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BIOGEOCHEMISTRY OF REDOX-SENSITIVE ELEMENTS IN NATURAL WATERS: CHEMICAL SPECIATION OF MOLYBDENUM AND VANADIUM

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ABSTRACT OF THE DISSERTATION

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The transition elements, molybdenum (Mo) and vanadium (V), are essential micronutrients for plants, animals and microorganisms. Mo and V both form part of the active sites of metalloenzymes that execute key transformations in the metabolism of nitrogen, sulfur, carbon and halide compounds. These two elements have a rich redox chemistry (+II to +V for V, and +II to +VI for Mo), which partly explains why they are so biologically active. Mo and V are also relatively abundant in the ocean, with Mo (VI) and V (V) dominating under oxic conditions, while Mo (V) and V (IV) are expected to exist as the soluble forms under anoxic reducing conditions. Biologically both Mo and V belong to the group of trace elements that organisms need in minute amounts and both metals are also toxic at high concentrations. On the other hand, lack of Mo and V is also

lethal for organisms. Toxicity and bioavailability are dependent on the speciation of the two metals rather than simply the total concentration: Mo (V) is expected to be the more bioavailable form than Mo (VI) in aqueous environments, while V (V) is much more toxic than V (IV).

Despite the importance of reduced species of Mo and V in marine environments, few studies have been conducted on the speciation of these two elements. This is mainly due to the lack of direct methods for the determination of different redox states of Mo and V in seawater. Therefore, this dissertation research combined laboratory experiments and field investigations to develop direct methods of measuring reduced and oxidized species of Mo and V in seawater, and then successfully applied the new methods to examine the existence of the species in coastal waters of Long Island, New York. This research also investigated the early diagenetic behaviors of Mo in porewater at Flax Pond, and hence furthered our understanding of the biogeochemical cycles and paleoceanographic implications of these elements in the marine systems.

New methods for the determination of reduced Mo and V in seawater were developed first. The new protocol for determining V (V) and V (IV) involves Chelex 100 solid-phase extraction of both species, and then stripping off V (V) with ammonium solution and finally V (IV) was removed with acid. The new protocol for reduced Mo involves complexation of Mo (V) with tartrate at pH=7.0, subsequent XAD-7 solid-phase extraction of Mo (V) complexes, and elution by acidic acetone. All V and Mo species were quantified via Graphite Furnace Atomic Absorption Spectrometry (GF-AAS). The detection limits of the protocols are approximately on the order of 0.5 nM and 0.2 nM for V and Mo respectively. Analytical precision are ~10% in the concentration range of 10

nM for both elements. The methods were successfully applied to the determination of V (IV) and Mo (V) in coastal waters around Long Island, New York, including filtered seawater, sediment pore water, and river water samples. Both methods are sensitive, simple and reproducible, but careful handling and operation under nitrogen are critical because both reduced V and Mo may be oxidized quickly under atmospheric conditions.

By applying the new methods, field investigations of reduced forms of these two elements, V (IV) and Mo (V), along with hydrological parameters, were carried out at the head of Peconic River Estuary and in Long Island Sound to examine the existence of reduced forms of both metals. Consistent with thermodynamic calculations, reduced forms of Mo and V exist in these natural waters. Field investigations showed that V (IV) and Mo (V) were favored under low pH and low dissolved oxygen conditions. Mo and V concentrations and speciation changed dynamically both seasonally and spatially in estuarine waters in response to redox conditions. V (IV) in the Peconics and LIS apparently was formed under suboxic conditions, which may be related to sewage inputs as well as reducing environments. Mo (VI) was rapidly mobilized from carrier phases (Fe, Mn-oxides and organic particles), reduced to Mo (V), under nonsulfidic or low sulfide conditions, while Mo (V) was further reduced to Mo (IV) and precipitated as MoS_2 in highly sulfidic sediments during early diagenetic processes. Mo (V), and presumably V (IV), are released as transient dissolved intermediates during the reduction and oxidation of particulate carrier phases (Fe, Mn-oxides, organic matter and Fe-sulfides). The implications of the reduced forms of Mo and V on biological processes are still unknown.

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CHAPTER ONE: INTRODUCTION

GENERAL DESCRIPTION AND JUSTIFICATION

The field of marine trace metal biogeochemistry was revolutionized with the development and implementation of trace metal clean techniques for sampling and analysis developed in the 1970s (Broecker, 1974; Bruland et al., 1979; Broecker and Peng, 1982). Since then, all of the naturally occurring elements of the periodic table have been measured at least once, and their oceanic distribution patterns reflect the impact of both physical and biological processes (Broecker and Peng, 1982; Bruland, 1983; Quinby-Hunt and Turekian, 1983; Li, 1991; Bruland and Lohan, 2003). Another major research effort has been in isolating and measuring the different chemical species found within the so-called dissolved phase as their bioavailability (and toxicity) often depends more on their speciation than on the total concentration (e.g., Sunda, 1989; Allen and Hansen, 1996; Templeton et al., 2000). However, most speciation research has focused on differentiating the amount of metal chelated by organic ligands from the inorganic metal pool (e.g., Kozelka and Bruland, 1998; Wells et al., 1998). Another area of chemical speciation has focused on estimating the concentrations and cycling of metals that exist in seawater in more than one oxidation state (e.g., Morel and Price, 2003). These studies, however, have focused on only a few of the first-row transition metals of the periodic table, e.g., Mn, Cr, and Se (German et al., 1991; Rue et al., 1997; Christian et al., 2002). The speciation of a large number of redox sensitive elements (e.g., Cu, Mo, Tl and V) in seawater is still unknown, mainly because the analytical techniques to isolate and measure their different valences in seawater have not yet been developed.

The lack of techniques to measure the different metal species of redox-sensitive elements has hindered our understanding of how some bioactive elements are being

biologically utilized in the ocean, as well as how their chemical speciation reflects changes in the redox conditions of the ocean through geological time. An example of this is the limited understanding of the cycling of different chemical species of molybdenum (Mo) and vanadium (V) in aquatic environments. Both elements exist in different oxidation states (Mo, +II to +VI, and V, +III to +V; Lovell et al., 2002), and they easily switch between different valences at low potentials (~0.0 V), facilitating their utilization in several biochemical pathways (Williams and Fraústo da Silva, 2002; Rehder, 2003). In fact, more than 70 Mo and V containing enzymes have been identified so far that catalyze essential metabolic reactions in the global cycles of carbon, nitrogen, sulfur and halides (e.g., Hille, 1996; Rehder, 2003; all Mo and V containing enzymes are listed in Appendix I: Tables 1&2). These two elements may be adsorbed onto particles and accumulated in sediments under strongly reducing environments (e.g., Bertine, 1972; Wehrli and Stumm, 1989), and therefore have been used as paleoenvironmental indicators of oceanic anoxic events and oxygenation processes (e.g., Nijenhuis et al., 1998; Yang et al., 2002; Algeo and Maynard, 2004). Reduced forms of these two elements, V (IV) and Mo (V), are expected to be involved in these processes, but no research has yet attempted to study the speciation in response to redox changes in the water column of the world ocean. In large part, this is simply because of the difficulties isolating and analyzing the extremely low levels of reduced Mo and V species in seawater. Therefore there is a need for successful chemical protocols for determining the different species of these metals in seawater. The main objective of this research is then to develop the methodology for separating and quantifying the different chemical species of Mo and V in seawater. These techniques are not only required to further our

understanding of biogeochemical cycles of these redox sensitive elements in the ocean, but also to shed light on the mechanisms of their utilization in paleoceanographic studies.

This chapter provides a general introduction to the importance of reduced forms of V and Mo, summarizes current studies, and highlights the main constraints that contribute to the scarcity of information on the ecological role and biogeochemical cycling of reduced V and Mo in the marine environment. In Chapter Two, the development of a new method is described for the determination of different chemical species of dissolved vanadium, V (IV) and V (V) in seawater. The existence of V (IV) was examined at the head of Peconic River Estuary and in the Long Island Sound. Chapter Three focuses on the new method development of measuring dissolved Mo (V) in seawater, and the existence of Mo (V) was examined at the head of Peconic River Estuary. In Chapter Four, early diagenesis of Mo in porewater at Flax Pond was investigated, and incubation experiments were also conducted to verify the dynamics of concentration and speciation of Mo during early diagenesis. The final chapter summarizes the major findings and implications in this dissertation and indicates directions for future work.

IMPORTANCE OF MO AND V IN DIFFERENT METABOLIC PATHWAYS

a) Enzymatic reactions

It is very striking that just a handful of trace elements, as essential micronutrients, have been selected in the evolution of life. Virtually all of the biogeochemical cycles of carbon, nitrogen, sulfur and halides on Earth depend today upon several rare, transition trace metals such as Mo and V (Hille, 1996; Kisker et al., 1997; Rehder, 2003).

Biologically these elements are part of the active sites of metalloenzymes that execute key transformations in many metabolic processes (reviewed by Stiefel, 1993; 1997; Hille, 1996; Rehder, 2003). Lack of these two elements is lethal to an organism, while a higher level of uptake is also toxic (e.g., Barceloux, 1999; Davies et al., 2005). More than 70 enzymes have been identified that contain either Mo or V (some examples are listed in Table 1 and all Mo and V containing enzymes are listed in Appendix I: Tables 1&2). Mo and V containing enzymes catalyze hydroxylation or oxygen atom transfer on various carbon-, sulfur-, nitrogen- and halide-compounds in a one-electron or two-electron transfer reaction (e.g., Wever et al., 1995; Kisker et al., 1997). Based on their biological functions, all these enzymes are classified into four groups: N-cycle, S-cycle, C-cycle involving enzymes, as well as halogen-cycle involving enzymes that contain only V.

b) Relevance of Mo and V in the nitrogen cycle

Nitrogen is an essential component of all biopolymers, such as amino acids, proteins and nucleic acids, and it exists in the biosphere in several oxidation states, ranging from +V to –III. Although N_2 accounts for roughly 80% of the Earth atmosphere, and nitrate is relatively abundant in seawater, ammonia is the only form of nitrogen that can be assimilated into biomass directly. Therefore, reduction of binitrogen and nitrate to bioavailable ammonia, catalyzed by nitrogenases and nitrate reductases, becomes critical in the biological system (e.g., Marquez and Kaspar, 1983). Mo and V are essential and constitute part of the active centers of these enzymes. The biogeochemical cycle of nitrogen (as shown in Figure 1; Einsle and Kroneck, 2004) generally includes several metabolic pathways: 1) nitrogen fixation, 2) denitrification, 3)

nitrification, 4) assimilatory and 5) dissimilatory nitrate ammonification, 6) anaerobic ammonia oxidation (anammox). A large family of Mo enzymes are also involved in N-heterocyclic metabolism.

Based on their structures, Mo enzymes are further classified into two types (e.g., Lovell, 2002; Williams and Fraústo da Silva, 2002): 1) Mo-cofactor containing enzymes; and 2) Mo-Fe cofactor containing enzymes (Figure 2). The first contains a Mo-cofactor (Mo-co, Figure 2a), that is a mononuclear Mo atom coordinated to the sulfur atoms of a pterin (e.g., nitrate reductase, xanthine oxidase, aldehyde oxidase and formate dehydroferases as listed in Table 1), and the second contains a unique poly-metallic cofactor (Mo-Fe-co, Figure 2b) as its active site (only found now in nitrogenases, e.g., Burgess and Lowe, 1996; Howard and Rees, 1996; Kisker et al., 1997). Nitrogen fixation provides an important source of biologically available nitrogen to ocean surface waters (Capone et al., 1997; Karl et al., 1997), and it stimulates phytoplankton productivity, therefore influencing the global carbon cycle (Falkowski, 1997; Sañudo-Wilhelmy et al., 2001). Three different types of nitrogenases have been traditionally found in all N_2 fixing organisms: Mo containing nitrogenase, V containing nitrogenase, and Fe containing nitrogenase (e.g., Burris, 1991; Pau et al., 1993; Chatterjee et al., 1996; Loveless et al., 1999). Mo nitrogenase is dominant and has been intensively studied for the last several decades (e.g., Shah et al., 1978; Robson et al., 1986). As it is physiologically required in nitrogen fixation (e.g., Howarth et al., 1988), Mo has been considered as a potential limiting factor in soils (Anderson and Spencer, 1949), in lakes (Howarth and Cole, 1985; Howarth et al., 1988), in estuaries (Brattberg, 1977), in oceans (Capone and Carpenter, 1982), and especially in regions with lower Mo availability

(Howarth et al., 1988). Fe and V nitrogenases, however, have only been discovered in recent years (e.g., Hales et al., 1986; Siemann et al., 2002). Generally, Mo nitrogenase is more efficient in nitrogen fixation than V nitrogenase, which is more efficient than Fe nitrogenase under normal conditions (e.g., Joerger and Bishop, 1988; Eady, 1996; Tsygankov et al., 1997). V and Fe nitrogenases have traditionally been proposed to serve as alternative routes for nitrogen fixation in situations where Mo (and V) is less available (e.g., Robson et al., 1986; Anbar and Knoll, 2002; Oda et al., 2005). Miller and Eady (1988), however, further showed that V nitrogenase was more efficient than Mo nitrogenase in the reduction of nitrogen to ammonia under lower temperatures (between 5-10^{o} C). Tsygankov et al. (1997) also showed that, among these nitrogenases, only V nitrogenase exhibited resistance to alkaline pHs, and a culture of *Anabaena variabilis* with V nitrogenase grew even at pH=10, while Mo and Fe nitrogenases did not. Hence although still largely unknown, V nitrogenase might have special characteristics and play a critical role under extreme conditions such as high alkalinity and/or low-temperature.

