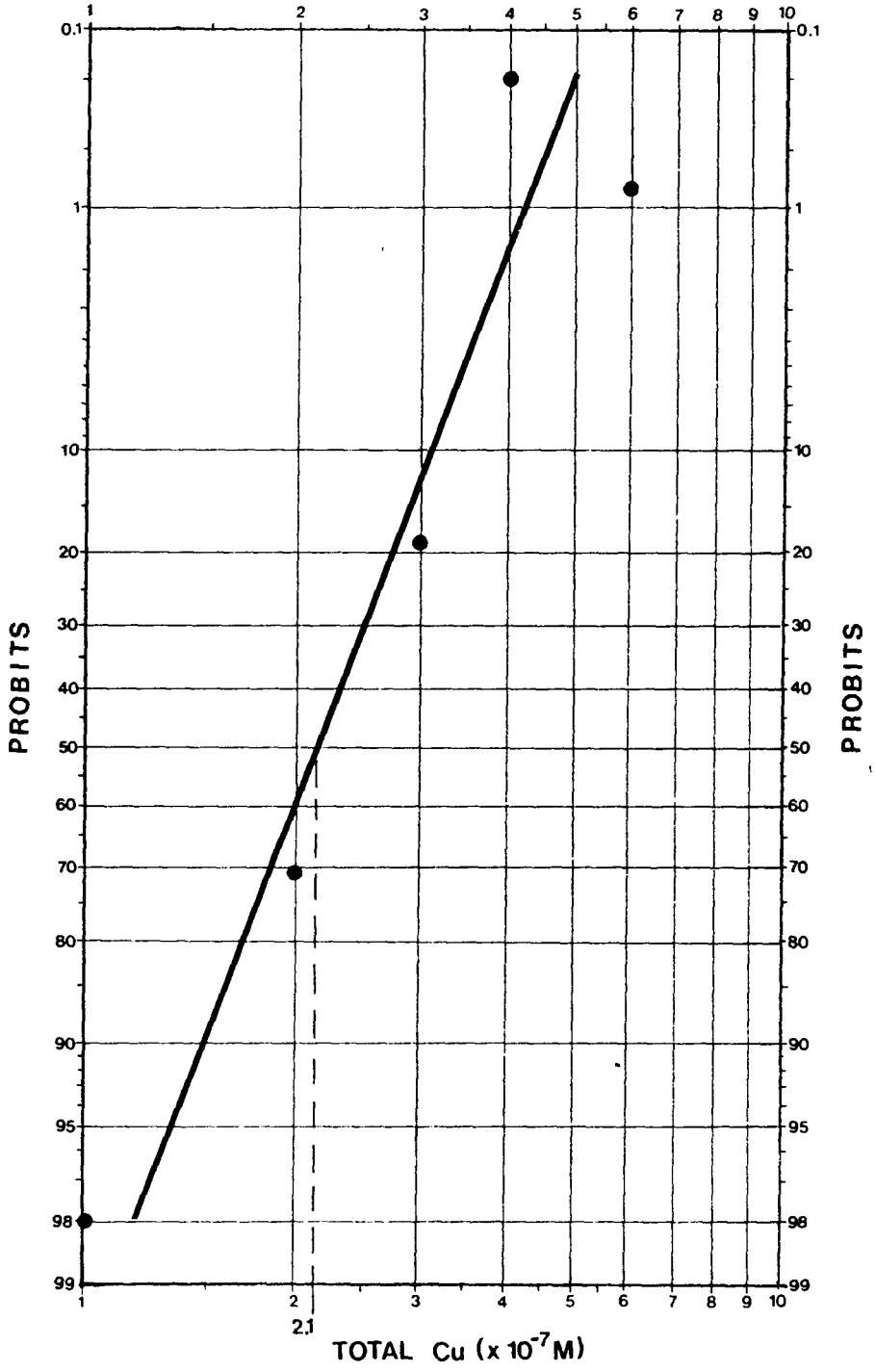
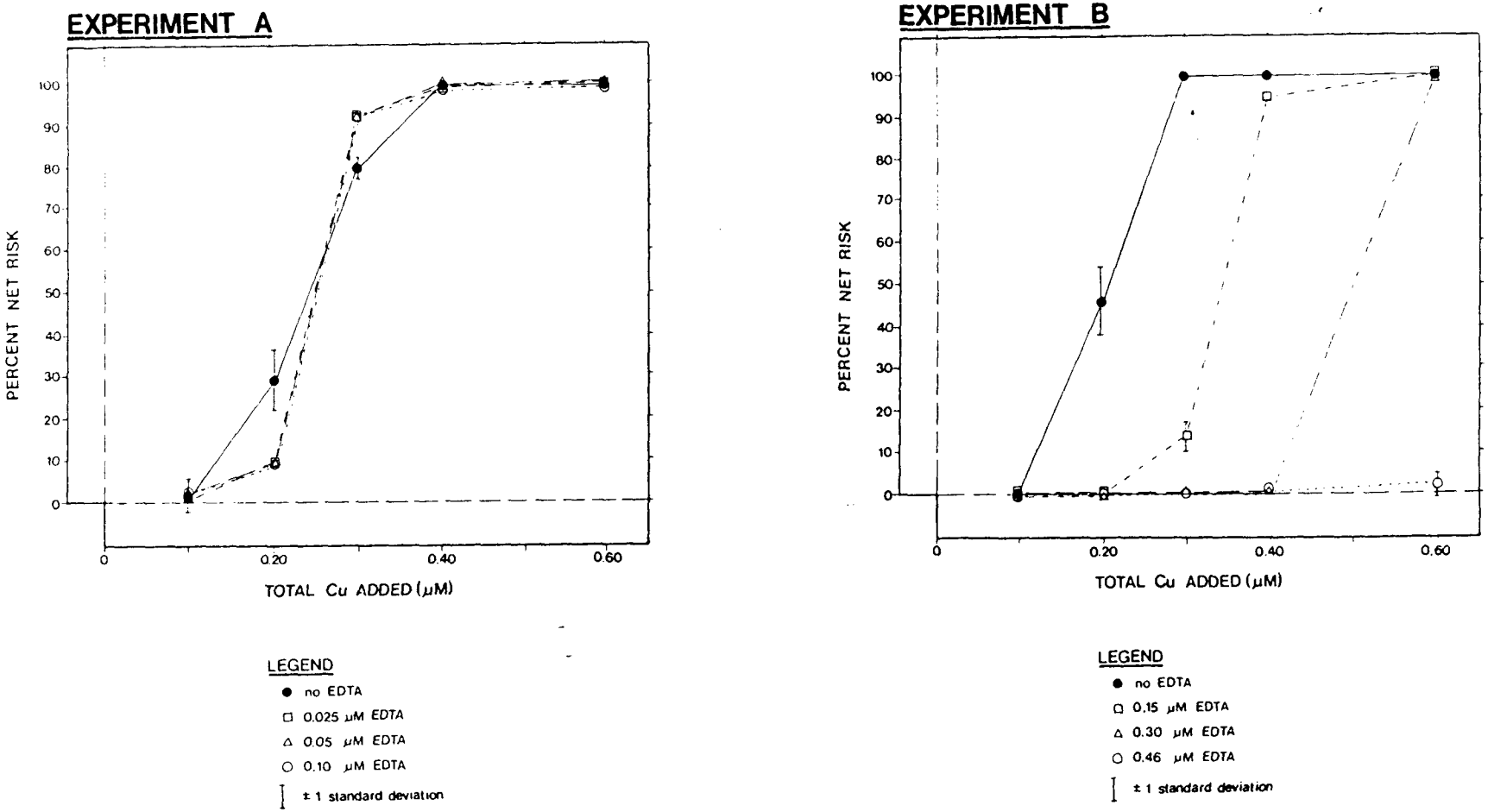


FIGURE 18: **ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs THE TOTAL ADDED COPPER CONCENTRATION IN THE PRESENCE OF EDTA**



However, increasing the concentration of EDTA above 0.10 uM had a dramatic effect. Figure 18B presents toxicity curves for embryos exposed to Cu in the presence of 0.00, 0.15, 0.30 and 0.46 uM EDTA. As the EDTA concentration increased, the toxicity at a given metal concentration decreased. For example, net risk obtained at 0.30 uM Cu was reduced from 99.7% to less than 1% by the addition of 0.30 uM EDTA. An addition of 0.46 uM EDTA practically eliminated Cu toxicity at all concentrations studied, including a Cu level of 0.60 uM. In conclusion, adding EDTA in a concentration equivalent to that of the Cu concentration appeared to be sufficient to reduce its toxicity.

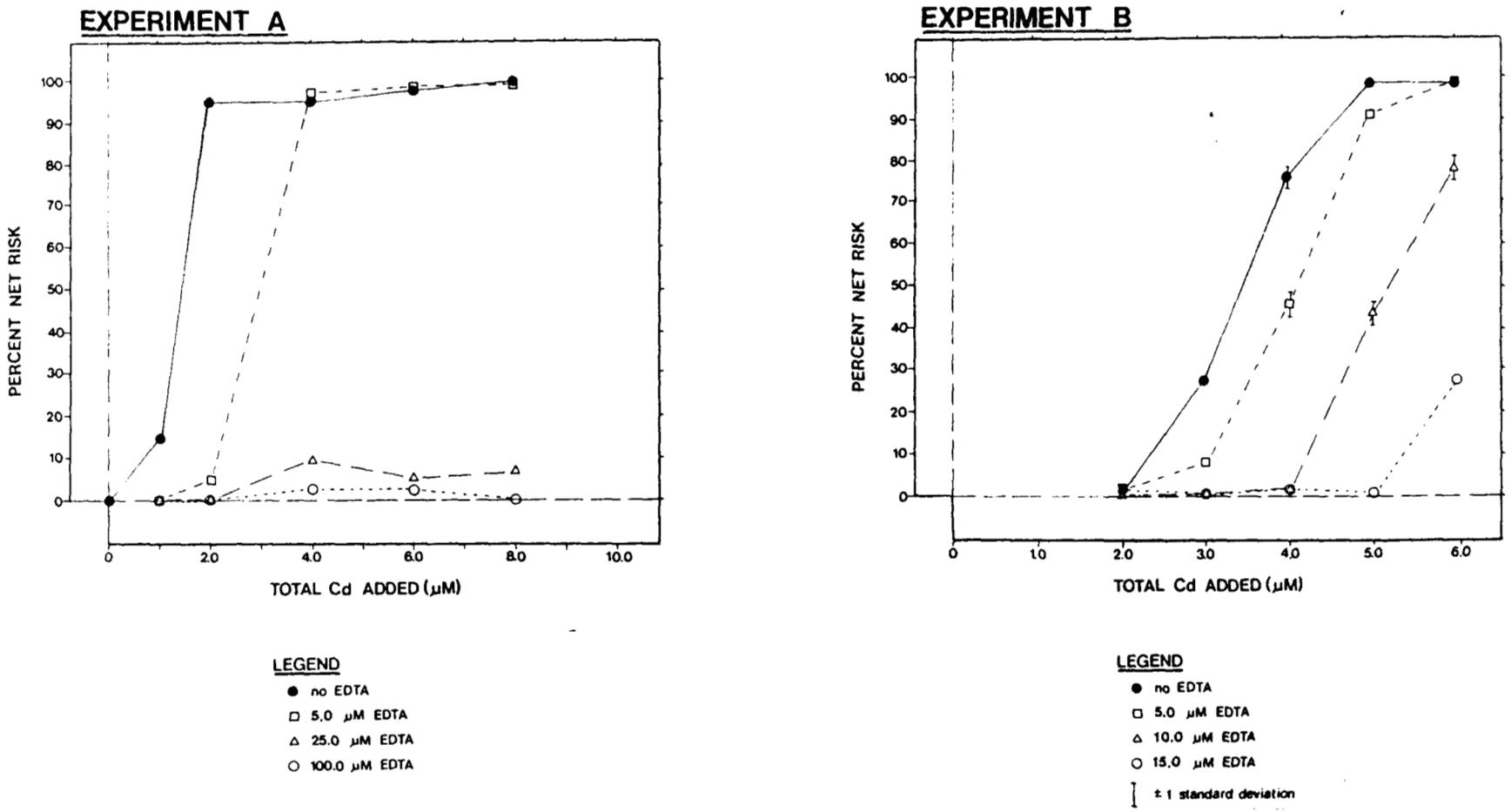
Cadmium

The amount of Cd required to initiate a toxic response was greater than that required for Cu. Total metal concentrations of 2.0 uM or more were needed to affect the development of the embryos as compared with only 0.2 uM for Cu.

Figure 19 presents the relationship between toxicity and total Cd concentrations in the presence of EDTA. As with Cu, the addition of EDTA resulted in a decrease in Cd toxicity and the extent of this reduction was related to ligand concentration. However, the amount of ligand needed to reduce Cd toxicity was proportionally much greater than that needed for Cu (i.e. the concentration of ligand added had to be much higher than the concentration of metal before a reduction in toxicity was realized). This can be attributed to the relative stability of the Cd-EDTA complex and the strong complexation of Cd by the chloride ion. (As the affinity of EDTA for Cd ($\log K_1$ = 16.5; I = 0.5M) is lower than for Cu ($\log K_1$ = 18.1), more EDTA is required to reduce the free cadmium ion concentration than would be required to reduce the free cupric ion concentration.)

2886

FIGURE 19: **ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs THE TOTAL ADDED CADMIUM CONCENTRATION IN THE PRESENCE OF EDTA**



Lead and Zinc

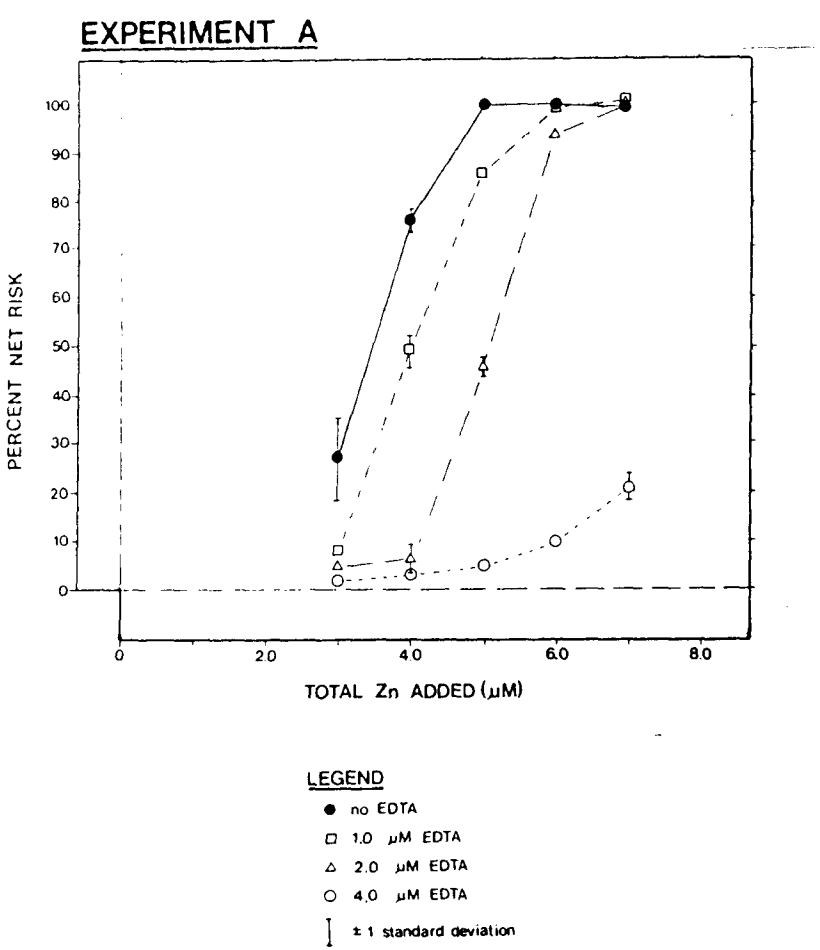
Figures 20 and 21 presents the toxicity curves for the experiments conducted with Zn and Pb. The amount of Zn required to induce a toxic response was similar to that found for Cd. Total Zn concentrations between 2.0-3.0 μM were found to affect embryo development and a 100% response was found at a total metal concentration of approximately 5.0 μM.

In the Pb experiments, the sensitivity of the oyster embryos to total inorganic Pb concentrations varied. For example, in one experiment (Figure 21A) the addition of 4.0 μM Pb resulted in a net risk of 0.5%, while in another (Figure 22B) the same addition of metal resulted in a net risk of approximately 60%. As was demonstrated for Cd, the change in sensitivity to Pb was partly attributed to variability in the sensitivity of embryos from different parent stocks.

As with the other metals, the addition of EDTA to the culture water reduced the toxicity of both Pb and Zn and the extent of this reduction was related to the ligand's concentration. Moreover, as was found for Cu, a metal-ligand concentration ratio of approximately 1:1 was sufficient to reduce the metal's toxicity.

In conclusion, the relationship between toxicity and total metal concentration changed dramatically with the addition of EDTA for all metals studied. When one extrapolates these findings to the natural environment, it is evident that prediction of a metal's toxicity in a natural seawater sample containing complexing agents would be limited if only total or dissolved metal concentration data were available.

FIGURE 20: **ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs THE TOTAL ADDED ZINC CONCENTRATION IN THE PRESENCE OF EDTA**



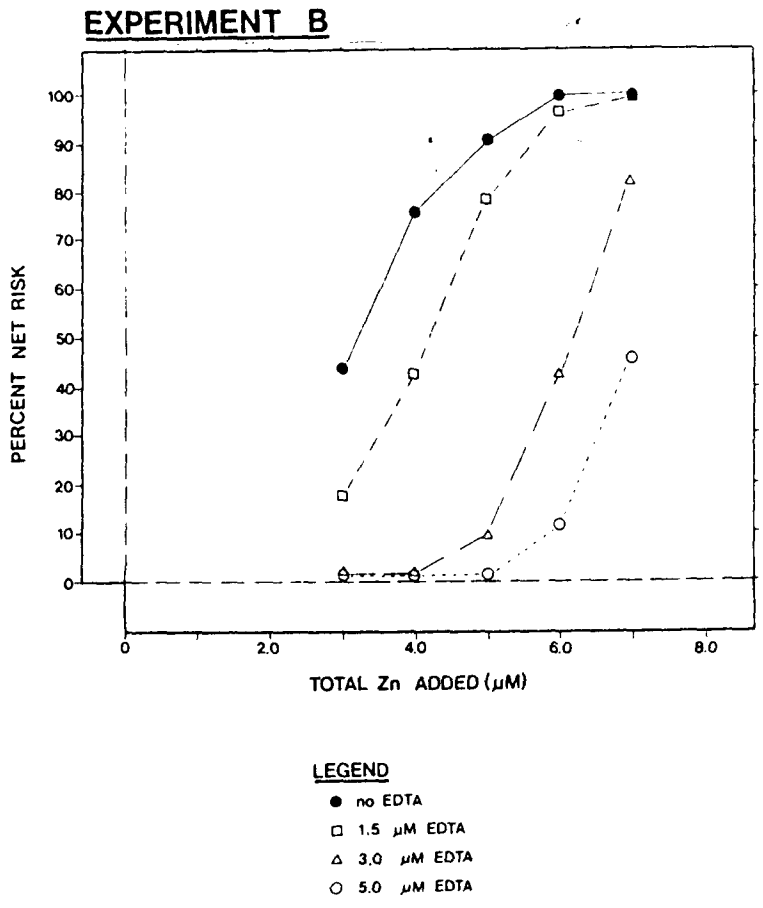
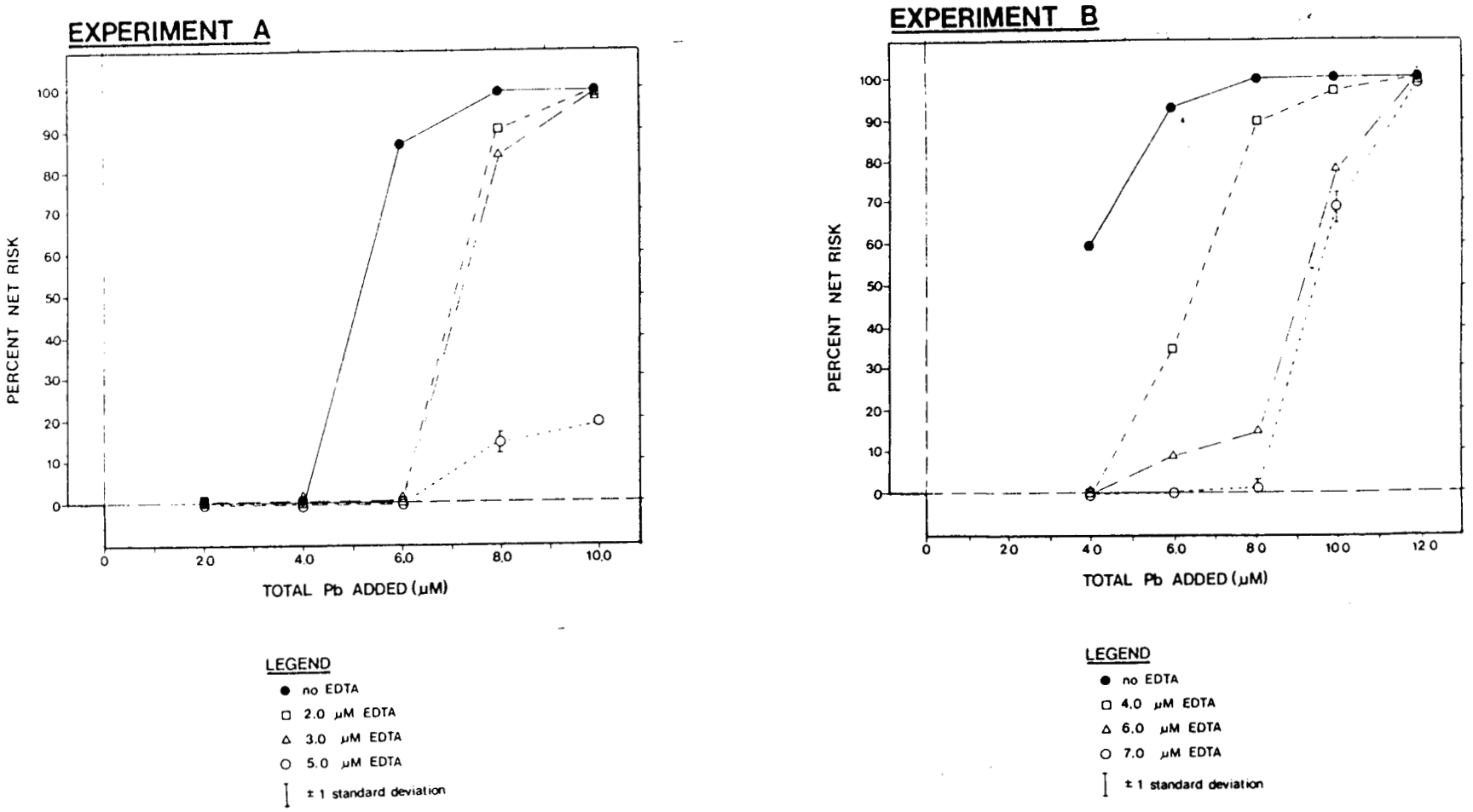


FIGURE 21: **ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs THE TOTAL ADDED LEAD CONCENTRATION IN THE PRESENCE OF EDTA**



3.1.3 Ion-Exchange Results

Aliquots of the metal-EDTA samples analyzed in the oyster assay also underwent analysis by the ion-exchange procedure to determine an EMC for each sample. The results of the ion-exchange analyses are presented in Tables 15-18.

As EMC is equated to the inorganic metal concentration of a sample (see Section B.1.0), a sample having no EDTA addition should have an EMC value similar to the total concentration of metal added to that sample provided the natural seawater used in the experiment did not contain organic ligands in sufficient quantity to affect the chemistry of the metal.

Although the EMC's for Cu, Cd and Zn in samples having no EDTA added were generally similar to the total added metal concentration (Tables 15-17), there were a few instances where EMC's were lower than the total added metal concentration. For example, in an experiment with Cu (Table 15: Expt. B), an EMC value of 0.139 uM was obtained when 0.200 uM Cu was added to the sample. Such discrepancies were attributed to experimental error.

Lead, on the other hand, had EMC values lower than the amount of Pb added for all concentrations studied (Table 18). As this occurred in both experiments, the decrease in EMC appeared to be due to factors affecting Pb speciation in the natural seawater. Such factors include the presence of colloidal matter that could adsorb the metal or complexation of the metal by organic ligands.

