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The shipboard determination of zinc in seawater using flow injection analysis with fluorometric detection

Jocelyn Lindsey Nowicki
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injection analysis with fluorometric detection**

Nowicki, Jocelyn Lindsey, M.S.

San Jose State University, 1992

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THE SHIPBOARD DETERMINATION OF ZINC IN SEAWATER
USING FLOW INJECTION ANALYSIS WITH FLUOROMETRIC DETECTION

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Marine Sciences

By

Jocelyn Lindsey Nowicki

December, 1992

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ABSTRACT

The Shipboard Determination of Zinc in Seawater Using Flow Injection Analysis with Fluorometric Detection

by Jocelyn Lindsey Nowicki

A sensitive technique for the shipboard determination of zinc in seawater has been developed. The technique couples flow injection analysis with fluorometric detection (FIA-FL). A cation exchange column is used to separate Zn from interfering alkaline earth metals and to concentrate Zn from pH 4.5-5.0 seawater. The organic indicator ligand, p-tosyl-8-aminoquinoline (pTAQ), is used to form a fluorescent complex with Zn. The detection limit is 0.12 nM for a 4.4 mL sample. A relative error of 6.4% was obtained for replicate analyses. A single sample can be analyzed in 6 minutes. The technique was determined to be accurate based on the analysis of standard seawater solutions CASS-2 and NASS-2 and by comparison with previous reliable investigations. Shipboard analyses performed at several stations in the equatorial Pacific show high linear correlation of Zn to reactive silica and have proved oceanographically consistent. Zinc profiles reported here support the present understanding of Zn biogeochemical cycling in seawater. Box model calculations indicate a surface water residence time for Zn in the equatorial Pacific of around 9 days and about 1 year in the central North Pacific.

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PREFACE

This thesis presents a novel method to measure zinc in seawater together with results from its shipboard application. It begins with an introduction to zinc biogeochemistry as it pertains to the marine environment and this study (Chapter 1). A description of the laboratory development of the method to measure zinc in seawater follows (Chapter 2). The thesis next presents results of the shipboard application of the method and a comparison to previous investigations of zinc in seawater (Chapter 3). Chapters 2 and 3 will be, or are being, submitted to refereed journals of our trade.

The format of each chapter reflects that required by the journal to which it is being submitted. Chapter 2, submitted to *Analytical Chemistry*, presents the development of the method to measure zinc in seawater and gives detailed information on laboratory procedures and test results. Brief results of the field application of the method are included in support of its accuracy and applicability. Chapter 3 is a full report of the shipboard application of the method. Results are interpreted with respect to measured oceanographic properties and constituents, and current theories of zinc biogeochemical cycling. This chapter will be submitted to *Marine Chemistry*.

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CHAPTER 1

Introduction

Background

A revolution in seawater trace metal chemistry has occurred since the mid 1970s. During this time, the first reliable analyses of dissolved trace metals in seawater were performed. Accurate analyses became possible due to identification of contamination sources, advances in clean sample collection, handling, storage and analysis, and increased sensitivities of the analytical methods themselves. These advances have resulted in studies which describe the concentrations and distributions of picomolar to nanomolar quantities of metals in seawater. These studies have been reviewed by Bruland (1983) and Burton and Statham (1990). Zinc has only been measured accurately in the open ocean by a few investigators (Bruland et al. 1978; Bruland 1980; Danielsson 1980; Bruland and Franks 1983; Danielsson and Westerlund 1983; Magnusson and Westerlund 1983) because of its low concentrations in seawater (<0.1 nM at the surface) and the ease with which a sample can become contaminated. The uncertainty of all zinc data prior to these investigations, and the paucity of reliable data since, has left us little information to unravel the biogeochemical cycle of zinc in the marine environment.

It is essential to evaluate whether or not new trace metal data accurately represent water column concentrations. Bruland et al. (1979) described their methods for collection, storage, handling and analysis of trace metal clean seawater samples. Extreme precautions must be taken throughout the process

of collection and analysis to insure contaminant free seawater samples. This is especially true for zinc. It is not enough to claim meticulously clean procedures or extremely low values. Criteria used to evaluate the validity of metal measurements have been set forth by Boyle (1977) and are exemplified by Bruland (1980). These criteria include the need for interlaboratory agreement and oceanographically consistent data. To be considered oceanographically consistent, profiles must show smooth variations related to various chemical and physical parameters determined by conventional means. Regional variations should coincide with known large-scale physical and chemical circulation patterns. These criteria must be addressed when presenting new data or analytical methods.

The widespread use of zinc in industry, especially marine oriented industries, along with its demand by biological systems and the potential for environmental contamination, requires more be known about the distribution and behavior of zinc in the marine environment. Zinc is a major component of galvanized metal, paint, rubber and is the sole material in sacrificial anodes, which are used extensively on ships and marine instrumentation. Common laboratory materials can contain high levels of zinc including glass, plastics, rubber, and filter paper (Cammarota 1980). The ubiquity of sources of contaminant zinc, combined with extremely low concentrations in seawater, makes zinc, like lead and iron, one of the most difficult metals to measure accurately in seawater (Martin et al. 1980).

Dissolved zinc concentrations have been determined in seawater by

preconcentration using organic extraction (such as a liquid-liquid extraction using dithiocarbamates, dithizone or co-precipitation with Co dithiocarbamate), or binding metals to immobilized chelating resins such as Chelex-100 followed by graphite furnace atomic absorption spectrometry (Bruland 1980; Sturgeon et al. 1980; Bruland and Franks 1983) or isotope dilution mass spectrometry (Mykytiuk et al. 1980). These methods necessarily involve much time and utilize delicate, expensive instruments which need trained technicians in ultra-clean laboratory facilities for their operation. The expense and amount of handling and manipulation required by traditional methods, make the determination of zinc in seawater a formidable task. Conditions at sea can be extreme and limiting, and such things as sea state, vibrations, and power requirements can affect the feasibility of shipboard analyses. These factors virtually eliminate traditional methods for use aboard ship. An increased understanding of the behavior of zinc in marine systems now requires analytical methods that are rapid, inexpensive, less prone to contamination, more sensitive and (optimally) can be performed at sea. The goal of this study was to develop a system to determine zinc that satisfies these requirements and that provides accurate and oceanographically consistent data.

Biogeochemistry of Zinc

The reliable studies of zinc in seawater have shown that the concentration of zinc in the Northeast Pacific ocean ranges from about 0.1 nM in surface waters to approximately 3 nM at 500 meters, increasing steadily to reach 9 nM at depths of two or more kilometers (Bruland et al. 1978; Bruland 1980). Coastal levels of zinc can be up to 40 times higher in surface waters due to fluvial runoff and anthropogenic inputs to coastal waters and air masses (Bruland and Franks 1983). The main inputs of zinc to the world ocean include atmospheric fallout (natural and anthropogenic sources) and fluvial runoff (Chester and Murphy 1988). Hydrothermal vents have been shown to introduce substantial amounts of zinc to the ocean at mid-depths. However, most is precipitated as sulfur compounds upon mixing with seawater either in the vent or near the sea floor (Von Damm et al. 1985).

The reactivity of a metal in the water column influences how long it will spend there before being removed to the sediments. The average time that an element remains in the ocean is known as the residence time. In a steady state system this is defined by the average concentration in seawater divided by the input rate (or removal rate). Shiller and Boyle (1985) in an extensive study of dissolved zinc in rivers, calculated an oceanic residence time of about 50,000 years. This estimate must be lowered somewhat because of the recent direct measurement

and recognized importance of atmospheric input of zinc to the open ocean not accounted for in their study (Arimoto et al. 1985, 1987; Chester and Murphy 1988). These findings indicate that on a basin wide scale, atmospheric input is about three times the river input (Bruland 1980). Bruland (1980) calculated a minimum oceanic residence time of 5000 years for zinc which is long with respect to the ocean mixing cycle. Collier and Edmond (1984) arrive at a oceanic residence time of 10,000 years for zinc.