Since all three nitrogenases reduce N_2 to NH_3, C_2H_2 to C_2H_4, and H^+ to H_2, their activities are traditionally assayed by indirectly measuring hydrogen and/or ethylene production. In addition to reducing C_2H_2 to C_2H_4, only V and Fe nitrogenases can catalyze the formation of C_2H_6 (as a minor product) from C_2H_2 (e.g., Dilworth et al., 1987; Chatterjee et al., 1996). Schneider et al. (1997) reported that Fe nitrogenase had a H_2-producing activity (which, combined with acetylene reduction, is traditionally used to measure nitrogenase activity) and this activity was much less inhibited by competitive substrates in Fe nitrogenese than in Mo nitrogenase. Hence these alternative nitrogenases (V and Fe) might somehow play special roles in the metabolisms of many organisms.

Nitrate reductases are required by virtually every plant that uses nitrate as a N source (assimilatory nitrate reduction) (e.g., Eppley et al., 1969; Solomonson and Barber, 1990; Campbell, 1999). Nitrate reductases catalyze the first step of nitrate assimilation in all autotrophs, e.g., higher plants, algae, and fungi (as showed in Figure 1; e.g., Campbell, 1999; Einsle and Kroneck, 2004). Since nitrate is the most significant source of nitrogen in many nitrate rich regions (e.g., agricultural farms, coastal runoff), nitrate metabolism and nitrate reductases have been studied over the past several decades (e.g., Solomonson and Barber, 1989; Crawford, 1995). Mo is required in almost all nitrate reductases, which contain the Mo-cofactor as a structural unit (Zumft, 1997; Moreno-Vivian et al., 1999; Philippot and Højberg, 1999). Nicholas et al. (1963) reported that bacterial growth and nitrate reductase activity were markedly reduced in cells deficient in Mo. New nitrate reductases with V or Fe have also been discovered in sulfur reducing bacteria that do not contain a Mo co-factor (Antipov et al., 1998; Murillo et al., 1999; Antipov, 2003). It is still unclear how widespread these alternative nitrate reductases are, or which type of bacteria are able to produce them and under which conditions.

Denitrification, an important step in the nitrogen cycle, refers to the dissimilatory reduction of nitrate sequentially to nitrite, nitric oxide, nitrous oxide and nitrogen gas (Figure 1; e.g., Zehr and Ward, 2002; Einsle and Kroneck, 2004). Denitrification is a significant N sink, especially in oxic/anoxic interfaces in both sediments and suboxic waters (Bonin et al., 1998; Christensen et al., 2000; Zehr and Ward, 2002). Dissimilatory nitrate reductase, which also contains Mo co-factor, catalyzes the reduction of nitrate to nitrite during dinitrification. Dissimilatory nitrate reductase has been well studied in sediments (e.g., Kaspar, 1983; Koike and Sorensen, 1988; Nielsen and Glud, 1996), but

less so in the water column (Ward et al., 1989). Zehr and Ward (2002) further suggested that dissimilatory nitrate reductase could be important in suboxic waters where dinitrification occurs. In addition, the Mo enzyme, nitrite oxido-reductase, also widely exists in chemoautotrophs, and it oxidizes ammonia in the nitrification process (Kroneck and Abt, 2002). Therefore, it is reasonable to hypothesize that bacteria and/or phytoplankton growing in suboxic regions of the ocean may have a higher Mo requirement due to the fact that Mo-containing dissimilatory nitrate reductase and nitrite oxido-reductase are required for denitrification and nitrification.

c) Relevance of Mo and V in the sulfur and carbon cycles

Sulfur occurs in two amino acids, cysteine and methionine, and sulfur is also a component of a couple of vitamins and essential metabolites. Microbes can transform sulfur from its most oxidized form, sulfate, to its most reduced state, sulfide. In spite of a few Mo and V enzymes involved, these enzymes are essential in the sulfur cycle (as in Figure 3; Hagedorn, 2006; Todar, 2006). As one of the most important containing Mo-cofactor, the sulfite oxidase, catalyzes conversion of sulfite to sulfate, which is the terminal step in metabolizing sulfur containing compounds (e.g., cysteine and methionine; Kisker, 2002). In sulfite oxidase catalyzed reactions, Garner et al. (1982) suggested that Mo (VI) possibly first reduced to Mo (IV) by two electron transfer, and further switched to Mo (V) by transferring a reducing equivalent. Hill and Massey (1985) further pointed out that about 50% of Mo was found present as Mo (V) in sulfite oxidase enzymes. Although sulfite oxidase has been mainly studied in mammalian

tissues, especially liver (Kisker et al, 1997; 2002), it can also exist in other living organisms (e.g., bacteria and plants; Cramer et al., 1979).

Besides sulfite oxidase, Mo containing dimethylsulfoxide (DMSO) reductases are another large and diverse group of enzymes found in bacteria and archaea (McEwan et al., 2002), and they play a particularly important role in anaerobic respiration (including the dissimilatory reduction of certain toxic oxoanions; e.g., McEwan et al., 2002). Generally, Mo-DMSO reductases catalyze dimethylsulfoxide (DMSO) to dimethylsulfide (DMS), by switching states from Mo (IV) to Mo (VI) within the enzyme. The Mo (IV) state was regenerated by two subsequent one-electron transfer reactions after passing through the Mo (V) state (Kisker et al., 1997), and therefore all three states of Mo-DMSO reductases exist naturally as the following: Mo (IV), Mo (V) and Mo (VI). Mo is also involved in polysulfide reductase, which converts polysulfide (as sulfur) to H_2S (Stiefel, 1993; 1997; 2002). Compared to Mo, V is less involved in sulfur cycling, and vanadium-dependent haloperoxidases can mimic sulfideperoxidases, converting thioethers to sulfoxides (ten Brink et al., 2001; Rehder, 2003).

As the backbone of all organic molecules, carbon is the most prevalent element in cellular material. Many Mo containing enzymes are essential in the biological carbon cycle (as listed in Table 1). Generally, Mo-based enzymes are required in the formation of methane and in the oxidation of formate, carbon monoxide, and various aldehydes (Kisker et al., 1997; Stiefel, 1997; Mendel and Schwartz, 1999; Mendel and Hansch, 2002). For example, xanthine oxidase and aldehyde oxidase (or oxidoreductase) catalyze oxidative hydroxylation reactions of aldehydes and aromatic heterocyclic compounds by switching different oxidation state of Mo in the enzymes (Hille and Massey, 1985; Hille,

1996). Especially, xanthine oxidase is one of the most extensively studied proteins due primarily to the fact that large amounts of this enzyme can be conveniently isolated from cow's milk (Hille and Massey, 1985). Xanthine oxidase, containing the Mo-cofactor as the active site, is also able to hydroxylate a wide range of purines, pteridines and other aromatic heterocycles (Hille, 1999; Mendel and Schwartz 1999). However, xanthine oxidase is not only widely found in animal tissues (e.g., Al Khalidi and Chaglassian, 1965; Rajagopalan and Handler, 1967), but also in xanthine-metabolizing bacteria (Woolfolk and Downard, 1977). Aldehyde oxidase (or oxidoreductase) has been extensively investigated since it is ubiquitous in animals, plants, and microorganisms (e.g., Sekimoto et al., 1997). Aldehyde oxidase catalyzes the oxidation of a variety of aldehydes and N_2-containing heterocyclic compounds in the presence of O_2 or certain dyes (Rajagopalan and Handler, 1966; Hall and Krenitsky, 1986; Krenitsky et al., 1986). In plants, aldehyde oxidase, containing Mo co-factor, is possibly involved in plant hormone biosynthesis (e.g., indole-3-acetine and abscisic acids; Sekimoto et al., 1997).

d) Relevance of V in halide cycles

Besides the occurrence in V nitrogenase and nitrate reductases, V-dependent haloperoxidases oxidize halides in almost all classes of marine algae, e.g., brown alga *Ascophyllum nodosum* and the red alga *Corallina officinalis* (Wever et al., 1985; Sakurai and Tsuchiya, 1990; Butler, 1998; Butler et al., 2004). Vanadium-containing haloperoxidases are able to catalyze oxidation by peroxide of a halide (X-) to the corresponding hypohalous acid (Plass, 2002; Rehder, 2003) according to the following equation:

$$H_2O_2 + X^- + H^+ \rightarrow H_2O + HOX$$

HOX may further react with a broad range of organic substrates (e.g., nucleophilic substances) to form a diversity of halogenated compounds (Plass, 2002; Rehder, 2003), some of which are volatile (e.g., Moore et al., 1995; 1996; Scarratt and Moore, 1999). These haloperoxidases require V as an essential cofactor for enzyme activity (Krenn et al., 1989; Rehder, 2003), but both V (V) and V (IV) exist in these algae (Crans et al., 1989; 2004).

These V-containing haloperoxidases are named after the most electronegative halide they can use, and thus a V chloroperoxidase (V-CPO) is able to oxidize chloride, bromide and iodide; bromoperoxidase oxidizes bromide and iodide; and iodoperoxidase oxidizes iodide (Plass, 2002; Ohshiro et al., 2004). Bromoperoxidase has been characterized in *Corallina* (Itoh et al., 1986; Sheffield et al., 1993), *Ascophyllum nodosum* (Wever et al., 1985), and *Ulvella lens* (Ohshiro et al., 1999), while iodoperoxidase has been characterized in *Laminaria digitata* (Colin et al., 2003). Chloroperoxidase is the only one that is not characterized in marine macroalgae, but from a terrestrial fungus *Curvularia inaequalis*, which also evolved from a marine ancestor (Messerschmidt and Wever, 1996; Messerschmidt et al., 1997; Butler, 1998).

In addition to macrophytes, a variety of halogenated compounds are also found in many marine phytoplankton species (e.g., Butler, 1988; Moore et al., 1995). It has been reported that volatile halogenated organic compounds (e.g., halogenated methanes) were produced by marine phytoplankton in polar areas (Sturges et al., 1992; Tokarczyk and Moore, 1994; Tait and Moore, 1995; Moore et al., 1995; 1996). Moore et al. (1996) further reported that, under laboratory conditions, marine diatoms could potentially

produce various brominated and iodinated volatile compounds (e.g., $CHBr_3$, CH_2Br_2, CH_2I_2, and CH_2ClI), and V dependent bromoperoxidases and iodoperoxidases were further identified in these marine phytoplankton cultures. As carriers of chlorine, bromine, and iodine into the atmosphere, these halogenated methanes play an important role in transporting halides to the troposphere, causing the destruction of tropospheric ozone (Barrie et al., 1988; Kritz et al., 1993). For example, these organic halides were strongly correlated with the Arctic troposphere ozone layer destruction (e.g., Barrie et al., 1988). Therefore, V containing haloperoxidases are important for controlling the cycles of halides and volatile halogenated methanes, and even regulating the ozone concentrations in the atmosphere, although the function of these V containing haloperoxidases, especially in marine phytoplankton, is still not entirely understood.

In summary, Mo-containing enzymes hold key positions in the biogeochemical cycles of carbon, nitrogen and sulfur (Stiefel, 2002) and in the metabolism of every organism. Vanadium can replace Mo in some enzymes (e.g., nitrogenases and nitrate reductaces), which becomes critical when Mo availability is low (Howarth et al., 1988), or under extreme conditions (e.g., low temperature and high alkalinity; Antipov and Sorokin, 2003). In addition, another group of V-containing enzymes, V dependent haloperoxidases, play a critical role in cycles of halides by catalyzing the production of volatile halogenated methanes, which as carriers of halides, transfer halides from the ocean to the atmosphere and contribute to the regulation of ozone found in the troposphere (e.g., Moore et al., 1995; 1996).

MO AND V IN NATURAL WATERS

a) Concentration range and distributions

Concentrations, sources and sinks of Mo and V in the ocean are presented in Table 2. Mo and V are the most abundant transition elements in the modern ocean (~105 nM Mo and 34-45 nM V; Morris, 1975; Collier, 1984; 1885), and far more abundant than Fe (0.01-1 nM, Butler, 1998). The principal Mo chemical species found in oxic seawater, molybdate (MoO_4^{2-}), behaves relatively conservative (with salinity normalized concentrations) in the open ocean (Figure 4; Morris, 1975; Sherrell and Boyle, 1988). Although Mo is widely used in several critical enzymatic systems in marine organisms (e.g., nitrogenase and nitrate reductases; Falkowski, 1983), this requirement may not be necessarily reflected by the distribution of the total dissolved Mo concentrations in oligotrophic oceans (e.g., Morris, 1975; Tuit and Ravizza, 2003).