When EDTA was added to the samples, the EMC value of the sample was decreased and the extent of this reduction was related to ligand concentration. The reduction of the EMC values was attributed to the complexation of the free metal ion by EDTA and thus the reduction of the cationic metal concentration in solution; it is the concentration of cationic metal that determines the amount of metal sorbed by the resin and hence the EMC value.

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lts of the Ion-Exchange Analyses for Copper.
:s are Means (One Standard Deviation) of EMCs (uM) for Duplicates

er-nt[a]	EDTA Conc.[b] (uM)	Copper Conc. Added[c] (uM) 0.100	0.200	0.300	0.400	0.600
	0.0	0.089 (0.005)	0.198 (0.027)	0.302 (0.036)	0.438 (0.043)	0.687 (0.032)
	0.025	0.050 (0.004)	0.117 (0.015)	0.190 (0.025)	0.265 (0.004)	0.609 (0.020)
	0.050	0.074 (0.002)	0.172 (0.007)	0.288 (0.042)	0.381 (0.018)	0.518 (0.033)
	0.100	0.017 (0.001)	0.100 (0.003)	0.223 (0.023)	0.308 (0.029)	0.456 (0.020)
	0.00	0.090 (0.009)	0.139 (0.029)	0.302 (0.073)	0.374 (0.002)	0.491 (0.011)
	0.15	0.008 (0.001)	0.057 (0.002)	0.156 (0.041)	0.240 (0.003)	0.352 (0.013)
	0.30	N.D.[d]	0.007 (0.000)	0.040 (0.007)	0.145 (0.003)	0.357 (0.025)
	0.46	N.D.	N.D.	0.006 (0.000)	0.013 (0.002)	0.222 (0.003)

esin: AG 50W X-8 (#27947).
oncentrations of total EDTA added to the bioassay water (Seawater Batch #4).
oncentrations of total Cu added to the bioassay water.
on-Detectable: Limit of Quantification - 0.003 uM; Limit of Detection - 0.001 uM

86

TABLE 16

Results of the Ion-Exchange Analyses for Cadmium.
Values are Means (One Standard Deviation) of EMCs (uM) for Duplicates

Experiment[a]	EDTA Conc.[b] (uM)	Cadmium Conc. Added[c] (uM)				
		1.0	2.0	4.0	6.0	8.0
A	0.0	0.94(0.02)[d]	2.00(0.03)	4.02(0.07)	5.98(0.11)	8.11(0.03)
	5.0	0.37(0.01)	0.81(0.04)	2.35(0.26)	4.37(0.08)	6.48(0.38)
	25.0	0.26(0.06)	0.70(0.13)	1.37(0.03)	2.01(0.13)	3.10(0.30)
	100.0	0.25(0.03)	0.59(0.03)	1.22(0.01)	1.74(0.01)	2.43(0.40)
		2.0	3.0	4.0	5.0	6.0
B	0.0	1.78(0.06)	3.01(0.06)	4.04(0.15)	4.99(0.03)	6.80(0.64)
	5.0	0.71(0.04)	1.11(0.06)	1.59(0.00)	2.42(0.13)	3.04(0.11)
	10.0	0.29(0.01)	0.70(0.01)	1.11(0.01)	1.48(0.05)	1.77(0.13)
	15.0	0.49(0.13)	0.81 (-)	0.94(0.07)	1.40(0.11)	1.63(0.11)

[a]Resin: AG 50W X-8 (#27947).

[b]Concentrations of total EDTA added to the bioassay water (Seawater Batch #4).

[c]Concentrations of total Cd added to the bioassay water.

[d]Limit of Quantification: 0.005 uM; Limit of Detection: 0.002 uM.

2886

TABLE 17

Results of the Ion-Exchange Analyses for Zinc.
Values are Means (One Standard Deviation) of EMCs (uM) for Duplicates

Experiment[a]	EDTA Conc.[b] (uM)	Zinc Conc. Added[c] (uM)				
		3.0	4.0	5.0	6.0	7.0
A	0.0	2.73(0.00)	3.74(0.07)	4.60(0.00)	5.58(0.00)	6.45(0.07)
	1.0	1.58(0.26)	2.67(0.03)	3.51(0.14)	4.43(0.04)	5.14(0.04)
	2.0	0.84(0.21)	1.71(0.04)	2.64(0.00)	3.23(0.21)	3.71(0.11)
	4.0	N.D.[d]	N.D.	0.94(0.06)	1.63(0.14)	2.24(0.10)
B	0.0	2.95(0.03)	4.05(0.00)	5.04(0.06)	6.14(0.09)	6.92(0.00)
	1.5	1.58(0.11)	2.62(0.03)	3.47(0.06)	4.39(0.30)	5.61(0.00)
	3.0	N.D.	1.36(0.40)	2.37(0.00)	3.33(0.09)	4.05(0.09)
	5.0	N.D.	N.D.	N.D.	1.47(0.00)	2.47(0.09)

[a]Resin: AG 50W X-8 (#27947)

[b]Concentrations of total EDTA added to the bioassay water (Seawater Batch #4).

[c]Concentrations of total Zn added to the bioassay water.

[d]Non-Detectable: Limit of Quantification - 0.68 uM; Limit of Detection - 0.14 uM.

2886

TABLE 18

Results of the Ion-Exchange Analyses for Lead.
Values are Means (One Standard Deviation) of EMCs (uM) for Duplicates

Experiment[a]	EDTA Conc.[b] (uM)	Lead Conc. Added [c] (uM)				
		2.0	4.0	6.0	8.0	10.0
A	0.0	1.69(0.08)[d]	3.62(0.08)	4.65(0.35)	7.00(0.17)	8.78(0.07)
	2.0	0.11(0.03)	2.11(0.11)	3.73(0.31)	5.27(0.21)	6.72(0.08)
	3.0	0.06(0.04)	0.97(0.04)	2.81(0.03)	4.30(0.25)	5.98(0.09)
	5.0	0.05(0.01)	0.04(0.01)	0.85(0.11)	2.53(0.15)	4.09(0.05)
		4.0	6.0	8.0	10.0	12.0
B	0.0	2.96(0.04)	4.34(0.13)	6.16(0.33)	8.27(0.22)	8.38(0.16)
	4.0	0.25(0.04)	2.18(0.07)	3.71(0.01)	5.68(0.04)	6.22(0.00)
	6.0	0.06(0.01)	0.53(0.08)	2.37(0.01)	4.23(0.27)	5.92(0.00)
	7.0	0.06(0.02)	0.15(0.01)	1.50(0.02)	3.25(0.33)	4.68(0.11)

[a]Resin: AG 50W X-8 (#27947).

[b]Concentrations of total EDTA added to the bioassay water (Seawater Batch #4).

[c]Concentrations of total Pb added to the bioassay water.

[d]Limit of Quantification - 0.027 uM; Limit of Detection - 0.008 uM.

2886

Although Cd EMC values could be decreased with the addition of EDTA, the relationship between its concentration and the effect on EMC was different than the other metals. Apparently, as a threshold concentration of EDTA was reached, the ability of the ligand to reduce EMC was dramatically reduced. For example, in the first Cd experiment (Table 16: Expt. A), the addition of 5 uM EDTA to a sample containing 4.0 uM Cd resulted in a 42% reduction in EMC. However, adding 5 times the quantity of EDTA (25 uM) resulted in a decrease of EMC by an additional 24%. Increasing the EDTA concentration to 100 uM resulted in an EMC value slightly lower than was obtained with 25 uM EDTA. As this high level of EDTA should have dramatically reduced the EMC value, it appeared that other factors were affecting the ion-exchange analysis of Cd. These results suggest that either the complexing ability of EDTA for Cd decreased as its concentration increased, or there was adsorption of some of the Cd-EDTA complex. Further work is necessary to discover the reason(s) for these results.

It should be noted that there were some inconsistencies in the data. In one experiment with Cu (Table 15: Expt. A), samples containing 0.025 uM EDTA had lower measured EMC values than EMCs measured for samples containing a higher EDTA concentration (0.050 uM EDTA). Since increasing the concentration of EDTA should decrease the EMC value of the sample, the reasons for the discrepancy are attributed to experimental error, possibly with the initial spike of the metal, or in the GFAAS analysis.

2886

3.1.4 Comparison of Oyster Assay and Ion-Exchange Results

To determine the relationship between toxicity and EMC, the % net risk statistic was plotted as a function of the EMC values. Data for all metal-EDTA concentration combinations analyzed in a given experiment were plotted on a single graph. By plotting the data in this manner, all data points should fall along a single sigmoidal curve regardless of the EDTA concentration assuming that EMC is proportional to the toxic metal concentration in solution. Furthermore, as EMC is equivalent to the truly dissolved inorganic metal concentration in a sample, plotting the toxicity data as a function of EMC should result in a toxicity curve similar to the curve obtained when the embryos were exposed to only inorganic metal (i.e. no EDTA addition) provided no natural complexing agents were present.

Figure 22 shows the relationship between toxicity and EMC for two experiments conducted with Zn. The data could be best described by a single sigmoidal curve suggesting the relationship between toxicity and EMC was independent of the EDTA concentration and EMC was proportional to the toxic metal concentration in solution.

A similar relationship between toxicity and EMC was also found for Pb in that the % net risk statistic appeared to be a direct function of EMC for all ligand concentrations studied (Figure 23). This was in contrast to the separate functions obtained for each ligand concentration when the same toxicity data were plotted against total metal concentrations (Figure 21). It was thus concluded that EMC was proportional to the concentration of toxic metal and EMC could be used to predict the metal's toxicity more accurately than was possible using total metal concentration data.

FIGURE 22: ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs EMC VALUES FOR ZINC

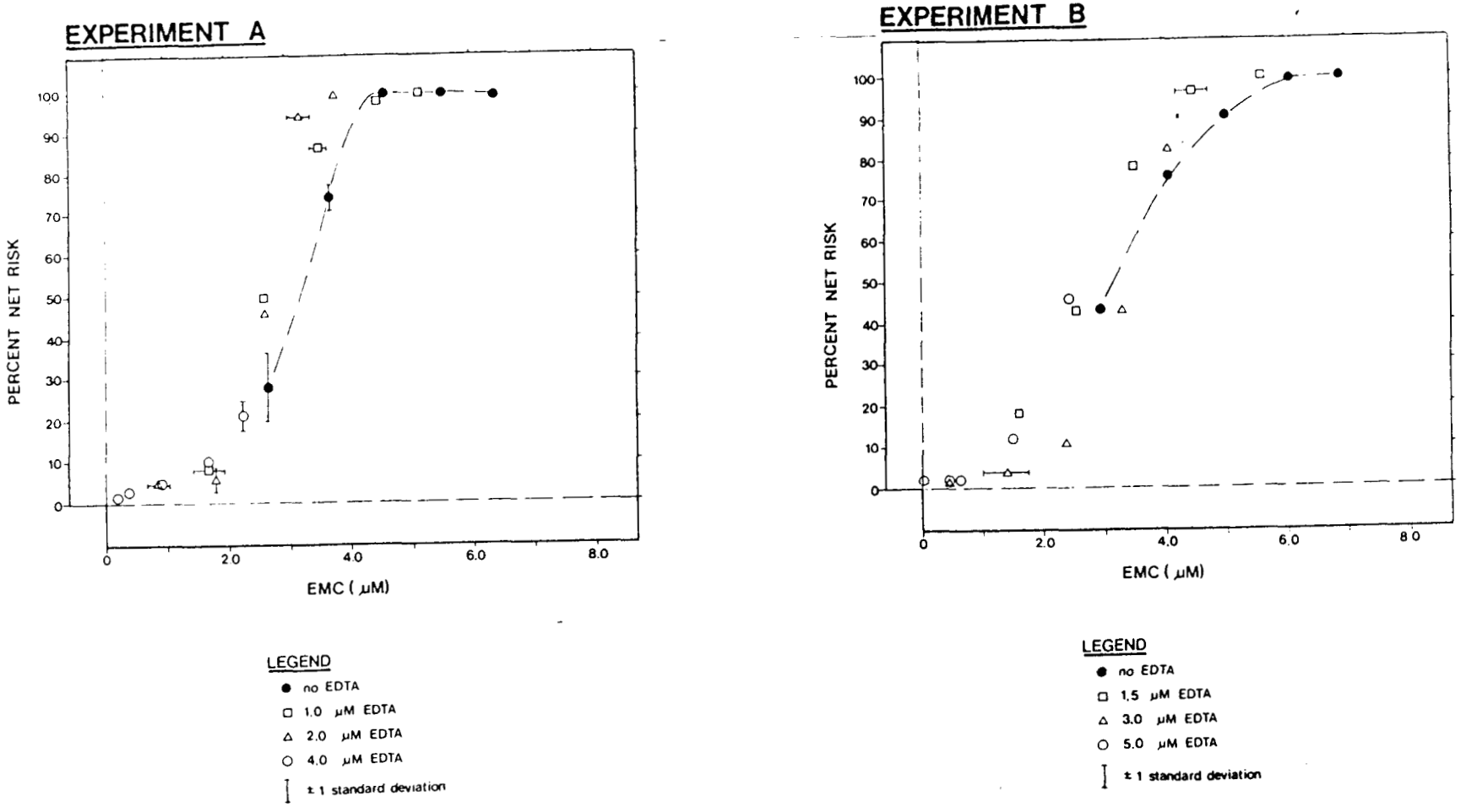


Figure 24 shows the relationship between toxicity and EMC for Cu (Expt. B). This relationship was not as well defined as that found for Pb and Zn primarily because of the lack of data points between the region of no toxicity (<5% net risk) and full toxicity (>95% net risk). However, the % net risk statistic appeared to be proportional to EMC provided the 46% net risk data point is ignored. As discussed earlier, this data point is probably in error as the EMC value and the amount of inorganic metal added were not similar (EMC was only 0.14 μM for 0.20 μM total metal added); these two values should be similar in the absence of EDTA as almost all of the metal added is in the inorganic form. This argument is strengthened by the fact that other samples containing Cu and no EDTA had EMC values similar to the total metal concentration.

Cadmium did not display the same relationship as the other metals (Figure 25). As shown in Figure 25A, the plot of % net risk and EMC was not described by a single sigmoidal curve but appeared to be divided into two curves; one curve was associated with samples containing EDTA and the other associated with samples containing no EDTA. As the data points of samples with EDTA additions were to the left of the data points of no EDTA addition, this indicated that either the ion-exchange procedure was underestimating the amount of toxic Cd in a sample or the Cd-EDTA complex was contributing to toxicity.

The plot of % net risk and EMC for a Cd experiment using higher EDTA concentrations gave a different curve than described above. In Figure 25B, a number of data points are to the right of the data points associated with samples having no EDTA addition, indicating that the ion-exchange procedure was overestimating the amount of toxic Cd in a sample. These results indicate that Cd-EDTA adsorption may be influencing the ion-exchange results.

FIGURE 23: **ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs EMC VALUES FOR LEAD**

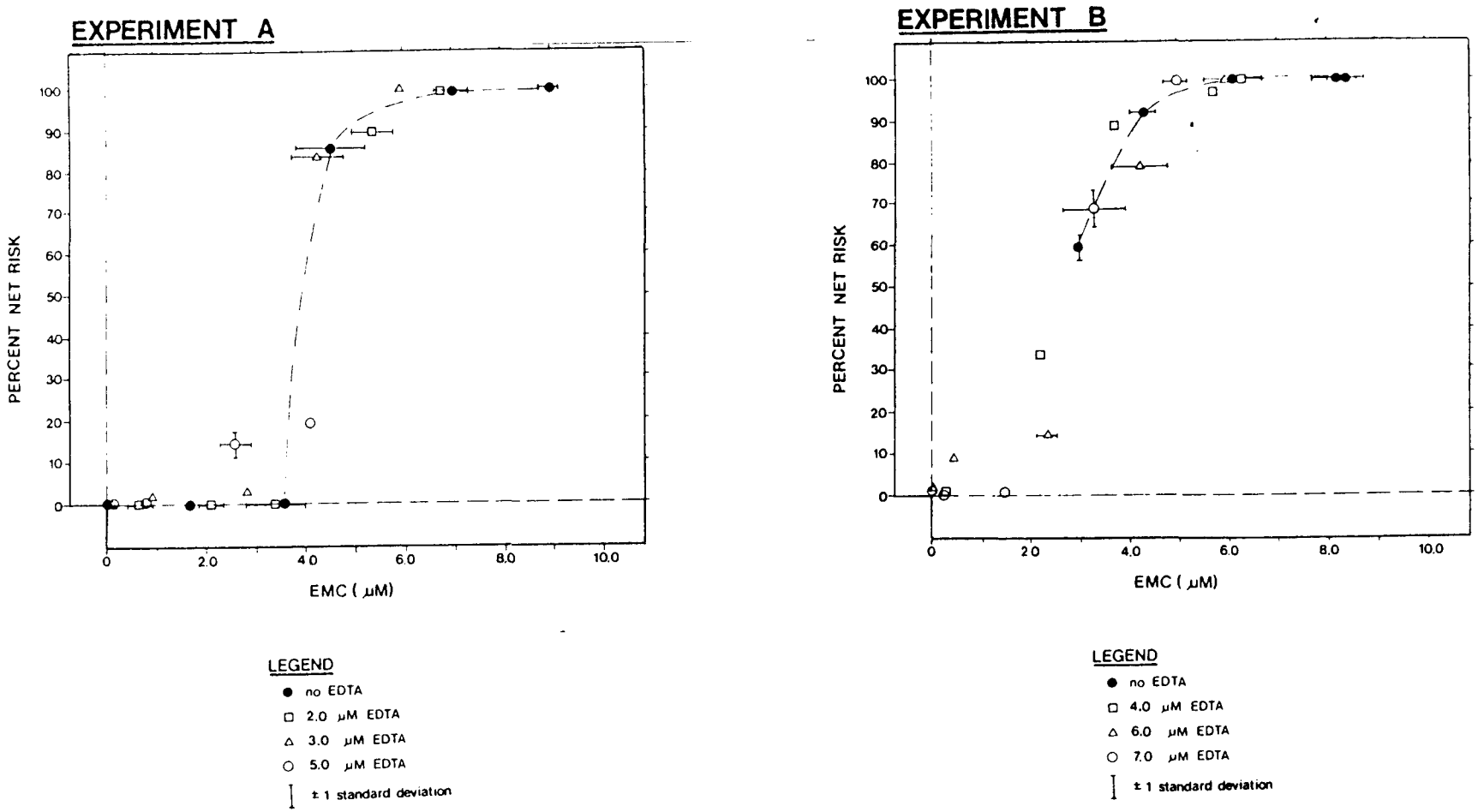
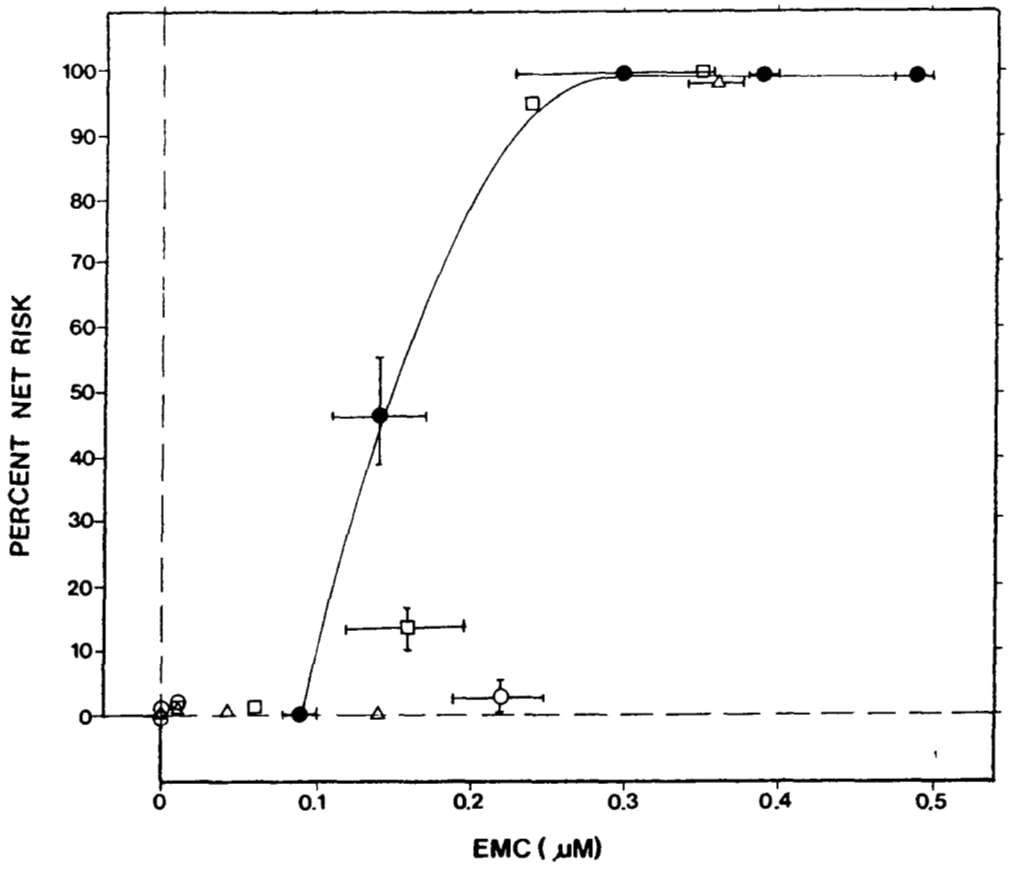


FIGURE 24

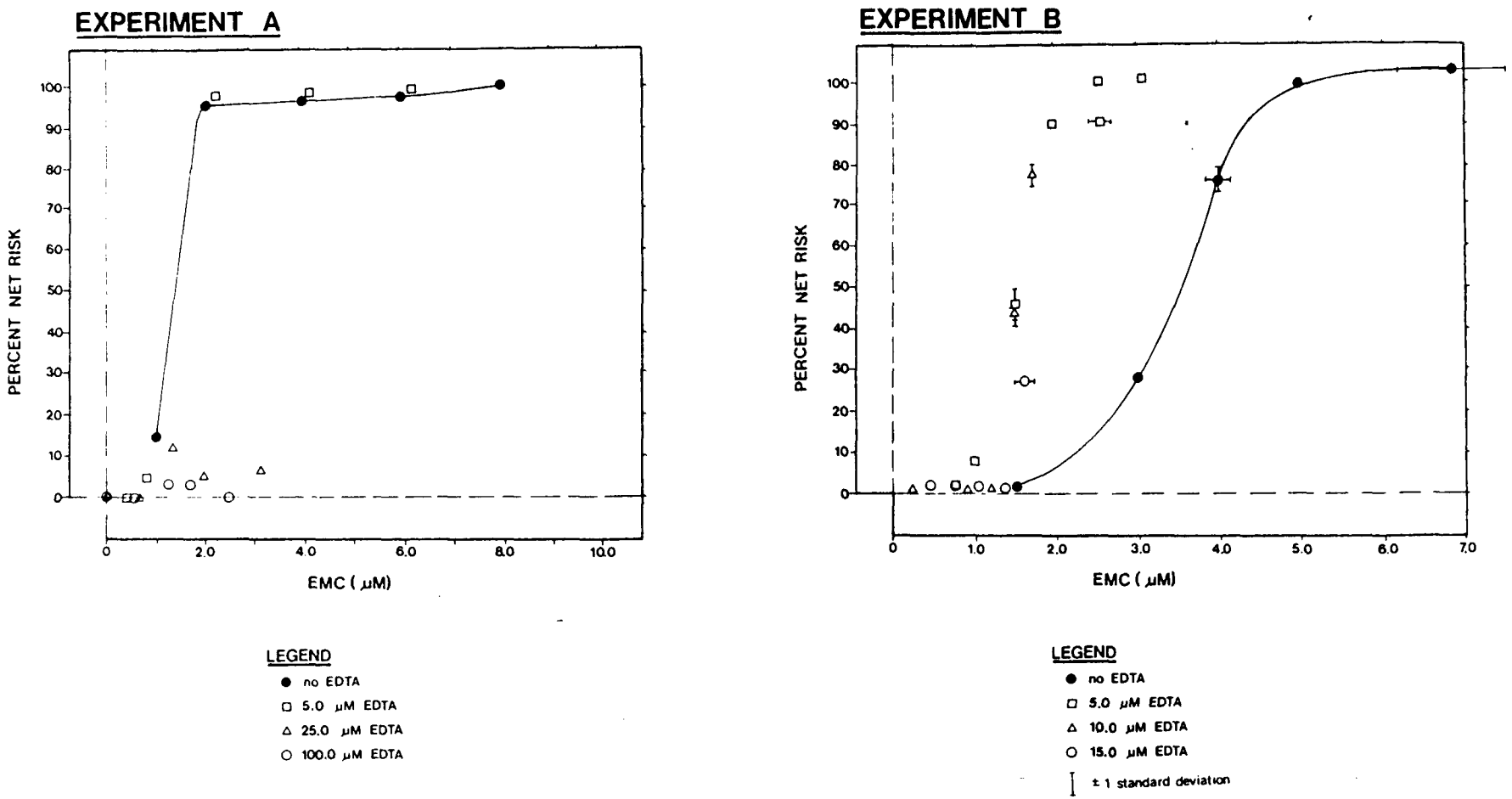
ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs EMC VALUES FOR COPPER



LEGEND

- ● no EDTA
- □ 0.15 µM EDTA
- △ 0.30 µM EDTA
- ○ 0.46 µM EDTA
- ⊥ ± 1 standard deviation

FIGURE 25: **ABNORMAL EMBRYONIC DEVELOPMENT (% net risk) OF C. gigas vs EMC VALUES FOR CADMIUM**



3.2 Algal Assays

3.2.1 Toxicity Curves

A series of experiments were conducted to determine the sensitivity of the algal organism to Cd, Cu, Pb and Zn. These experiments involved exposing the organism to a wide range of metal concentrations and determining the cell yield after a 96 hour exposure period. The results were then used to define concentration ranges for subsequent toxicity experiments. Duplicate cultures were run for each metal concentration.