The oceanographically consistent open ocean profiles reveal a nutrient-like behavior of zinc in seawater evidenced by surface depletion with enrichment at depth. Zinc concentrations are linearly related to dissolved silicic acid, (H_2SiO_4) the probable main species of Si in oxygenated seawater (Bruland 1980; Bruland 1983; Bruland and Franks 1983). Similarly, other metals have been shown to have distributions similar to nutrients. Such element/nutrient pairs include cadmium and phosphate, barium and silicate, and iron and nitrate. Although these correlations suggest that these metals are involved in biogeochemical cycles similar to those of the major nutrients, the exact removal mechanisms are not known.

Our present understanding of the biogeochemical cycling of Zn is that its distribution is controlled by phytoplankton uptake in the surface waters and regeneration at depth (Bruland et al. 1991). The influence of biological interactions is evidenced by depletion of zinc in surface waters of both oceans to the same degree, about 0.1 nM (Bruland and Franks 1983; Sunda and Huntsman 1992).

Zinc appears to be carried to depth in "biological fallout" in association with particulate organic matter and is regenerated at depth by oxidation of organic material and remineralization of inorganic particles. The regeneration depth of zinc closely mimics that of silica. Zinc, like silica, also exhibits a fractionation between ocean basins, the Atlantic concentrations being about 1/5 those at similar depths in the Pacific (Bruland and Franks 1983). The Atlantic concentrations, however, are twice the levels that the nutrient-metal relationship in the Pacific would predict. That is, the deep Pacific:deep Atlantic enrichment factor is different, 10:1 for silica and only 5:1 for zinc (Bruland 1983). This fractionation represents internal cycling of zinc superimposed upon the deep circulation patterns of the entire ocean. The difference between deep water values of the Atlantic and Pacific represents an accumulation of zinc regenerated in the progressively older waters moving toward the Northeast Pacific. The covariation of zinc with silica could result from the incorporation of zinc in the silicious matrices of diatom and radiolarian tests. Although the role of zinc in biological systems is well known, the specific biological role in controlling zinc distributions in seawater has not been definitively resolved. The role of zinc in regulating biological processes is, however, better known.

Zinc is a required trace element in all living organisms and therefore a necessary nutrient for marine biological processes. It is an important cofactor involved in over 100 different enzyme systems (e.g., carbonic anhydrase) (Lehninger 1982) and therefore potentially limiting to phytoplankton growth in

seawater. It is difficult, if not impossible, to discuss algal trace metal requirements without mention of speciation or complexation. The metal available to phytoplankton in seawater is controlled by the activity (proportional to concentration) of the free ion or "aquo-ion", rather than the total concentration (Anderson et al. 1978). Therefore, the degree of complexation of zinc and the various complexes it forms are critical to phytoplankton success in the ocean. Van den Berg (1985) showed, using organic ligand competition followed by cathodic stripping voltammetric detection, that natural zinc-organic complexes are the dominant dissolved zinc species in the Irish Sea. Bruland (1989) showed that greater than 98% of dissolved zinc in the upper 500 meters of North Pacific open ocean surface waters is strongly complexed by a zinc-selective organic ligand. So while dissolved zinc varies from 0.1 nM in surface waters to about 3 nM at 500 meters, complexation reduces the inorganic Zn species to ~2 pM and the free $[Zn^{2+}]$ to as low as 1 pM.

Anderson et al. (1978) showed that low free zinc ion activity could limit coastal diatom growth. Likewise, Brand et al. (1983) demonstrated that neritic species of phytoplankton were growth limited by open ocean levels of free zinc ion activity while open ocean species were either not limited or only slightly limited by the lowest attainable ion activities. This demonstrates that zinc requirements can play a role in phytoplankton community composition and distribution and possibly in the selective forces effecting evolutionary patterns. Therefore, any shifts in available

zinc, due to changes in ligand or total zinc concentrations, could affect community structure. An accurate assessment of available zinc would aid in determining if an area, coastal or oceanic, has been substantially altered due to pollution and what possible effects it has had or could have on the algal community there. Similarly, any serious depletion of free zinc ion activity has the potential to limit the growth of algae.

An assessment of how much zinc is available as free ions is only the beginning of what is being discovered as a complex interaction among phytoplankton, macronutrients and essential trace metals. Morel et al. (1991) estimate that Zn, like Fe, may limit phytoplankton productivity under certain circumstances. Morel and Hudson in 1985 hypothesized that various metals and macronutrients may act in concert to limit, and therefore control, biological production in the sea. Bruland et al. (1991) presented a combination of field data and laboratory studies to address the potential synergistic and antagonistic behavior of biolimiting metals on phytoplankton productivity. Some laboratory studies give indications that the ratios of free ion activities may be just as important as the absolute free ion concentrations in their effect on growth rates (Cu:Zn, Reuter and Morel 1981; Mn:Zn, Sunda 1986).

Sunda and Huntsman (1992) investigated the uptake kinetics of zinc and its effect on growth of neritic and oceanic phytoplankton which previously had shown differences in zinc requirements (Anderson et al. 1978; Brand et al. 1983). They

performed radiotracer experiments to examine the nature of these differences. Specifically, they wanted to determine if the differences in zinc requirements were due to intracellular transport capabilities or to differences in growth requirements. Results of their study showed that oceanic species had a lower cellular growth requirement for zinc and not an increased ability to take up zinc intracellularly. The reason for this reduced need is not known. They concluded that Zn can be an important factor controlling phytoplankton species composition, but at the same time, $[Zn^{2+}]$ ion activity is itself regulated by algal uptake and regeneration in surface waters.

Coale and Bruland (1990) have shown that free cupric ion activity, $\{Cu^{2+}\}$, appears to be under the control of organic ligands produced by phytoplankton. Ligand production appears to be inducible and regulated, controlling $\{Cu^{2+}\}$ at 10^{-14} M, a physiological optimum. This sort of ligand control has not been demonstrated for zinc, but is a possibility. The complex interactions and influences of metals and nutrients on phytoplankton are only beginning to be studied. Advances in analytical capabilities that can be applied in the field and to natural algal populations are needed if this web of interactions is to be understood. One promising technique for metal analysis is through measurement of the fluorescence of a Zn-ligand complex.

Fluorescence

Fluorescence spectroscopy is a powerful analytical tool with many applications in the areas of DNA and RNA determinations, protein-ligand interactions, fluoro-immunoassays and fiber-optic-based sensing (Bright 1988). Initial observations of fluorescence lead back to the Spanish botanist and physician Nicolas Monardes in 1565 (Bright 1988). He noticed that water held in a cup of a specific wood (*Ligirium nephiticiem*) exhibited a strange blue shimmer. George Stokes was the first to coin the phrase "fluorescence" and to propose its use as an analytical tool around 1852. Stokes is also credited with the observation that emission wavelengths are longer than excitation wavelengths, a phenomenon referred to as "Stoke's shift".

Fluorescence involves absorption of discrete quanta of electromagnetic radiation of a certain wavelength by a molecular species. Absorption of energy and promotion of electrons to a higher electronic state occurs if the absorbed photon energy is the same as the energy difference between two allowed energy states. A molecule will rapidly lose this photon-induced energy and return to the stable ground state. Relaxation generally occurs via the fastest pathway, which for most species is through molecular vibrational and rotational modes of decay and collisions with solvent molecules. In some cases (usually involving rigid species with conjugated double bonds) the decay through vibration and rotation is slowed,

and the excited molecule releases energy as photons. Depending on the lag time between excitation and emission, the decay will occur as phosphorescence or fluorescence. All molecules absorb energy, but only a few fluoresce, and even fewer phosphoresce, at ambient temperatures. Fluorescence lifetimes are on the order of 10^{-9} - 10^{-7} seconds while phosphorescence lifetimes are longer, ranging from milliseconds to 10 seconds (Lakowicz, 1983; Christian and O'Reilly, 1986).

Fluorescence is a very sensitive and selective technique for quantifying molecular species compared to absorption techniques. Because the excitation and emission do not occur at the same wavelength, fluorescence is essentially a zero background technique. Absorbance, however, is a measure of the difference between incident and transmitted energy, which are both large signals. Fluorescence, therefore, is in general a technique of much higher sensitivity. Selectivity in fluorescence arises from the relatively low number of fluorescent species and the selection (by filters or diffraction gratings) of both excitation and emission wavelengths particular to the analyte of interest.