V exists mainly in the form of vanadate ($H_2VO_4^-$), and its distribution is nearly conservative. V distribution is characteristic of other algal nutrients, with a slight depletion in surface waters of the open ocean (Figure 4; Morris, 1975; Collier, 1984; 1985; Sherrell and Boyle, 1988). V can replace Mo in nitrogenases and nitrate reductases, and these enzymes are mainly expressed when Mo is not abundant, or under some extreme conditions (e.g., Loveless et al., 1999; Antipov and Sorokin, 2003). On the other hand, V is also required in haloperoxidases not only by macrophytes, but also by marine phytoplankton (Moore et al., 1995; 1996). As an essential element, V is virtually required by all plants (e.g., V ions can play a role in biology as counter-ions for proteins, DNA, RNA, and in various biological organelles; Crans et al., 2004). Vanadium ions also have many structural roles in plants due to structural and electronic analogy to

phosphorus in plants (e.g., Chasteen, 1983; Crans et al., 2004). Hence, it is reasonable to expect that phytoplankton uptake could be one of reasons for the surface depletion of V observed in the oligotrophic oceans as shown in Figure 4.

Mo and V are also enriched in strongly reduced sediments. In enclosed basins, such as the Cariaco Trench, the Black Sea and the Saanich Inlet, concentrations of Mo and V in sediments can be as high as 140 and 400 μg/g respectively (Berrang and Grill, 1974; Emerson and Huested, 1991). Both Mo and V have been proposed as a paleoceanographic proxy of oceanic anoxic events in sediment cores (Emerson and Huested, 1991; Thomson et al., 1995; Dean et al., 1999). Nijenhuis et al. (1998) showed an organic matter peak in sediment cores, indicative of an oceanic anoxic event associated with increased surface phytoplankton productivity; Mo and V were also strongly correlated with that peak (as shown in Figure 5; Nijenhuis et al., 1998). Under strongly reducing conditions, molybdate is likely reduced to Mo (V) or Mo (IV) and may precipitate as MoS_2 (e.g., Vorlicek and Helz, 2002; Nameroff et al., 2002; Algeo and Maynard, 2004), while vanadium is reduced to V (IV), or even solid V_2S_3 or V_2O_3 (e.g., Breit and Wanty, 1991; Wanty and Goldhaber, 1992). Although this behavior may explain the observed enrichments in reducing sediments, there are no direct studies on the speciation of these two elements in the ocean yet.

The oceanic residence times of Mo and V are very long, being approximately 800 kyr for Mo and 100 kyr for V with respect to riverine input (Emerson and Huested, 1991). These calculations, however, only apply to the total dissolved pool, and the residence time of the different species of these metals is still unknown. Although river input is one of the major sources of Mo and V to the ocean (Table 2; Morford and

Emerson, 1999), the average Mo and V concentrations in river water are considerably lower than in seawater (5 nM Mo and 15 nM V in world rivers on average; Table 2) (Morford and Emerson, 1999; References therein). Lower concentrations of Mo and V have also been reported in lake waters (e.g., 52 nM Mo in the Lake Donk, Belgium; Dumont, 1972; and 5.1 nM V in the Lake Biwa, Japan; Okamura et al., 2001).

Generally concentrations of Mo and V in freshwater systems are balanced between several major sources and sinks. Weathering reactions are the major sources of Mo and V into freshwater systems (Kaback and Runnells, 1980; van der Sloot, 1985; Shiller and Boyle, 1987; Shiller and Mao, 2000), while sinks involve a combination of algal uptake, and reductive processes followed by removal via coprecipitation with Fe and Mn oxides/ oxyhydroxides (Crusius et al., 1996; Elbaz-Poulichet et al., 1997; Johannesson et al., 2000).

Natural iron hydroxides have a minimum surface charge within the pH range of 6-8 (Parks, 1967), and manganese oxides within the pH range of 3-7 (Harriss and Troup, 1970). In contrast to the small variation in pH observed in seawater (~8.2), pH in freshwater systems varies largely from 5 to 7 (Harriss and Troup, 1970). The most abundant vanadyl species, thermodynamically in equilibrium in natural waters (Table 3), are VO^{2+} and HVO_2^{+} (Coughlan, 1980), and the vanadate species are $H_2VO_4^{-}$ and HVO_4^{2-} (Wehrli and Stumm, 1989). The thermodynamically favored forms of molybdate in natural waters are Mo (VI): MoO_4^{2-} and $HMoO_4^{-}$, and Mo (V): likely MoO_2^{+}, MoO^{3+},$Mo_2O_4^{2+}$ and Mo_3O_8 (as a mixture of Mo (V) and Mo (VI)) (e.g., Bertine, 1972; Loach, 1970; Szilágyi, 1967; Coughlan, 1980). Low particle-reactive oxyanions (e.g., $H_2VO_4^{-}$ and MoO_4^{2-}) are the most abundant chemical species of Mo and V in oxic water,

and the low reactivity of these oxyanions is mainly due to the fact that they are less absorbed onto ferromanganese mineral particles, which are negatively charged at the higher pH values characteristic in seawater (e.g., Morris, 1975). On the contrary, these thermodynamically favored oxyanions tend to be associated with mineral particles in river water, and therefore decrease levels of total Mo and V are observed in rivers. However, although thermodynamically unfavorable under oxic conditions, V(IV) and Mo (V) cations (HVO_2^+ and $Mo_2O_4^{2+}$; Coughlan, 1980) are expected to be less particle-reactive at the lower pH values characteristic of freshwater (e.g., Harriss and Troup, 1970). This is because pH in freshwater is much closer to the point of zero charge of mineral particles, and therefore particle surfaces are much less negatively charged in freshwater (e.g., Parks, 1967; Harriss and Troup, 1970). Nakano et al. (1990) and Hirayama et al. (1992) showed the possible existence of V (IV) cations in natural waters. Howarth and Cole (1985) expected Mo (V) (as in cations) to exist in freshwater as a bioavailable form of Mo.

b) Thermodynamic considerations for the presence of reduced Mo and V species in aquatic systems

Based on oxygen and sulfide concentrations, there are four types of environments that can potentially influence the chemical speciation of Mo and V in natural waters: 1) oxic (> 10 μM O_2), 2) suboxic (1-10 μM O_2), 3) anoxic or nonsulfidic (< 1 μM O_2 and < 1 μM H_2S) and 4) sulfidic (> 1 μM H_2S) (e.g., Nameroff et al., 2002; Zheng et al., 2000).

However, in order to use published Eh-pH diagrams to identify the predominant Mo and V species present in each environment, oxic seawater is defined as a solution

with a pH of 7.9~8.3 and Eh of > 0.6 V (e.g., Vismann, 1996). Suboxic seawater is simply defined as an environment where nitrate reduction (dinitrification) occurred with a Eh is ~ 0.6 V (range: 0.2~0.6 V) and pH ranging from 7.4~7.9 (Zirino, 1975). Anoxic (or nonsulfidic) as an environment where Fe reduction occurred with a pH of 7.2~7.6 and Eh of ~0.0 V (range: 0.0~0.2 V) (Mylyono et al., 1988; Lee et al., 2004). Sulfidic as an environment where sulfate reduction occurred with pH of 6.9~7.4 and Eh of ~-0.2 V (range: -0.4~0.0 V) (e.g., Postgate, 1959; Vismann, 1996). Nameroff et al. (2002) reported that direct Mo sulfide precipitation occurs at above 100 μM H_2S, and therefore two types of sulfidic conditions are further classified: low sulfide (< 100 μM H_2S) and highly sulfidic (> 100 μM H_2S) have also been discussed in this dissertation research in order to show the different redox reactions of Mo. The different redox conditions are shown in the Eh-pH diagrams of V and Mo (Figures 6 and 7).

Theoretically, both pH and Eh strongly dictate the speciation of Mo and V in natural environments: Mo (VI) and V (V) generally dominate under oxic conditions (pH=~8.0, and Eh=0.7 V), while reduced Mo and V, Mo (V) and V (IV) dominated in anoxic environments (pH=7.2~7.6 and Eh=~0.0 V) (Figures 6 and 7; Brookins, 1988; Peacock and Sherman, 2004). Under sulfidic conditions (pH=6.8~7.4, and Eh=~-0.2 V), Mo and V was further reduced to solid forms as MoS_2 and V_2O_3 or V_2S_3.

The Eh dependence of Mo, V and some other elements in seawater and freshwater are presented in Figure 8 (modified from Turner et al., 1981). In natural seawater (with pH of 8.2), the Eh of V (V)/(IV) is relatively high (~0.2 V), and slightly above the biologically relevant potential of 0.1 V for the Fe (III)/(II) couple (Rehder, 2003), suggesting that reduced V could exist in the presence of Fe (II). The Eh of Mo (VI)/(V)

is relatively low (Eh= ~-0.2 V) in seawater (Figure 8), similar to anoxic conditions when sulfate reduction occurs, suggesting that reduced Mo exists under sulfidic conditions.

In freshwater, the Eh values of Mo (VI)/(V) and V (V)/(IV) are ~0.2 and 0.4 V respectively (as shown in Figure 8; modified from Turner et al., 1981), higher than the values for seawater, suggesting that reduced forms of both elements are more likely to be present in freshwater systems under anoxic (Eh: 0.0~0.2 V as defined above) or suboxic (Eh: 0.2~0.6 V) conditions.

Within the range of pH and Eh values of suboxic conditions (as defined above), reduced V(IV) as $H_2VO_4^-$ and VO^{2+} can exist in seawater (Wehrli and Stumm, 1989; Peacock and Sherman, 2004). In addition, organic chelation may extend the stability field of V(IV) to Eh values of about +0.4 V (Wehrli and Stumm, 1989; Elbaz-Poulichet et al., 1997). Therefore, reduced forms of V(IV) could likely be found with significant concentrations under higher Eh conditions (e.g., where Fe reduction occurred as in Figure 8). Elbaz-Poulichet et al. (1997) suggested the possible existence of reduced V forms, complexed with organic ligands in Lake Balaton, Hungary. Ladd (1974) reported that V (IV) accounted for about 1% of total V in natural seawater, and Emerson and Huested (1991) also detected the existence of V (IV) under natural seawater conditions.

The redox potential of the Mo (VI)/(V) couple could increase by 0.55 V after complexing with organic ligands (Lee et al., 2004) as Mo is easily complexed with organic matter (Brumsack and Gieskes, 1983; Coveney et al., 1991; Elbaz-Poulichet et al., 1997). The (pseudo-) redox potential for Mo (VI)/(V) could be as high as 0.14 V at pH=7.0 in DMSO reductases (Bastian et al., 1991; McMaster and Enemark, 1998). Williams and Fraústo da Silva (2002) also reported a redox potential for Mo (VI)/(V) of

0.18 V at pH=7.0 in nitrate reductases. These (pseudo-) redox potentials fall within anoxic conditions (Eh: 0.0 ~ 0.2 V as defined in this dissertation).

OBJECTIVES OF THIS DISSERTATION

In this chapter the biological importance of Mo and V in different enzymatic pathways in the C, N, S and halide cycles was established. However, chemical speciation studies have ignored these trace metals for many years, and successful techniques for isolating the different chemical species of Mo and V in fresh and seawater have not yet been reported. Thermodynamic calculations suggest that reduced forms of Mo and V are thermodynamically favored under suboxic and anoxic environments, such as those found in suboxic and anoxic waters, and reducing sediments. These environments, possibly as sources, contribute to the existence of reduced Mo and V in natural waters. Therefore, the main objectives of this dissertation are to establish chemical protocols for separating the different chemical species of Mo and V in seawater, and to examine the existence and sources of reduced Mo and V in specific waters, e.g., at the head of Peconic River Estuary, and in Long Island Sound. Early diagenetic processes of Mo have been carefully examined to study the dynamics of concentration and speciation of Mo in sediment porewater at Flax Pond.

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TABLE CAPTIONS

Table 1: Major enzymes of molybdenum and vanadium in the biological systems.

Enzymes	Co-factor	Reactions catalyzed
N cycle		
Mo nitrogenase[a]	Mo-Fe-co	Dinitrogen to ammonia
Mo Nitrate Reductase[b,c,d]	Mo-co	Nitrate to nitrite
Nitrite oxidase[f]	Mo-co	Nitrite to nitrate
V nitrogenase[e]	V-Fe-co	Dinitrogen to ammonia
V Nitrate reductase[e]	V-co	Nitrate to nitrite
N-Heterocyclic metabolism		
Xanthine dehydrogenase[f]	Mo-co	Hypoxanthine and xanthine to uric acid
Xanthine oxidase[b,c,d]	Mo-co	Xanthine to uric acid
Trimethylamine N-oxide reductase[f]	Mo-co	Trimethylamine N-oxide to trimethylamine
S cycle		
Sulphite oxidase[b,c,d]	Mo-co	Sulphite to sulphate
Dimethylsulfoxide reductase[b,c,d]	Mo-co	DMSO to DMS
Sulfideperoxidase[e]	V-co	Thioethers to sulfoxides
Polysulfide reductase[f]	Mo-co	Polysulfide or S to H_2S
Biotin sulfoxide reductase[f]	Mo-co	Biotin sulfoxide to biotin
Tetrathionate reductase[f]	Mo-co	Tetrathionate to thiosulfate
C cycle		
Formate dehydroferase[b,c,d]	Mo-co	Formate to CO_2
Carbon monoxide oxidoreductase[f]	Mo-co	CO to CO_2
N-formyl methanofuran dehydrogenase[f]	Mo-co	CO_2 and methanofuran to CHO-methanofuran
2-furoyl dehydrogenase[f]	Mo-co	2-furoyl-CoA to S-(5-hydroxy-2-furoyl)-CoA
Aldehyde oxidase[f]	Mo-co	Aldehyde to carboxylic acid
Aldehyde dehydrogenase[f]	Mo-co	Chloroacetaldehyde to chloroacetate
Pyridoxal oxidase[f]	Mo-co	Pyridoxal to 4-pyridoxate
Halide cycle		
Haloperoxidases[e]	V-co	Halide to hypohalous acid

a: Williams and Fraústo da Silva, 2002; b: Kisker et al., 1997; c: McMaster and Enemark, 1998; d: Mendel and Hansch, 2002; e: Rehder, 2003; f: Stiefel, 1997

Table 2: Critical concentrations for Mo and V in the ocean (Morford and Emerson, 1999; references therein; n=negligible).