Figure 26 shows the relationship between cell yield expressed as a percentage of the control cultures and total added Cu concentrations. The metal was found to be quite toxic as only 20 nM Cu was required to cause a decrease in cell yield. A metal concentration of 100 nM resulted in greater than a 95% reduction in cell yield.

Lead was toxic at concentrations similar to those of Cu. When Pb was added at 10, 25, 50, 75 and 100 nM, a reduction in cell yield was evident at 25 nM (Figure 27) and an addition of 100 nM Pb caused a decrease in cell yield of over 80%. Cell yield was found to decrease by 20% for each 25 nM increase in Pb concentration.

Figure 28 shows the response of the organism to Cd concentrations between 100 and 6000 nM. Cadmium was not as toxic as Cu or Pb. A Cd level of 250 nM was required to reduce cell yield as compared to approximately 20 nM for Pb and Cu. A Cd concentration of 1000 nM reduced cell yield below 10% of the control cultures.

FIGURE 26

RESPONSE OF T. pseudonana TO COPPER

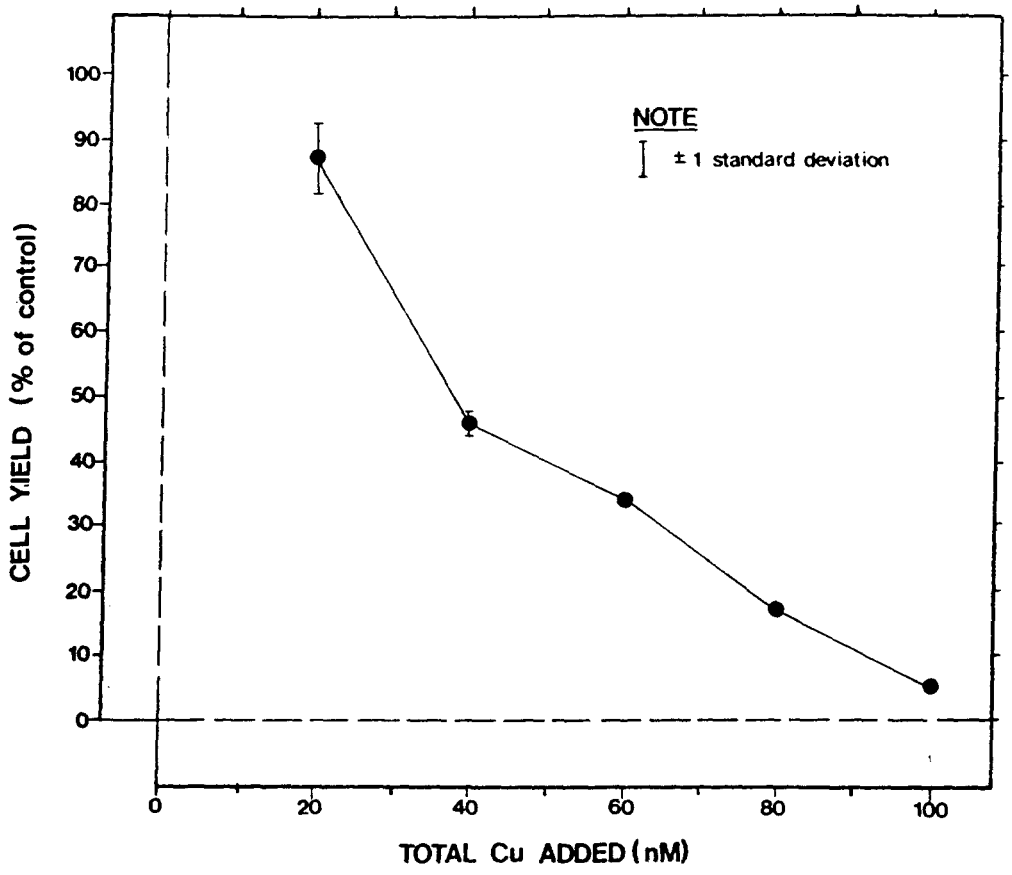


FIGURE 27

RESPONSE OF T. pseudonana TO LEAD

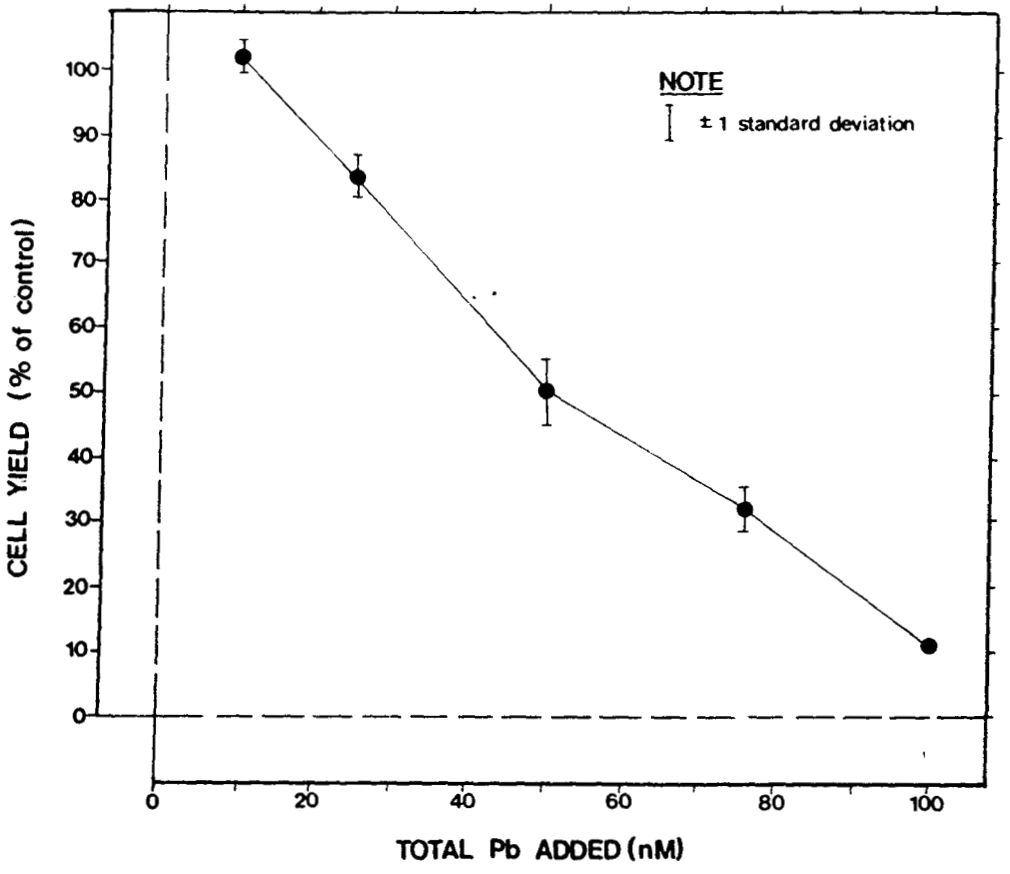
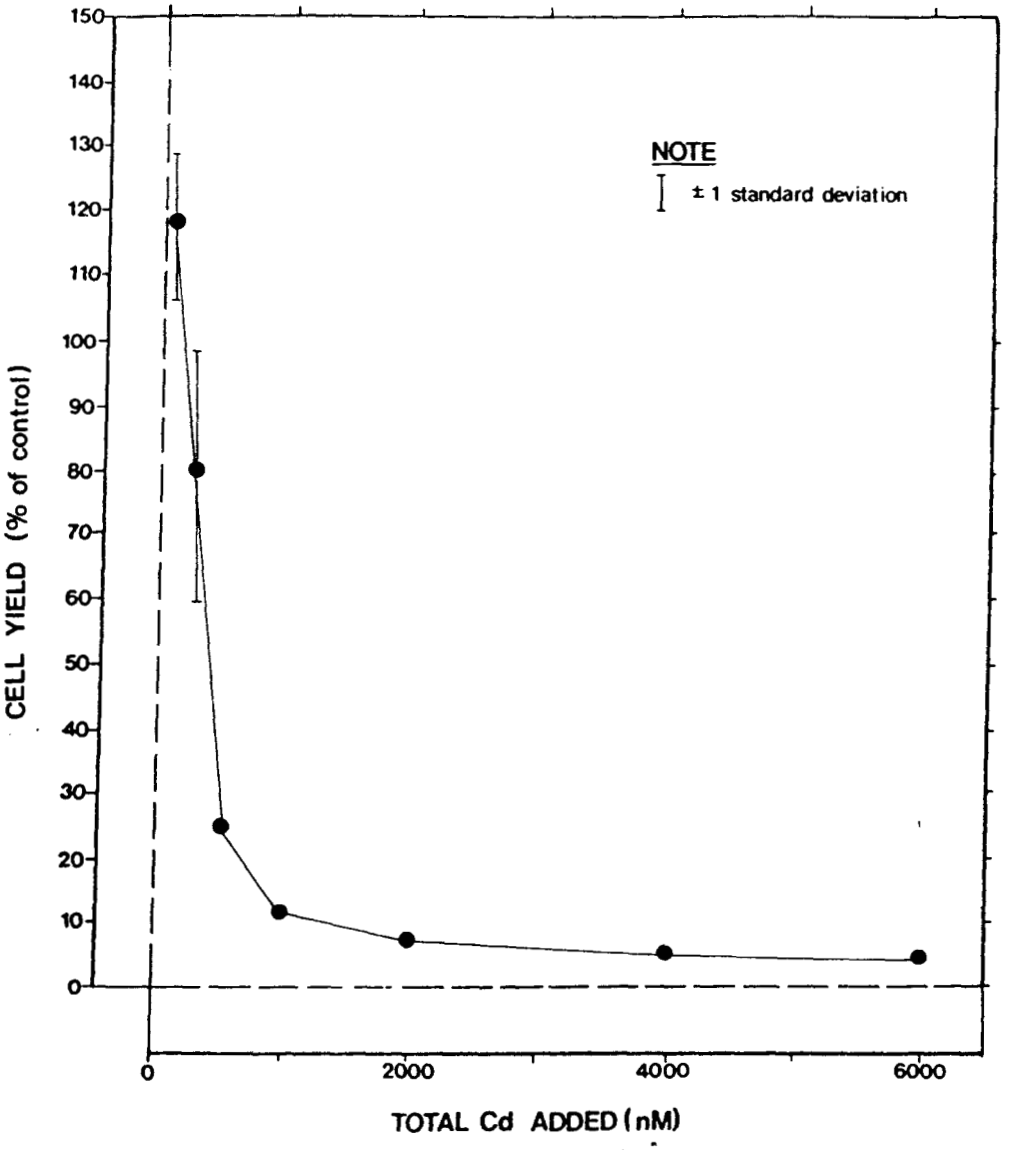


FIGURE 28

RESPONSE OF T. pseudonana TO CADMIUM



The organism was exposed to Zn concentrations from 100 to 6000 nM to determine toxicity. The concentration of zinc required to initiate a toxic response was found to be between 250 and 500 nM (Figure 29), with a Zn concentration of 1000 nM reducing cell yield by 90%. However, interpretation of the results was complicated by the fact that some test cultures had cell yields higher than those of the controls. For example, cultures with 100 nM Zn added had cell yields 33% higher than the controls. This suggested that Zn was a limiting nutrient in the culture medium. Zinc limitation will be discussed further in a later section.

3.2.2 Toxicity Experiments

A series of metal-EDTA experiments were performed using the same experimental design as was used in the oyster embryo assays; i.e. a 6 x 4 factorial design that used six metal concentrations and four EDTA concentrations per assay.

Copper

The response of the organism when exposed to Cu in the presence of 0, 50, 100 and 150 nM EDTA is presented in Figure 30A. Toxicity is represented here as cell yield expressed as a percentage of control cultures. At all Cu concentrations studied the addition of EDTA resulted in a reduction of Cu toxicity. In a culture containing 100 nM Cu and no EDTA, for example, the cell yield was only 5% of the controls, but by adding 100 nM EDTA the cell yield could be increased to over 90% of the controls. The effect that EDTA had on toxicity was also related to ligand concentration.

A further experiment was conducted using a lower and narrower concentration range of Cu and EDTA. In this experiment, Cu concentrations of 0-100 nM at 20

FIGURE 29

RESPONSE OF T. pseudonana TO ZINC

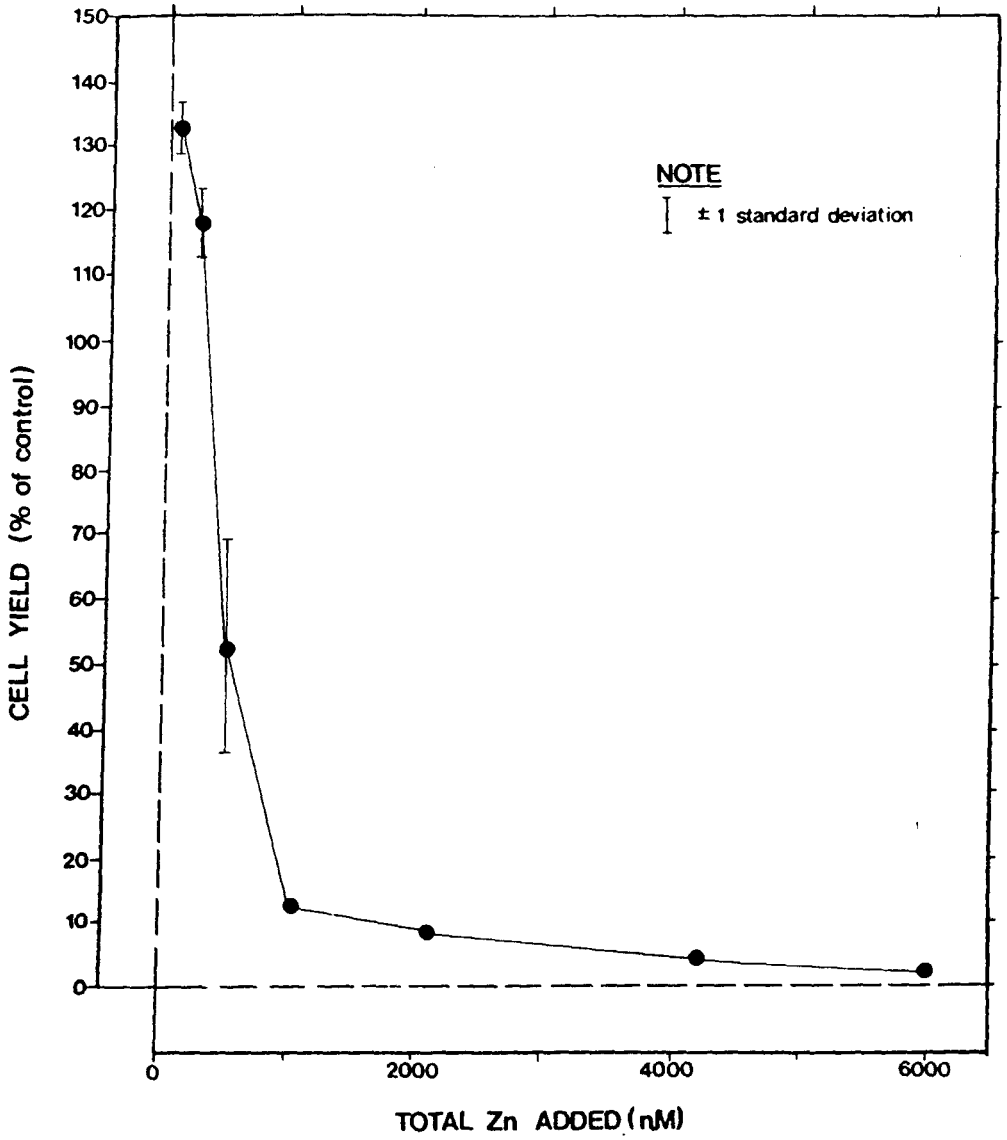
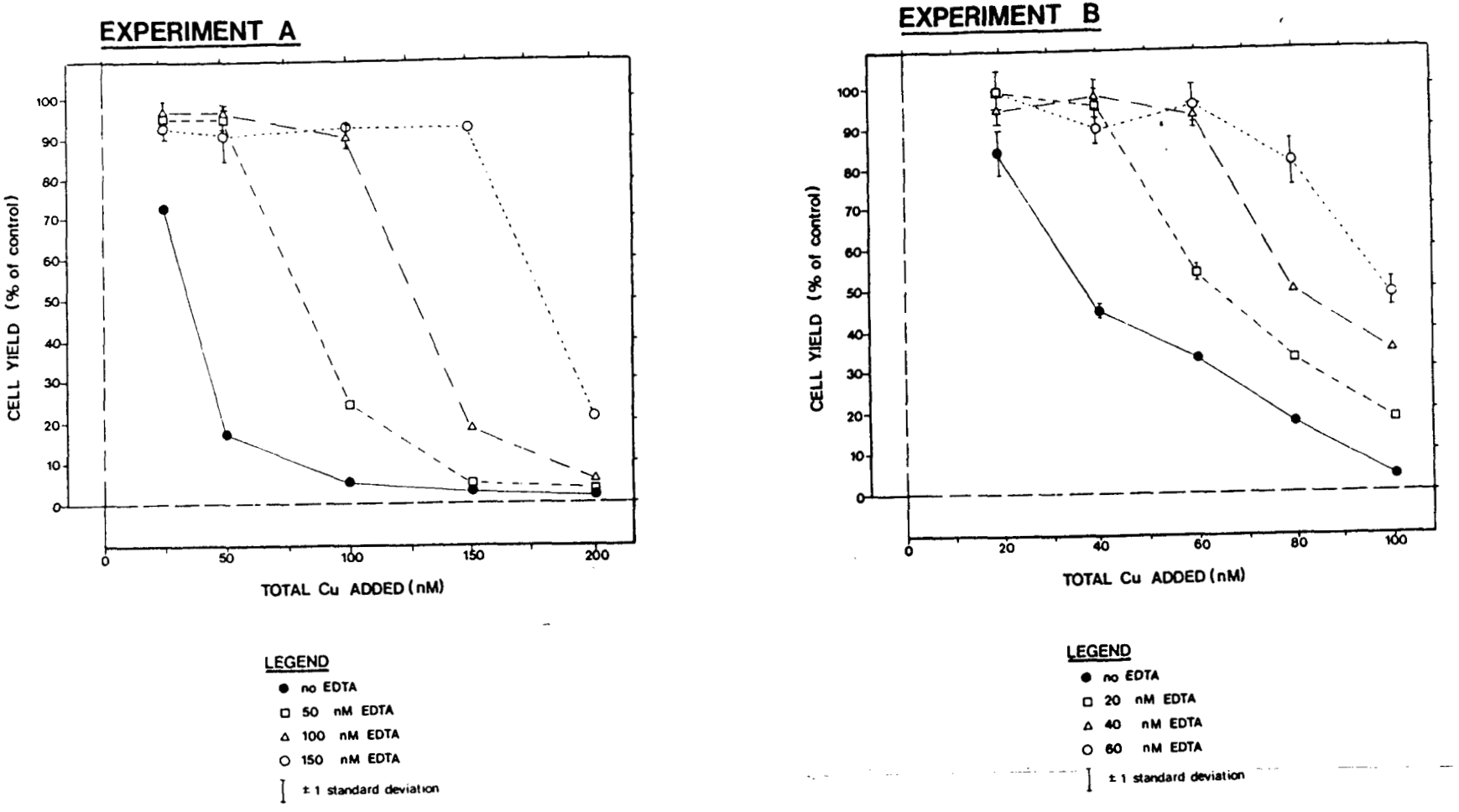


FIGURE 30: CELL YIELD (% of control) OF T. pseudonana vs TOTAL ADDED COPPER CONCENTRATION IN THE PRESENCE OF EDTA



nM increments were studied in the presence of 0, 20, 40 and 60 nM EDTA. As in the first experiment, the toxicity of Cu decreased with the addition of EDTA and the extent of this reduction was related to the ligand's concentration (Figure 30B). As in the oyster assays, the addition of EDTA at a concentration equivalent to the metal concentration was sufficient to dramatically reduce the metal's toxicity.

Lead and Cadmium

Figures 31 and 32 present the relationships between cell yield (% of control) and total metal concentrations for Pb and Cd in the presence of EDTA. As with Cu, the addition of EDTA reduced both Pb and Cd toxicity and the extent of this reduction was related to ligand concentration. With respect to Cd, however, a proportionally much higher concentration of EDTA was required to reduce its toxicity than was required for Cu or Pb.

An anomaly was also observed in the experiment with Cd. From Figure 32, it appeared that EDTA, at the concentrations studied, could not completely reduce Cd toxicity. For example, an addition of 1500 nM EDTA to cultures containing only 150 nM Cd had cell yields 20% below that of the control cultures. As 1500 nM EDTA increased cell yields from 25% to 75% in cultures containing a Cd concentration of 600 nM, this level of EDTA at a Cd concentration of only 150 nM should have resulted in a complete reduction in the metal's toxicity. However, upon close examination of the data, this effect appeared to be an artifact of the experimental procedure and not to a true Cd effect.