Molecular structure is one factor that will determine if a substance will fluoresce. Fluorescence is enhanced in molecules that possess rigid structures, such as those with conjugated double bonds (Skoog 1985). Complexation of a ligand with a metal ion reduces the vibrational and rotational mobility of the ligand, thereby increasing the potential for electron decay to the ground state through emission of photons. Radiationless deactivation of the unbound ligand is more

likely than when it is complexed by a metal. This creates a high signal to noise ratio for analytical determinations using fluorescence as an indicator of analyte concentration.

The fluorescent ligand used in this study, p-tosyl-8-aminoquinoline (Figure 1, Chapter 2) was first described by Boshewolnow (1965). It was shown to be a promising tool by Ditzler (1983) and Seitz et al. (1985) as a silica gel immobilized fluorogenic ligand and has recently been used on fiber-optic-based sensors for Zn and Cd in aqueous solutions (Lieberman et al. 1987, 1989). The difficulty in utilizing this ligand for metals in seawater with a fiber-optic system is the inability to separate the signal due to the fluorescence of the Zn complex from the interference due to the alkaline earth metal complexes of pTAQ. Application of this chemistry to a flow injection system allowed for the separation of the major salts from seawater.

Flow Injection Analysis

Flow injection analysis (FIA) is a technique based on the automation of sample handling procedures and detection schemes. The principles and applications of FIA have been reviewed extensively (Betteridge 1978, Ruzicka and Hansen 1978, 1981). Computer controlled valves and a peristaltic pump allow sample to be automatically injected into a moving, unsegmented reagent stream. Chemical reactions take place inside small diameter tubing (<1 mm i.d.) which is plumbed

directly to a detector. Signal detection schemes are specific for the chemistry involved and include chemiluminescence, molecular and atomic absorbance, and fluorescence. Flow injection analysis has recently been successfully applied to the shipboard determination of NO_3 (Johnson and Petty 1983), Co (Sakamoto-Arnold and Johnson 1987), Fe (Elrod et al. 1991), Mn (Chapin et al. 1991), and Cu (Coale et al. 1992). These methods combined FIA with chemiluminescence or an absorbance detection scheme. The detection limits of these methods allowed for determination of the analyte at nanomolar to picomolar quantities in seawater. In these studies, flow injection was used to automate laboratory procedures which would previously have to be done manually, thereby greatly reducing the risk of sample contamination. Other advantages include high sample throughput, relatively low cost, and the ability to obtain results aboard ship, allowing for the alteration of sampling regimes if needed.

Compañó et al. (1991) have described a flow injection method for the determination of zinc. Their method was based on the fluorescence of a zinc-5,7-dichloro-2-methylquinoline-8-ol chelate in a Brij 35 medium. This technique was adapted for determination of zinc in pharmaceutical preparations and in tap water, and had a detection limit (50 nmol/L) which is above that necessary for evaluation of zinc in the open ocean. FIA methods utilizing various other detection methods have been described for the determination of zinc (Burguera et al. 1981; Zagatto et al. 1983; Brennan and Svehla 1989). These methods, however, have not been

adapted for the detection of open ocean levels of zinc in a seawater matrix. Flow injection analysis is a technique which can achieve the low detection limits of traditional methods yet can be easily brought into the field.

In this study FIA is used to optimize the chemistry of the pTAQ fluorescence reaction and adapt it for automated analysis of zinc on board ship. This technique was then applied to the study of zinc in seawater, rain water, and pore water and was used on cruises to the equatorial Pacific and the continental margin of the California coast.

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CHAPTER 2

The Determination of Zinc in Seawater Using Flow Injection Analysis with Fluorometric Detection.

Abstract

A sensitive technique for the shipboard determination of zinc in seawater has been developed. The technique couples flow injection analysis with fluorometric detection (FIA-FL). A cation exchange column is used to separate zinc from interfering alkali and alkaline earth metals and to concentrate Zn from pH 4.5-5.0 seawater. The organic indicator ligand, p-tosyl-8-aminoquinoline (pTAQ), is used to form a fluorescent complex with zinc. The detection limit (defined as three times the standard deviation of the blank) is 0.12 nM for a 4.4 mL sample. An average relative error of 6.4% is obtained for replicate analyses. A single sample can be analyzed in 6 minutes. Lower detection limits can be achieved by lengthening the preconcentration step. The technique was determined to be accurate based on the analysis of the standard seawater solutions CASS-2 and NASS-2 and by comparison with previous reliable investigations. A typical profile of 12 samples along with standards and blanks can be completed in triplicate in 5.5 hours.

Introduction

Zinc has only been measured accurately in the open ocean by a few investigators (1-6). Few data are available because of very low Zn concentrations in seawater and the ubiquity of sources of Zn contamination. The uncertainty of all Zn measurements prior to these investigations, and the paucity of reliable data since, leaves little information to unravel the biogeochemical cycle of Zn, or detect waters perturbed by anthropogenic inputs.

Interest in Zn concentrations and distributions stems from its dual role as a required nanonutrient and as a potential toxicant due to its widespread industrial and marine usage (7, 8). Zinc is required as a cofactor in a wide variety of enzymes, including carbonic anhydrases, carboxypeptidases, and dehydrogenases. Modes of zinc toxicity may involve the competitive displacement of other metal cofactors. Indirect toxicity may result from the displaced metals binding to other cellular components (8).

Zinc exists in North Pacific surface water at a total concentration of about 0.1 nM, increasing to 3 nM at 500 meters, and reaching a maximum of about 9 nM at depths greater than 2000 meters (1, 2). Our present understanding of Zn biogeochemical cycling is that Zn is actively incorporated into phytoplankton as an essential nutrient and by passive adsorption in the surface waters and carried to depth in association with particulate organic matter. It is regenerated deep in the

water column in concert with silica and has a long deep water residence time as revealed by its profile and its linear relationship to silica (2). The major inputs of Zn to seawater include atmospheric deposition (both natural and anthropogenic in origin), fluvial runoff and upwelled waters (9).

Dissolved Zn concentrations in seawater have been determined by preconcentration using organic extraction or chelating resins such as Chelex-100 followed by graphite furnace atomic absorption spectrometry (3, 4, 10, 11) or isotope dilution mass spectrometry (12). These procedures must be performed by trained personnel in ultra clean facilities. The time, expense and sensitivity of the instruments and procedures used in these analyses precludes them from being performed at sea. An increased understanding of the behavior of Zn in marine systems now requires analytical methods that are rapid, less expensive, less prone to contamination, more sensitive and that (optimally) can be performed at sea (13). The goal of this study was to develop a flow injection system to determine Zn that satisfies these requirements and provides accurate data.

The organic ligand p-tosyl-8-aminoquinoline (pTAQ) (Figure 1) which forms a fluorescent complex with Zn, was first described by Boshewolnow (14). This ligand has since been utilized in aqueous solutions as part of a fiber optic based sensor system for the determination of Zn (15, 16). Although the pTAQ-Zn complex is highly fluorescent, the ligand also forms weakly fluorescent compounds with the major salts in seawater. The interference from alkaline earth ion complexes with

pTAQ prevents this compound from being used to directly determine Zn in seawater.

This paper describes a flow injection method for the determination of Zn at low nanomolar concentrations in seawater. Flow injection analysis with chemiluminescence detection has previously been successfully adapted to measure Co (17), Fe (18), Mn (19) and Cu (20) in seawater at nanomolar to picomolar levels. With limited handling and high sample throughput, this technique is well suited for trace metal determinations. A FIA system is described here that incorporates an in-line cation exchange column to separate Zn from alkaline earth metals. The column also serves to concentrate Zn from seawater before analysis of the pTAQ-Zn complex. The technique presented here is a novel method for the shipboard determination of Zn in seawater at open ocean levels. It is rapid, relatively inexpensive, capable of operation at sea and produces accurate and precise results.