	Mo	V
Concentrations		
Ocean, dissolved (nM)	105	35–45
River, dissolved (nM)	5	15
Marine sed. oxic (ppm)	8	130
Marine sed. anoxic (ppm)	50	200–400
Source fluxes (10^8 mol/yr)		
River	1.8	5.4
Dust	n	0.1
Continental margin sediments	0.08-0.17	3-8
Hydrothermal: high and low temperature	n	n
Total sources	1.88-1.97	8.5-13.5
Sink fluxes (10^8 mol/yr)		
Sediments: oxic: deep ocean basin	0.9	4.3
Sediments: continental margin	n	n
Sediment anoxic:	0.2-0.8	0.5
Mn nodules and metalliferous sediments	n	5.4
Hydrothermal: high and low temperature	0.04-0.17	5.4
Total sinks	1.1-1.9	11.1
Steady state residence time (kyr)	800	100
w.r.t. River inflow (kyr.)		

FIGURE CAPTIONS

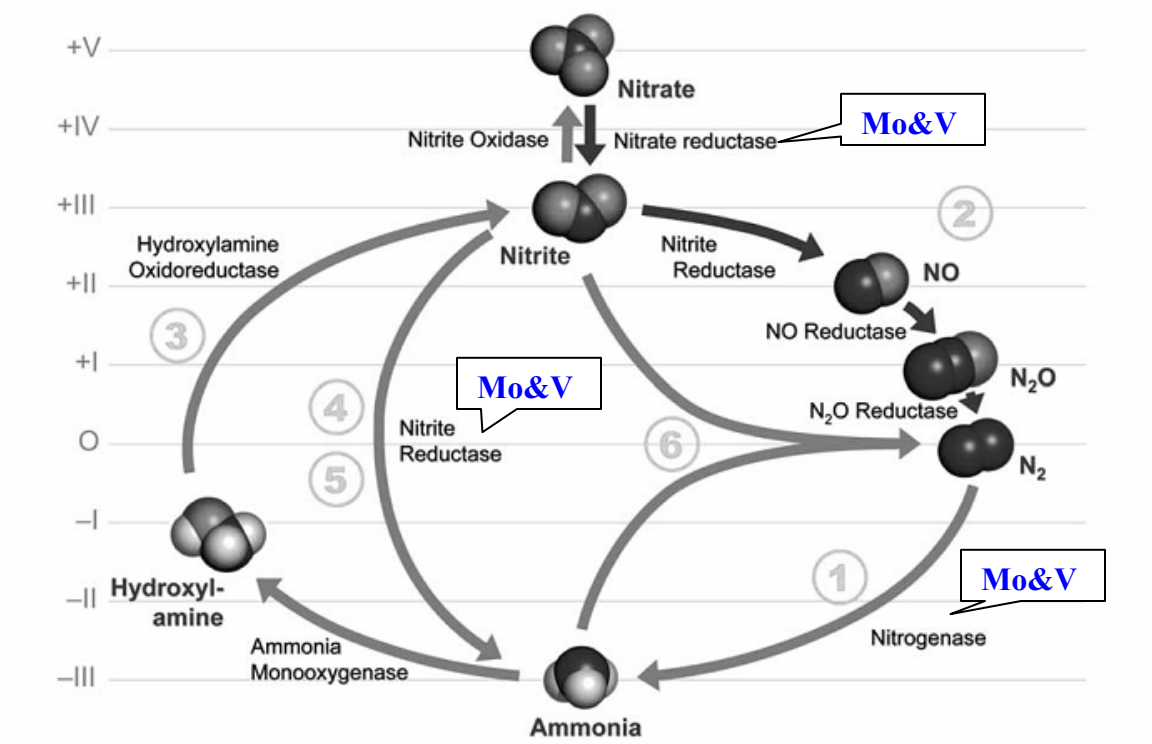
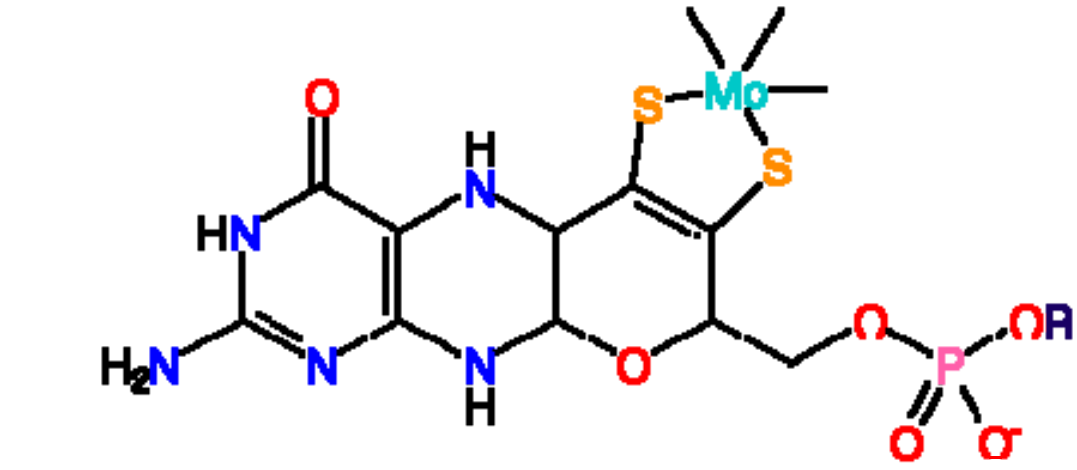
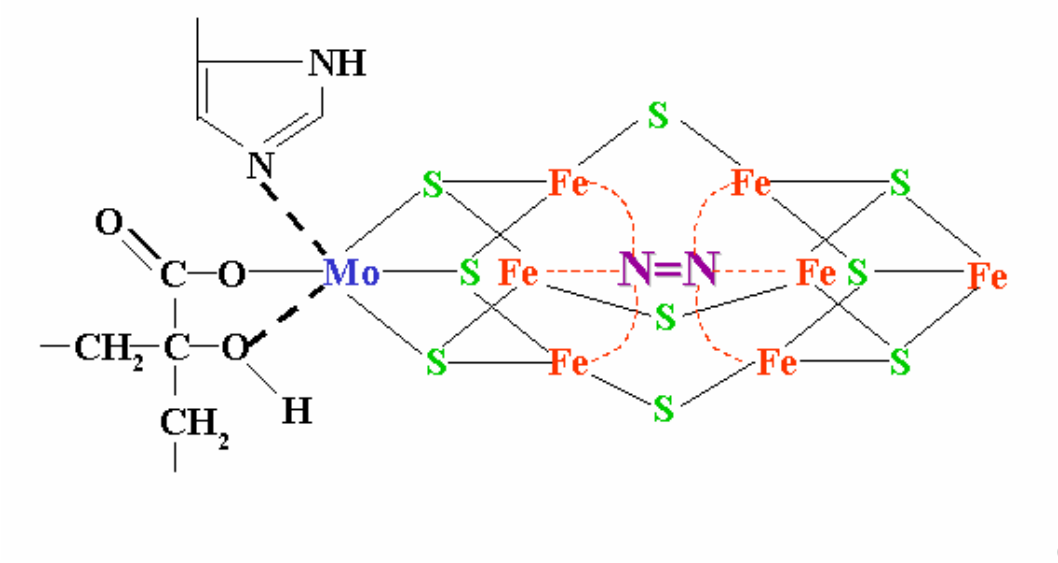


Figure 1: Mo, V and their roles in the biogeochemical cycle of nitrogen (Adapted from Einsle and Kroneck, 2004).



(a)



(b)

Figure 2: Schemes of Mo cofactor containing enzymes (Mo-co) (a) and Mo-Fe cofactor (b) containing enzymes (Mo-Fe-co) where Mo could be replaced by V or Fe (Modified from Hille, 1996; Kisker et al., 1997; Heldt, 1998).

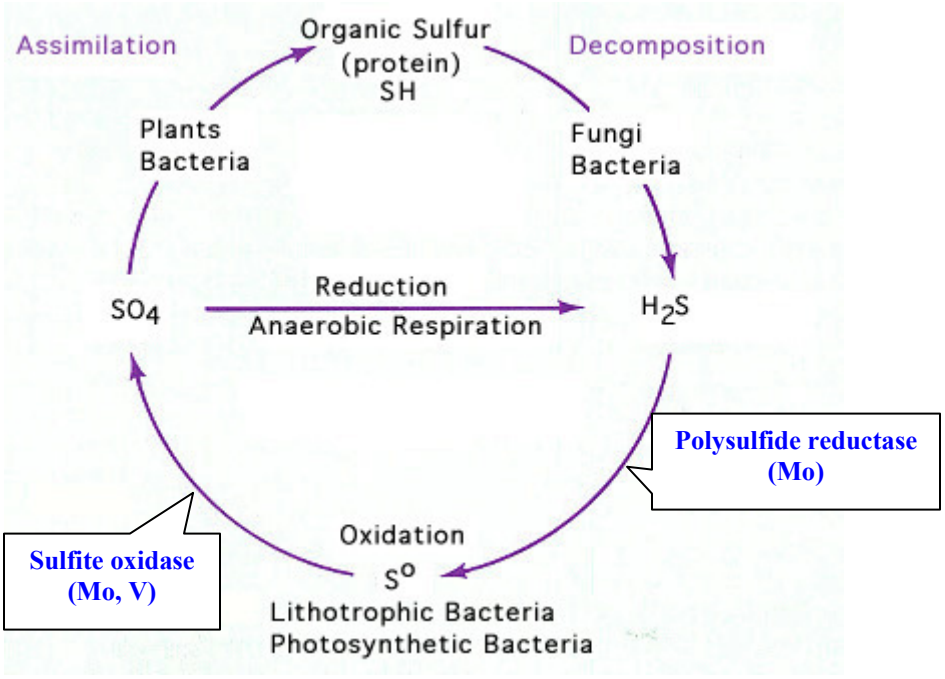


Figure 3: Mo, V and their roles in the biological sulfur cycle (modified from Hagedorn, 2006 and Todar, 2006).

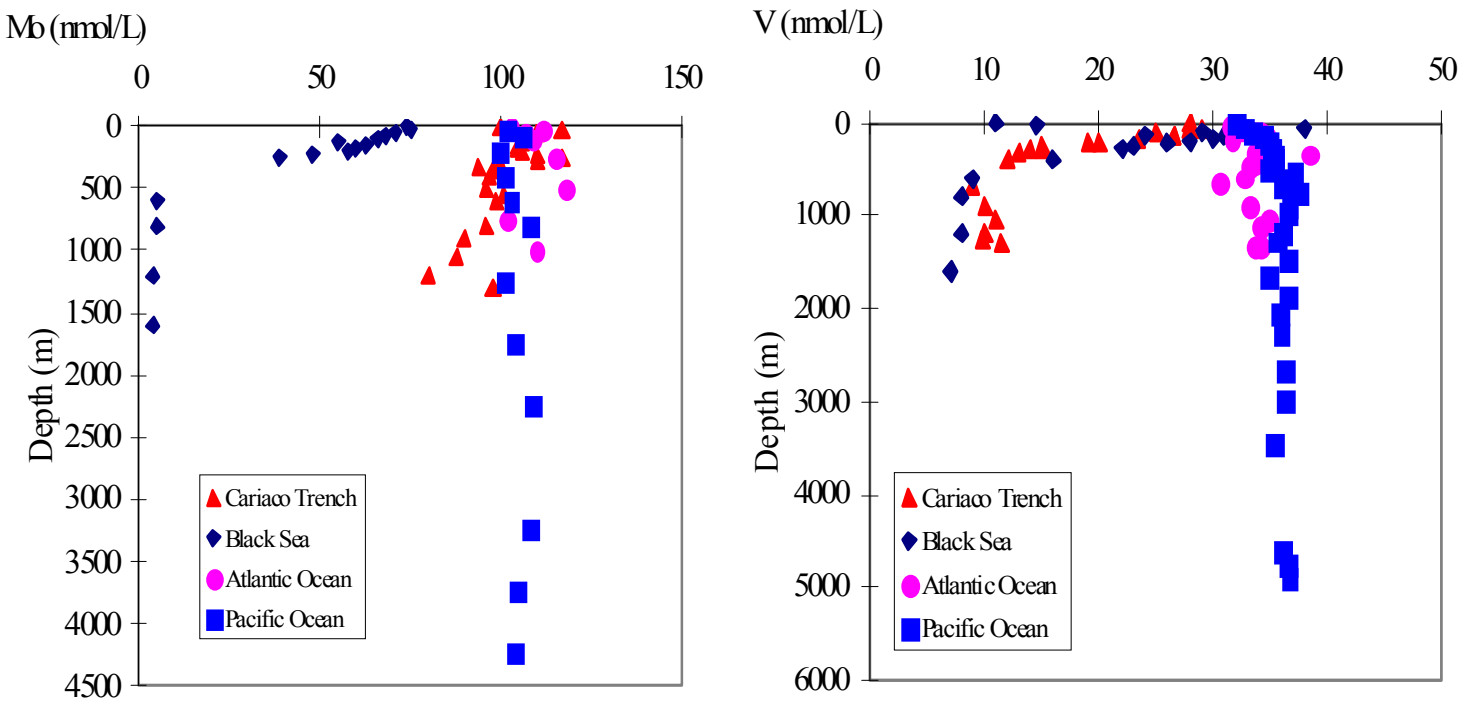


Figure 4: Vertical profiles of molybdenum (left) and vanadium (right) in the ocean (both Mo and V data in the Cariaco Basin and Black Sea are from Emerson and Huested, 1991; molybdenum data in the Atlantic and Pacific Ocean from Morris, 1975; vanadium data in the Atlantic and Pacific Ocean from Collier, 1984 and Sherrell and Boyle, 1988).