If one examines the daily cell numbers for the Cd experiment (Appendix V), cultures with low Cd concentrations (with and without EDTA added) had similar cell numbers to that of the controls for the first 72 hours of growth. It is only in the last 24 hour period that control values exceeded those of the

FIGURE 31 : **CELL YIELD (% of control) OF T. pseudonana vs TOTAL ADDED LEAD CONCENTRATION IN THE PRESENCE OF EDTA**

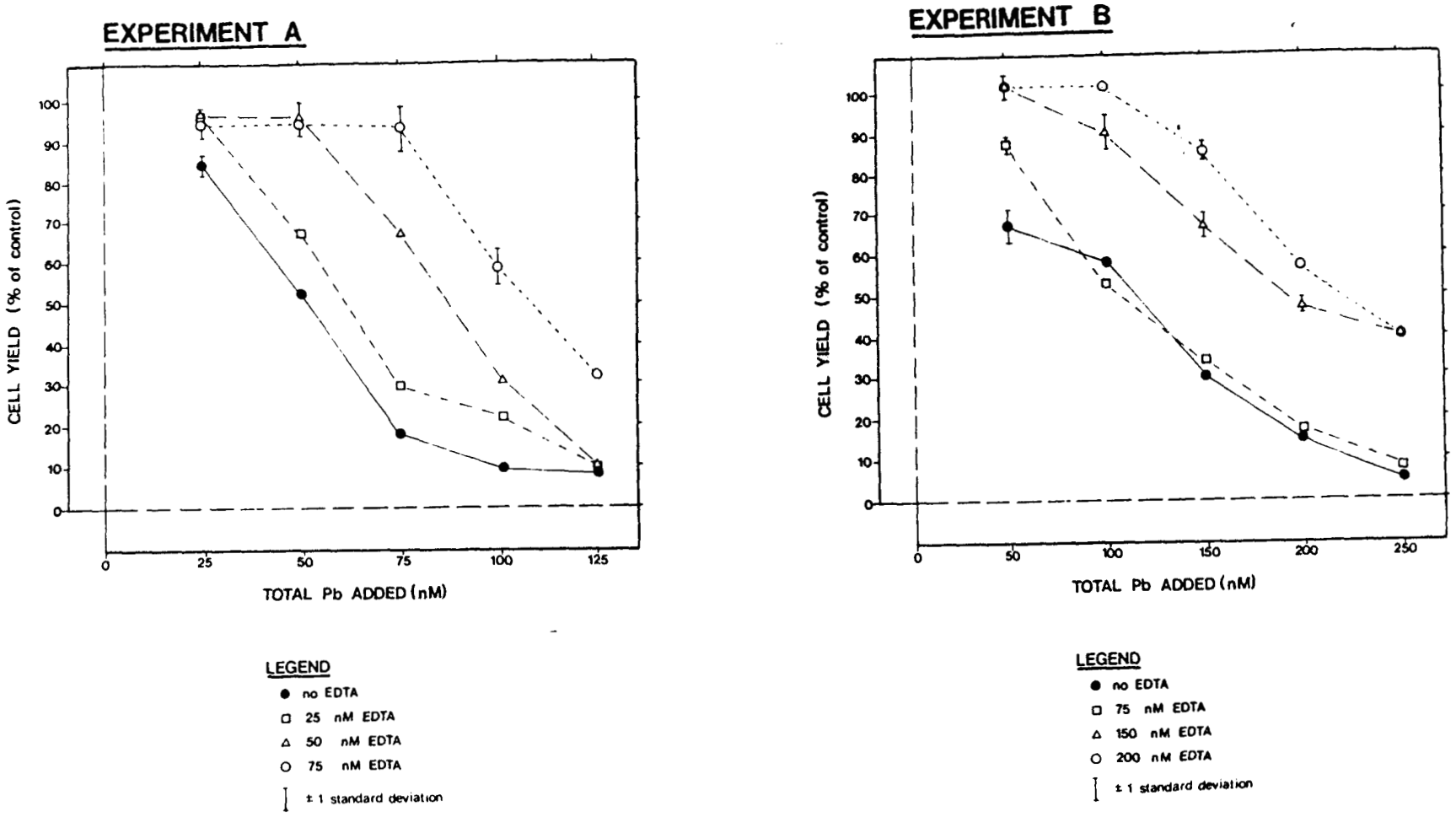
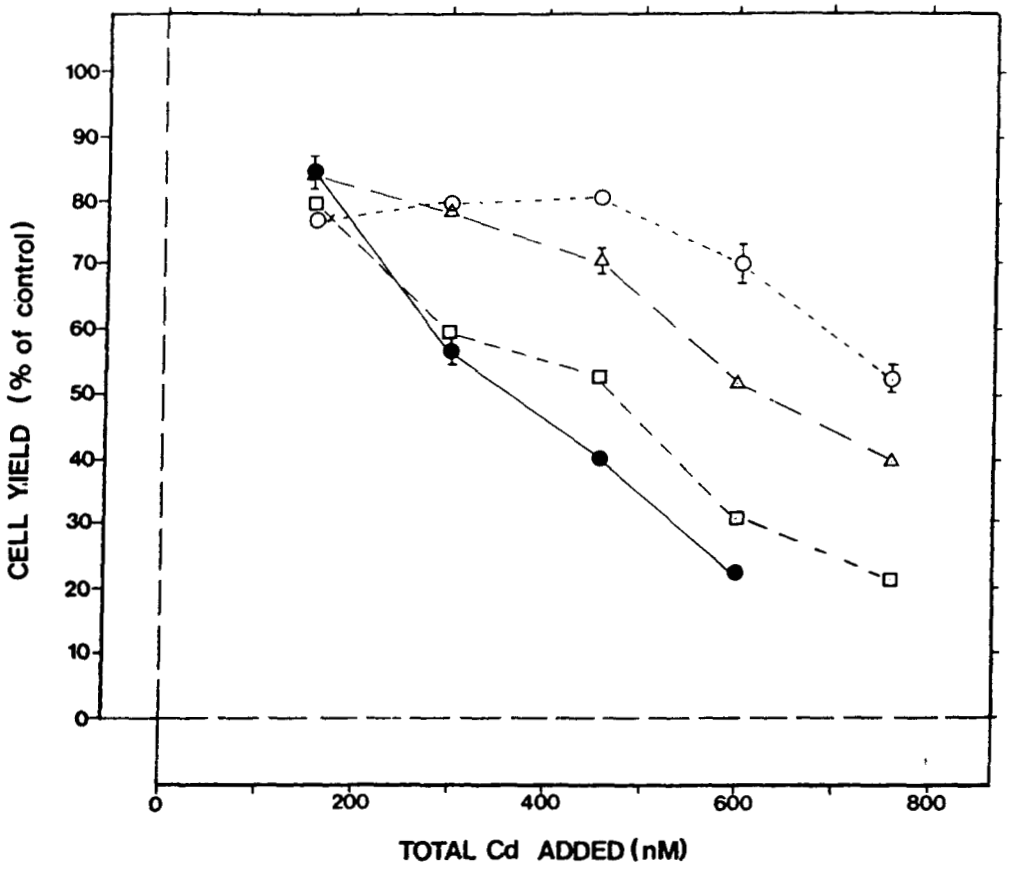


FIGURE 32

CELL YIELD (% of control) OF T. pseudonana vs TOTAL ADDED CADMIUM CONCENTRATION IN THE PRESENCE OF EDTA



LEGEND

- ● no EDTA
- □ 500 nM EDTA
- △ 1000 nM EDTA
- ○ 1500 nM EDTA
- I ± 1 standard deviation

treatment cultures. As a metal's effect was generally apparent throughout the growth period for all other metals studied, the slower growth of the treatment cultures only during the last 24 hours indicates that either it took 72 hours for Cd at low concentrations to affect the cultures or there was an increase cell number in the control cultures due to other unidentified factors. The latter is believed to be the more plausible explanation.

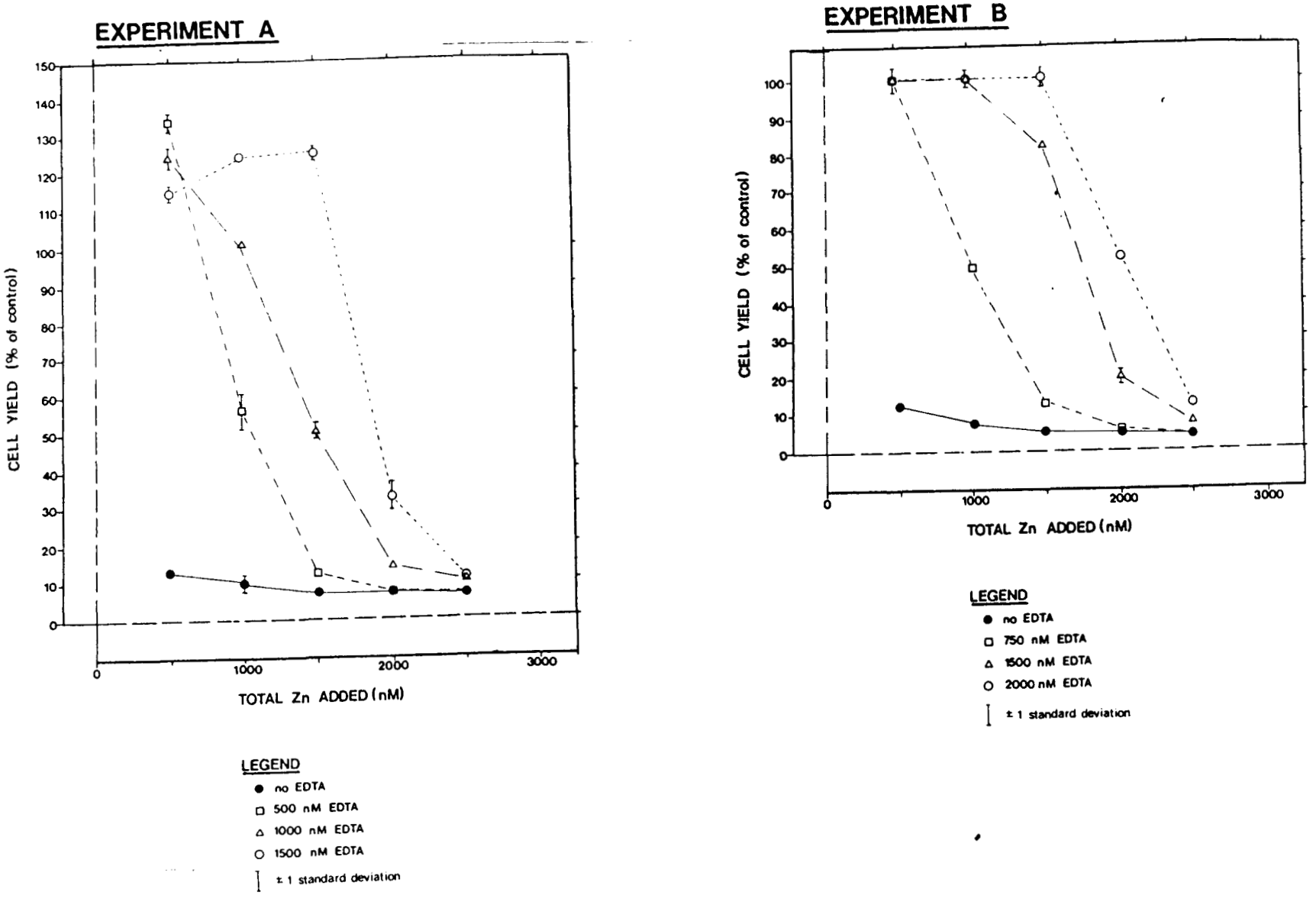
Zinc

Figure 33A presents the response of the organism when exposed to Zn in the presence of 0, 500, 1000 and 1500 nM EDTA. As expected, the addition of EDTA decreased Zn toxicity and the extent of this reduction was related to ligand concentration. However, interpretation of the results was complicated by the fact that some of the treatment cultures had cell yields much higher than the control cultures. This was further evidence to suggest a Zn limitation in the control cultures. Zinc is important to algal growth because it appears to function as an enzyme activator or cofactor in physiological processes such as protein synthesis and silicic acid uptake (Anderson et al., 1978; Rueter & Morel, 1981).

To test for zinc limitation, a further algal assay was conducted where a baseline Zn concentration of 50 nM was added to all control cultures, which is a concentration below the metal's toxic concentration range. Figure 33B presents the results from this experiment. The absence of cell yields greater than 100% of the controls indicated that Zn limitation had been occurring in the previous experiments and a 50 nM spike to the control cultures was sufficient to eliminate the Zn deficiency.

2886

FIGURE 33 : **CELL YIELD (% of control) OF T. pseudonana vs TOTAL ADDED ZINC CONCENTRATION IN THE PRESENCE OF EDTA**



3.2.3 Ion-Exchange Results

Aliquots of the metal-EDTA samples analyzed by algal assay were also analyzed by the ion-exchange procedure to determine an EMC value for each sample. The results of the ion-exchange analyses are given in Tables 19-22.

For all metals studied, the EMC value of a sample decreased with the addition of EDTA and the extent of the decrease was related to the ligand's concentration. With cadmium, a proportionally higher EDTA concentration was required to decrease a sample's EMC value than was required for the other metals.

However, there were some inconsistencies in the data. In an experiment with Pb (Table 19: Expt. B), a sample containing 25 nM Pb and 25 nM EDTA had a lower EMC value than a similar sample containing a higher EDTA (50 nM), while in a Cd experiment (Table 20) an EMC value of 1068 nM was obtained when only 750 nM total metal ws added to the sample. As these were isolated anomalies, these data were attributed to experimental error.

As previously discussed, an EMC for a sample containing no EDTA should be similar to the total added metal concentration provided there are no naturally occurring complexing agents in the natural seawater used in the experiment. However, this was not the case for Cu (Table 21). The EMC values of samples containing no EDTA were much lower than amount of metal added. For example, an EMC value of only 47 nM was obtained when 100 nM Cu was added to the sample. Low EMC values were also found in a Pb experiment (Table 19: Expt. A) that used similar seawater as was used in the Cu experiments (Seawater Batch #5). When a second Pb experiment was conducted using a different batch of seawater (Table 19: Expt. B; Batch #6), the EMC values were similar to the total added metal concentrations, suggesting the low EMC values for both Pb and Cu were batch related.

TABLE 19

Results of the Ion-Exchange Analyses for Lead..
Values are Means (One Standard Deviation) of EMCs (nM) for Two Replicates.

Experiment[a]	EDTA Conc.[b] (nM)	Lead Conc. Added[c] (nM)				
		25	50	75	100	125
A	0.0	19 (0)	35 (1)	53 (2)	68 (2)	89 (3)
	25.0	10 (0)	21 (1)	36 (1)	56 (4)	71 (4)
	50.0	12 (0)	14 (1)	29 (0)	51 (1)	72 (0)
	75.0	N.D.[d]	(0)	7 (1)	18 (0)	31 (0)
		50	100	150	200	250
B	0.0	50 (1)	100 (4)	139 (4)	170 (5)	243 (5)
	75.0	21 (1)	68 (1)	102 (1)	145 (5)	174 (7)
	150.0	11 (2)	23 (1)	55 (5)	90 (9)	141 (5)
	200.0	N.D.	13 (4)	42 (1)	70 (1)	92 (3)

[a]Resin: AG 50W X-4 (#21934) used in Expt. A; AG 50W X-4 (#30008) used in Expt. B.

[b]Concentrations of total EDTA added to the bioassay water (Expt. A - Seawater Batch #5; Expt. B - Seawater Batch #6).

[c]Concentrations of total Pb added to the bioassay water.

[d]Non-Detectable: Limit of Detection - 4 nM.

2886

TABLE 20

Results of the Ion-Exchange Analysis for Cadmium.
Values are Means (One Standard Deviation) of EMCs (nM) for Two Replicates

Experiment[a]	EDTA Conc.[b] (nM)	Cadmium Conc. Added[c] (nM)				
		150	300	450	600	750
A	0	135 (13)[d]	266 (3)	395 (2)	522 (23)	1068 (49)
	500	111 (6.3)	221 (15)	361 (6)	468 (19)	577 (11)
	1000	99 (1.1)	219 (10.2)	329 (25)	466 (55)	610 (18)
	1500	81 (2.5)	189 (0.0)	319 (5)	426 (13)	553 (11)

[a]Resin: AG 50W X-4 (#21934)

[b]Concentrations of total EDTA added to the bioassay water (Seawater Batch #5).

[c]Concentrations of total Cd added to the bioassay water.

[d]Limit of Quantification - 1.4 nM; Limit of Detection - 0.4 nM.

TABLE 21

Results of the Ion-Exchange Analyses for Copper.
Values are Means (One Standard Deviation) of EMCs (nM) for Two Replicates.

Experiment[a]	EDTA Conc.[b] (nM)	Copper Conc. Added[c] (nM)				
		25	50	100	150	200
A	0.0	17 (0.3)	26 (1.2)	47 (1.0)	91 (0.4)	132 (0.6)
	50.0	N.D.[d]	13 (0.4)	26 (1.0)	46 (11.0)	107 (5.2)
	100.0	N.D.	N.D.	11 (1.4)	22 (0.2)	40 (2.2)
	150.0	N.D.	N.D.	7 (0.1)	11 (1.3)	27 (0.9)
		20	40	60	80	100
B	0.0	20 (0.5)	37 (1.4)	46 (4.5)	57 (2.6)	65 (1.3)
	20.0	15 (1.6)	20 (0.5)	34 (18.0)	47 (0.3)	54 (4.9)
	40.0	12 (0.4)	19 (0.8)	27 (2.3)	37 (2.6)	52 (6.9)
	60.0	N.D.	6 (0.2)	7 (1.0)	29 (3.3)	31 (1.6)

[a] Resin: AG 50W X-4 (#21934)
[b] Concentrations of total EDTA added to the bioassay water (Seawater Batch #5).
[c] Concentrations of total Cu added to the bioassay water.
[d] Non-Detectable: Limit of Quantification - 3.8 nM; Limit of Detection - 1.1 nM.

2886

TABLE 22

Results of the Ion-Exchange Analyses for Zinc.
Values are Means (One Standard Deviation) of EMCs (nM) for Two Replicates

Experiment[a]	EDTA Conc.[b] (nM)	Zinc Conc. Added[c] (nM) 500	1000	1500	2000	2500
A	0	342 (50)	1088 (26)	1465 (50)	2132 (50)	2202 (50)
	500	N.D.[d]	342 (149)	1237 (174)	1570 (149)	1833 (29)
	1000	N.D.	200 (-)	478 (52)	913 (56)	1212 (59)
	1500	N.D.	N.D.	430 (124)	1009 (99)	1147 (124)
B	0	333 (44)	748 (22)	1255 (0)	1687 (131)	2133 (65)
	500	N.D.	348 (65)	794 (87)	1256 (44)	1687 (174)
	1500	N.D.	N.D.	213 (0)	698 (39)	1227 (20)
	2000	N.D.	N.D.	N.D.	345 (17)	597 (0)

[a]Resin: AG 50W X-4 (#29934) used in Expt. A; AG 50W X-4 (#30008) used in Expt. B.

[b]Concentrations of total EDTA added to the bioassay water: Expt. A - Seawater Batch #5; Expt. B - Seawater Batch #6.

[c]Concentrations of total Zn added to the bioassay water.

[d]Non-Detectable: Limit of detection - 101 nM.

One possible explanation for low EMC values from Seawater Batch #5 is that the natural seawater may have contained natural complexing agents in sufficient quantity to affect the metals' speciation. As an extensive organic association of Cu and Pb in coastal seawater has been reported by a number of researchers (see Hasle and Abdullah, 1981; Duinker and Kramer, 1977; Whitfield, 1975), it is likely that organic complexing agents were responsible for the complexation of these metals.

3.2.4 Comparison of Algal Assay and Ion-Exchange Results

To compare the results from the algal assays to the ion-exchange analyses, cell yields (% of control) were plotted as a function of the EMC values. As with the oyster embryo assays, all data obtained in a given algal experiment were plotted on a single graph. The data were expected to be described by a single function provided EMC was proportional to the toxic metal concentration.

Figure 34 describes the relationship between toxicity and EMC for two experiments conducted with Cu. The data in both figures can be described by a single function suggesting that the relationship between toxicity and EMC was independent of the EDTA concentration and EMC was proportional to the toxic metal fraction in solution. A similar relationship between toxicity and EMC was found for Pb (Figure 35) and Zn (Figure 36), as cell yields appeared to be a direct function of EMC for all the EDTA concentrations studied.

In one experiment with Zn, the relationship between toxicity and EMC was not as well defined as was found for the Cu and Pb primarily due to scatter in the data above cell yields of 100%. This was attributed to Zn limitation in the control cultures (see Section 3.2.2). By eliminating the Zn deficiency (Figure 36B), the scatter in data was reduced considerably.