Methods

Reagents. All reagents were used as received. Reagents were prepared in acid washed polyethylene 1 L volumetric flasks using purified $18\text{ M}\Omega\text{ cm}^{-1}$ water (MQ water) from a Milli-Q® deionization system (Millipore Corp.). All sample and reagent bottles were made of polyethylene or polypropylene and acid washed, stored and handled using trace metal clean techniques (10). Bottles were soaked in Micro® detergent for one week, transferred to 10% HCl for another week, and

thoroughly rinsed with MQ water. The triple bagged sample bottles were filled with 10% HCl for storage, and rinsed three times with seawater before a sample was taken. Specific reagent preparations are as follows:

40 μ M p-Tosyl-8-aminoquinoline (pTAQ). The reagent pTAQ was used to form a fluorescent complex with Zn. A 0.05 M stock solution was made by dissolving 0.291 g of pTAQ (Chemica Inc., Gardena, CA) in 20 mL non-ionic surfactant Triton-X-100 (polyoxyethylene isooctylphenol) (Fisher Scientific). This was stirred for 72 hours to insure complete dissolution. From this stock solution a 40 μ M working reagent was made by adding 800 μ L of 0.05 M pTAQ to 1000 mL of a sodium tetraborate buffer solution.

0.1 M Sodium Tetraborate. The 0.1 M buffer solution was made by dissolving 6.2 g of H_3BO_3 (Fisher Scientific) in MQ water, adding 50 mL of 1 N NaOH (Fisher Scientific) and bringing the mixture up to 1000 mL with MQ water.

0.08 N HCl. The eluent acid carrier was prepared by adding 13.3 mL of 6 N reagent grade HCl (Fisher Scientific) to approximately 500 mL of MQ water, and bringing it up to 1000 mL with MQ water.

Zinc Standards. All Zn standards were stored in 0.01 M HCL which was prepared by adding 167 μ L of 6 N GFS double-distilled HCl (GFS Chemicals) to 100 mL of MQ water. A 100 ppm (183 μ M) stock standard was prepared by diluting 10 mL of 1000 ppm AA standard solution (Fisher Scientific) to 100 mL of 0.01 M HCl. A working standard of 10 μ M was prepared by adding 0.55 mL of the

100 ppm stock standard to a 100 mL volumetric flask and diluting to 100 mL with 0.01 M HCl. Working standards were made daily in acid washed Teflon® bottles.

FIA System. A schematic diagram of the FIA manifold used for the determination of Zn is shown in Figure 2. This system is similar to that described by Sakamoto-Arnold and Johnson (17). Sample and reagent were propelled by a Rainin Rabbit-Plus® eight channel peristaltic pump. Fisher PVC pump tubing, coded white/white (0.7 mm i.d.) was used for the sample and eluent acid carrier. Fisher PVC tubing, coded blue/yellow (1.52 mm i.d.) was used for the reagent/buffer solution. A pump setting of 15 rpm, resulted in a flow rate of 2.3 mL min⁻¹ for the blue/yellow and 1.1 mL min⁻¹ for the white/white tubing. All other manifold lines were of 0.8 mm i.d. Teflon® tubing. The ion exchange column of 8-hydroxyquinoline (8-HQ) immobilized on Fractogel® (21) was used to concentrate Zn and separate it from alkaline earth metals. The cation exchange column consisted of 35 µL of 8-HQ resin. The slurry was packed between two plugs of glass wool inside a 4.0 cm length of 1.5 mm i.d. Teflon® tubing, and fitted into the sample loop of the injection valve (Figure 2 inset).

A 6-port selection valve, V1 (Figure 2), was used to control the sequence of solutions flowing to the injection valve, V2, and then through the 8-HQ column in the sample loop of V2. Solutions pumped through V1 were used to strip, load and rinse the column.

The timing cycle of V1 was as follows: with the injection valve in load position

a 0.1 minute column wash with strong acid (1N HCl) was passed through the column, followed by a four minute sample load period and then a 1.0 minute column rinse period in which salts were rinsed from the column with MQ water. The strong acid removed all metals from the column, while the MQ water rinse eliminated the alkaline earth metals prior to sample elution. The injection valve was then switched to the elute position and a 1.0 minute elution/detection period using 0.08 N HCl followed. The injection valve would then switch back to the load position and the cycle would start again. The load time can be varied to accommodate higher or lower expected Zn concentrations.

The eluted sample slug merged with the reagent stream and was sent through a 200 μ L quartz flow-through cuvette in a Perkin-Elmer LS-5 Fluorescence Spectrophotometer. The fluorometer is equipped with a pulsed xenon discharge lamp, and has two reflection grating monochromators (excitation and emission) in the optical system with reference and sample photomultiplier detectors. Fluorescence was continually monitored during the elution cycle and recorded by a Soltec® 1242 chart recorder and saved onto computer disk. All valve positions and data acquisition were controlled by a Hewlett-Packard Vectra computer equipped with a Metrabyte DAS-8PGA 12 bit A/D converter and a digital interface.

Fluorometer settings are as follows: Excitation wavelength, 377 nm; emission wavelength 495 nm; excitation and emission slits, 5 nm, and a response setting of 4. This corresponds to a 16.8 second signal averaging setting.

Sample Collection and Treatment. Seawater samples were collected aboard the R/V Wecoma on the FeLINE 92 cruise to the equatorial Pacific. Thirty liter Teflon® coated Go-Flo® bottles (General Oceanics, Inc.) suspended on a Kevlar® wire were used for all water collection. Bottles were soaked in acid and flushed with seawater prior to sampling. Seawater samples were filtered with two, in-line acid washed 0.2 μm Sartopure Mini Capsule® polypropylene filters (Sartorius, Inc.) directly into 125 mL sample bottles. They were immediately adjusted to pH 4.5 - 5.0 using 35 μL of double distilled GFS® HCl (GFS Chemicals) per 125 mL sample. Unfiltered samples were also collected and acidified. Filtration and sampling activities were carried out inside a positive pressure, class 100 portable laboratory. Samples were drawn into the FIA manifold directly from sample bottles. Standard additions used to calibrate the method were prepared in acid-washed Teflon® vials and run identically to the samples. All open sample, reagent and standard bottles were contained within a class 100 laminar flow hood (Environmental Air Control, Inc.). Sample concentrations were determined using least squares regression analysis of standard additions, to every 10th sample.

Results and Discussion

Initial studies using pTAQ and Zn spiked MQ water in batch mode showed that the chemistry involved is relatively simple, rapid, and that the pTAQ-Zn complex is stable over time periods of minutes to hours. Adaptation to a flow-

injection system was, therefore, straightforward. The greatest concern was preventing sample contamination and eliminating interfering alkaline earth metals from the analysis. Contamination of the sample could be easily avoided during analysis because the analytical manifold is nearly all Teflon® and acid washing of the manifold lines between samples effectively eliminated contaminant Zn from the system. Interferences were eliminated by incorporating the 8-HQ column in the sample loop. Acidification of the sample kept most of the alkali and alkaline earth ions from binding to the column, and a 1.0 min MQ water wash (approximately pH 5-6) insured the removal of major seawater cations from column interstices before the sample was eluted (17).

Optimization of reaction conditions. Excitation and emission spectra for the pTAQ-Zn complex are shown in Figures 3a and 3b. The maximum signal was produced using wavelengths of 377 nm and 495 nm for the excitation and emission, respectively. Indicator ligand concentration, buffer pH, and eluent acid concentration were all optimized for the FIA-FL system using the manifold shown in Figure 2. In most cases, a trial run using MQ water was performed in order to assess any matrix effects. A 40 μ M concentration of pTAQ was used for all field and laboratory determinations. This ligand concentration was optimized for the concentrations of zinc expected in the open ocean (Figure 4). Higher pTAQ concentrations may be needed if this method is applied to near shore or polluted waters.

The reagent needed to be buffered to neutralize the acid carrier used to elute the sample from the column. After trying several types of buffers, (e.g., ammonium hydroxide and sodium phosphate), a 0.1 M sodium tetraborate buffer was chosen because it was easy to use and gave the lowest baseline signal. The concentration of the borate buffer was varied to determine if reaction pH affected the zinc signal (Figure 5). There was apparently no effect on the signal over a wide range of reaction pH's; however, the signal decreased at lower pH values. This was probably due to dissociation of the pTAQ-Zn complex.

The placement of Zn in the Irving-Williams order indicates that a relatively strong acid should be used to elute Zn from the 8-HQ column. Eluent acid concentration was varied over a range of 0.01-0.2 M to determine the minimum amount of acid required to elute Zn (Figure 6). An eluent HCl concentration of 0.08 N gave the best signal, and this was used for all subsequent field and laboratory analyses. This combination of buffer and 0.08 N acid carrier resulted in a reaction pH of about 8.1.