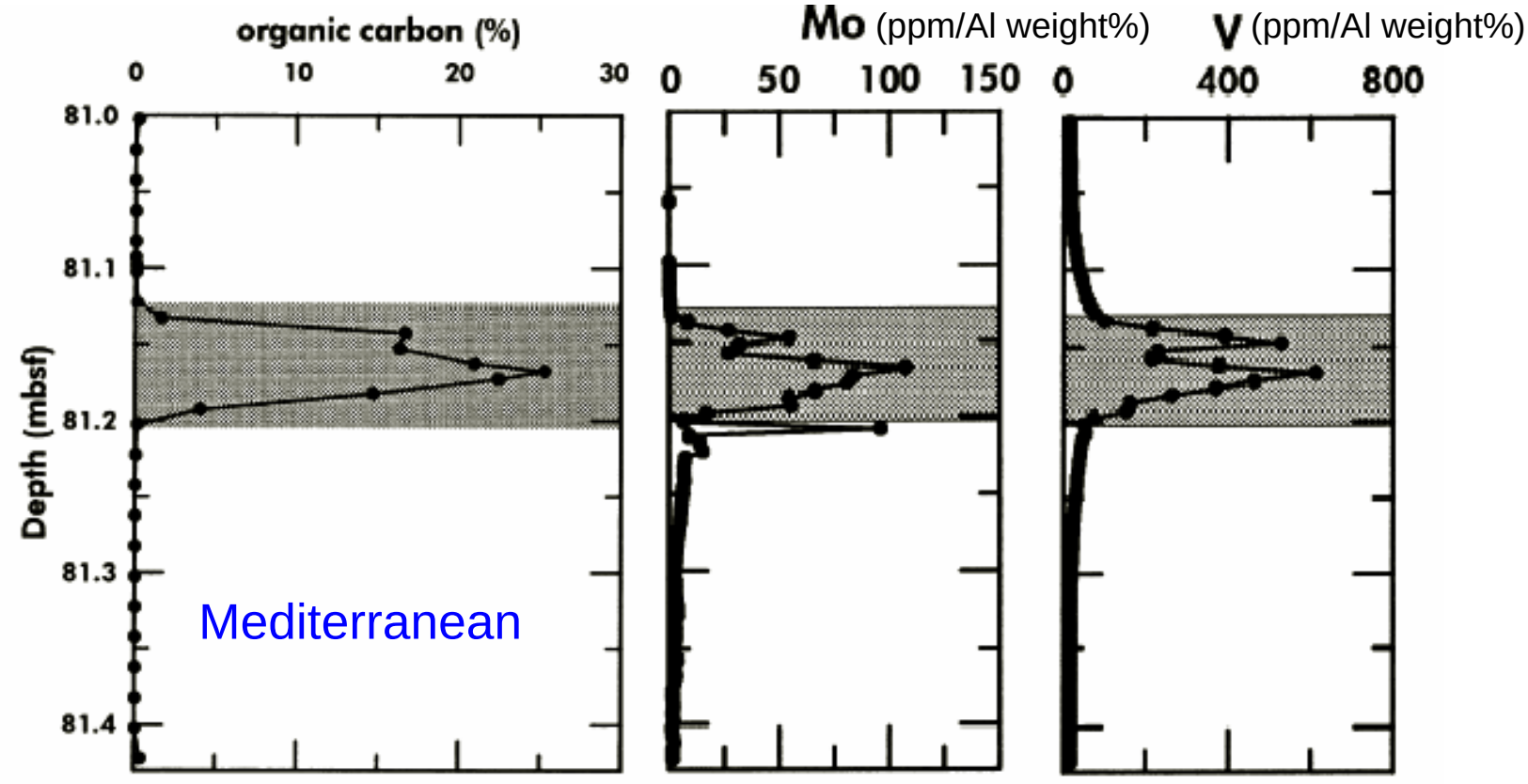


Figure 5: Organic carbon content (a), Mo (b) and V (c) profiles in a sediment core from the Mediterranean (Mo and V are shown as ratios to Al: ppm/wt%; percentage of organic carbon is weight percentage; mbsf: meters below seafloor) (Adapted from Nijenhuis et al., 1998).

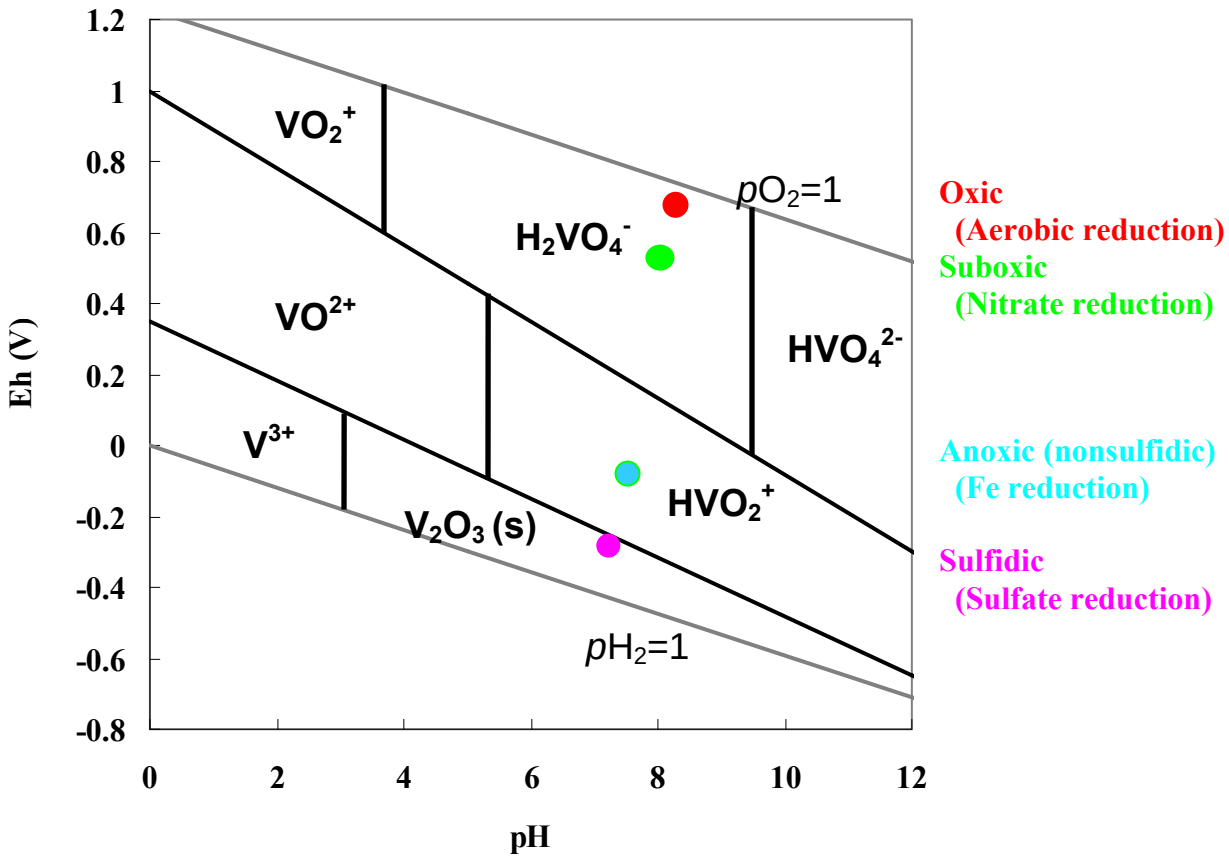


Figure 6: Eh-pH diagram for vanadium species in aqueous environments (The pH-Eh calculated for different redox conditions are also shown; Assuming activity for dissolved V=10^{-7} M; modified from Peacock and Sherman, 2004; V(IV) forms are from Pope et al., 1980).

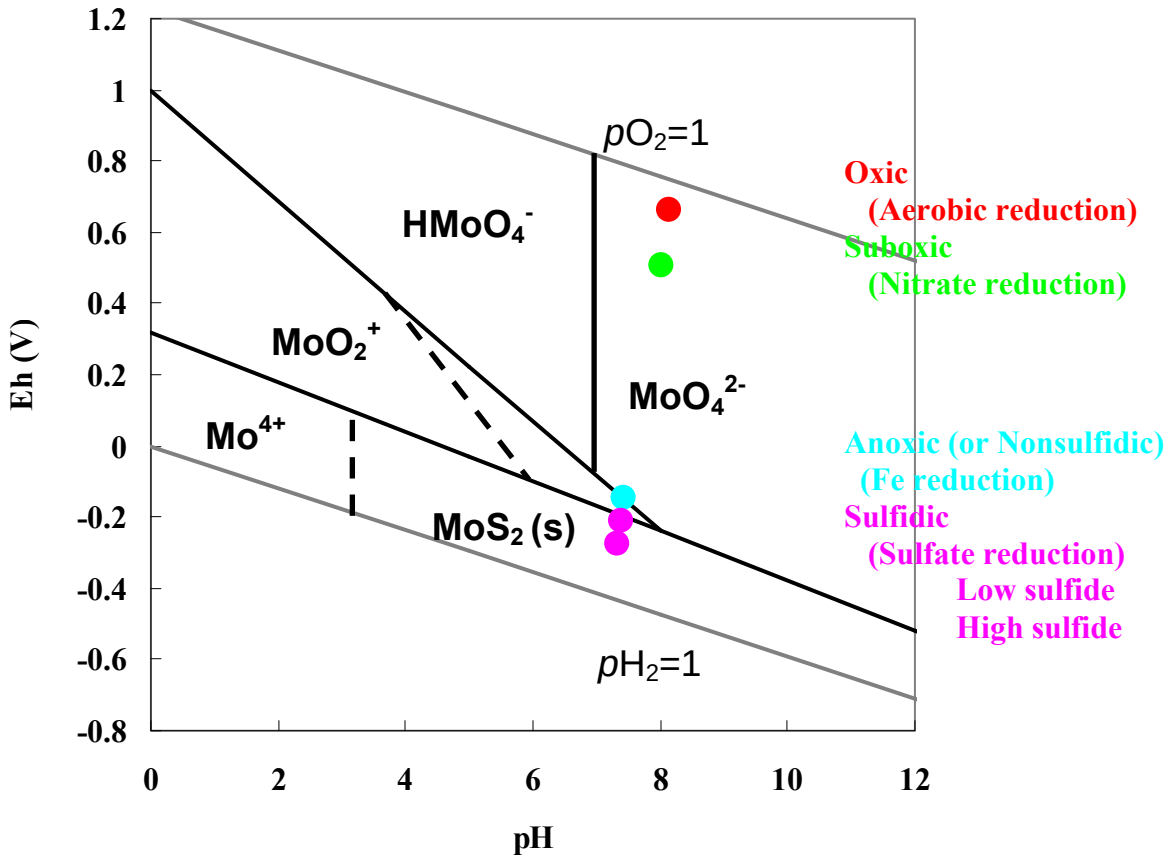


Figure 7: Eh-pH diagram for molybdenum species in aqueous solutions (The pH-Eh calculated for different redox conditions are also shown; Assuming activity of dissolved Mo=10^{-7} M, and Sulfide=10^{-3} M, modified from Brookins, 1988).