FIGURE 34: CELL YIELD (% of control) OF T. pseudonana vs EMC VALUES FOR COPPER

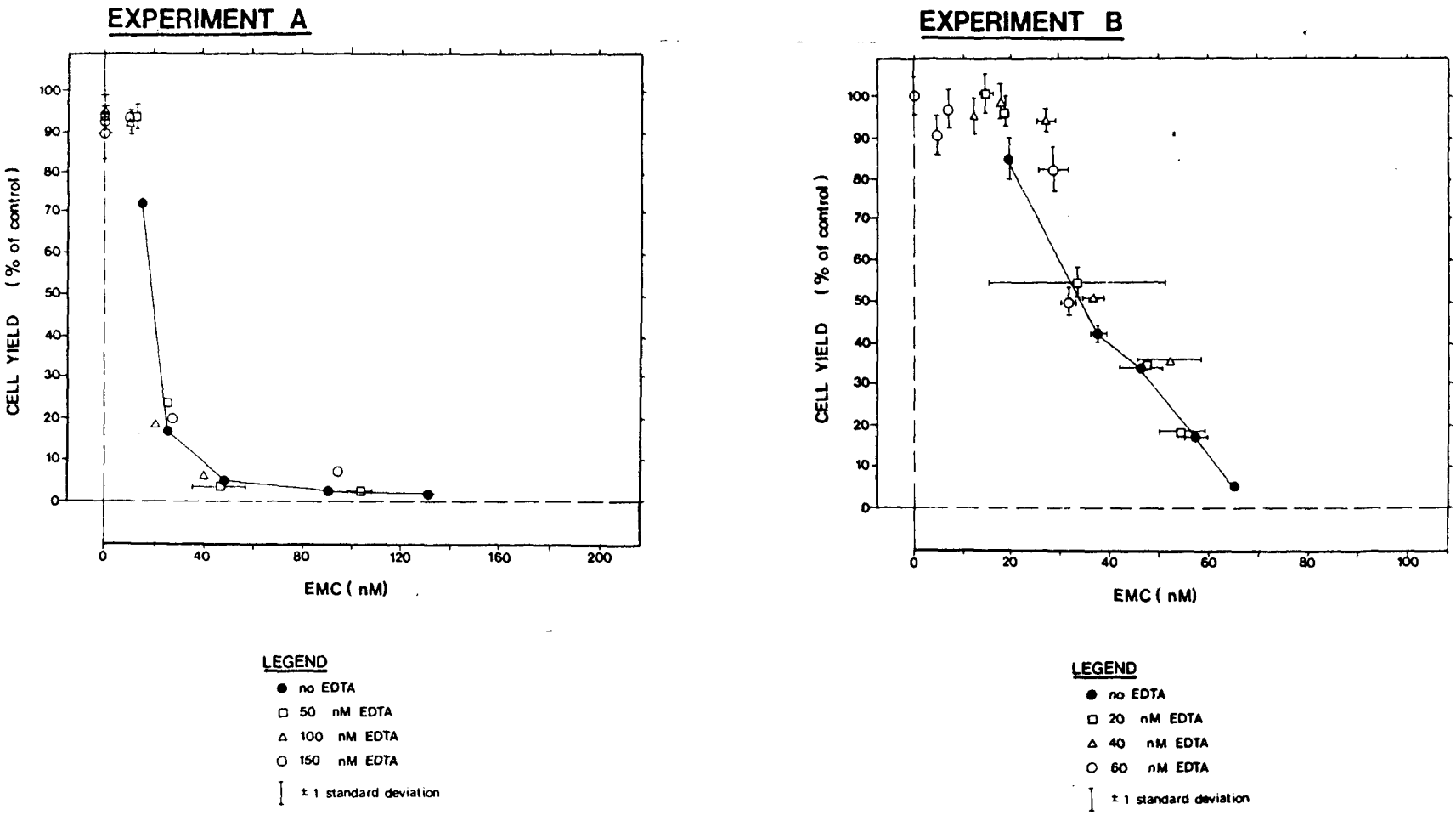


FIGURE 35 : CELL YIELD (% of control) OF T. pseudonana vs EMC VALUES FOR LEAD

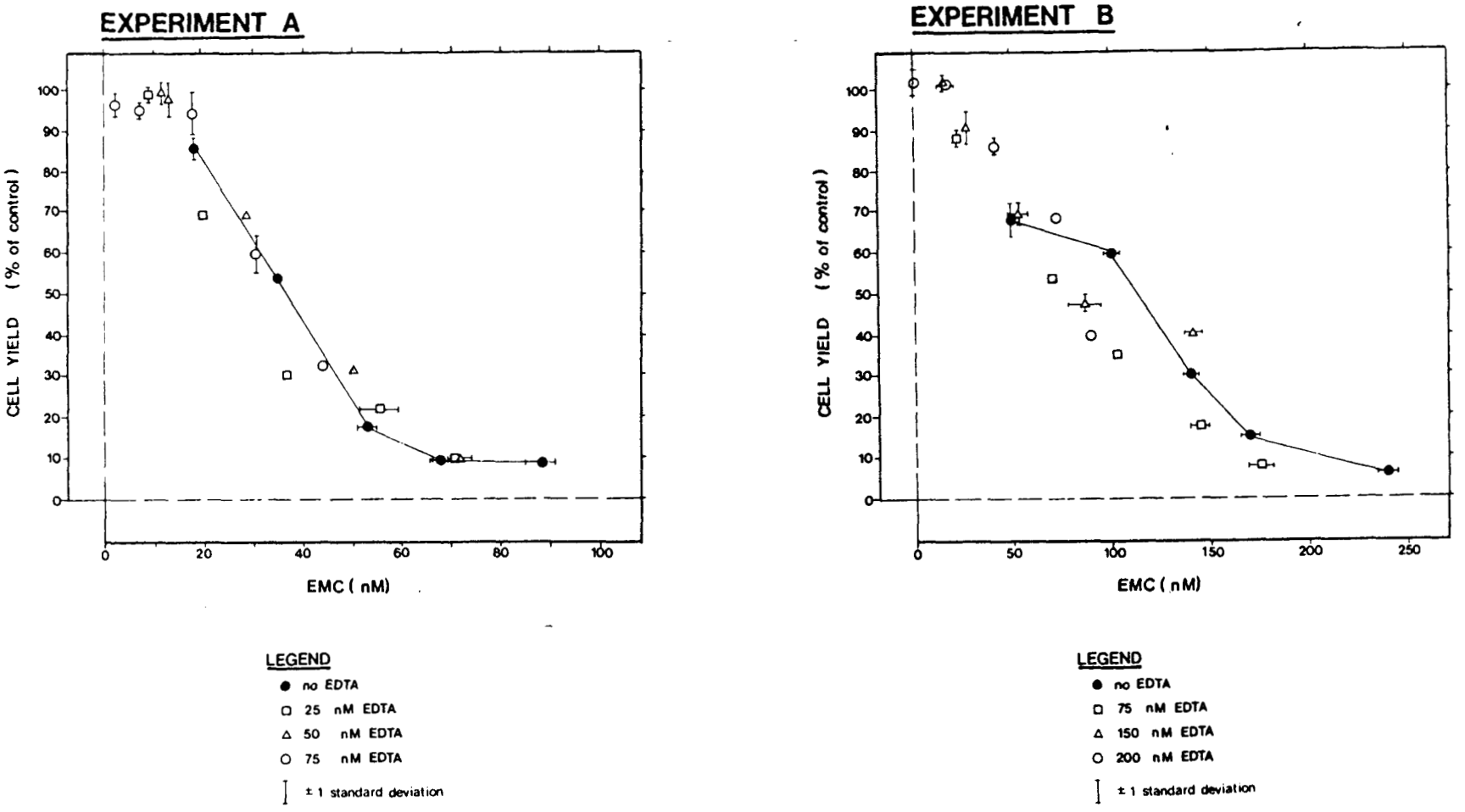
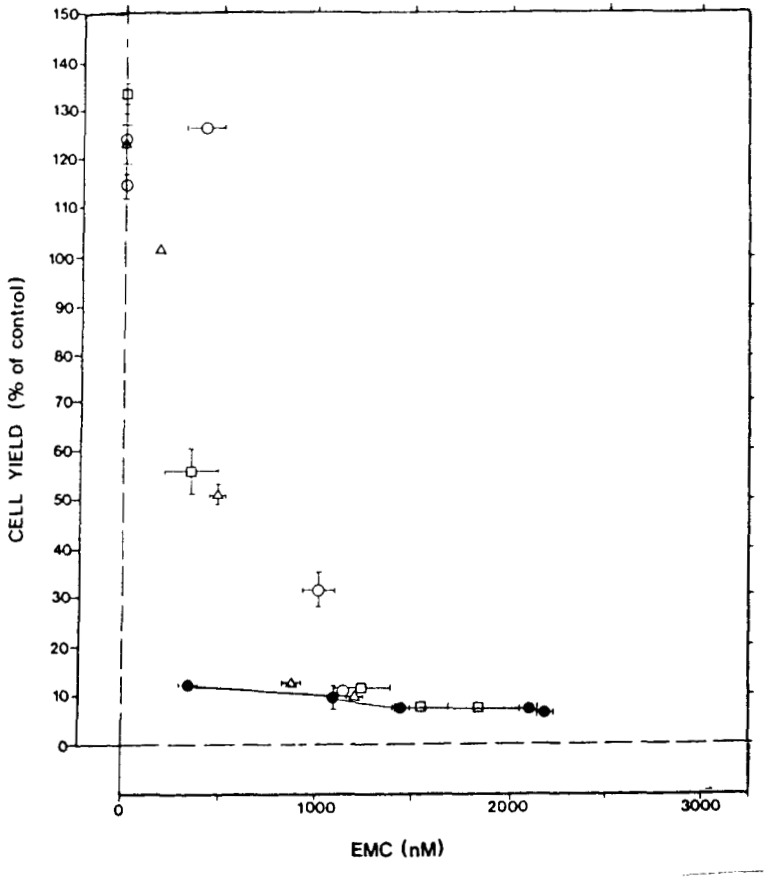


FIGURE 36 : CELL YIELD (% of control) OF T. pseudonana vs EMC VALUES FOR ZINC

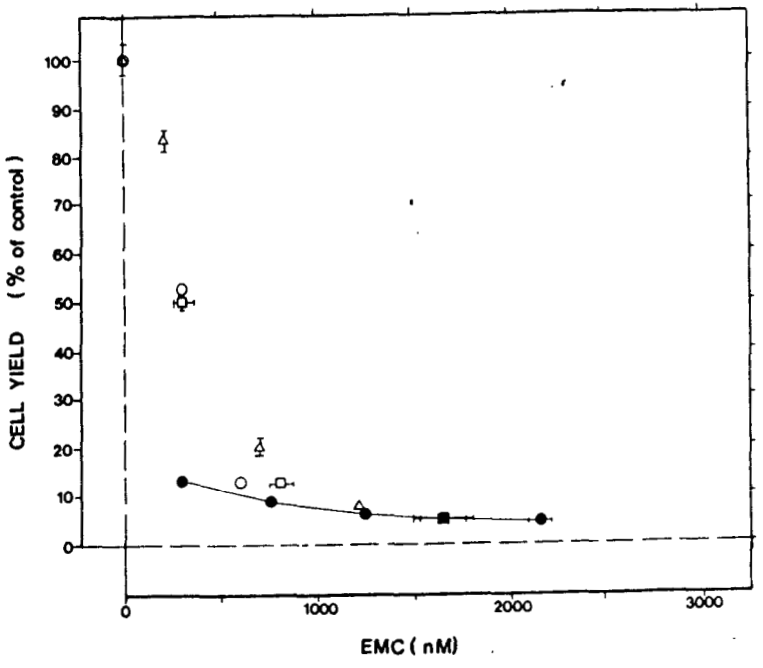
EXPERIMENT A



LEGEND

- ● no EDTA
- □ 500 nM EDTA
- △ 1000 nM EDTA
- ○ 1500 nM EDTA
- ⊥ ± 1 standard deviation

EXPERIMENT B



LEGEND

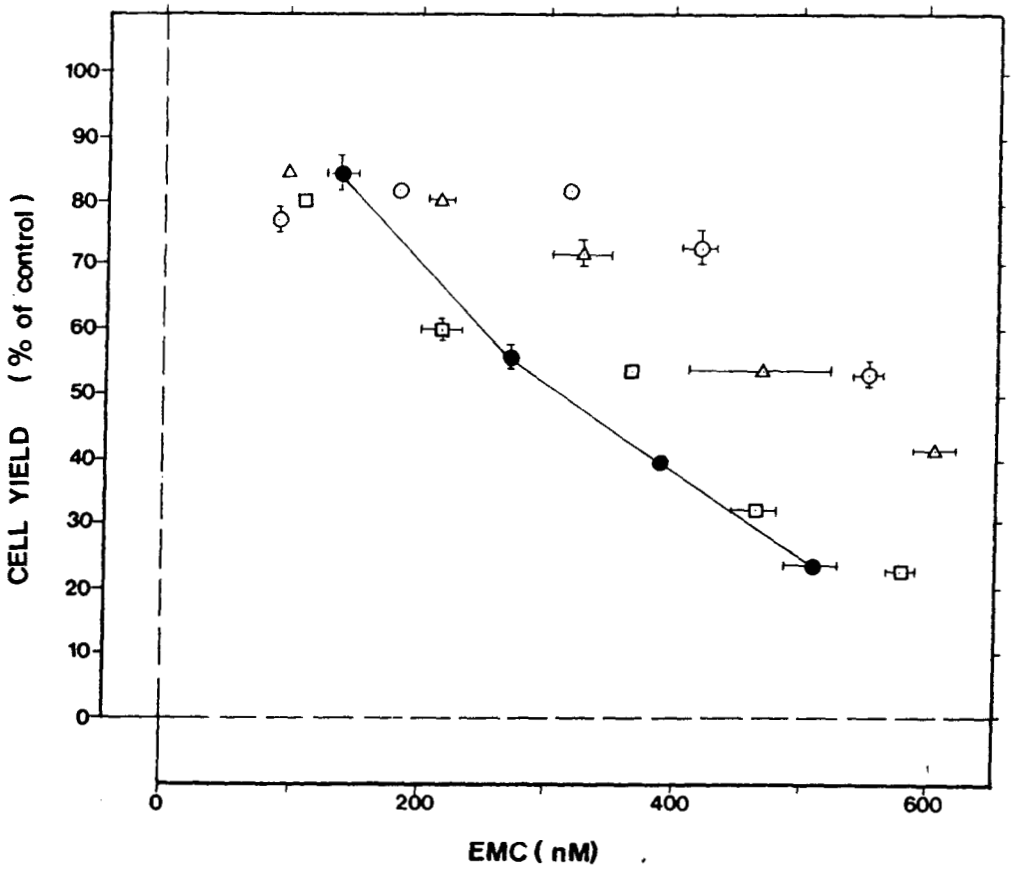
- ● no EDTA
- □ 750 nM EDTA
- △ 1500 nM EDTA
- ○ 2000 nM EDTA
- ⊥ ± 1 standard deviation

Cadmium did not display the same relationship between toxicity and EMC as the other metals (Figure 37). The scatter in the data was similar to the scatter obtained when the same cell yield data was plotted as a function of the total added Cd concentration (Figure 32). As the data points of samples containing EDTA were to the right of the data points of samples containing no EDTA, this implied that the ion-exchange procedure was overestimating the amount of toxic metal in solution. In other words, the amount of EDTA added to the samples did not appear to reduce the EMC values while the response of the algal organism indicated a reduction in the amount of toxic metal due to EDTA. This effect could be explained if adsorption of the Cd-EDTA complex by the resin was occurring.

These results combined with the findings from the oyster assay strongly suggest that the concentration of Cd sorbed to the resin is not solely due to cationic metal concentrations in solution and adsorption of the Cd-EDTA complex is occurring. These problems will have to be resolved before the ion-exchange procedure can be applied to the measurement of biologically effective Cd concentrations in seawater.

FIGURE 37

CELL YIELD (% of control) OF T. pseudonana vs EMC VALUES FOR CADMIUM



LEGEND

- ● no EDTA
- □ 500 nM EDTA
- △ 1000 nM EDTA
- ○ 1500 nM EDTA
- I ± 1 standard deviation

D. RECOMMENDATIONS FOR FUTURE STUDIES

As the potential of the ion-exchange procedure to measure biologically active heavy metal concentrations in seawater has been demonstrated, further studies with the ion-exchange procedure are warranted. The research studies that are recommended are as follows:

1. The ion-exchange procedure should be applied to natural seawater samples. A study would be designed to take the ion-exchange procedure in the field and use it for on-site measurements. These measurements would then be compared to measurements made under laboratory conditions.

2. Examine the use of natural seawater for the preparation of the seawater metal standards rather than using artificial seawater to calibrate the resin. It is believed that natural seawater pretreated to remove organics and background metal concentrations may provide a better medium for the seawater metal standards. Other advantages include preparation of large seawater batches at a reduced cost.

3. Conduct further experimentation with cadmium to determine the factors that affect the metal's sorption to the resin. This would be required before the ion-exchange could be applied to cadmium measurements in natural seawater.

4. Incorporate the ion-exchange procedure into pre-screening methods for heavy metals such as those used for dredging spoils or drilling muds.

5. Examine ways of automating the ion-exchange procedure.

2886

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2886

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2886

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2886

APPENDIX I

CHELEX PREPARATION

Chelex-100 Preparation

Due to a low degree of cross linkage, Chelex is subject to large changes in volume as the cationic form of the resin is changed. Therefore, resin preparation and regeneration is performed in the batch mode prior to being added to a column. Chelex-100 is supplied in the Na^+ form and should be converted to the ionic composition and pH of SOW before use. This will avoid any alkalinity changes that might occur in the medium if the resin gained or lost protons as the solution was passed through. The method of new resin preparation is different from the method used when the resin is regenerated. The two methods are presented below.

New Chelex: A 40 g portion of the resin is placed in a 500 mL polypropylene disposable beaker (acid cleaned), rinsed with 100 mL of methanol to remove any residual organics, and rinsed 3 times with 200 to 300 mL of GDW. The resin is slurried two to three times in 300 mL of SOW and titrated with acid or base to a pH of ca. 8.0. The solution is decanted, fresh solution added and the titration procedure repeated. Fresh solution is added four or five times or until the pH is stable after the addition of fresh solution. The resin is then ready to be poured into the column.

Regenerated Chelex: The columns should be regnerated after ca. 40 L of SOW has been passed through. The top 5 cm of the resin was generally discolored and removed before regeneration. The resin was removed from the column, rinsed with 200 mL GDW, then slurried in 200 mL of 1N HCl and stirred for 30 min. The acid was decanted and the resin was rinsed 3 times with 200 mL of GDW. To convert the resin to the Na^+ form, 200 mL of 0.5 NaOH was added, stirred for 30 min and rinsed 3 times with GDW. The resin then underwent the same procedure as for new chelex.

The final step in the resin preparation was the preparation of the columns. A 5.0 x 60.0 cm glass column fitted with glass wool was filled with the solution to be cleaned. While the column was dripping, a slurry of resin was poured into the column at a constant rate. A slurry was used so as to prevent any bubbles from being trapped as the resin was packed. A fluid head of 25 cm was maintained over the resin at all times.

APPENDIX II

ATOMIC ABSORPTION SPECTROPHOTOMETRY OPERATING CONDITIONS

METAL: Mn

Program

Step	1	2	3	4	5
Temp (°C)	110	150	500	1000	2700
Ramp Time (s)	5	5	5	5	0
Hold Time (s)	10	20	5	15	5

Injection Volume: 10 µl
Wavelength: 279.5 nm
Lamp Current: 12 ma
Slit Width: 0.2 nm (Normal)

METAL: Cu

Program

Step	1	2	3	4	5
Temp (°C)	110	150	250	900	2500
Ramp Time (s)	8	30	10	5	0
Hold Time (s)	10	20	10	10	5

Injection Volume: 25 µl
Wavelength: 324.8 nm
Lamp Current: 12 ma
Slit Width: 0.7 nm (Alternate)

METAL: Pb

Program

Step	1	2	3	4	5
Temp (°C)	110	550	1,900	2,500	
Ramp Time (s)	8	10	1	1	
Hold Time (s)	20	30	5	3	

Injection Volume: 10 µl
Wavelength: 217.0 nm
Lamp Current: 8 ma
Slit Width: 0.7 nm (Alternate)

* Pyrolytic coated tubes were used for all metals

METAL: Cd

Program

Step	1	2	3	4	5
Temp (^{o}C)	110	120	350	1900	2500
Ramp Time (s)	5	5	5	2	1
Hold Time (s)	10	20	10	5	3

Injection Volume: 10 µl
Wavelength: 228.8 nm
Lamp Current: 8 ma
Slit Width: 0.7 nm (Alternate)

METAL: Zn

Flame AAS

Wavelength: 213.9 nm
Air-Acetylene Mixture

APPENDIX III

Determination of EMC Values

Conversion of Absorbance Readings of the GFAAS Analysis to EMC Values

The purpose of this exercise is to outline the methods used to determine effective metal concentration (EMC) of a sample from the analysis of column eluates by atomic absorption spectrophotometry. The worksheet given below is taken from an algal experiment that studied the Pb-EDTA concentration combinations given in Table III-1. To aid in the explanation of the worksheet, the first page has been divided into sections which represent different components of the analysis.

In Section 1, a portion of the experimental conditions used in the ion-exchange procedure are described including the dry weight of the resin used in each column, the volume of EDTA passed through the columns to remove the sorbed metals, and the sample injection volume used in the GFAAS analysis. The resin weight and eluate volume are required for certain calculations and their use will be given below.

Section 2 outlines the analysis of metal-EDTA standards. These standards were made up in 0.01 M EDTA (i.e. the same EDTA concentration as was used to elute the columns) and were used to quantify the metals in the column eluates and to monitor the GFAAS analysis for precision, day to day variation and linearity of readings. They were stable for a long period of time as the strong complexation of the metal by EDTA prevented adsorption and precipitation losses.

Three absorbance readings were taken for each metal-EDTA standard. Absorbance readings were also taken for an EDTA solution having no metal added (standard blank). The standard blank was kept stored with the metal standards to determine any metal contamination over time. The absorbance readings for the standards were averaged and corrected for the background metal in the standard blank (heading "Corr Mean" in worksheet). Linear

regression analysis of the corrected readings was then used to generate a correlation coefficient (r), the slope (m) of the standard curve and the y-intercept (b). This component of the analysis was performed before any subsequent analysis of sample eluates to provide early warning of any procedural or equipment problems (e.g. worn out graphite tubes, alignment of graphite furnace or spectrophotometer, etc.). These metal-EDTA standards were also run during the analysis of the sample eluates to determine if changes in instrument sensitivity was occurring during the analysis.

Section 3 describes the analysis associated with the sample eluates. Generally, 28 eluates were analyzed at one time, which was half the number of eluates generated in any one algal or oyster experiment. Of the 28 eluates, six were associated with seawater metal standards, one with a column blank and 21 with samples. In this example, columns 1, 2, 3 and 15, 16, 17 were associated with eluates of the seawater metal standards (duplicates of three metal concentrations). The seawater metal standards were used to calibrate the resin to inorganic metal concentrations and were made up in artificial seawater that matched the salinity and pH of the samples undergoing analysis. Column 18 was used as a column blank. The column blank was a resin-column prepared in the same manner as the other resin-columns except that no standard or sample was passed through. The column blank was used to indicate if any metal contamination occurred during the preparation of the resin-columns. All other eluates were from the natural seawater samples. Generally, duplicate resin-columns were run for each metal-EDTA concentration combination (see Table III-1).

Three absorbance readings were taken for each eluate. Absorbance readings were also taken for the EDTA solution used to elute the columns to determine an eluent blank. The readings for the column eluates were then averaged and corrected for the eluent blank. The corrected mean was then used in the

regression equation derived from the metal-EDTA standard curve (from Section 2) to solve for the concentration of the metal in the eluates. This is given under the heading "nM" in Section 3 on the worksheet. The eluate metal concentration in nM was then converted to the number of nanomoles (nmol) in the eluate volume (e.g. nmol/10 mL) and using the weight of resin, to nmol/g. (The nmol/g value is calculated by dividing nmol/10 mL value by the weight of resin used.) The nmol/g value is independent of the volume of eluate or weight of resin and therefore can be used for comparison between analyses.

Although any of the values generated in the analysis (e.g. nM, nmol/10 mL or nmol/g) could be used to generate EMC values, generally the nmol/g values were used. This involved generating a standard curve (Figure III-1) from the seawater metal standard (columns 1, 2, 3, 15, 16, 17) nmol/g values and applying polynominal or linear regression analysis, depending on the curve's shape, to determine an equation for the curve. Generally, linear regression was suitable for Cd and Zn standard curves and polynominal regression for Cu and Pb standard curves.

To derive an EMC value, the coefficients of the regression equation of the standard curve could be used to solve for the concentration of inorganic metal in a sample with the following equation:

$$\text{Conc. of inorganic metal in nM.} = \frac{-b + \sqrt{b^2 - 4a(c-y)}}{2a} = \text{EMC}$$

where a, b, c are the coefficients of the regression equation and y is the nmol/g value of the sample. The EMC value was equivalent to the inorganic metal concentration as defined by the seawater metal standards and had the same concentration units as these standards.

Detection Limits

The limit of detection of a method is defined as the lowest concentration level that can be determined to be statistically different from a blank (ACS Committee, 1983). The method used to calculate LODs in the present study is given in Section B.2.3.4.

The LOD of the ion-exchange procedure for a metal was strongly dependent on the operating conditions of the analysis. Conditions such as type (e.g. crosslinkage, batch number) and weight of resin used, flow rate, eluate volume and the sample injection volume used in the GFAAS analysis, all affected the LOD level. It is therefore difficult to define an LOD for each metal unless the conditions of the analysis are strictly monitored and kept constant. Even then, there is some variability in the sorption characteristics of the resin between experiments. However, under the operating conditions of 0.75 g of AG 50W X-8 resin, 10 mL of eluate volume, and 10 uL sample injection volume for GFAAS, conservative estimates of LODs were as follows:

Cu - 2 nM
Cd - 2 nM
Pb - 4 nM
*Zn - 100 nM

* Zinc was determined by flame atomic absorption spectroscopy. This LOD could be reduced considerably by using ASV instead of AAS - after appropriate treatment to free metal from complex.

TABLE III.1
Metal-EDTA Concentration Combinations for an Experiment Conducted with Lead

SET # 1[a]

Column #	Metal Conc. (nM)	EDTA Conc. (nM)	Column #	Metal Conc. (nM)	EDTA Conc. (nM)
1	50	Standards	15	50	Standards
2	150	made up in	16	150	made up in
3	250	diluted SOW	17	250	diluted SOW
4	Column Blank		18	0	-
5	50	0	19	50	75
6	50	0	20	50	75
7	100	0	21	100	75
8	100	0	22	100	75
9	150	0	23	150	75
10	150	0	24	150	75
11	200	0	25	200	75
12	200	0	26	200	75
13	250	0	27	250	75
14	250	0	28	250	75

SET # 2[a]

Column #	Metal Conc. (nM)	EDTA Conc. (nM)	Column #	Metal Conc. (nM)	EDTA Conc. (nM)
1	50	Standards	15	50	Standards
2	150	made up in	16	150	made up in
3	250	diluted SOW	17	250	diluted SOW
4	Column Blank		18	0	0
5	50	150	19	50	200
6	50	150	20	50	200
7	100	150	21	100	200
8	100	150	22	100	200
9	150	150	23	150	200
10	150	150	24	150	200
11	200	150	25	200	200
12	200	150	26	200	200
13	250	150	27	250	200
14	250	150	28	250	200

[a] The experiment was conducted with seawater Batch #6; Resin, AG 50W X-4 (#30008). These Metal-EDTA concentrations were added to the 500 mL of medium and allowed to equibrate 12 hours before analyses.