Timing parameters were adapted from previous flow injection analysis methods for trace metals (17-19) and only adjusted slightly for this system. Sample load time was found to be linear ($r^2 = 0.99$) for a 10 nM addition to seawater up to, and possibly beyond, 40 minutes (Figure 7). After a load time of this length, however, some column compaction occurs. This can lead to non-linearity in flow characteristics and can rupture the column tubing connection. A

load time of 4 minutes was adopted. This load time allowed sufficient concentration of zinc from seawater, while keeping the total analysis time to a minimum. Load time can be varied to optimize the technique for high or low Zn concentrations, e.g., 10 minute load times for rainwater or 2 min load times for sediment interstitial waters (unpublished data).

A 1 minute MQ water rinse was added to the cycle to insure all alkaline earth metals were removed from the column before the elute period. The rinse can be shortened to reduce total analysis time. The elution/detection time was kept at 1 minute. This allowed sufficient time to read the fluorescence signal and reestablish a baseline after the sample peak had eluted.

Zinc is strongly bound to organic ligands in seawater making it necessary to acidify seawater samples before analysis. A range of sample pH's was tested (Figure 8) with the optimum falling at 4.5-5.0. At lower pH values, Zn was not efficiently retained on the column, whereas at pH values higher than 5.5, complexation by organic ligands (22) allowed Zn to pass through the column. The signal was constant following a 10 minute delay after acidification indicating rapid dissociation of organic-Zn complexes in seawater. Analyses performed at sea were completed within 24-36 hours of sample collection and acidification.

Interference Studies. Alkaline earth metals exist in seawater at concentrations up to 5×10^8 times greater than zinc. It has been shown that the alkaline earth metals Ca and Mg form weakly fluorescent complexes with the ligand pTAQ

(Shimuzu, Inman and Lieberman, unpublished data) and could potentially account for up to 99% of the fluorescence signal if not eliminated from the seawater sample. Sodium and potassium also fluoresce weakly when complexed by the ligand. At pH values less than 7, however, Ca^{2+} , Mg^{2+} , and other salts are not retained on the 8-HQ column (17). The additional MQ water rinse removed any interfering ions from column interstices.

Interference experiments with other metals added to MQ water showed that only Cd gave substantial interference at 10 times normal seawater concentration (Figure 9a). A slight signal was detected from Ni in this interference study; however, no signal was detected in similar studies. This could be the result of Zn contamination in the nickel standard. The potential for zinc contamination is always present and may be the cause of this particular result.

PTAQ also forms a fluorescent complex with cadmium. It is possible to discriminate between pTAQ bound Zn and Cd by their fluorescent decay times (15). However, it is not possible to discriminate between those complexes based on their emission spectra alone. Essentially, any Cd in the seawater sample will also fluoresce, thereby increasing the Zn signal.

Laboratory experiments showed that Cd gave a 30% smaller response than Zn at equal concentrations in seawater (Figure 9b). Cadmium exists in seawater at 1/10 the concentration of zinc (2, 23). Therefore, a Cd interference, while most likely present, was not substantial and could be corrected by determining Cd

separately. Cadmium interference was minimal in surface waters because the signal from the picomolar levels of this metal is below the detection limit of this system. Interference from Cd is highest in deep water where concentrations increase to about one nanomolar. At that level of Cd, the interference can be equivalent to 0.7 nM Zn, and a correction is necessary.

Cadmium has a nutrient-like distribution with depletion in surface waters and enrichment at depth (1, 2, 4, 24, 25). It has been linearly correlated with phosphate in all oceans. A compilation of available data from all oceans by Boyle (26) has resulted in two equations describing the Cd:P relationship. These are:

$$\text{Cd (nM)} = 0.21 \text{ P } (\mu\text{M})$$

for P concentrations below 1.3 μM . For higher P values:

$$\text{Cd (nM)} = 0.40 \text{ P } (\mu\text{M}) - 0.25$$

This relationship, which is relatively constant ($\pm 7\%$) through all the ocean basins, allows Cd concentration to be estimated from PO_4 concentration. Phosphate levels were determined using a standard colorimetric technique for all samples taken on the FeLINE 92 cruise. Cadmium concentrations were calculated using these PO_4 values and the equations from Boyle (26). Cadmium interferences were calculated and corrected for accordingly.

Analytical Figures of Merit. The fluorescence response was linear ($r^2 = 0.99$) from 0-500 nM Zn added to pH 4.5-5.0 seawater using a 4 minute load time (Figure 10). Typical standard additions at sea were +3.33, +6.66, and +8.33 nM

Zn. A strip chart recording of fluorescence output for a similar addition to 500 m water from the equatorial Pacific is shown in Figure 11.

The detection limit (defined as three times the standard deviation of the blank) was 0.12 nM for a 4.4 mL sample (equivalent to a 4 minute column load time). An average relative error of 6.4% was obtained for replicate analyses. Detection limits can be reduced by increasing the load time of the sample. The blank was determined by analyzing seawater that had been passed through two cation exchange columns (both of 8-HQ immobilized on Fractogel®) stripping all Zn from the seawater prior to analysis by FIA-FL. An alternative method to determine the blank was achieved by setting valve 1 to load sample for only 0.5 minutes. This was enough time for the sample to reach the column in the sample loop at V2, but only allowed minimal sample to pass through the column. In this way, no detectable Zn was adsorbed to the column. Results from both blank determination schemes indicate the presence of a fairly large, but reproducible blank. The value ranged from 1.5-3.0 nM over the course of this work. The blank may be the result of an undetectable pH shift, a change in flow rate, or a pressure change due to valve rotation. Whatever the mechanism, blanks were constant and reproducible and did not compromise the integrity of the analyses.

The accuracy and precision of the method was determined by evaluation of standard seawater solutions CASS-2 (Coastal Atlantic Surface Seawater) and NASS-2 (North Atlantic Surface Seawater) obtained from the National Research

Council of Canada, Marine Analytical Chemistry Standards Program (27). Agreement was good between the FIA-FL method and the expected values. Results are given in Table 1. These values represent zinc concentrations uncorrected for Cd interference. In both cases, the interference from Cd (0.1 nM for NASS-2; 0.2 nM for CASS-2) was small and within the error of the measurement.

Comparison with oceanographically consistent profiles. The reliability of a new technique can be evaluated by comparing results with previously published reliable data. Figure 12a shows the profile of total Zn vs depth from the equator station on the FeLINE 92 cruise and includes northeast Pacific data from Bruland (2) for comparison. The silica depth profile (Figure 12b) shows a typical oceanic distribution for this nutrient. The Zn:Si relationship was found to be linear (slope = 0.06 ± 0.005 nmol Zn: $\mu\text{mol Si}$ with an r^2 value of 0.97) and resembles that of Bruland (1980), who obtained a slope of 0.05 ± 0.003 ($r^2 = 0.99$) for data in the North Pacific (Figure 12c). The similarity of these zinc profiles with those previously published, the results of the standard seawater analyses, and the oceanographic consistency of the data, demonstrate the accuracy and reliability of this method.

The flow injection method described here has potential for adaptation to other natural waters. It has been used to measure zinc in rain water as well as interstitial waters from bottom sediments (unpublished data). This technique can be utilized

for pollution mapping, point source monitoring and industrial process control. Adaptation of the technique for these purposes would increase our understanding of zinc concentrations and distributions in anthropogenically influenced waters.

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Figure Captions

Figure 1. Structure of indicator ligand p-tosyl-8-aminoquinoline (pTAQ) and the fluorescent complex it forms with Zn.

Figure 2. Flow injection manifold used for the shipboard determination of zinc in seawater. Inset depicts dimensions of cation exchange column (8-Hydroxyquinoline immobilized on Fractogel®) included in the sample loop of the injection valve, V2.

Figure 3. Excitation (a) and emission (b) spectra for a 20 nM Zn addition to MQ water using a 40 μ M pTAQ concentration.

Figure 4. PTAQ concentration vs fluorescence signal for blank, +5 and +10 nM additions of Zn in seawater. A 40 μ M pTAQ concentration was sufficient for the levels of Zn in open ocean waters and maximized the difference between the sample and blank signal. This concentration was utilized for all subsequent analyses.