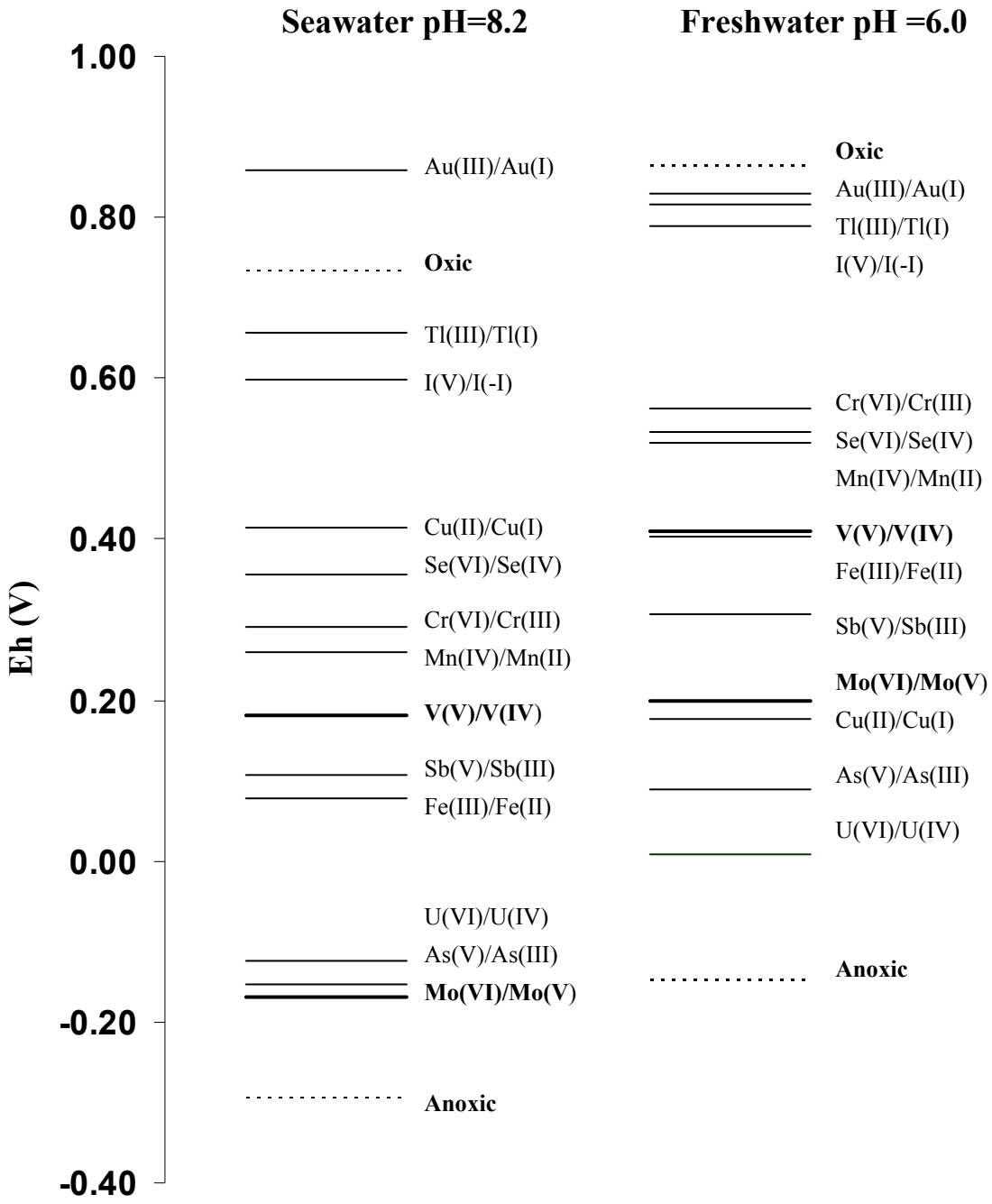


Figure 8: Eh values with potentially variable oxidation states in seawater and freshwater (Redrawn after Turner et al., 1981; Mo and V data are from Brookins, 1988 and Peacock and Sherman, 2004).

CHAPTER TWO: DEVELOPMENT OF AN ANALYTICAL PROTOCOL FOR THE DIRECT DETERMINATION OF V (IV) AND V (V) IN SEAWATER

ABSTRACT

A new method is developed for differentiating and determining V (IV) and V (V) in seawater by using anion exchange separation procedure. The developed method includes Chelex 100 resin solid-phase extraction at pH=4.5 using Chelex 100 resin, elution with a base (for V (V)) and an acid (for V (IV)), and subsequent quantification by graphite furnace atomic absorption spectrometry. The recovery of V species in synthetic seawater was >95% for V (V) and 92% for V (IV) at a concentration of 40 nM. Stability experiments showed V (VI) was relatively stable over 24 h. The detection limit for V (IV) and V (V) was on the order of 0.5 nM. The analytical precision is ~10% in the concentration range of 10 nM.

This new method was applied to coastal water samples, collected at the head of Peconic River Estuary and Long Island Sound during spring and summer 2005. Concentrations of dissolved total vanadium, ranged from 6.0 to 35 nM in both the Peconic River Estuary and Long Island Sound. Vanadium speciation varied seasonally and spatially in response to redox changes. During spring 2005, reduced V (IV) accounted for less than 10% in coastal waters of LIS (with pH=7.8) to more than 50% of total V at the head of Peconic River Estuary (with pH=~7.5). During summer 2005, V (IV) accounted for less than 10% in central LIS (pH=8.0-8.3) to more than 30% in waters near the New York City (pH=7.5). Possible reducing agents influencing the reduction of V (V) in our study areas include H_2S, Fe^{2+}, Mn^{2+} and peroxide. Additional sources of reduced V to the LIS could be diffusion from reducing sediments, and input from sewage effluents near the NY city.

INTRODUCTION

Both V (IV) and V (V) exist in well-aerated freshwater and seawater (e.g., Evans and Garrels, 1958; Kalk, 1963), although theoretically V (V) is expected to be the thermodynamically stable form under oxic conditions and V (IV), the stable form under reducing conditions (e.g., Peacock and Sherman, 2004). Vanadium is very abundant in the open ocean with concentrations ranging between 34 and 45 nM in the Pacific and Atlantic Oceans (Collier, 1984; Morris, 1975). Vanadium concentrations, however, are much lower in reducing waters (e.g., about 10 nM in water below 600 m in the Cariaco Trench and Black Sea; Emerson and Huested, 1991). In contrast, V levels are very high in anoxic sediments in those basins reaching concentrations as high as 200-400 μg/g (Emerson and Huested, 1991). In mildly reducing (suboxic) environments, V (V) as vanadate is first reduced to V (IV) as vanadyl, which may be adsorbed onto organic particles, and therefore removed from the water column (Szalay and Szilágyi, 1967; Emerson and Huested, 1991; Morford and Emerson, 1999). Under more strongly reducing (sulfidic) conditions, sulfides causes V to be further reduced to V(III), as solid V_2O_3 or hydroxide $V(OH)_3$ (Breit and Wanty, 1991; Wanty and Goldhaber, 1992; Algeo and Maynard, 2004), which led to high V enrichment observed in anoxic sediments (e.g., Emerson and Huested, 1991). While thermodynamic calculations have been used to describe these V speciation changes, the different chemical redox species of V in the ocean have never been reported.

V is an essential element, i.e., lack of this element is lethal, but at high levels it is toxic. Toxicity and bioavailability of vanadium, however, are much more dependent on speciation than on the total pool: V (V) being the more toxic form (e.g., Patel et al., 1990;

Willsky et al., 1984; Taylor and van Staden, 1994). Both species of V are used by plants and animals, and both V (IV) and V (V) exist in phytoplankton and bacteria (e.g., Crans et al., 1989; Baran, 2000). However, V (IV) is expected to be the dominant form in intercellular media (Willsky et al., 1985). Vanadium forms part of active centers involved in V-dependent enzymes, e.g., V nitrogenases and V haloperoxidases (e.g., Rehder, 2003) and play an important role in nitrogen fixation in some diazotrophs (e.g., *Azotobacter*; Dilworth et al., 1987), and the cycle of halides in diatoms and marcoalgae (Moore et al., 1996; Butler, 1998).

Vanadium can be released into the environment from burning fossil fuels and from various industrial processes (Bertine and Goldberg, 1971; Zoller et al., 1974; Shiller and Mao, 2000; Pyrzyńska and Wierzbicki, 2004) as this element is widely used in several industrial processes such as in the production of special steels, temperature-resistant alloys, pigments and paints (e.g., Pyrzyńska and Wierzbicki, 2004).

In recent years, several studies have reported measurements of total vanadium in environmental samples such as in seawater (Emerson and Huested, 1991), river water (Shiller and Mao, 2000), biological and sediment samples (Blotcky et al., 1979; Colina et al., 2005), using an array of different analytical techniques including radiochemical neutron activation analysis (Repinc et al., 2005; Repinc and Benedik, 2005), electro-thermal atomic absorption spectrometry (Aucelio et al., 2004; Nukatsuka et al., 2002), high performance liquid chromatography (Vachirapatama et al., 2002; Haddad et al., 2005), inductively coupled plasma atomic emission spectrometry (Coetzee et al., 2002; Fan et al., 2005), mass spectrometry (Krushevska et al., 1998; Yang et al., 2002), and neutron activity (e.g., Arroyo and Brune, 1972; Blotcky et al., 1979).

Several studies have also attempted to measure vanadium speciation in environmental samples (e.g., Starczewska, 2002; Filik et al., 2004; Fan et al., 2005) using different methods such as coprecipitation (Weisel et al., 1984; Fujiwara et al., 1986), solvent extraction (Remya and Reddy, 2004) ion exchange chromatography (Khuhawar et al., 2002; Huang et al., 2002; Zhang et al., 2003), capillary electrophoresis (Chen and Naidu, 2002), and liquid and solid phase extraction (e.g., Nakano et al., 1990; Minelli et al., 2000; Cowan et al., 2000; Okamura et al., 2001; Pyrzyńska and Wierzbicki, 2004). However, most studies indirectly obtained V (IV) concentrations by subtracting V (V) levels from the total vanadium (e.g., Zhao et al., 2006). In addition, all the above studies were conducted under oxic conditions without taking in consideration the kinetics of oxidation due to long periods of sample manipulation and analysis. Therefore, the objective of this study was to develop a quick and precise method of measuring different chemical species of vanadium, V (IV) and V (V) in oxic and suboxic seawater.

The technique to separate the different redox species of V is based on solid-phase extraction. Solid phase extraction has been widely used for differentiating metal species in aqueous solution (e.g., Minelli et al., 2000; Pyrzyńska and Wierzbicki, 2004) due to its high enrichment factor, efficiency and handling simplicity (Fang, 1991; Pyrzyńska and Trojanowicz, 1999). For example, the chelating resin Chelex 100 is a styrene divinylbenzene copolymer containing iminodiacetate ions, with a very strong attraction for transition metals, even in highly concentrated salt solution (e.g., Chan and Riley, 1966; Chandra et al., 1988; Dupont et al., 1991). Chelex 100 also acts as a cation exchanger at higher pH, and an anion exchanger at lower pH. Both cations and anions can be adsorbed onto the Chelex 100 resin in the pH range of 4.0-7.4 (as described in the

manufacturer's manual). Soldi et al. (1996) showed that, in a batch method, both vanadium species (V (V) as in the form of $H_2VO_4^-$ and V (IV) as in VO^{2+}) were sorbed on Chelex 100 at pH of 3-6 in a nitrogen-enriched atmosphere. Furthermore, V (V) and V (IV) were eluted under basic (pH>10), and acidic (pH<0.8) conditions respectively. However, the choice of the best operational conditions for sorption and elution of different V ions is still a problem which has been mostly dealt with on a purely experimental basis (Soldi et al., 1996; Pyrzyńska and Wierzbicki, 2004). In addition, the batch method requires a very long sorption time of ~2 h, which increase the possibility of redox changes of V during the extraction protocol. Based on the method of Soldi et al. (1996), a quick and precise column procedure for separating V (V) and V (IV) in seawater was established with optimized conditions. The method was successfully applied to measure V (IV) and V (V) in coastal waters at the head of the Peconic River Estuary and in Long Island Sound where summer hypoxia create suboxic areas (Parker and O'Reilley, 1991; Breuer et al., 1999) suitable for the reduction of V (V) and the stability of V (IV).

MATERIALS AND METHODS

a) General description of the method for separating V (V) and V (IV)

A general protocol for separating V (V) and V (IV) species in natural water was established and it is summarized in Figure 1. Basically, 100 ml of a water sample was first adjusted to a pH of 4.5 with concentrated perchloric acid or with an acetate buffer (pH=4.5 in 0.1 M acetic acid and sodium acetate). After the pH adjustment, 10 ml of the water sample was immediately loaded, at a rate of 2 ml/min, onto a poly-prep column

with H^+ form Chelex 100 resin (100-200 mesh, Bio-Rad) using a peristaltic pump with Teflon tubing extending from the head of the column to the water sample (Figure 1).

Both V (V) and V (IV) species absorbed onto Chelex 100 resin as in R-V(V)O_3 and R=V(IV)O (R: iminodiacetic groups in chelex 100 resin). After rinsing with 20 ml of the acetate buffer and 20 ml of Milli-Q water, V (V) was eluted with 10 ml of 0.1 M ammonium hydroxide (0.1 M, pH=11.2) (10 elutions of 1 ml each). Following the V (V) elution, V (IV) was eluted with 10 ml (10 elutions of 1 ml each) of 0.2 N perchloric acid (pH=0.8). To avoid any changes in the V speciation during the handling of the samples, the loading and elution protocols were conducted under a nitrogen atmosphere. All the collected eluents for both V (V) and V (IV) analyses were dried on a hot plate and re-dissolved in 2 ml 0.1 N HNO_3. The quantification of both species of V was carried out by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS).