WORKSHEET EXAMPLE

Experiment#: 41 | Metal: Pb Set#1 | Date: Feb 26/86

Resin wt.: 0.75 g
Eluate volume: 10 mL
Inject Vol.: 10 µL

Section 1

Standards:

nM	Absorbance ($x10^{-3}$) Rd#1	Rd#2	Rd#3	Mean	Corr Mean
Std Blk	4	3	4	4	
50	43	40	39	41	37
100	84	83	79	82	78
200	156	161	160	159	155

r= 0.9998
m= 0.780
b= -1.5

Section 2

Column Eluates:

Column #	Absorbance ($x10^{-3}$) Rd#1	Rd#2	Rd#3	Corr Mean	nM	nmol/ 10 ml	nmol/ g	EMC (nM)
1	43	37	41	37	49	0.49	0.65	Metal Standards
2	134	129	130	128	165	1.65	2.20	
3	190	184	186	184	237	2.37	3.16	
4	4	5	2	1	0	0.00	0.00	0
5	47	46	48	44	58	0.58	0.77	52
6	46	48	44	43	57	0.57	0.77	52
7	91	92	90	88	114	1.14	1.52	103
8	88	86	87	84	109	1.09	1.45	98
9	118	115	116	113	146	1.46	1.95	136
10	119	122	121	118	152	1.52	2.03	143
11	144	140	141	139	179	1.79	2.39	175
12	139	134	138	134	173	1.73	2.30	167
13	180	179	178	176	226	2.26	3.01	243
14	184	188	186	183	235	2.35	3.13	260
Eluent Blank	3	4	2					

Section 3

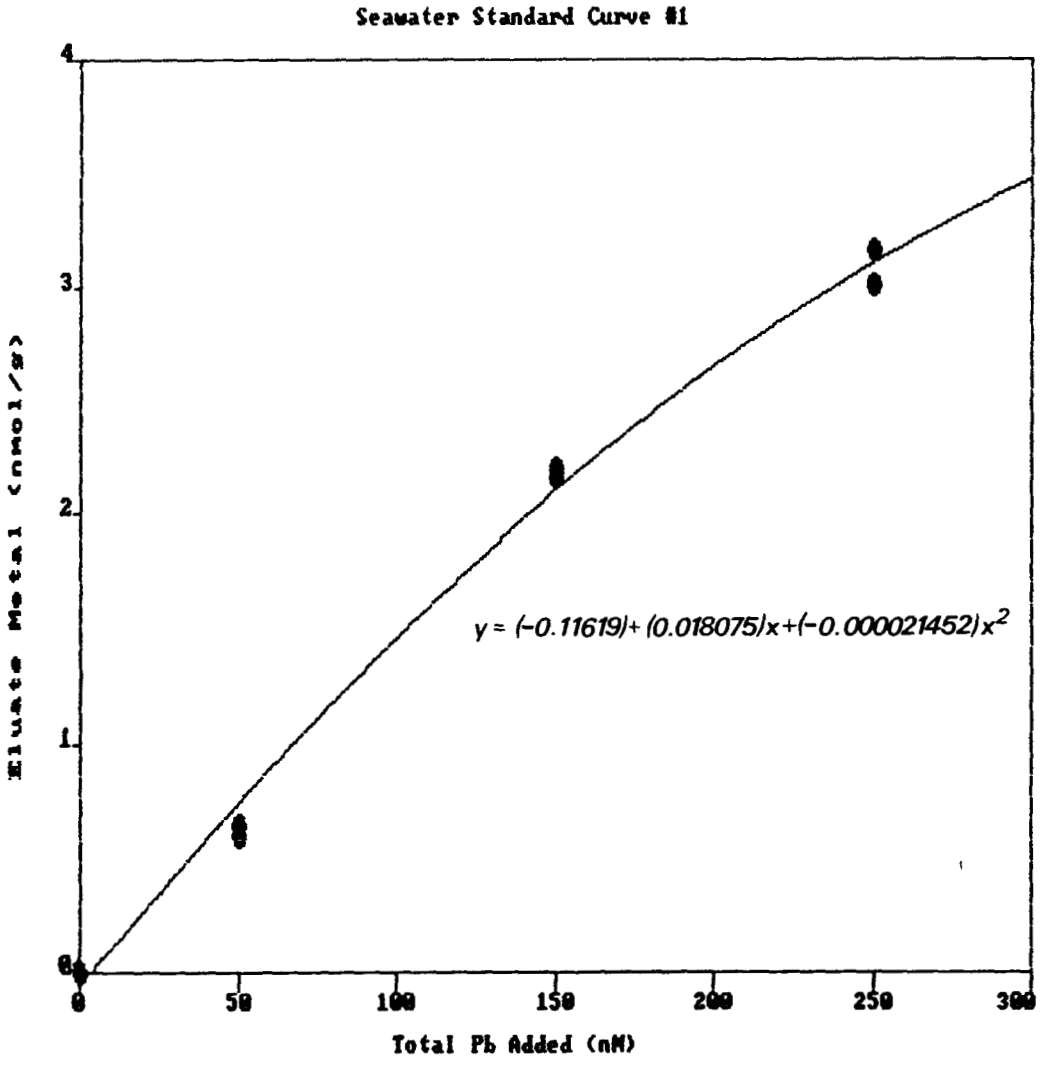
Experiment #: 41 (con't) Set #1 Date: Feb 26/86

Column Eluates:

Column #	Absorbance ($x10^{-3}$) Rd#1	Rd#2	Rd#3	Corr Mean	nM	nmol/ 10	nmol/ g	EMC (nM)
15	36	36	38	34	45	0.45	0.60	
16	127	128	131	126	163	1.63	2.17	Metal Standards
17	175	174	170	170	219	2.19	2.92	
18	5	3	3	1	0	0.00	0.00	
19	21	22	20	18	25	0.25	0.33	26
20	18	19	22	17	24	0.24	0.32	25
21	59	65	63	59	77	0.77	1.03	69
22	63	61	61	59	77	0.77	1.03	69
23	90	90	88	86	112	1.12	1.49	101
24	88	91	86	85	110	1.10	1.47	100
25	121	121	125	119	154	1.54	2.05	145
26	119	116	117	114	147	1.47	2.09	148
27	140	141	143	138	179	1.79	2.39	175
28	137	135	134	132	170	1.70	2.33	169

Section 3(cont.)

Figure III.1. Seawater Metal Standards Calibration Curve



Experiment#: 41 (con't) Set #2 Date: Feb 27/86

Resin wt.: 0.75 g
Eluate vol.: 10 mL
Inject vol.: 10 µl

Standards:

nM	Absorbance ($x10^{-3}$) Rd#1	Rd#2	Rd#3	Mean	Corr Mean
Std Blk	3	2	3	3	0
50	39	41	40	40	37
100	80	79	80	80	77
200	161	158	160	160	157

R= 0.9999
m= 0.787
b= -1.1

Column Eluates:

Column #	Absorbance ($x10^{-3}$) Rd#1	Rd#2	Rd#3	Corr Mean	nM	nmol/ 10	nmol/ g	EMC (nM)
1	44	41	44	41	53	0.53	0.71	
2	116	119	120	116	149	1.49	1.94	
3	180	181	183	179	229	2.29	3.05	
4	2	4	3	1	2	0.02	0.03	
5	8	8	9	6	9	0.09	0.12	10
6	10	11	12	9	12	0.12	0.17	13
7	22	18	19	18	23	0.23	0.31	23
8	21	21	19	18	24	0.24	0.32	24
9	42	46	44	42	54	0.54	0.73	52
10	51	51	49	48	62	0.62	0.84	59
11	71	73	70	69	89	0.89	1.19	85
12	80	81	82	79	101	1.01	1.36	97
13	116	118	117	115	147	1.47	1.96	144
14	114	112	111	110	141	1.41	1.89	139
Eluent Blank	3	2	2					

Experiment #: 41 (con't) Set #2 Date: Feb 27/86

Column Eluates:

Column #	Absorbance ($x10^{-3}$) Rd#1	Rd#2	Rd#3	Corr. Mean	nM	nmol/ 10	nmol/ g	EMC (nM)
15	40	38	38	737	48	0.48	0.63	
16	125	126	127	124	159	1.59	2.12	
17	184	186	185	183	234	2.34	2.12	
18	4	5	4	2	4	0.04	-	
19	4	1	3	1	0	0.00	0.00	0.0
20	3	2	2	0	0	0.00	0.00	0.0
21	14	14	16	13	18	0.18	0.24	18
22	9	10	9	7	10	0.10	0.14	12
23	36	35	39	35	46	0.46	0.61	43
24	35	39	39	36	47	0.47	0.63	45
25	59	62	58	58	75	0.75	1.00	71
26	61	61	59	58	75	0.75	1.00	71
27	74	76	76	73	94	0.94	1.25	89
28	78	80	79	77	99	0.99	1.32	94

Figure III.2. Seawater Metal Standards Calibration Curve

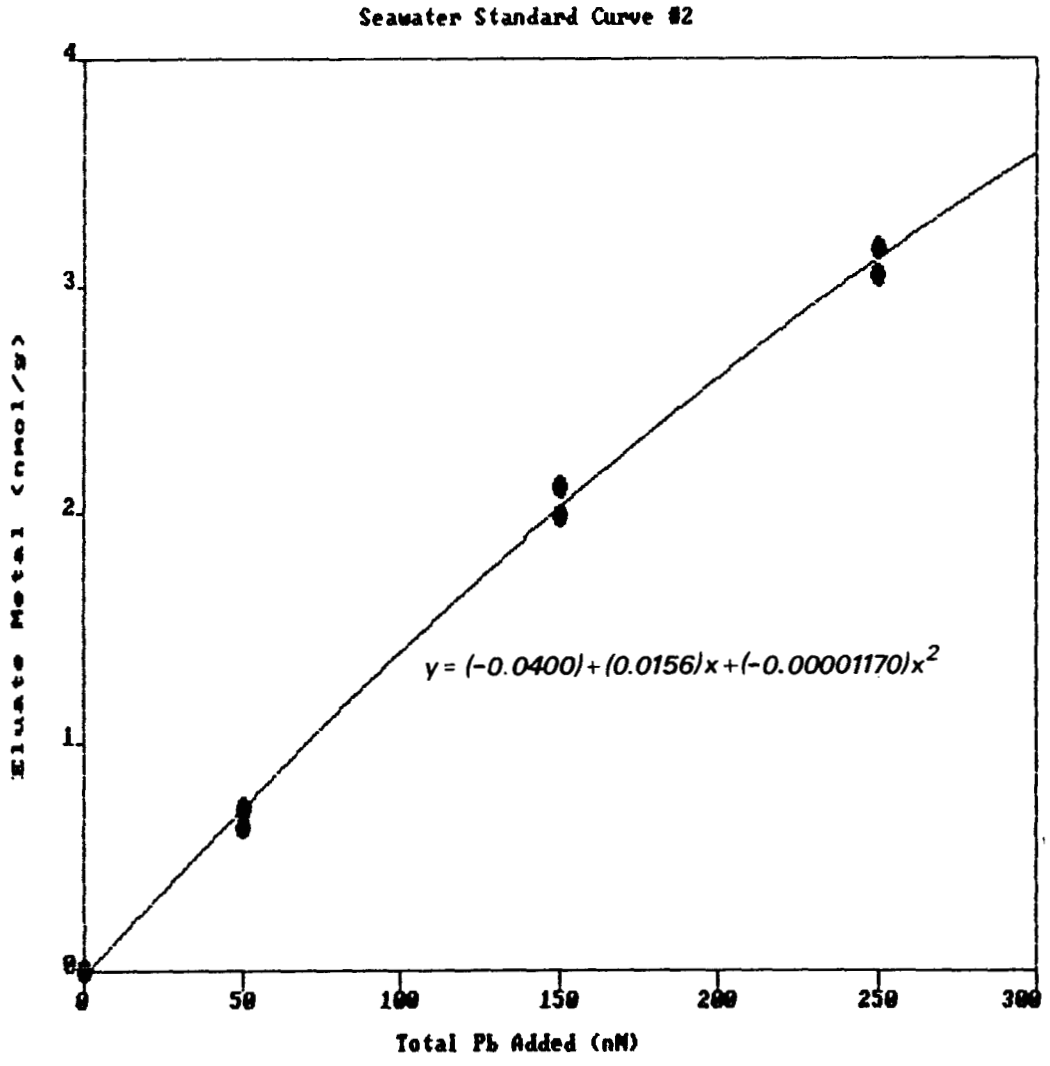
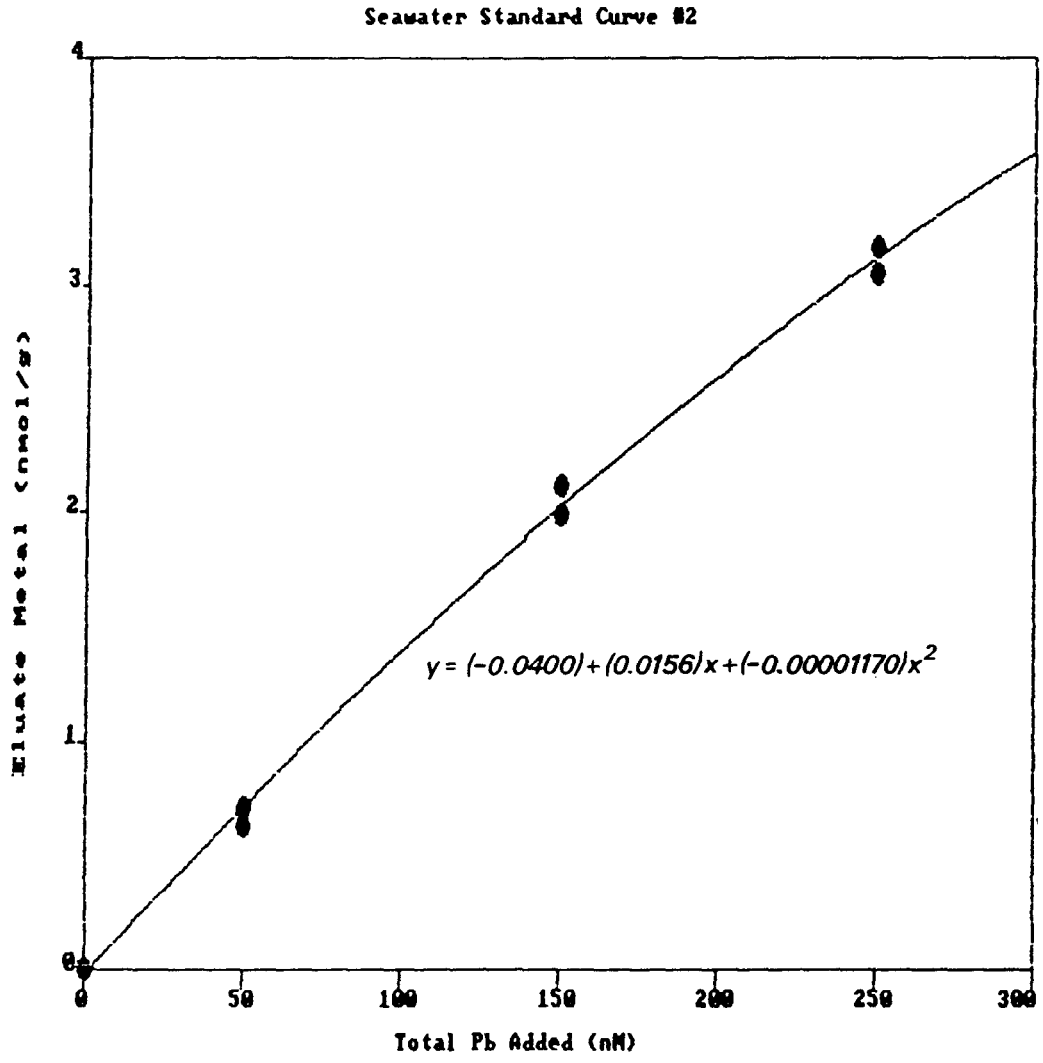


Figure III.2. Seawater Metal Standards Calibration Curve



APPENDIX IV

Oyster Embryo Assay Results

Experiment#: A (21) Metal: Cu Date: Aug/85

EDTA Conc.: 0.000 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	5.0	5.2	5.1	
	B	3.0	2.5	2.8	
	C	3.3	2.4	2.9	
	D	0.9	1.8	1.4	
	E	4.1	3.4	3.8	
	F	3.2	3.0	3.1	
			MEAN	3.2	
			SD	1.2	
0.10	A	6.8	6.3	6.6	3.5
	B	5.6	5.8	5.7	2.6
	C	2.4	3.8	3.1	0.0
			MEAN	5.1	2.0
			SD	1.8	1.9
0.20	A	31.0	31.3	31.2	28.9
	B	27.3	27.9	27.6	25.2
	C	33.8	34.6	34.2	32.1
			MEAN	31.0	28.7
			SD	3.3	3.4
0.30	A	81.7	80.2	81.0	80.3
	B	80.6	80.8	80.7	80.1
	C	84.9	80.7	82.8	82.2
			MEAN	81.5	80.9
			SD	1.1	1.2
0.40	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	99.1	100.0	99.6	99.5
			MEAN	99.9	99.8
			SD	0.3	0.3
0.60	A	99.2	98.3	98.8	98.7
	B	98.4	99.2	98.8	98.8
	C	100.0	100.0	100.0	100.0
			MEAN	99.2	99.2
			SD	0.7	0.7

EDTA Conc.: 0.025 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	5.0	5.2	5.1	
	B	3.0	2.5	2.8	
	C	3.3	2.4	2.9	
	D	0.9	1.8	1.4	
	E	4.1	3.4	3.8	
	F	3.2	3.0	3.1	
			MEAN	3.2	
			SD	1.2	
0.10	A	4.7	5.0	4.9	1.8
	B	5.1	5.8	5.5	2.4
	C	3.6	4.4	4.0	0.9
			MEAN	4.8	1.7
			SD	0.7	0.8
0.20	A	13.7	13.4	13.6	10.7
	B	13.5	12.4	13.0	10.1
	C	15.0	13.6	14.3	11.5
			MEAN	13.6	10.8
			SD	0.7	0.7
0.30	A	91.2	92.4	91.8	91.5
	B	93.4	95.5	94.5	94.3
	C	94.0	94.2	94.1	93.9
			MEAN	93.5	93.2
			SD	1.4	1.5
0.40	A	100.0	100.0	100.0	100.0
	B	99.1	100.0	99.6	99.5
	C	100.0	99.0	99.5	99.5
			MEAN	99.7	99.7
			SD	0.3	0.3
0.60	A	100.0	100.0	100.0	100.0
	B	98.2	100.0	99.1	99.1
	C	100.0	99.1	99.6	99.5
			MEAN	99.6	99.5
			SD	0.4	0.5

Experiment#: A (cont.) Metal: Cu

EDTA Conc.: 0.050 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	5.0	5.2	5.1	
	B	3.0	2.5	2.8	
	C	3.3	2.4	2.9	
	D	0.9	1.8	1.4	
	E	4.1	3.4	3.8	
	F	3.2	3.0	3.1	
			MEAN	3.2	
			SD	1.2	
0.10	A	7.0	6.5	6.8	3.7
	B	7.1	6.4	6.8	3.7
	C	5.9	6.0	6.0	2.9
			MEAN	6.5	3.4
			SD	0.5	0.5
0.20	A	14.7	14.9	14.8	12.0
	B	12.5	12.0	12.3	9.4
	C	13.5	13.2	13.4	10.5
			MEAN	13.5	10.7
			SD	1.3	1.3
0.30	A	92.1	92.4	92.3	92.0
	B	94.0	93.2	93.6	93.4
	C	92.4	92.9	92.7	92.4
			MEAN	92.8	92.6
			SD	0.7	0.7
0.40	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	100.0	100.0	100.0	100.0
			MEAN	100.0	100.0
			SD	0.0	0.0
0.60	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	100.0	100.0	100.0	100.0
			MEAN	100.0	100.0
			SD	0.0	0.0

EDTA Conc.: 0.100 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	5.0	5.2	5.1	
	B	3.0	2.5	2.8	
	C	3.3	2.4	2.9	
	D	0.9	1.8	1.4	
	E	4.1	3.4	3.8	
	F	3.2	3.0	3.1	
			MEAN	3.2	
			SD	1.2	
0.10	A	7.7	8.5	8.1	5.1
	B	8.7	7.2	8.0	5.0
	C	6.8	7.3	7.1	4.0
			MEAN	7.7	4.7
			SD	0.6	0.6
0.20	A	9.2	9.1	9.2	6.2
	B	9.9	10.2	10.1	7.1
	C	12.2	10.0	11.1	8.2
			MEAN	10.1	7.2
			SD	1.0	1.0
0.30	A	90.4	91.6	91.0	90.7
	B	91.9	90.6	91.3	91.0
	C	91.7	91.2	91.5	91.2
			MEAN	91.2	90.9
			SD	0.2	0.2
0.40	A	97.1	98.2	97.7	97.6
	B	98.1	99.1	98.6	98.6
	C	99.1	99.0	99.1	99.0
			MEAN	98.4	98.4
			SD	0.7	0.7
0.60	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	99.2	100.0	99.6	99.6
			MEAN	99.9	99.9
			SD	0.2	0.2

Experiment#: B (22) Metal: Cu Date: Aug/85

EDTA Conc.: 0.00 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.7	2.7	3.2	
	B	6.4	4.8	5.6	
	C	5.7	6.0	5.9	
	D	3.8	2.7	3.3	
	E	3.5	4.2	3.9	
	F	5.8	5.9	5.9	
			MEAN	4.6	
			SD	1.3	
0.10	A	5.9	5.2	5.6	1.0
	B	4.0	4.7	4.4	0.0
	C	5.3	4.2	4.8	0.2
			MEAN	4.9	0.3
			SD	0.6	0.6
0.20	A	53.8	52.6	53.2	50.9
	B	46.8	46.7	46.8	44.2
	C	45.8	48.1	47.0	44.4
			MEAN	49.0	46.5
			SD	3.7	3.8
0.30	A	99.0	100.0	99.5	99.5
	B	100.0	100.0	100.0	100.0
	C	100.0	99.0	99.5	99.5
			MEAN	99.7	99.7
			SD	0.3	0.3
0.40	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	100.0	100.0	100.0	100.0
			MEAN	100.0	100.0
			SD	0.0	0.0
0.60	A	100.0	99.1	99.6	99.5
	B	99.0	99.0	99.0	99.0
	C	100.0	100.0	100.0	100.0
			MEAN	99.5	99.5
			SD	0.5	0.5

EDTA Conc.: 0.15 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.7	2.7	3.2	
	B	6.4	4.8	5.6	
	C	5.7	6.0	5.9	
	D	3.8	2.7	3.3	
	E	3.5	4.2	3.9	
	F	5.8	5.9	5.9	
			MEAN	4.6	
			SD	1.3	
0.10	A	6.1	5.0	5.6	1.0
	B	5.8	6.0	5.9	1.4
	C	4.7	4.4	4.6	0.0
			MEAN	5.3	0.8
			SD	0.7	0.7
0.20	A	6.3	6.5	6.4	1.9
	B	5.4	5.5	5.5	0.9
	C	6.0	4.9	5.5	0.9
			MEAN	5.8	1.2
			SD	0.5	0.6
0.30	A	19.2	18.3	18.8	14.8
	B	15.8	17.1	16.5	12.4
	C	18.7	19.2	19.0	15.0
			MEAN	18.1	14.1
			SD	1.4	1.5
0.40	A	95.9	95.1	95.5	95.3
	B	96.0	94.7	95.4	95.1
	C	94.6	95.9	95.3	95.0
			MEAN	95.4	95.1
			SD	0.1	0.1
0.60	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	100.0	100.0	100.0	100.0
			MEAN	100.0	100.0
			SD	0.0	0.0

Experiment#: B (22) (cont.) Metal: Cu

EDTA Conc.: 0.30 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.7	2.7	3.2	
	B	6.4	4.8	5.6	
	C	5.7	6.0	5.9	
	D	3.8	2.7	3.3	
	E	3.5	4.2	3.9	
	F	5.8	5.9	5.9	
			MEAN	4.6	
			SD	1.3	
0.10	A	6.4	4.5	5.5	0.9
	B	5.2	4.5	4.9	0.3
	C	3.9	4.8	4.4	0.0
			MEAN	4.9	0.3
			SD	0.6	0.6
0.20	A	4.5	3.4	4.0	0.0
	B	5.0	4.8	4.9	0.3
	C	5.4	5.5	5.5	0.9
			MEAN	4.8	0.2
			SD	0.8	0.8
0.30	A	4.8	5.2	5.0	0.4
	B	6.3	4.0	5.2	0.6
	C	5.5	5.7	5.6	1.0
			MEAN	5.3	0.7
			SD	0.3	0.3
0.40	A	4.9	4.3	4.6	0.0
	B	5.5	4.9	5.2	0.6
	C	4.5	3.7	4.1	0.0
			MEAN	4.6	.0
			SD	0.6	0.6
0.60	A	98.2	99.0	98.6	98.5
	B	100.0	100.0	100.0	100.0
	C	99.0	99.1	99.1	99.0
			MEAN	99.2	99.2
			SD	0.7	0.7

EDTA Conc.: 0.46 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.7	2.7	3.2	
	B	6.4	4.8	5.6	
	C	5.7	6.0	5.9	
	D	3.8	2.7	3.3	
	E	3.5	4.2	3.9	
	F	5.8	5.9	5.9	
			MEAN	4.6	
			SD	1.3	
0.10	A	4.0	4.1	4.1	0.0
	B	5.4	4.5	5.0	0.4
	C	5.0	6.0	5.5	0.9
			MEAN	4.8	0.2
			SD	0.7	0.8
0.20	A	4.4	5.8	5.1	0.5
	B	5.8	4.9	5.4	0.8
	C	5.0	4.4	4.7	0.1
			MEAN	5.1	0.5
			SD	0.3	0.3
0.30	A	6.2	5.6	5.9	1.4
	B	4.2	4.9	4.6	0.0
	C	4.1	6.0	5.1	0.5
			MEAN	5.2	0.6
			SD	0.7	0.7
0.40	A	5.1	4.3	4.7	0.1
	B	5.1	5.2	5.2	0.6
	C	5.5	5.0	5.3	0.7
			MEAN	5.0	0.5
			SD	0.3	0.3
0.60	A	7.3	6.0	6.7	2.1
	B	9.3	8.5	8.9	4.5
	C	7.0	6.2	6.6	2.1
			MEAN	7.4	2.9
			SD	1.3	1.4

Experiment A (17) Metal: Cd Date: Aug /85

EDTA Conc.: 0.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	4.9	2.8	3.9	
	B	6.1	3.5	4.8	
	C	4.1	4.5	4.3	
	D	5.4	5.9	5.7	
	E	3.0	3.9	3.5	
	F	4.2	3.1	3.7	
			MEAN	4.3	
			SD	0.8	
1	A	7.0	8.2	7.6	3.5
	B	23.6	22.3	23.0	19.5
	C	24.3	24.8	24.6	21.2
			MEAN	18.4	14.7
			SD	9.4	9.8
2	A	96.0	94.6	95.3	95.1
	B	95.0	96.8	95.9	95.7
	C	95.7	95.1	95.4	95.2
			MEAN	95.5	95.3
			SD	0.3	0.3
3	A	93.3	95.3	94.3	94.0
	B	96.8	96.0	96.4	96.2
	C	97.0	97.2	97.1	97.0
			MEAN	95.9	95.8
			SD	1.5	1.5
4	A	94.8	96.0	95.4	95.2
	B	100.0	97.9	99.0	98.9
	C	99.0	98.0	98.5	98.4
			MEAN	97.6	97.5
			SD	1.9	2.0
5	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	100.0	100.0	100.0	100.0
			MEAN	100.0	100.0
			SD	0.0	0.0