Figure 5. Reaction pH vs fluorescence signal for a zero addition and a +5 nM addition of Zn in seawater. P indicates the use of a sodium phosphate buffer, a sodium tetraborate buffer was used for all others points. Buffering capacities were altered by serial dilutions of stock buffers in order to arrive at a different reaction pH.

Figure 6. Eluent acid concentration (HCl) vs fluorescence signal for a 4 minute load of a 10 nM Zn addition to seawater. An eluent acid concentration of 0.08 N HCl was optimal.

Figure 7. Load time vs fluorescence signal for a 10 nM Zn addition to seawater. A 35 μ L 8-HQ column was used for all samples.

Figure 8. Sample pH vs fluorescence signal for seawater after acidification at time intervals of 1 hour, 4 hours and 24 hours. A sample pH of 4.5-5.0 and a 4 minute load period was optimal and used for all field analyses.

Figure 9. Interference study results: (a) Instrumental response to zinc and various metals in seawater at 10X average seawater concentrations (Zn also at 10X avg. seawater concentration) (b) Instrumental response to Zn and Cd additions in seawater.

Figure 10. Zinc concentration in seawater vs fluorescence signal using a 4 minute load time and an indicator ligand concentration of $40\ \mu\text{M}$. The Zn signal was linear up to $500\ \text{nM}$ with an r^2 value of 0.99 .

Figure 11. Strip chart recording for a 500 m sample with typical standard additions, and a 750 m water sample from the equatorial Pacific. Additions were +0.0, +3.33, and +6.33 nM Zn.

Figure 12. Results of shipboard FIA-FL analyses: (a) Depth profile of total Zn at 0°N , 140°W . (b) Depth profile of silica at 0°N , 140°W . (c) Relationship of total zinc to silica. Bruland (1980) data from the North Pacific included for comparison. $\text{Zn (nM)}:\text{Si}(\mu\text{M}) = 0.06 \pm 0.005$ ($r^2 = 0.97$)

Table 1. Results of standard seawater analysis. Errors represent 95% confidence interval. Concentrations in nM.

	NASS-2	CASS-2
Reported Value	2.72 $\pm$ 0.38 nM	30.1 $\pm$ 1.80 nM
FIA-FL	2.78 $\pm$ 0.20 nM	25.6 $\pm$ 0.73 nM

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12.. 0°N , 140°W

CHAPTER 3

Zinc in the Equatorial Pacific

Abstract

A novel method using flow injection analysis with fluorometric detection (FIA-FL) was used to determine Zn in the equatorial Pacific. Filtered and unfiltered samples from 25-2000 meters were taken at three stations (0°N, 6°N, 15°N) near 140° W. Results show characteristic nutrient-like profiles for total zinc and a high linear correlation to reactive silica. The equatorial Zn:Si relationship observed in this study was $\text{Zn}(\text{nM}) = 0.06 (\pm 0.005) \text{PO}_4(\mu\text{M}) - 0.24 (\pm 0.8) (r^2 = 0.98)$, which is consistent with results of other investigators. Using available data to estimate atmospheric flux and upwelling velocities in this region, a residence time for Zn in equatorial surface waters was calculated to be on the order of 9 days at 0°N. Residence times calculated for the other stations ranged from 1.0-1.5 years, reflecting greater new production and particle scavenging at the equator. Data presented here demonstrate the utility of the FIA-FL method and support previous studies on the concentration and distribution of Zn in the ocean.

Introduction

The biogeochemical cycles of some trace metals can be understood by relating their distribution to that of an analog nutrient and other water column parameters. This approach was taken by Chan et al. (1977) to study the distribution of dissolved barium in the Atlantic. They used the distribution of SiO_4 to interpret the factors affecting the distribution of Ba, after finding similar depth profiles and a high linear correlation between them. Zinc has a high linear correlation to silica in the open ocean (Bruland et al., 1978; Bruland, 1980; Bruland and Franks, 1983). This suggests that Zn is sequestered by phytoplankton in surface waters as an essential nanonutrient and is transported to depth in association with a biogenic carrier phase and by passive adsorption onto particles. It appears to be regenerated at a depth similar to silica. Zinc exhibits fractionation between the Pacific and Atlantic oceans, with concentrations reaching 9 nM in the deep Pacific (greater than 2000 meters) while only reaching about 3 nM at similar depths in the deep Atlantic (Bruland and Franks, 1983). Interpretation of Zn distributions with respect to only physical or chemical oceanographic parameters in surface waters may not accurately account for the processes that control Zn. Sunda and Huntsman (1992) and Bruland et al., (1991) have shown that the Zn:Si relationship must be produced by a complex interaction of biological feedbacks. Excursions in the relationship between a metal and other physical and chemical

parameters in the water column may indicate the existence of controls particularly affecting the distributions of that metal. It is with this approach that our data from the equatorial Pacific is interpreted.

Background

The equatorial Pacific at 140°W is a dynamic region characterized by high productivity and variable chemical and physical oceanographic structure from north to south. Increased levels of surface nutrients at the equator are supplied by upwelling. Upwelling is a result of the divergence of westward moving surface currents and subsequent intrusion of nutrient enriched waters from between 50-150 meters (Wyrski, 1981). The zonal circulation pattern driven by easterly trade winds is responsible for north-south gradients in biological productivity and nutrient levels across the equator. The locations of these boundaries migrate depending on the season and subsequent wind stress (Knauss, 1963). Physical oceanographic data also show an increase in surface temperatures along the equator from east to west indicating advection of a tongue of cold, upwelled water from the east toward the central equatorial Pacific (Wyrski, 1981). The eastward decrease in temperature, which is strongest in August and September, is accompanied by an increase in nutrients in the surface waters. Nutrient availability makes primary productivity in the equatorial Pacific a major component of the global carbon and nitrogen cycles (Chavez and Barber, 1987; Feely et al., 1987).

Historical patterns show nutrient levels in surface waters at the equator are high, without the extreme depletion which occurs in central gyre areas. Productivity in the equatorial Pacific, however, is not as high as could be supported by the levels of nutrients. High nutrient levels with low productivity indicate another factor may be limiting phytoplankton growth. Limitation may be due to iron, a theory that has recently received much attention. Similar high nutrient, low productivity conditions exist in the subarctic Pacific with convincing evidence to support iron limitation as the cause (Martin and Fitzwater, 1988; Coale, 1991). Zinc is one of many trace elements, including Fe, which is a required nanonutrient for biological production (see review by Sunda, 1991). This requirement may control phytoplankton distributions (Brand et al., 1983) as well as rates of primary productivity.

Methods

Samples were collected during the FeLINE 92 cruise to the equatorial Pacific at 140°W aboard the R/V Wecoma, in March and April of 1992 (Fig. 1). Samples were retrieved from depths ranging from 25-2000 meters at three stations (0°N, 140°W; 6°N, 140°W; and 15°N, 149°W). Teflon® coated 30 L Go-Flo® bottles (General Oceanics, Inc.) suspended on a Kevlar® wire were used to collect water from at least 8 depths at each station. Filtered (two acid rinsed, in-line 0.2 μ M polypropylene filters from Sartopure®, Sartorius, Inc.) as well as unfiltered samples

were taken inside a positive pressure class 100 portable laboratory. Seawater samples were collected in acid washed polyethylene bottles and immediately acidified to pH 4.5-5.0 using GFS (double-distilled) HCl (GFS Chemicals). All samples were analyzed at sea within 24-36 hours after collection using flow injection analysis with fluorometric detection (Nowicki et al. submitted).

Samples from the Go-Flo® bottles were also taken from each depth for analysis of nutrients. Reactive silica and nitrite plus nitrate concentrations were determined using a standard colorimetric technique aboard ship. Extreme heat in the laboratory ($>38^{\circ}\text{C}$) prevented phosphate analyses from being run; therefore, these samples were frozen and analyzed later at Moss Landing Marine Laboratory. CTD/rosette casts were performed at each station using a Neil Brown CTD and 12, ten liter Niskin bottles.