Compared to previous studies (e.g., Soldi et al., 1996; Pyrzyńska and Wierzbicki, 2004), several analytical conditions needed to be optimized to achieve the best separation of V (V) and V (IV) species at the actual nanomolar concentrations that V exists in seawater. Those modifications include sample volume, amount of Chelex 100 resin used in the separation, optimum pH of the water sample to do the chemical separation of both species, sample loading rate onto the resin, elution volumes required to obtain full extraction, and so on. The different analytical conditions are listed in Table 1, and a detailed description is provided in Appendix II.

The GF-AAS was also optimized for V analysis according to the following: thermal program or sequence (Table 2): drying at 130^o C for 30 s, ashing in ramp at 1600^o C for 10 s, hold for 20 s at 1600^o C, atomization at 2500^o C for 5 s, cleaning at 2550^o C

for 3 s. The accuracy and precision of the V speciation analysis was obtained by analyzing certified reference seawater CASS-4 from the National Research Council, Canada.

b) Solid-phase extraction

Chelex 100 (particle size range of 100-200 mesh) in a Na form was converted to a H^+ form by the procedure described by Pesavento et al. (1993). Two grams of Chelex-100 resin were transferred into a 10-ml poly-prep column (BIO-RAD) and washed with ~60 ml of Milli-Q water followed with ~20 ml of 0.1 N $HClO_4$. In order to obtain a full conversion to the H^+ form, the resin was exposed to the acid for at least an hour. The resin was then rinsed with three 20 ml Milli-Q water aliquots each before starting the solid-phase extraction as described in Figure 1. The solid phase extraction was carried out using a peristaltic pump with rotor heads (Cole-Parmer, Masterflex, Model 7553-12, 1-100 rpm), poly-prep columns containing the pre-prepared resin and Teflon tubing that extended from the head of the column into the bottom of a 30 ml acid-washed polycarbonate bottle reservoir (as shown in Figure 1).

c) Recovery of different species of vanadium

In order to establish the operational conditions for the solid-phase extraction of the different V species, recovery experiments were carried out in synthetic seawater. In contrast to previous studies where several mM concentrations of V were used (Soldi et al., 1996; Pyrzyńska and Wierzbicki, 2004), in the recovery experiments, the synthetic

seawater was spiked with V concentrations typical of open ocean environments (40 nM of V (IV) only; 40 nM of V (V) only; and 40 nM of both V (V) and V (IV)).

Standard solutions of V (IV) and V (V) were prepared by diluting stock solutions (4 mM $NaVO_3$ in Milli-Q water, and 4 mM $VOSO_4 \cdot 5H_2O$ in 0.1 N perchloric acid) respectively to the desired concentration in synthetic seawater immediately before experiments. Synthetic seawater was prepared by dissolving sea salt (Sigma) in Milli-Q water (18.2 Ω purity) with final salinity of 35, and pH was titrated to 4.5 by adding concentrated perchloric acid. The synthetic seawater was then passed through an acid-washed polypropylene filter (0.2 μm), and then through H^+ Chelex 100 resin column (as described earlier) to remove any possible vanadium ions. The synthetic seawater was finally degassed at least 1 h with nitrogen gas until use.

d) Stability of V (IV)

Stability of V (IV) at pH=4.5 was assessed in order to establish whether changes in V speciation occurs during the solid-phase extraction. A bottom water sample (Temp.=22^o C, Salinity=14, pH=~7.5, and DO = ~10 μM) collected at the head of the Peconic River estuary in September 2007. The water samples (250 ml, two replicates) were adjusted pH=4.5 immediately with perchloric acid, and incubated at room temperature (20^o C). V (IV) was determined at different time intervals (up to 4 h from collection). All other operation and handling follow the method described above.

Stability of naturally occurring V (IV) was also assessed in order to determine whether biological activity occurring inside the sample bottles could affect V speciation during the handling and operation. Water samples were collected from the Peconics

(with the same hydrological condition as above). Water samples (1 L in each bottle) are incubated at 20^0 and at 0^0 C (two replicates). V (IV) concentrations were also separated and determined at different time intervals over 24 h.

e) Coastal seawater sample collections at the head of Peconic River Estuary and in Long Island Sound

Once the optimum operational conditions for the best recovery of both V (V) and V (IV) were established, the method was applied to the determination of both V species in filtered coastal seawater. The seawater samples were collected in April 2005 at the head of Peconic River Estuary and in April and September 2005 in the Long Island Sound (Figure 2).

The head of the Peconic River Estuary is located at Riverhead in eastern Long Island, NY. Due to damming at the head of Peconic River, minimal riverine flow allows in-situ processes (e.g., groundwater seepage, benthic remobilization and tidal exchange) to dominate trace metal geochemistry in this area (Schubert, 1988; Wilson, 1996; Breuer et al., 1999; Gobler, 1999). The local wastewater treatment plant in Riverhead, NY contributes the largest freshwater inputs to the estuary (NYDEC, 1992), and suboxic conditions are often observed at the head of the estuary (Breuer et al., 1999) (Figure 2). The Long Island Sound (LIS) extends from the East River at New York City to the Race at the eastern end (Figure 2), and it is the third-largest estuary in the United States with a mean depth of 20 m and a total volume of 6.2×10^{10} m^3 (Wolfe et al., 1991; EPA, 1994). Long Island Sound exchanges with lower salinity water through the East River at its western headwaters, and receives about 70% of its freshwater from its largest tributary,

the Connecticut River. At the east, LIS is connected with the Atlantic Ocean, and deeper, more saline ocean water exhibits a net westerly flow as a bottom layer, while fresher surface water generally moves eastward in a surface layer through the Race (Riley, 1967). Periodic bottom water hypoxia events occur from June to September in western LIS, and hypoxia has been observed even in eastern LIS (e.g., Parker and O'Reilly, 1991). The Long Island Sound and the head of the Peconic River estuary are suitable places for studying V speciation, as V (IV) is expected to exist under suboxic/anoxic conditions.

The water samples used in this study were collected at the head of Peconic River Estuary (water depth of ~1 m), using a peristaltic pump equipped with Teflon tubing on a plastic pole and lowered to a depth of 0.2 m above the bottom. In the LIS, seawater samples were collected during two R/V Seawolf cruises; 6 bottom samples in April 2005 and surface and bottom samples at 11 locations covering from the East River to eastern LIS (Middle layer water samples were also collected at stations in central and eastern LIS) in September 2005 (Figure 2). Seawater samples in Long Island Sound were also collected using a peristaltic pump equipped with trace metal clean Teflon tubing attached to 10 m trace metal clean boom.

For V speciation, about 100 ml water samples from each station were obtained by filtration through trace metal clean, polypropylene capsule filters (0.2 μm), and then processed immediately in situ according to the method described above. Once V (IV) and V (V) were isolated, samples were stored at -10^{o} C in freezers until they were brought back to laboratory and analyzed via GF-AAS within a week. All sampling

material used in this study was prepared using trace metal clean techniques (Flegal et al., 1991).

RESULTS AND DISCUSSION

a) Recovery experiments of different vanadium additions

The results of recovery experiments are summarized in Figure 3. With both V (IV) and V (V) spiked, the cumulative recovery was 98% for V (V) and 96% for V (IV). With spiked V (V) only, the cumulative recovery was 95% for V (V) and no V (IV) was detected (Figure 3). However, with spiked V (IV) only, the cumulative recovery of total V was 92% V (IV) and 9% V (V) (Figure 3). The formation of a small amount of V (V) when only V (IV) spike was initially present shows that oxidation of free V (IV) ions occurred during handling under laboratory conditions although steps were taken to prevent the introduction of oxygen. The detection limit for both V (V) and V (IV) was on the order of 0.5 nM. The precision is ~10% with concentrations of 10 nM.

b) Stability of V (IV)

The short- term stability study showed that the levels of V (IV) in the water sample collected in the Peconics remained unchanged for at least 4 h after collection and pH adjustment to 4.5 (Figure 4). The results suggest that at pH 4.5, the reduced form of V is stable for several hours. Furthermore, the similar results obtained in the different temperature incubation experiments (0^0 and 20^0 C) (Figure 5), suggest that, for at least 24 hrs, biological activity did not cause the reduction of V (V) or oxidation of V (IV), In contrast to free V (IV) ions (made in lab) with only 7-15 min of oxidation half time once

added to oxic seawater or lake river (Okamura et al., 2001), the naturally occurring V (IV) was relatively stable over 24 h presumably because V(IV) was stabilized in natural environments as complexing with organic ligands (e.g., Szalay and Szilágyi, 1967; Baran, 2000; Zhou et al., 2005).

c) Variation of V speciation with pH in certified reference seawater CASS-4 and in the LIS

Since there are no certified reference materials for V (IV) yet, the certified reference seawater (SRSW) CASS-4 (23.1 ± 3.1 nM V) from National Research Council, Canada was analyzed for vanadium speciation. Consistent with the fact that the CASS-4 has been acidified (pH = 1.6), the speciation protocol recovered 93% of the total V as V (IV). The amount of V (V) found in the acidified SRSW (~7%) was within the 10% of the uncertainty of the analysis (Table 3). In order to measure V (V) in the SRSW, the pH was adjusted to 8.5 for 5 h and reanalyzed for both V species. At this basic pH, V (V) is the dominant species, accounting for 96% of the total V (Table 3). The amount of V (IV) in the oxidized CASS-4 was only 4% of total V and within the uncertainty of the measurements (Table 3). The results on the effect of pH on V speciation are consistent with previous studies (e.g., Okamura et al., 2001; Nukatsuka et al., 2002) and validated the speciation protocol.

The accuracy of the new speciation method was also established by comparing the concentration of total dissolved V (adding both V (IV) and V (V) species) measured in the LIS cruise using the new protocol with the V levels obtained using the APDC/DDDC organic extraction and ICPMS quantification (Figure 6). The highly significant linear

regression obtained between the two different protocols (r^2=0.98; V (IV+V) = 0.998 * (V measured by APDC/DDDC extraction) + 1.10) clearly demonstrated that the new technique produces very reliable V results.

d) Redox speciation of vanadium at the head of Peconic River Estuary and in Long Island Sound

Total dissolved V concentrations in both study areas ranged from 6.1 to 18.0 nM in April 2005 (Figure 7). Total dissolved V at the head of Peconic River Estuary was more variable and relatively low (6.1-11.0 nM) in comparison to coastal waters of Long Island Sound measurements (10.0-18.0 nM; April 2005) (Figure 7). When plotting against dissolved oxygen (DO), the lowest total V concentrations were measured in the Peconics at DO levels < 250 μM while relatively high levels were measured in oxic waters of the LIS (Figure 7). A similar pattern was also observed in bottom waters off the Gulf of Mexico by Shiller et al. (1998). However, salinity in the LIS and the Peconic was lower than in the Louisiana Shelf bottom water, and consequently, total V concentrations in our area of study were also lower (Figure 7).

The results of the V speciation in the Peconics and in the LIS measured in April 2005 are shown in Figure 8. Consistent with the redox conditions of the different locations, V (V) was the dominant species under the oxic conditions of the LIS (range: 8-16.0 nM); reduced V (IV) (range: 0.5- 3.0 nM) concentrations were < 10% of the oxidized form. In contrast, in the suboxic Peconic River Estuary, dissolved V (V) levels ranged between 4.0 to 10.0 nM and the levels of V (IV) (range: 2-5.5 nM) were about 40% of the oxidized V (Figure 8).

Low levels of total dissolved V under low oxygen conditions have been associated with V enrichments in sediments (Shiller et al., 1998). Our study suggests that the reduction of V (V) to V (IV) occurred under low oxygen, and therefore V (IV) cations (VO^{2+} or HVO_2^{+}; Pope et al., 1980) could be adsorbed onto particles and removed from the water column, leading to lower total V levels in seawater and high enrichments in sediments.

Distributions of V (IV) and V (V) in LIS measured in September 2005 are shown in Figure 9. The average concentration of V (IV) was 3.0 nM (± 1.4 nM) ranging from undetectable to 6.1 nM. The average concentration of V (V) was 22.1 nM (± 4.0 nM), ranging from 11.6 to 32.2 nM. Overall, V (V) was the dominate V species found in the LIS, and V (IV) accounted for only 0 ~ 20% of the total V. The highest levels of V (V) were detected in the eastern and central regions of the LIS, especially in deep waters, which likely resulted from the input of oxic waters from the Atlantic Ocean (Figure 9). In contrast, relatively low levels of V (V) were found in surface waters near river discharges and near New York City in the western region of the LIS (Figure 9). To some extent, this is consistent with the low levels of dissolved V reported in rivers (e.g., Shiller and Mao, 2000). The highest levels of V (IV) were measured in two areas: near New York City in western LIS, and near the Connecticut River (Figure 9). Higher levels of V (IV) in WLIS may be related to inputs from sewage inputs with high levels of dissolved Mn and Fe because Fe and Mn may be required as reductants for reduction of V (V) to V (IV) (e.g., Myers et al., 1993; 1997; 2004), while high levels near the Connecticut River may be related to the river runoff there. Also V (V) may be reduced to V (IV) due to the

existence of peroxides produced by photoreduction (e.g., Voelker and Sedlak, 1995; Spokes and Liss, 1995) in eastern LIS.