EDTA Conc.: 5.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
CTR	A	4.9	2.8	3.9	
	B	6.1	3.5	4.8	
	C	4.1	4.5	4.3	
	D	5.4	5.9	5.7	
	E	3.0	3.9	3.5	
	F	4.2	3.1	3.7	
			MEAN	4.3	
			SD	0.8	
1	A	4.0	3.9	4.0	0.0
	B	5.9	7.0	6.5	2.3
	C	5.0	3.1	4.1	0.0
			MEAN	4.8	0.6
			SD	1.4	1.5
2	A	8.5	7.5	8.0	3.9
	B	9.4	7.3	8.4	4.2
	C	10.4	10.2	10.3	6.3
			MEAN	8.9	4.8
			SD	1.2	1.3
3	A	98.2	97.1	97.7	97.5
	B	98.1	99.0	98.6	98.5
	C	99.1	98.0	98.6	98.5
			MEAN	98.3	98.2
			SD	0.5	0.5
4	A	100.0	100.0	100.0	100.0
	B	100.0	99.1	99.6	99.5
	C	97.9	99.1	98.5	98.4
			MEAN	99.4	99.3
			SD	0.8	0.8
5	A	97.8	99.0	98.4	98.3
	B	99.0	100.0	99.5	99.5
	C	100.0	100.0	100.0	100.0
			MEAN	99.3	99.3
			SD	0.8	0.9

Experiment A (17) Metal: Cd Date: Aug /85

EDTA Conc.: 25 uM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
CTR	A	4.9	2.8	3.9	
	B	6.1	3.5	4.8	
	C	4.1	4.5	4.3	
	D	5.4	5.9	5.7	
	E	3.0	3.9	3.5	
	F	4.2	3.1	3.7	
			MEAN	4.3	
			SD	0.8	
1	A	6.0	4.6	5.3	1.1
	B	6.1	3.8	5.0	0.7
	C	7.5	2.9	5.2	1.0
			MEAN	5.2	0.9
			SD	0.2	0.2
2	A	2.9	3.7	3.3	0.0
	B	6.0	4.4	5.2	1.0
	C	6.7	3.5	5.1	0.9
			MEAN	4.5	0.3
			SD	1.1	1.1
3	A	15.3	15.7	15.5	11.7
	B	18.6	16.3	17.5	13.8
	C	12.8	13.0	12.9	9.0
			MEAN	15.3	11.5
			SD	2.3	2.4
4	A	9.3	8.9	9.1	5.0
	B	6.9	7.9	7.4	3.3
	C	12.5	9.4	11.0	7.0
			MEAN	9.2	5.1
			SD	1.8	1.9
5	A	11.7	12.4	12.1	8.1
	B	8.1	11.6	9.9	5.8
	C	11.3	8.6	10.0	5.9
			MEAN	10.6	6.6
			SD	1.2	1.3

EDTA Conc.: 100 uM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
CTR	A	4.9	2.8	3.9	
	B	6.1	3.5	4.8	
	C	4.1	4.5	4.3	
	D	5.4	5.9	5.7	
	E	3.0	3.9	3.5	
	F	4.2	3.1	3.7	
			MEAN	4.3	
			SD	0.8	
1	A	6.3	4.1	5.2	1.0
	B	4.5	1.9	3.2	0.0
	C	6.1	4.5	5.3	1.1
			MEAN	4.6	0.3
			SD	1.2	1.2
2	A	3.1	4.0	3.6	0.0
	B	5.0	3.8	4.4	0.1
	C	2.7	4.6	3.7	0.0
			MEAN	3.9	0.0
			SD	0.5	0.0
3	A	8.4	8.6	8.5	4.4
	B	7.6	5.9	6.8	2.6
	C	5.8	4.8	5.3	1.1
			MEAN	6.9	2.7
			SD	1.6	1.7
4	A	11.0	9.8	10.4	6.4
	B	6.7	6.4	6.6	2.4
	C	4.5	5.5	5.0	0.7
			MEAN	7.3	3.2
			SD	2.8	2.9
5	A	5.9	4.9	5.4	1.2
	B	5.5	4.0	4.8	0.5
	C	4.2	4.8	4.5	0.2
			MEAN	4.9	0.6
			SD	0.5	0.5

Experiment#: B (27) Metal: Cd Date: Sept/85

EDTA Conc.: 0.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.1	2.3	2.7	
	B	3.6	3.0	3.3	
	C	2.5	2.6	2.6	
	D	3.3	3.4	3.4	
	E	2.0	1.9	2.0	
	F	3.3	4.2	3.8	
			MEAN	2.9	
			SD	0.7	
2.0	A	5.4	5.0	5.2	2.3
	B	4.9	4.2	4.6	1.7
	C	5.1	4.3	4.7	1.8
			MEAN	4.8	1.9
			SD	0.3	0.4
3.0	A	29.4	28.7	29.1	26.9
	B	29.6	31.5	30.6	28.5
	C	29.3	30.1	29.7	27.6
			MEAN	29.8	27.6
			SD	0.8	0.8
4.0	A	76.4	78.2	77.3	76.6
	B	73.9	74.1	74.0	73.2
	C	77.5	79.2	78.4	77.7
			MEAN	76.6	75.8
			SD	2.3	2.3
5.0	A	98.2	99.1	98.7	98.6
	B	99.1	100.0	99.6	99.5
	C	98.3	98.3	98.3	98.2
			MEAN	98.8	98.8
			SD	0.6	0.7
6.0	A	100.0	99.1	99.6	99.5
	B	100.0	100.0	100.0	100.0
	C	99.2	99.2	99.2	99.2
			MEAN	99.6	99.6
			SD	0.4	0.4

EDTA Conc.: 5.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.1	2.3	2.7	
	B	3.6	3.0	3.3	
	C	2.5	2.6	2.6	
	D	3.3	3.4	3.4	
	E	2.0	1.9	2.0	
	F	3.3	4.2	3.8	
			MEAN	2.9	
			SD	0.7	
2.0	A	5.0	4.9	5.0	2.1
	B	4.6	3.8	4.2	1.3
	C	5.3	4.8	5.1	2.2
			MEAN	4.7	1.9
			SD	0.5	0.5
3.0	A	10.6	10.6	10.6	7.9
	B	10.9	10.8	10.9	8.2
	C	11.9	11.2	11.6	8.9
			MEAN	11.0	8.3
			SD	0.5	0.5
4.0	A	42.6	46.9	44.8	43.1
	B	49.0	47.4	48.2	46.6
	C	51.9	48.3	50.1	48.6
			MEAN	47.7	46.1
			SD	2.7	2.8
5.0	A	91.0	90.6	90.8	90.5
	B	90.4	91.3	90.9	90.6
	C	91.2	90.0	90.6	90.3
			MEAN	90.8	90.5
			SD	0.1	0.1
6.0	A	100.0	99.1	99.6	99.5
	B	100.0	100.0	100.0	100.0
	C	100.0	100.0	100.0	100.0
			MEAN	99.9	99.8
			SD	0.3	0.3

Experiment#: B (27) (cont.) Metal: Cd

EDTA Conc.: 10.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.1	2.3	2.7	
	B	3.6	3.0	3.3	
	C	2.5	2.6	2.6	
	D	3.3	3.4	3.4	
	E	2.0	1.9	2.0	
	F	3.3	4.2	3.8	
			MEAN	2.9	
			SD	0.7	
2.0	A	4.1	4.8	4.5	1.6
	B	4.7	4.5	4.6	1.7
	C	4.0	5.1	4.6	1.7
			MEAN	4.5	1.6
			SD	0.1	0.1
3.0	A	5.1	4.5	4.8	1.9
	B	4.4	4.2	4.3	1.4
	C	5.2	4.1	4.7	1.8
			MEAN	4.6	1.7
			SD	0.3	0.3
4.0	A	5.1	5.4	5.3	2.4
	B	4.4	4.9	4.7	1.8
	C	4.4	4.8	4.6	1.7
			MEAN	4.8	2.0
			SD	0.4	0.4
5.0	A	48.8	47.1	48.0	46.4
	B	46.5	47.7	47.1	45.5
	C	44.2	43.0	43.6	41.9
			MEAN	46.2	44.6
			SD	2.3	2.4
6.0	A	80.8	81.1	81.0	80.4
	B	77.5	77.6	77.6	76.9
	C	76.7	76.1	76.4	75.7
			MEAN	78.3	77.6
			SD	2.4	2.4

EDTA Conc.: 15.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.1	2.3	2.7	
	B	3.6	3.0	3.3	
	C	2.5	2.6	2.6	
	D	3.3	3.4	3.4	
	E	2.0	1.9	2.0	
	F	3.3	4.2	3.8	
			MEAN	2.9	
			SD	0.7	
2.0	A	5.0	5.4	5.2	2.3
	B	4.4	4.5	4.5	1.6
	C	5.3	4.8	5.1	2.2
			MEAN	4.9	2.0
			SD	0.4	0.4
3.0	A	4.6	4.9	4.8	1.9
	B	4.4	4.8	4.6	1.7
	C	3.9	4.8	4.4	1.5
			MEAN	4.6	1.7
			SD	0.2	0.2
4.0	A	5.3	4.6	5.0	2.1
	B	5.0	5.2	5.1	2.2
	C	4.5	5.1	4.8	1.9
			MEAN	5.0	2.1
			SD	0.1	0.2
5.0	A	5.2	4.5	4.9	2.0
	B	5.0	4.8	4.9	2.0
	C	3.8	3.9	3.9	0.9
			MEAN	4.5	1.6
			SD	0.6	0.6
6.0	A	26.7	29.3	28.0	25.8
	B	29.5	29.6	29.6	27.4
	C	29.4	30.3	29.9	27.7
			MEAN	29.1	27.0
			SD	1.0	1.0

Experiment#: A (23) Metal: Zn Date: Aug/85

EDTA Conc.: 0.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	1.6	2.5	2.1	
	B	0.9	1.9	1.4	
	C	1.9	3.5	2.7	
	D	1.9	1.9	1.9	
	E	2.5	1.6	2.1	
	F	2.6	3.3	3.0	
			MEAN	2.2	
			SD	0.6	
3.0	A	20.2	18.9	19.6	17.8
	B	36.6	35.6	36.1	34.7
	C	34.9	32.8	33.9	32.4
			MEAN	29.8	28.3
			SD	9.0	9.2
4.0	A	73.5	74.5	74.0	73.4
	B	75.2	77.1	76.2	75.6
	C	77.6	77.0	77.3	76.8
			MEAN	75.8	75.3
			SD	1.7	1.7
5.0	A	100.0	99.0	99.5	99.5
	B	98.9	100.0	99.5	99.4
	C	99.1	98.3	98.7	98.7
			MEAN	99.2	99.2
			SD	0.4	0.5
6.0	A	100.0	99.2	99.6	99.6
	B	100.0	99.1	99.6	99.5
	C	100.0	100.0	100.0	100.0
			MEAN	99.7	99.7
			SD	0.2	0.3
7.0	A	100.0	99.1	99.6	99.5
	B	99.1	99.0	99.1	99.0
	C	100.0	99.0	99.5	99.5
			MEAN	99.4	99.4
			SD	0.3	0.3

EDTA Conc.: 1.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	1.6	2.5	2.1	
	B	0.9	1.9	1.4	
	C	1.9	3.5	2.7	
	D	1.9	1.9	1.9	
	E	2.5	1.6	2.1	
	F	2.6	3.3	3.0	
			MEAN	2.2	
			SD	0.6	
3.0	A	11.6	11.4	11.5	9.5
	B	10.0	10.6	10.3	8.3
	C	9.4	9.6	9.5	7.5
			MEAN	10.4	8.4
			SD	1.0	1.0
4.0	A	51.7	50.8	51.3	50.2
	B	47.2	48.1	47.7	46.5
	C	53.1	53.8	53.5	52.4
			MEAN	50.8	49.7
			SD	2.9	3.0
5.0	A	85.1	85.9	85.5	85.2
	B	87.5	87.2	87.4	87.1
	C	86.8	86.3	86.6	86.3
			MEAN	86.5	86.2
			SD	0.9	0.9
6.0	A	98.0	99.0	98.5	98.5
	B	99.1	98.1	98.6	98.6
	C	97.4	96.7	97.1	97.0
			MEAN	98.1	98.0
			SD	0.9	0.9
7.0	A	100.0	100.0	100.0	100.0
	B	100.0	100.0	100.0	100.0
	C	100.0	100.0	100.0	100.0
			MEAN	100.0	100.0
			SD	0.0	0.0

Experiment#: A (23) (cont.) Metal: Zn

EDTA Conc.: 2.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	1.6	2.5	2.1	
	B	0.9	1.9	1.4	
	C	1.9	3.5	2.7	
	D	1.9	1.9	1.9	
	E	2.5	1.6	2.1	
	F	2.6	3.3	3.0	
			MEAN	2.2	
			SD	0.6	
3.0	A	8.9	8.0	8.5	6.4
	B	6.1	6.8	6.5	4.4
	C	7.2	7.1	7.2	5.1
			MEAN	7.4	5.3
			SD	1.0	1.0
4.0	A	3.7	5.2	4.5	2.3
	B	11.5	10.2	10.9	8.9
	C	9.2	9.3	9.3	7.2
			MEAN	8.2	6.1
			SD	3.3	3.4
5.0	A	47.6	47.7	47.7	46.5
	B	44.4	44.6	44.5	43.3
	C	48.6	49.1	48.9	47.7
			MEAN	47.0	45.8
			SD	2.2	2.3
6.0	A	95.7	95.0	95.4	95.2
	B	93.2	93.8	93.5	93.4
	C	94.3	94.4	94.4	94.2
			MEAN	94.4	94.3
			SD	0.9	0.9
7.0	A	100.0	99.1	99.6	99.5
	B	99.0	99.1	99.1	99.0
	C	100.0	99.2	99.6	99.6
			MEAN	99.4	99.4
			SD	0.3	0.3

EDTA Conc.: 4.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	1.6	2.5	2.1	
	B	0.9	1.9	1.4	
	C	1.9	3.5	2.7	
	D	1.9	1.9	1.9	
	E	2.5	1.6	2.1	
	F	2.6	3.3	3.0	
			MEAN	2.2	
			SD	0.6	
3.0	A	4.5	3.3	3.9	1.8
	B	5.2	4.8	5.0	2.9
	C	3.7	4.5	4.1	2.0
			MEAN	4.3	2.2
			SD	0.6	0.6
4.0	A	5.9	6.6	6.3	4.2
	B	4.3	5.4	4.9	2.7
	C	4.9	5.1	5.0	2.9
			MEAN	5.4	3.3
			SD	0.8	0.8
5.0	A	8.1	8.0	8.1	6.0
	B	6.8	7.1	7.0	4.9
	C	7.6	6.8	7.2	5.1
			MEAN	7.4	5.3
			SD	0.6	0.6
6.0	A	13.1	12.3	12.7	10.8
	B	10.6	11.1	10.9	8.9
	C	13.4	12.1	12.8	10.8
			MEAN	12.1	10.1
			SD	1.1	1.1
7.0	A	27.2	25.0	26.1	24.5
	B	19.6	23.1	21.4	19.6
	C	23.0	21.3	22.2	20.4
			MEAN	23.2	21.5
			SD	2.5	2.6

Experiment#: B (28) Metal: Zn Date: Sept/85

EDTA Conc.: 0.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.8	4.1	4.0	
	B	2.9	2.8	2.9	
	C	4.6	4.3	4.5	
	D	2.7	3.5	3.1	
	E	2.7	3.4	3.1	
	F	4.2	2.5	3.4	
			MEAN	3.5	
			SD	0.6	
3.0	A	47.4	46.7	47.1	45.2
	B	46.5	45.7	46.1	44.2
	C	45.7	45.4	45.6	43.6
			MEAN	46.2	44.3
			SD	0.8	0.8
4.0	A	76.9	76.7	76.8	76.0
	B	78.3	76.5	77.4	76.6
	C	77.1	77.5	77.3	76.5
			MEAN	77.2	76.3
			SD	0.3	0.3
5.0	A	91.5	90.8	91.2	90.8
	B	91.0	91.7	91.4	91.0
	C	92.2	91.9	92.1	91.8
			MEAN	91.5	91.2
			SD	0.5	0.5
6.0	A	99.1	100.0	99.6	99.5
	B	100.0	100.0	100.0	100.0
	C	98.2	99.0	98.6	98.5
			MEAN	99.4	99.4
			SD	0.7	0.7
7.0	A	100.0	100.0	100.0	100.0
	B	100.0	99.2	99.6	99.6
	C	100.0	100.0	100.0	100.0
			MEAN	99.9	99.9
			SD	0.2	0.2

EDTA Conc.: 1.5 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.8	4.1	4.0	
	B	2.9	2.8	2.9	
	C	4.6	4.3	4.5	
	D	2.7	3.5	3.1	
	E	2.7	3.4	3.1	
	F	4.2	2.5	3.4	
			MEAN	3.5	
			SD	0.6	
3.0	A	19.4	19.0	19.2	16.3
	B	21.2	21.8	21.5	18.7
	C	21.0	20.6	20.8	18.0
			MEAN	20.5	17.7
			SD	1.2	1.2
4.0	A	44.9	44.5	44.7	42.7
	B	45.9	44.8	45.4	43.4
	C	46.2	45.4	45.8	43.9
			MEAN	45.3	43.3
			SD	0.6	0.6
5.0	A	78.7	78.4	78.6	77.8
	B	80.6	80.8	80.7	80.0
	C	77.8	78.9	78.4	77.6
			MEAN	79.2	78.5
			SD	1.3	1.3
6.0	A	95.5	96.3	95.9	95.8
	B	94.9	95.8	95.4	95.2
	C	95.6	95.7	95.7	95.5
			MEAN	95.6	95.5
			SD	0.3	0.3
7.0	A	100.0	99.1	99.6	99.5
	B	99.2	100.0	99.6	99.6
	C	100.0	100.0	100.0	100.0
			MEAN	99.7	99.7
			SD	0.2	0.3

Experiment#: B (28) (cont.) Metal: Zn

EDTA Conc.: 3.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.8	4.1	4.0	
	B	2.9	2.8	2.9	
	C	4.6	4.3	4.5	
	D	2.7	3.5	3.1	
	E	2.7	3.4	3.1	
	F	4.2	2.5	3.4	
			MEAN	3.5	
			SD	0.6	
3.0	A	5.9	6.3	6.1	2.7
	B	4.4	5.7	5.1	1.6
	C	3.5	4.2	3.9	0.4
			MEAN	5.0	1.6
			SD	1.1	1.2
4.0	A	8.3	6.8	7.6	4.2
	B	6.6	6.1	6.4	3.0
	C	6.5	5.6	6.1	2.7
			MEAN	6.7	3.3
			SD	0.8	0.8
5.0	A	14.3	13.8	14.1	11.0
	B	13.8	13.9	13.9	10.8
	C	13.6	12.7	13.2	10.0
			MEAN	13.7	10.6
			SD	0.5	0.5
6.0	A	46.7	45.1	45.9	44.0
	B	46.1	44.2	45.2	43.2
	C	44.3	42.5	43.4	41.4
			MEAN	44.8	42.8
			SD	1.3	1.3
7.0	A	84.2	84.0	84.1	83.5
	B	82.1	80.5	81.3	80.6
	C	83.2	84.9	84.1	83.5
			MEAN	83.2	82.5
			SD	1.6	1.7

EDTA Conc.: 5.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	3.8	4.1	4.0	
	B	2.9	2.8	2.9	
	C	4.6	4.3	4.5	
	D	2.7	3.5	3.1	
	E	2.7	3.4	3.1	
	F	4.2	2.5	3.4	
			MEAN	3.5	
			SD	0.6	
3.0	A	6.0	5.5	5.8	2.4
	B	5.6	5.2	5.4	2.0
	C	5.6	5.3	5.5	2.1
			MEAN	5.5	2.1
			SD	0.2	0.2
4.0	A	6.4	5.6	6.0	2.6
	B	5.3	5.9	5.6	2.2
	C	5.4	4.8	5.1	1.7
			MEAN	5.6	2.2
			SD	0.5	0.5
5.0	A	5.8	6.2	6.0	2.6
	B	5.7	4.5	5.1	1.7
	C	5.7	4.6	5.2	1.8
			MEAN	5.4	2.0
			SD	0.5	0.5
6.0	A	15.5	14.9	15.2	12.2
	B	15.3	14.8	15.1	12.0
	C	15.1	15.6	15.4	12.3
			MEAN	15.2	12.2
			SD	0.1	0.2
7.0	A	47.4	46.9	47.2	45.3
	B	52.6	51.5	52.1	50.3
	C	42.6	46.2	44.4	42.4
			MEAN	47.9	46.0
			SD	3.9	4.0

Experiment#: A (25) Metal: Pb Date: Aug/85

EDTA Conc.: 0.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	2.5	1.6	2.1	
	B	1.6	2.7	2.2	
	C	1.8	2.5	2.2	
	D	1.0	2.8	1.9	
	E	1.6	1.6	1.6	
	F	2.5	2.5	2.5	
			MEAN	2.1	
			SD	0.3	
2.0	A	1.9	2.5	2.2	0.1
	B	4.7	3.6	4.2	2.1
	C	2.8	1.9	2.4	0.3
			MEAN	2.9	0.9
			SD	1.1	1.1
4.0	A	1.7	2.5	2.1	0.0
	B	3.5	3.4	3.5	1.4
	C	2.6	1.8	2.2	0.1
			MEAN	2.6	0.5
			SD	0.8	0.8
6.0	A	85.3	85.0	85.2	84.8
	B	86.8	87.9	87.4	87.1
	C	87.3	86.4	86.9	86.6
			MEAN	86.5	86.2
			SD	1.2	1.2
8.0	A	98.1	99.1	98.6	98.6
	B	99.0	100.0	99.5	99.5
	C	100.0	99.1	99.6	99.5
			MEAN	99.2	99.2
			SD	0.5	0.5
10.0	A	100.0	100.0	100.0	100.0
	B	99.0	100.0	99.5	99.5
	C	100.0	100.0	100.0	100.0
			MEAN	99.8	99.8
			SD	0.3	0.3

EDTA Conc.: 2.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	2.5	1.6	2.1	
	B	1.6	2.7	2.2	
	C	1.8	2.5	2.2	
	D	1.0	2.8	1.9	
	E	1.6	1.6	1.6	
	F	2.5	2.5	2.5	
			MEAN	2.1	
			SD	0.3	
2.0	A	1.9	2.7	2.3	0.2
	B	2.6	1.8	2.2	0.1
	C	1.8	1.9	1.9	0.0
			MEAN	2.1	0.1
			SD	0.2	0.2
4.0	A	3.1	2.5	2.8	0.8
	B	2.5	2.5	2.5	0.5
	C	2.4	1.7	2.1	0.0
			MEAN	2.5	0.4
			SD	0.4	0.4
6.0	A	1.9	1.9	1.9	0.0
	B	1.8	2.6	2.2	0.1
	C	2.5	2.5	2.5	0.5
			MEAN	2.2	0.1
			SD	0.3	0.3
8.0	A	89.2	88.7	89.0	88.7
	B	91.2	90.0	90.6	90.4
	C	92.2	92.4	92.3	92.1
			MEAN	90.6	90.4
			SD	1.7	1.7
10.0	A	98.1	100.0	99.1	99.0
	B	99.0	100.0	99.5	99.5
	C	99.1	99.1	99.1	99.1
			MEAN	99.2	99.2
			SD	0.2	0.3