Results

Depth profiles of total Zn reveal characteristic nutrient-like profiles with extreme surface depletion and enrichment at depth (Figs. 2, 3, 4). These plots correspond well with previous Zn profiles in the northeast Pacific (Bruland, 1980). Dissolved values, however, exceeded total values for the equator station and 15°N station. A plot of filtered (dissolved) minus unfiltered (total) versus order of sample drawn reveals a systematic decrease in level of Zn contamination over time (Fig. 5). Apparently, the filters released contaminant Zn, and as more seawater flushed the

filter, less Zn was leached. Dissolved Zn values from the 6° N station appear not to have been contaminated. The cause of such high contamination of filtered samples from the 15°N station is unknown, but not uncommon to the determination of Zn. The need for extreme care in cleaning all apparatus in trace metal determinations is exemplified in these results. Results from the 6°N station also indicate that particulate Zn is not a large fraction of the total. Total Zn values are used for interpretation and comparisons.

Surface water nitrite plus nitrate values were detectable ($3\ \mu\text{M}$) at 0°N and 3°N, but fell to an undetectable level ($<0.1\ \mu\text{M}$) at the 6°N station and 15°N station (Figs. 2, 3, 4). Similar trends occurred for other nutrients. Temperature profiles at 0°N revealed slightly warmer than expected sea surface temperatures (SST) (28°C measured vs 25°C mean temperature), while stations 6°N, and 15°N had SSTs near the climatological mean (25°C). Salinity profiles, as well as density profiles (Figs. 6, 7, 8), show typical surface values for regions on and near the equator (Tchernia, 1980). At both 6°N and 15°N a tongue of high salinity water occurs near 100 meters, with salinity returning to just above surface water values at about 125 meters. CTD failure at 0°N precluded our attempt to obtain salinity and temperature data deeper than 120 m.

Discussion

Measurements of temperature, salinity, and density taken at all stations show

surface values within the average range of these parameters for the equatorial Pacific in March. Surface temperatures at the equator were at the high end of the expected range (28°C measured vs 25°C mean temperature, Tchernia, 1980) possibly indicating a lower than normal upwelling rate. Surface temperatures for the two other stations (6°N and 15°N) appeared normal, with the thermocline between 75-200 meters. High salinity at the base of the mixed layer indicates lateral mixing of a higher salinity source and the freshening of surface waters by rainfall. This high salinity water may originate in tropical regions north and east of the equator, where evaporation exceeds precipitation.

Nutrient concentrations were slightly lower than expected for the equatorial region, coinciding with warmer surface temperatures and a reduced upwelling rate. Zinc and silica depth profiles collected during the FeLINE 92 cruise agree with data from the northeast Pacific collected by Bruland, (1980). Figures 9, 10, and 11 show a slightly sharper nutricline at the 6°N and 15°N stations and a more gradual increase of nutrient concentration with depth. Plots of Zn vs SiO₄ for stations at 0°N, 6°N, and 15°N reveal highly linear relationships with slopes of 0.06 ($\pm$ 0.005), 0.04 ($\pm$ 0.003), and 0.04 ($\pm$ 0.007) nM Zn/ μ M Si (r^2 = 0.96, 0.92, and 0.74 respectively). Errors expressed represent the standard error of the estimate. These results agree with those of Bruland, (1980) for the central northeast Pacific (slope = 0.05 ($\pm$ 0.003), r^2 = 0.99) and support observations of deep Zn regeneration and long deep water residence times. Zinc data from the Atlantic

(Bruland and Franks, 1983) show a higher slope (slope = $0.09 (\pm 0.016)$, $r^2 = 0.79$) than any of the Pacific Ocean stations.

The depletion of zinc to the same concentration in surface waters of both oceans (Bruland and Franks, 1983), may indicate that phytoplankton uptake reduces Zn to around 0.2 nM and this activity then becomes limited. Limitation can result from lack of major nutrients or a nanonutrient (in the case of iron), or by more than one component simultaneously. Although the Zn:Si relationship is consistently linear, the difference in slopes in the regions investigated here, and elsewhere, may reflect differences in several factors. These include community structure (synechococcus vs diatom or coccolithophorid dominated regions), source of the metal, (anthropogenic vs natural atmospheric aerosols) and biogeochemical cycling (Zn is required by all phytoplankton, yet silica is not). Zinc may be incorporated into siliceous tests but overall cycling may differ due to differences in the fraction in which it is incorporated.

A box model can be used to estimate the average time Zn remains in surface waters (surface residence time, τ_{sf}) and is available for uptake by phytoplankton. Several assumptions are necessary to simplify the calculation. First, a steady state system is assumed in which inputs balance outputs and there is no change in Zn concentration. Further, it is assumed that the only flux out of the box is due to scavenging (i.e., no downwelling), lateral advection into the box equals that out, and any upwelling originates from 100 meters (Wyrki, 1981), which is the depth

of our box. The estimate for atmospheric flux at the 6°N and 15°N stations was taken from Chester and Murphy (1988) for the North Pacific ($0.029 \mu\text{g Zn cm}^{-2} \text{ yr}^{-1}$). Vertical advection was calculated from the measured Zn concentration at 100 meters (approximately 0.4 nM at both stations) multiplied by the estimated upwelling velocity of 4 m yr^{-1} (Broecker and Peng, 1982). Box models for 6°N and 15°N will have important input from eddy diffusion. Anderson (1978), using an empirical formula, estimated the vertical eddy diffusion coefficient (K_z) in this region to range from $0.05\text{-}0.51 \text{ cm}^2 \text{ s}^{-1}$. K_z multiplied by the depth rate of change ($d\text{Zn}/dz$) gives the diffusive flux of Zn. The existing reservoir was calculated as the average surface concentration (0.1 nM) integrated over the upper 100 meters. The flux of zinc into surface waters can be summarized by the following equations:

$$\text{upwelled flux} = 0.4 \text{ nmol L}^{-1} * 4 \text{ m yr}^{-1} = (8.8 \text{ nmol m}^{-2} \text{ d}^{-1})$$

$$\text{atmospheric flux} = 0.029 \mu\text{g cm}^{-2} \text{ yr}^{-1} = (12.2 \text{ nmol m}^{-2} \text{ d}^{-1})$$

$$\text{diffusive flux} = K_z * d\text{Zn}/dz = (1.5\text{-}15.9 \text{ nmol m}^{-2} \text{ d}^{-1})$$

$$\text{reservoir} = 0.1 \text{ nmol}/1000 \text{ cm}^3 * 10000 \text{ cm} = (10,000 \text{ nmol m}^{-2})$$

Using the equation:

$$\tau_{\text{sfc}} = \text{reservoir}/\text{flux}$$

the residence time for Zn in surface waters at 6°N and 15°N is 360-560 days (1.0-1.5 years). This estimate agrees with that of Bruland (1980) for the northeast Pacific. He calculated a residence time of 1 year for Zn in surface waters. Jannasch (1990) estimated a residence time for Zn (in water from 80-190 meters)

ranging from 500 to 1100 days (1.4-3.1 years) for the eastern equatorial Pacific. The 6°N and 15°N stations have relatively little Zn input compared to the equator station, which is influenced by upwelling. The following calculations demonstrate the difference.

The equatorial region is supplied with upwelled waters at a fairly high rate, 2 m d⁻¹ (Halpern et al., 1989). Since the upwelling is relatively constant, so should be the input of upwelled Zn. A similar box model can be used to estimate the residence time of Zn in the surface waters at the equator. The major inputs of Zn to the area are atmospheric fallout and upwelled water, again assuming lateral advection into the box is equal to that out. Using the measured Zn concentration at 100 m (0.53 nM) and the rate at which waters are upwelled, vertical advection can be calculated. The vertical eddy diffusion coefficient at the equator would most likely be at the low end of the range estimated by Anderson (1978) (0.05 cm² s⁻¹) reflecting a reduced nitrate gradient set up by upwelling. Using the same reservoir size (10,000 nmol cm⁻²) and the following fluxes:

$$\text{upwelled flux} = 0.53 \text{ nmol}/1000 \text{ cm}^3 * 2 \text{ m d}^{-1} = (1060 \text{ nmol m}^{-2} \text{ d}^{-1})$$

$$\text{atmospheric flux} = 0.0026 \text{ } \mu\text{g cm}^{-2} \text{ yr}^{-1} = (1 \text{ nmol m}^{-2} \text{ d}^{-1})$$

$$\text{diffusive flux} = 0.05 \text{ cm}^2 \text{ s}^{-1} * 0.3 \text{ nM}/100 \text{ m} = (2 \text{ nmol m}^{-2} \text{ d}^{-1})$$

a residence time for zinc in the upper 100 meters of the equatorial ocean is about 9 days. These calculations imply a high scavenging rate and demonstrate the rapid removal of Zn from surface waters. This residence time differs greatly from

that of Bruland (1980) as a result of differences in relative magnitudes of the inputs.