Experiment#: A (25) (cont.) Metal: Pb

EDTA Conc.: 3.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	2.5	1.6	2.1	
	B	1.6	2.7	2.2	
	C	1.8	2.5	2.2	
	D	1.0	2.8	1.9	
	E	1.6	1.6	1.6	
	F	2.5	2.5	2.5	
			MEAN	2.1	
			SD	0.3	
2.0	A	0.9	0.0	0.5	0.0
	B	1.7	0.9	1.3	0.0
	C	0.9	1.0	1.0	0.0
			MEAN	0.9	-1.2
			SD	0.4	0.4
4.0	A	4.7	3.5	4.1	2.1
	B	5.7	5.3	5.5	3.5
	C	3.5	4.0	3.8	1.7
			MEAN	4.5	2.4
			SD	0.9	0.9
6.0	A	4.7	4.1	4.4	2.4
	B	5.6	5.4	5.5	3.5
	C	4.2	5.4	4.8	2.8
			MEAN	4.9	2.9
			SD	0.6	0.6
8.0	A	84.6	84.7	84.7	84.3
	B	84.4	80.3	82.4	82.0
	C	86.1	86.0	86.1	85.8
			MEAN	84.4	84.0
			SD	1.9	1.9
10.0	A	98.3	99.2	98.8	98.7
	B	100.0	100.0	100.0	100.0
	C	99.1	99.1	99.1	99.1
			MEAN	99.3	99.3
			SD	0.6	0.7

EDTA Conc.: 5.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	2.5	1.6	2.1	
	B	1.6	2.7	2.2	
	C	1.8	2.5	2.2	
	D	1.0	2.8	1.9	
	E	1.6	1.6	1.6	
	F	2.5	2.5	2.5	
			MEAN	2.1	
			SD	0.3	
2.0	A	0.0	0.8	0.4	0.0
	B	0.8	0.9	0.9	0.0
	C	1.6	2.3	2.0	0.0
			MEAN	1.1	0.0
			SD	0.8	0.0
4.0	A	0.1	1.8	1.0	0.0
	B	2.5	1.6	2.1	0.0
	C	1.7	0.9	1.3	0.0
			MEAN	1.4	0.0
			SD	0.6	0.0
6.0	A	1.6	1.7	1.7	0.0
	B	1.7	1.8	1.8	0.0
	C	3.4	2.6	3.0	1.0
			MEAN	2.1	0.0
			SD	0.8	0.0
8.0	A	18.5	17.4	18.0	16.2
	B	16.7	17.7	17.2	15.5
	C	14.0	13.8	13.9	12.1
			MEAN	16.4	14.6
			SD	2.2	2.2
10.0	A	21.2	21.4	21.3	19.6
	B	20.9	21.4	21.2	19.5
	C	19.9	19.7	19.8	18.1
			MEAN	20.8	19.1
			SD	0.8	0.8

Experiment#: B (26) Metal: Pb Date: Aug/85

EDTA Conc.: 0.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	9.3	9.8	9.6	
	B	7.0	6.5	6.8	
	C	6.8	5.6	6.2	
	D	5.6	7.1	6.4	
	E	4.2	5.0	4.6	
	F	6.1	6.3	6.2	
			MEAN	6.6	
			SD	1.6	
4.0	A	59.2	63.0	61.1	58.3
	B	61.0	62.6	61.8	59.1
	C	66.0	64.1	65.1	62.6
			MEAN	62.7	60.0
			SD	2.1	2.3
6.0	A	93.7	93.9	93.8	93.4
	B	93.0	92.4	92.7	92.2
	C	92.5	92.0	92.3	91.7
			MEAN	92.9	92.4
			SD	0.8	0.9
8.0	A	100.0	99.1	99.6	99.5
	B	99.1	99.0	99.1	99.0
	C	100.0	100.0	100.0	100.0
			MEAN	99.5	99.5
			SD	0.5	0.5
10.0	A	100.0	100.0	100.0	100.0
	B	100.0	99.1	99.6	99.5
	C	100.0	99.2	99.6	99.6
			MEAN	99.7	99.7
			SD	0.2	0.3
12.0	A	100.0	99.2	99.6	99.6
	B	100.0	100.0	100.0	100.0
	C	99.2	100.0	99.6	99.6
			MEAN	99.7	99.7
			SD	0.2	0.2

EDTA Conc.: 4.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	9.3	9.8	9.6	
	B	7.0	6.5	6.8	
	C	6.8	5.6	6.2	
	D	5.6	7.1	6.4	
	E	4.2	5.0	4.6	
	F	6.1	6.3	6.2	
			MEAN	6.6	
			SD	1.6	
4.0	A	8.5	8.0	8.3	1.8
	B	8.0	6.8	7.4	0.8
	C	7.6	7.9	7.8	1.2
			MEAN	7.8	1.3
			SD	0.4	0.5
6.0	A	37.9	39.6	38.8	34.4
	B	41.0	39.4	40.2	36.0
	C	38.4	37.6	38.0	33.6
			MEAN	39.0	34.7
			SD	1.1	1.2
8.0	A	90.6	90.2	90.4	89.7
	B	88.7	89.1	88.9	88.1
	C	91.2	90.6	90.9	90.3
			MEAN	90.1	89.4
			SD	1.0	1.1
10.0	A	98.1	97.1	97.6	97.4
	B	97.3	96.4	96.9	96.6
	C	98.2	98.2	98.2	98.1
			MEAN	97.6	97.4
			SD	0.7	0.7
12.0	A	100.0	99.0	99.5	99.5
	B	100.0	100.0	100.0	100.0
	C	100.0	99.1	99.6	99.5
			MEAN	99.7	99.7
			SD	0.3	0.3

Experiment#: B (26) (cont.) Metal: Pb

EDTA Conc.: 6.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	9.3	9.8	9.6	
	B	7.0	6.5	6.8	
	C	6.8	5.6	6.2	
	D	5.6	7.1	6.4	
	E	4.2	5.0	4.6	
	F	6.1	6.3	6.2	
			MEAN	6.6	
			SD	1.6	
4.0	A	9.9	7.8	8.9	2.4
	B	9.2	7.5	8.4	1.9
	C	7.5	8.4	8.0	1.4
			MEAN	8.4	1.9
			SD	0.5	0.5
6.0	A	15.2	13.5	14.4	8.3
	B	15.2	14.9	15.1	9.0
	C	16.8	16.0	16.4	10.5
			MEAN	15.3	9.3
			SD	1.0	1.1
8.0	A	22.4	19.8	21.1	15.5
	B	20.4	19.8	20.1	14.4
	C	21.1	21.9	21.5	15.9
			MEAN	20.9	15.3
			SD	0.7	0.8
10.0	A	81.1	81.2	81.2	79.8
	B	79.0	78.0	78.5	77.0
	C	81.6	79.6	80.6	79.2
			MEAN	80.1	78.7
			SD	1.4	1.5
12.0	A	100.0	100.0	100.0	100.0
	B	99.2	100.0	99.6	99.6
	C	100.0	100.0	100.0	100.0
			MEAN	99.9	99.9
			SD	0.2	0.2

EDTA Conc.: 7.0 µM

Metal Conc. (µM)		% Abnormal Duplicates		Mean	% Net Risk
Ctr	A	9.3	9.8	9.6	
	B	7.0	6.5	6.8	
	C	6.8	5.6	6.2	
	D	5.6	7.1	6.4	
	E	4.2	5.0	4.6	
	F	6.1	6.3	6.2	
			MEAN	6.6	
			SD	1.6	
4.0	A	4.8	4.3	4.6	0.0
	B	5.9	5.2	5.6	0.0
	C	4.7	4.5	4.6	0.0
			MEAN	4.9	0.0
			SD	0.6	0.0
6.0	A	7.6	8.0	7.8	1.3
	B	5.6	5.1	5.4	0.0
	C	6.2	6.5	6.4	0.0
			MEAN	6.5	0.0
			SD	1.2	0.0
8.0	A	12.1	7.8	10.0	3.6
	B	7.6	7.8	7.7	1.2
	C	5.9	6.3	6.1	0.0
			MEAN	7.9	1.4
			SD	1.9	2.1
10.0	A	70.7	71.2	71.0	68.9
	B	66.9	68.0	67.5	65.1
	C	73.8	75.2	74.5	72.7
			MEAN	71.0	68.9
			SD	3.5	3.8
12.0	A	99.0	100.0	99.5	99.5
	B	100.0	99.1	99.6	99.5
	C	100.0	100.0	100.0	100.0
			MEAN	99.7	99.7
			SD	0.3	0.3

APPENDIX V

Algal Assay Results

Experiment#: A (35)
Metal: Cu

Metal Conc. (nM)		EDTA Conc. 0 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4	EDTA Conc. 50 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
Control	MEAN	2,067	10,323	77,906	236,880	479,319					
	SD	397	364	13,310	16,189	5,384					
25	MEAN	2,150	10,608	59,895	211,735	348,700	1,795	10,905	99,040	237,942	452,337
	SD	263	188	4,617	8,972	6,151	85	131	15,233	21,682	23,945
50	MEAN	2,037	9,097	22,997	49,688	84,537	1,907	10,673	98,367	234,545	449,343
	SD	86	265	2,805	6,068	3,227	45	1,478	16,410	37,096	14,599
100	MEAN	1,873	7,810	14,415	19,382	26,943	1,783	8,008	24,052	60,748	116,027
	SD	28	379	409	2,162	3,495	164	1,141	3,687	3,508	3,825
150	MEAN	1,712	6,127	11,770	13,330	13,420	1,727	6,723	11,775	16,737	18,480
	SD	16	551	1,034	728	683	43	1,190	442	285	626
200	MEAN	1,680	4,183	8,602	9,127	8,273	1,828	6,623	10,418	10,947	11,137
	SD	88	542	1,013	708	406	99	1,185	2,439	2,641	1,966
		EDTA Conc. 100 nM					EDTA Conc. 150 nM				
25	MEAN	1,960	10,850	83,512	225,565	457,548	1,907	11,968	86,035	225,388	447,497
	SD	36	797	22,946	18,838	2,455	84	1,106	4,256	5,745	9,201
50	MEAN	1,917	11,213	84,572	229,782	459,980	1,892	12,607	93,760	217,762	431,415
	SD	153	590	3,586	15,286	1,418	83	415	16,463	19,165	28,635
100	MEAN	1,808	12,477	92,143	233,063	442,000	1,940	11,123	85,853	215,732	452,633
	SD	60	343	9,878	12,213	14,720	69	976	9,850	11,953	6,748
150	MEAN	1,837	10,092	29,518	53,575	86,042	1,947	13,133	95,103	230,737	449,328
	SD	36	1,261	1,167	907	449	93	1,446	17,057	1,822	1,863
200	MEAN	1,862	7,577	14,403	19,388	24,767	1,885	12,060	24,802	49,203	99,868
	SD	24	1,012	1,855	1,968	1,595	48	948	461	3,561	2,604

Experiment#: B (39)
Metal: Cu

Metal Conc. (nM)		EDTA Conc. 0 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4	EDTA Conc. 20 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
Control	MEAN	1,597	10,436	80,068	241,083	468,765					
	SD	133	726	1,240	9,943	17,715					
20	MEAN	1,688	10,300	77,108	219,330	403,623	1,790	9,475	80,318	234,055	471,765
	SD	106	1,082	3,465	5,825	25,970	135	883	3,054	1,183	23,669
40	MEAN	1,683	9,960	76,012	137,522	216,437	1,703	9,158	79,587	200,267	453,655
	SD	85	987	1,430	2,790	8,106	29	474	1,679	13,641	17,032
60	MEAN	1,665	9,273	22,478	143,312	160,865	1,683	8,408	73,355	151,267	255,405
	SD	60	585	2,026	6,354	2,352	43	1,282	3,815	1,741	9,362
80	MEAN	1,765	10,157	16,515	46,345	81,835	1,723	8,730	22,377	140,413	163,900
	SD	75	1,018	822	2,637	665	15	893	313	5,223	1,481
100	MEAN	1,742	7,958	14,075	19,127	22,067	1,602	8,195	17,635	40,912	87,472
	SD	37	487	599	558	1,521	28	84	942	2,088	1,805

Metal Conc. (nM)		EDTA Conc. 40 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4	EDTA Conc. 60 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
20	MEAN	1,677	9,427	83,418	245,017	447,030	1,762	9,208	80,473	223,283	470,792
	SD	108	1,717	4,706	1,398	21,381	134	958	276	16,504	19,975
40	MEAN	1,778	10,360	77,508	231,660	464,250	1,680	9,143	80,380	233,887	427,067
	SD	158	793	2,361	13,453	21,268	61	1,009	1,316	2,212	24,178
60	MEAN	1,645	9,972	79,798	186,985	441,302	1,667	7,855	74,747	211,328	455,253
	SD	66	1,625	925	5,039	13,252	63	197	1,421	4,437	22,888
80	MEAN	1,692	10,192	68,375	141,837	240,928	1,708	8,305	69,903	176,232	390,423
	SD	60	722	1,715	3,370	986	179	850	233	4,690	26,106
100	MEAN	1,660	8,863	21,888	126,775	166,582	1,838	9,677	67,245	129,248	233,570
	SD	179	474	593	1,563	3,309	137	375	1,208	2,672	16,766

Experiment#: A (36)
Metal: Cd

EDTA Conc. 0 nM

Metal Conc. (nM)		Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
Control	MEAN	2,443	7,890	38,593	188,121	455,708
	SD	332	4,430	3,988	7,156	28,154
150	MEAN	2,075	5,365	44,975	178,937	387,102
	SD	169	417	3,855	938	12,788
300	MEAN	2,098	5,942	33,682	134,997	257,650
	SD	207	711	1,549	3,111	8,947
450	MEAN	2,422	5,558	28,053	109,788	181,542
	SD	103	215	1,461	1,488	837
600	MEAN	2,228	6,322	22,133	90,128	104,157
	SD	78	753	701	807	3,329
750	MEAN	2,318	4,900	19,307	28,597	31,232
	SD	35	305	630	2,141	1,398

EDTA Conc. 500 nM

Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
2,345	5,145	44,878	180,480	365,255
463	460	542	262	6,636
2,207	4,450	42,042	181,525	274,970
101	195	999	572	8,810
2,128	4,680	37,877	140,502	243,460
203	292	77	550	3,143
2,120	4,880	30,032	98,852	144,847
110	745	224	2,477	2,332
2,078	4,128	19,683	32,213	100,363
78	454	702	3,897	3,781

EDTA Conc. 1000 nM

150	MEAN	2,217	5,208	41,427	180,887	383,780
	SD	488	345	822	4,955	5,667
300	MEAN	2,263	5,713	40,492	180,590	362,492
	SD	128	323	1,004	487	2,197
450	MEAN	1,980	5,317	40,762	177,343	323,573
	SD	36	374	730	1,215	9,741
600	MEAN	1,983	5,480	34,178	133,275	239,450
	SD	121	218	155	2,805	2,883
750	MEAN	2,180	5,927	22,297	73,368	189,542
	SD	208	400	786	1,194	1,217

EDTA Conc. 1500 nM

1,962	6,175	44,547	180,683	357,267
173	48	326	3,771	8,896
2,618	6,373	40,535	179,792	371,108
368	578	2,659	1,253	6,754
2,202	6,522	41,230	180,273	372,123
171	326	1,660	1,297	3,167
2,100	6,268	42,485	170,185	329,925
33	134	352	2,505	13,612
2,122	6,087	31,887	91,768	244,372
172	355	116	137	9,356

Experiment#: A (38)
Metal: Pb

Metal Conc. (nM)		Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
EDTA Conc. 0 nM						
Control	MEAN	1,809	10,323	72,145	297,198	446,949
	SD	128	565	3,812	12,281	9,420
25	MEAN	1,765	5,675	69,105	304,237	384,535
	SD	133	1,903	4,407	7,968	13,037
50	MEAN	1,647	7,373	70,230	169,842	241,523
	SD	81	464	1,298	3,470	6,907
75	MEAN	1,768	5,442	32,480	43,188	84,203
	SD	45	399	1,083	2,574	2,233
100	MEAN	1,868	5,320	17,270	44,007	48,213
	SD	147	1,306	1,025	594	1,666
125	MEAN	1,683	3,620	14,553	41,850	42,978
	SD	100	88	413	204	467
EDTA Conc. 25 nM						
25	MEAN	1,812	7,035	68,875	307,122	440,673
	SD	140	983	2,089	5,513	10,628
50	MEAN	1,825	5,272	67,453	120,902	306,918
	SD	214	649	4,628	5,552	1,560
75	MEAN	1,782	4,353	44,492	83,292	139,067
	SD	83	1,184	1,213	960	3,608
100	MEAN	1,657	4,268	38,895	103,562	102,872
	SD	77	577	1,674	1,992	3,595
125	MEAN	1,653	3,548	15,603	46,778	47,735
	SD	81	501	1,322	2,665	2,112
EDTA Conc. 50 nM						
25	MEAN	1,710	5,522	93,748	261,688	441,663
	SD	87	1,579	6,017	19,030	12,767
50	MEAN	1,785	4,697	88,853	219,313	433,730
	SD	39	464	3,508	4,111	18,781
75	MEAN	1,708	3,475	72,408	114,245	301,878
	SD	114	198	1,725	6,335	3,941
100	MEAN	1,757	4,885	57,133	84,785	141,148
	SD	24	350	2,782	1,214	2,485
125	MEAN	1,687	3,587	27,227	41,695	45,047
	SD	160	661	1,224	1,387	3,919
EDTA Conc. 75 nM						
25	MEAN	1,705	5,520	115,912	209,585	429,725
	SD	51	306	4,281	14,532	13,657
50	MEAN	1,712	5,963	110,805	210,865	426,938
	SD	98	780	12,096	3,598	8,867
75	MEAN	1,985	5,285	94,693	214,947	420,833
	SD	88	193	4,008	22,617	26,103
100	MEAN	1,767	5,413	77,263	160,693	266,248
	SD	83	646	1,662	560	19,995
125	MEAN	1,672	5,082	29,620	93,463	147,045
	SD	80	26	1,151	3,123	3,340

Experiment#: B (41)
Metal: Pb

Metal Conc. (nM)		EDTA Conc. 0 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4	EDTA Conc. 75 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
Control	MEAN	2,058	11,453	105,504	309,926	445,712					
	SD	171	2,174	10,034	20,071	17,591					
50	MEAN	2,320	9,788	53,208	225,648	298,518	2,037	11,458	105,423	239,690	390,150
	SD	95	770	3,433	8,344	18,858	98	612	7,148	9,989	9,183
100	MEAN	2,017	8,880	40,188	151,703	261,992	1,895	11,157	58,697	143,975	240,843
	SD	61	850	472	9,311	6,504	92	553	2,986	2,481	51
150	MEAN	1,957	8,992	27,510	77,445	138,492	1,913	11,292	38,477	100,475	157,042
	SD	78	990	1,135	5,336	2,581	88	1,086	3,103	4,183	5,753
200	MEAN	2,067	9,005	23,362	44,692	65,675	1,928	9,592	26,492	40,227	73,537
	SD	143	319	573	5,293	2,795	99	771	1,418	2,241	710
250	MEAN	1,983	4,973	11,175	12,130	24,250	1,902	6,875	20,487	26,222	33,245
	SD	146	645	698	322	1,778	154	446	1,684	2,058	520

Metal Conc. (nM)		EDTA Conc. 150 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4	EDTA Conc. 200 nM Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
50	MEAN	1,948	12,712	103,448	299,033	454,267	1,875	10,198	110,762	306,618	456,422
	SD	49	331	1,142	4,521	10,139	71	992	1,287	4,821	13,544
100	MEAN	1,948	11,367	106,518	252,097	402,693	2,062	11,595	107,228	321,913	453,182
	SD	188	799	3,036	8,286	18,697	49	1,411	2,784	8,163	5,535
150	MEAN	1,978	11,765	67,270	148,558	301,307	1,982	10,920	100,103	287,435	382,957
	SD	84	553	2,730	1,068	14,027	95	1,424	3,421	4,046	9,710
200	MEAN	1,963	9,877	51,198	122,462	210,108	1,932	11,903	67,450	181,365	300,913
	SD	33	843	4,428	1,062	9,974	76	812	2,222	5,361	7,857
250	MEAN	2,030	9,428	32,198	93,023	180,660	1,967	12,227	46,348	140,357	179,925
	SD	116	1,171	1,727	1,677	2,995	51	1,015	2,765	1,701	1,317

Experiment#: B (40)
Metal: Zn

EDTA Conc. 0 nM

Metal Conc. (nM)		Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
Control	MEAN	1,917	7,873	44,944	199,369	382,700
	SD	118	1,290	7,660	14,322	12,380
500	MEAN	1,803	8,412	21,618	41,510	74,747
	SD	153	805	3,058	7,930	42,518
1000	MEAN	1,865	8,038	20,853	31,473	36,947
	SD	216	2,045	4,803	8,570	9,941
1500	MEAN	1,817	6,910	13,300	20,858	27,718
	SD	78	686	966	4,431	3,194
2000	MEAN	1,883	7,862	15,227	22,798	28,802
	SD	72	1,215	1,011	2,214	3,308
2500	MEAN	1,768	6,252	14,325	20,958	24,648
	SD	257	952	664	1,107	2,206

EDTA Conc. 500 nM

Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
1,907	8,903	52,915	254,308	512,645
156	1,418	2,507	4,650	9,114
1,765	7,942	32,933	76,368	214,262
87	667	1,322	5,247	19,568
1,715	7,308	19,730	37,297	48,400
61	803	576	834	1,355
1,907	6,617	15,297	24,185	28,602
88	537	175	436	1,069
1,828	7,115	14,717	25,547	29,920
112	959	1,272	3,699	3,306

EDTA Conc. 1000 nM

500	MEAN	1,787	7,553	50,860	292,105	558,082
	SD	159	1,401	377	61,727	141,547
1000	MEAN	1,962	8,753	44,645	286,408	557,100
	SD	191	1,132	4,575	74,238	150,967
1500	MEAN	1,813	8,732	30,137	85,675	189,597
	SD	182	863	417	4,973	7,562
2000	MEAN	1,745	7,948	20,015	34,088	49,637
	SD	73	174	770	2,131	2,694
2500	MEAN	1,790	8,355	19,418	27,348	37,588
	SD	13	129	870	969	477

EDTA Conc. 1500 nM

1,943	10,133	53,968	271,862	438,953
188	1,841	15,526	58,926	19,418
1,745	10,335	68,037	306,697	475,280
33	1,180	4,219	18,754	9,810
1,803	8,125	55,638	306,210	479,847
33	530	4,548	6,879	7,615
1,792	8,992	20,813	36,745	123,123
45	765	1,922	6,463	15,038
1,825	8,247	18,948	29,148	42,822
175	1,551	698	1,447	3,826

Experiment#: A (42)
Metal: Zn

EDTA Conc. 0 nM

Metal Conc. (nM)		Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
Control	MEAN	1,912	12,369	72,422	308,455	602,098
	SD	107	1,741	6,469	15,681	25,919
500	MEAN	1,803	11,770	36,522	68,697	79,447
	SD	105	320	1,455	3,422	3,500
1000	MEAN	1,868	9,490	16,193	23,020	46,483
	SD	98	983	525	1,113	1,235
1500	MEAN	1,865	9,158	14,733	20,268	34,983
	SD	50	505	118	1,328	757
2000	MEAN	1,875	9,272	12,497	17,095	30,450
	SD	72	762	950	487	844
2500	MEAN	1,738	7,662	11,477	13,617	26,110
	SD	94	288	765	1,310	786

EDTA Conc. 750 nM

Mean Cell Yield (cells/mL) Day 0	Day 1	Day 2	Day 3	Day 4
1,862	11,320	79,792	304,902	605,723
10	546	3,855	12,799	10,082
1,837	11,987	34,580	52,172	300,153
200	159	2,223	2,074	410
1,923	9,638	21,037	35,270	79,475
117	823	1,277	710	2,692
1,997	9,503	17,398	23,277	33,768
166	1,270	1,280	1,510	941
1,720	10,230	15,273	17,545	27,067
65	354	341	330	1,358

EDTA Conc. 1500 nM

500	MEAN	1,758	11,658	79,527	309,785	600,937
	SD	24	1,275	5,766	15,752	17,872
1000	MEAN	1,880	10,413	70,425	300,703	606,518
	SD	79	176	1,621	1,194	14,829
1500	MEAN	1,823	9,830	66,000	306,845	502,063
	SD	81	1,055	2,432	8,232	2,905
2000	MEAN	1,922	10,130	20,732	33,915	117,910
	SD	75	1,539	874	2,469	11,548
2500	MEAN	1,915	11,070	16,108	26,087	50,405
	SD	39	910	625	1,351	1,504

EDTA Conc. 2000 nM

1,913	10,747	79,007	315,342	602,020
58	399	2,694	13,311	16,670
1,937	10,775	76,798	312,022	609,442
118	699	2,240	17,759	13,690
1,858	12,263	80,597	321,880	606,710
78	968	1,482	6,887	16,261
1,783	10,820	66,543	213,873	319,487
103	419	1,176	9,024	7,815
2,042	11,493	26,245	58,553	80,065
37	875	854	2,590	1,974