Differences in residence times between the equator station and the 6°N and 15°N stations may be due to differences in the rate at which particles, and therefore zinc, are removed from surface waters. New production is "primary production associated with newly available nitrogen" (Dugdale and Goering, 1967), or as defined by Eppley and Peterson (1979) "quantitatively equivalent to the organic matter that can be exported from the total production in the euphotic zone without the production system running down". New production in the equatorial Pacific is thought to be a significant proportion of total production (on the order of 1 gigaton of C y⁻¹), whereas in the North Pacific gyre, the amount of material removed from surface waters as new production is relatively very small. If zinc is mainly removed by particles sinking from surface waters (after active uptake by phytoplankton or passive adsorption onto particles), its residence time should reflect the amount of new production in that region. This trend was found by Bruland and Coale (1986) in an investigation of spatial and temporal variations of ²³⁴Th in the Pacific Ocean. This study found scavenging of dissolved ²³⁴Th to be strongly correlated with particulate organic carbon flux throughout a wide range of marine environments (estuaries to the deep sea). Equatorial residence times for Zn are rapid, coinciding with high levels of new production. Central gyres and areas with low rates of new production (our 6°N, and 15°N stations) have longer Zn residence times, which reflect correspondingly low rates of biological fallout.

These calculations are sensitive to the relative magnitude of the input and removal terms. Zinc scavenging, assumed to be the only important removal in these models, is directly influenced by the amount of particles and the rate at which they are removed.

Conclusion

Identification and quantification of processes affecting zinc concentrations, especially free Zn ion, are necessary to completely understand Zn distributions, controlling factors and biogeochemical effects. Vertical biogeochemical cycling superimposed on large scale horizontal ocean circulation combine to give the observed metal distribution. By comparing Zn data to oceanographic and nutrient data, we can elucidate the processes that are affecting the distribution of Zn.

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Figure Captions

Figure 1. Location map showing stations where depth profiles for zinc were obtained.

Figure 2. Vertical profiles of zinc, silicate, phosphate and nitrate + nitrite for station 0°N, 140°W.

Figure 3. Vertical profiles of zinc, silicate, phosphate and nitrate + nitrite for station 6°N, 140°W.

Figure 4. Vertical profiles of zinc, silicate, phosphate, and nitrate + nitrite for station 15°N, 149°W.

Figure 5. Order of sample vs. filtered - unfiltered values of Zn concentration (nM) for each station. This plot shows the contamination introduced by filtering at the 0°N and 15° stations.

Figure 6. Vertical profiles of temperature (°C), salinity (PSU) and density (σ_t) for 0°N, 140°W.

Figure 7. Vertical profiles of temperature (°C), salinity (PSU) and density (σ_t) for 6°N, 140°W.

Figure 8. Vertical profiles of temperature (°C), salinity (PSU) and density (σ_t) for 15°N, 149°W.

Figure 9. Depth profiles of (a) zinc and (b) silica and (c) zinc vs silica at 0°N, 140°W. Northeast Pacific data from Bruland, 1980, is included for comparison.

Figure 10. Depth profiles of (a) zinc and (b) silica and (c) zinc vs silica at 6°N, 140°W. Northeast Pacific data from Bruland, 1980, is included for comparison.

Figure 11. Depth profiles of (a) zinc and (b) silica and (c) zinc vs silica at 15°N, 149°W. Northeast Pacific data from Bruland, 1980, is included for comparison.

Figure 1

Figure 2. 0°N, 140°W

Figure 3. 6°N, 140°W

Figure 4. 15°N, 149°W

Figure 5.

Figure 6. 0°N, 140°W

Figure 7. 6°N, 140°W

Figure 8. 15°N, 149°W

Figure 9. 0°N , 140°W

Figure 10. 6°N , 140°W

Figure 11. 15°N , 149°W

CHAPTER 4

Conclusion

The field of chemical oceanography has undergone a revolution since the mid-70s. Advances in analytical methods and chemistries, combined with extensive and persistent practices to avoid contamination, have enabled us to resolve inconsistent data and begin to understand oceanic trace metal concentrations and distributions. We have learned that many trace metals occur in seawater at picomolar to nanomolar concentrations, but can be essential to marine organisms. Zinc is one of these metals that influences, and is influenced by phytoplankton communities. Extensive use of zinc in industry, and its potentially harmful effects in the marine environment, make it necessary to characterize the concentration, distribution, and interactions of zinc in seawater. This demand for information requires analytical methods that are economical, reliable, accurate, and applicable to various natural systems. The project described in this paper is one effort to supply the means to collect this valuable information. The utility of this flow injection method can be extended to other natural systems, including lakes, rivers, and rain water. The technique is accurate, precise, inexpensive, rapid, and provides a means by which samples can be analyzed in the field.

APPENDIX

Metal, nutrient, and CTD data from stas 0°N, 140°W; 6°N, 140°W; 15°, 149°W from the FeLINE 92 cruise to the equatorial Pacific. Samples with probable metal contamination are designated with an asterisk (*).

Depth m	Zn nM	SiO ₄ μM	NO ₂ +NO ₃ μM	PO ₄ μM	Temp °C	Sal PSU	Density σ _t
0°N, 140°W							
25	0.4	1	3.9	0.35	28.022	35.113	22.475
99	0.5	5	11.4	0.75	22.617	35.152	24.160
248	0.8	22	26.9	1.80	-	-	-
498	1.6	47	39.5	2.72	-	-	-
750	3.1	66	41.1	2.89	-	-	-
1000	4.9	93	41.5	2.90	-	-	-
1499	7.3	131	40.9	2.86	-	-	-
2000	10.2	151	40.5	2.79	-	-	-
6°N, 140°W							
25	0.7	0	0	0.11	28.275	34.515	21.942
45	0.5	0	0	0.13	28.242	34.516	21.954
75	3.1*	0	0.40	0.19	27.965	34.621	22.123
100	0.8	6.1	9.20	0.47	22.144	34.832	24.051
145	0.4	26.6	26.13	1.89	14.939	34.579	25.661
200	0.6	32.8	33.36	2.32	11.066	34.654	26.495
250	0.9	34.2	33.83	2.31	10.131	34.661	26.665
349	1.3	37.0	35.12	2.41	9.350	34.644	26.783
500	2.8	50.0	40.12	2.86	7.985	34.592	26.956
747	7.4*	76.4	43.88	3.06	5.944	34.534	27.192
985	2.6	99.2	43.33	3.05	4.613	34.543	27.357
1239	4.7	117.7	43.07	3.05	3.738	34.566	27.468
1477	5.4	129.7	42.35	3.00	3.192	34.585	27.536
1748	6.2	144.8	41.34	2.87	2.687	34.605	27.598
1766	6.1	152.2	41.29	2.82	2.662	34.607	27.602

Appendix (cont'd)

Depth m	Zn nM	SiO ₄ μM	NO ₂ +NO ₃ μM	PO ₄ μM	Temp °C	Sal PSU	Density σ _t
15°N, 149°W							
25	0.8	0.3	0.1	0.06	24.494	34.147	22.851
50	1.3	0.3	0.2	0.04	24.461	34.147	22.861
75	1.3	0.4	0.3	0.09	23.541	34.338	23.277
100	1.7	0.7	2.9	0.38	20.510	34.610	24.330
175	1.5	16.5	18.3	1.22	12.209	34.305	26.010
249	2.5*	37.2	31.5	2.45	10.359	34.504	26.503
372	1.9	38.9	31.6	2.50	8.732	34.518	26.783
500	4.5	63.3	40.1	2.90	7.477	34.508	26.964
750	6.1	89.6	43.9	3.06	5.726	34.492	27.186
996	8.3	111.4	44.6	3.00	4.516	34.518	27.348
1241	8.6	123.9	43.5	3.02	3.742	34.548	27.453
1494	8.4	139.9	43.0	2.97	3.056	34.577	27.542
1743	11.1*	149.8	41.4	2.86	2.560	34.599	27.604
1992	12.1*	158.4	41.3	2.61	2.198	34.618	27.650