The field results confirmed the existence of V (IV) in coastal waters, although the reduced form of V was not the dominant species in this study. The existence of V (IV) in natural waters is not uncommon. For example, V (IV) was identified in well-aerated river waters (e.g., Evans and Garrels, 1958; Kalk, 1963; Willoud et al., 2001; Nakano et al., 1990), waste water from power stations (Akl et al., 2005), volcanic water (Minelli et al., 2000), seawater (e.g., Ladd, 1974; Hirayama et al., 1992; Wann and Jiang, 1997), groundwater (Veschetti et al., 1997) , sediment and biological samples (e.g., Colina et al., 2005).

e) Thermodynamic considerations regarding vanadium speciation in the LIS

The different redox-pH conditions shown in the Eh-pH diagram for V speciation (Figure 10) indicates that V (V) is the dominant species under oxic condition, while V (IV) predominates in anoxic environments. Based on pH and oxygen measurements carried out during the September 2005 cruise in the LIS, dissolved V should exists only as V (V) under the oxic conditions prevalent in the central and eastern LIS (oxygen levels > 350 μM; pH =8.0~8.3; Eh > 0.6 V). Near NY City in WLIS, V (IV) could also be detectable under the suboxic conditions prevalent in that region ($NO_3/NH_4 \sim 1$; pH=7.5~7.8; Eh ~0.4 V). These thermodynamic calculations are consistent with the geographical distributions of V (V) and V (IV) reported in Figure 9.

While the lowest levels of dissolved oxygen occur in bottom waters of WLIS, concentrations of reduced V (IV) were also relatively lower there (Figure 9). Because

bottom waters of WLIS are highly sulfidic (1~10 mM; Cuomo, 2005) and less basic than seawater (pH=7.5~7.8), the calculated Eh value for those waters is ~ -0.2 V (Figure 10). Those redox conditions are near the boundary between the V (IV)/(III) couple, suggesting that V (IV) was being reduced to solid V (III). Therefore, low levels of V (IV) in highly sulfidic bottom waters of the LIS are the result of sulfide precipitation of V (III) (e.g., Breit and Wanty, 1991; Wanty and Goldhaber, 1992). While these thermodynamic calculations are consistent with our field results, there are other important factors that can influence the concentrations of reduced V (IV) in some marine environments. For example, while free V (IV) ions are unstable under oxic conditions (oxidation half time of 7-15 min; Okamura et al., 2001), V (IV) is stabilized by organic ligands increasing the stability field of V (IV) to Eh values as high as +0.4 V under neutral pH (Wehrili and Stumm, 1989; Elbaz-Poulichet et al., 1997), close to the region where V (V) is stable (Figure 8).

A low proportion of V (IV) (~1%) has also been widely reported in oxic rivers water and seawater (e.g., Evans and Garrels, 1958; Kalk, 1963; Ladd, 1974; Emerson and Huested, 1991; Elbaz-Poulichet et al., 1997; Bosque-Sendra et al., 1998). V (IV), however, may be the dominant species in reducing environments. For example, Veschetti et al. (1997) reported that V (IV) accounted for >90% of total in anoxic ground water. Wann and Jiang (1997) reported more than ~35% of vanadium was V (IV) in acidified seawater, and > 90% as V (IV) in acidified river water. Wuilloud et al. (2001) found that V (IV) accounted for more than 50% of the total in river water.

In our study areas, V (IV) accounted for less than 1% to as high as 40% presumably because of the existence of a mildly reducing environment (suboxic conditions), where

reductants, e.g., H_2O_2, Fe^{2+}, Mn^{2+} are expected to exist (e.g., Voelker and Sedlak, 1995; Spokes and Liss, 1995; Myers et al., 1993; 1997; 2004).

SUMMARY

An analytical protocol has been developed for separating different redox species of V in sea water. The protocol is based on solid-phase extraction with Chelex and sequential elution with a base (for V (V) and an acid (for V (IV)). The detection limits for V (V) and V (IV) were on the order of 0.5 nM. The analytical precision was about 10%.

Consistent with thermodynamic calculations, the dominant V species in oxic waters was V (V), but the reduced form of V as V (IV) was also detected in coastal waters, being as high as 6.4 nM. The redox speciation of V changed spatially in LIS according to different ambient redox conditions, and V (IV) may account for up to 40% of the total dissolved V in some suboxic environments.

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TABLE CAPTIONS

Table 1: Comparison of analytical conditions for separating vanadium species between previous studies and this research.

	Previous studies	**In this research**
Water sample volume	400 ml (Soldi et al., 1996)	10 ml
Chelex 100 resin	0.2 g (Soldi et al., 1996)	2.0 g
Total operation time	> 2 h (Soldi et al., 1996)	~10 min
Handling and operation	Under nitrogen (Soldi et al., 1996) Under atmosphere (e.g., Pyrzyńska & Wierzbicki, 2004)	Under nitrogen
pH in water sample	pH = 3-5 (Chen et al., 1993) pH=4-6 (Soldi et al., 1996)	pH=4.5
Solution used for adjustment in water sample	Acetate buffer (Soldi et al., 1996)	Acetate buffer
Acid used adjusting pH in water samples and acetate buffer	Nitric or perchloric acid (Soldi et al., 1996) Nitric acid (Pyrzyńska & Wierzbicki, 2004)	Perchloric acid only
Water sample loading rate	2.0 ~ 4.0 ml/min (Chen et al., 1993)	2.0 ml/min
pH in ammonium solution to elute V^{5+}	> 10 (Soldi et al., 1996)	11.2
Volume of ammonium solution to elute V^{4+}	10 ml (Soldi et al., 1996)	10ml
Acid to elute V^{5+}	Nitric or perchloric acid (Soldi et al., 1996) Nitric acid (Pyrzyńska & Wierzbicki, 2004)	Perchloric acid only
pH in acid to elute V^{5+}	< 0.8 (Soldi et al., 1996)	0.8

Table 2: Optimized graphite furnace temperature program for the determination of vanadium.

Step	Temperature (^{o}C)	Ramp Time (s)	Hold Time (s)	Gas Flow (ml/min)
Dry 1	110	1	30	250
Dry 2	130	15	30	250
Pyrolysis	1600	10	20	250
Atomization	2500	0	5	0
Cleanout	2550	1	3	250

Table 3: Dissolved V (IV) and V (V) in CASS-4 reference material (All units for vanadium are nM).

Samples	V (IV)	V (V)	Total V
CASS-4 Reference Material (total V: 23.1±3.1) Analyzed immediately	24.1	1.8	25.9
CASS-4 Reference Material (total V: 23.1±3.1) After adjusting pH=8.5 for 5 h	1.0	21.8	22.7

FIGURE CAPTIONS

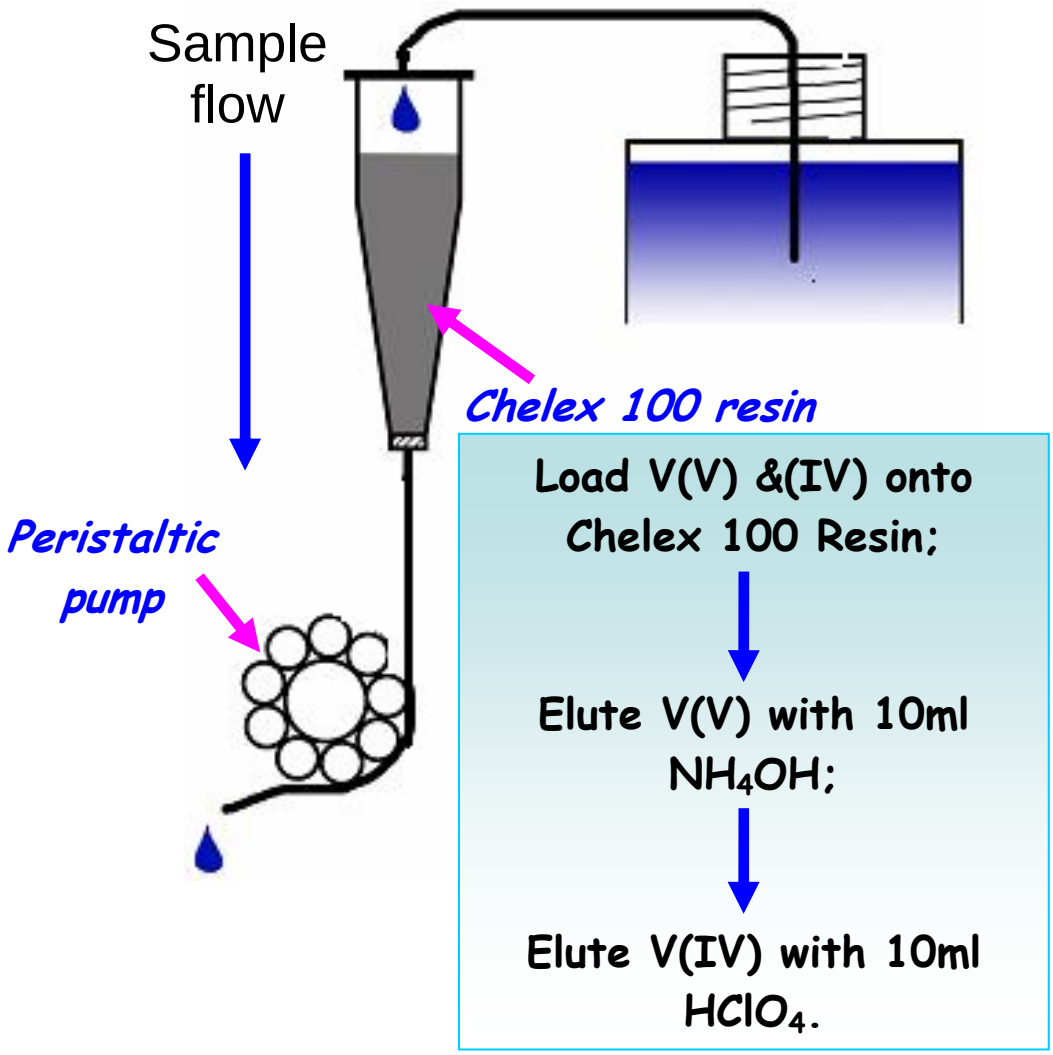

Figure 1: Schematics of the protocol for separating different redox species of vanadium in seawater.

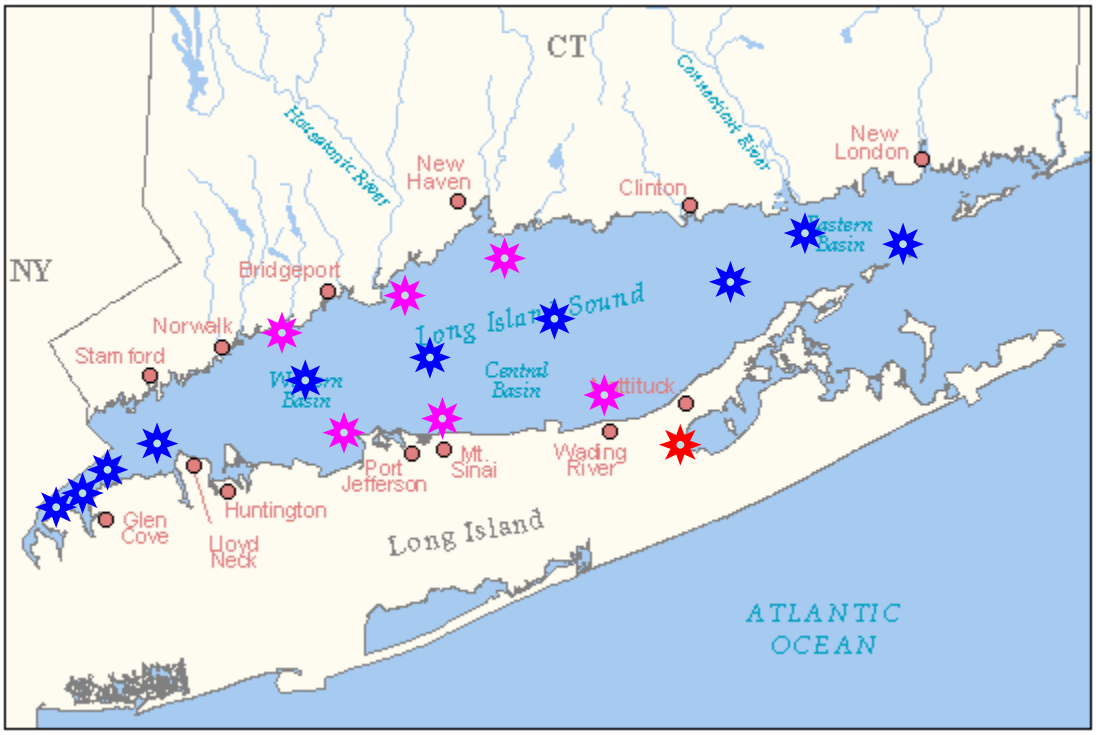

Figure 2: Sampling locations in this study (= samples collected in April 2005 at the head of Peconic River Estuary; =samples collected in April 2005 in Long Island Sound; =samples collected in September 2005 in Long Island Sound).

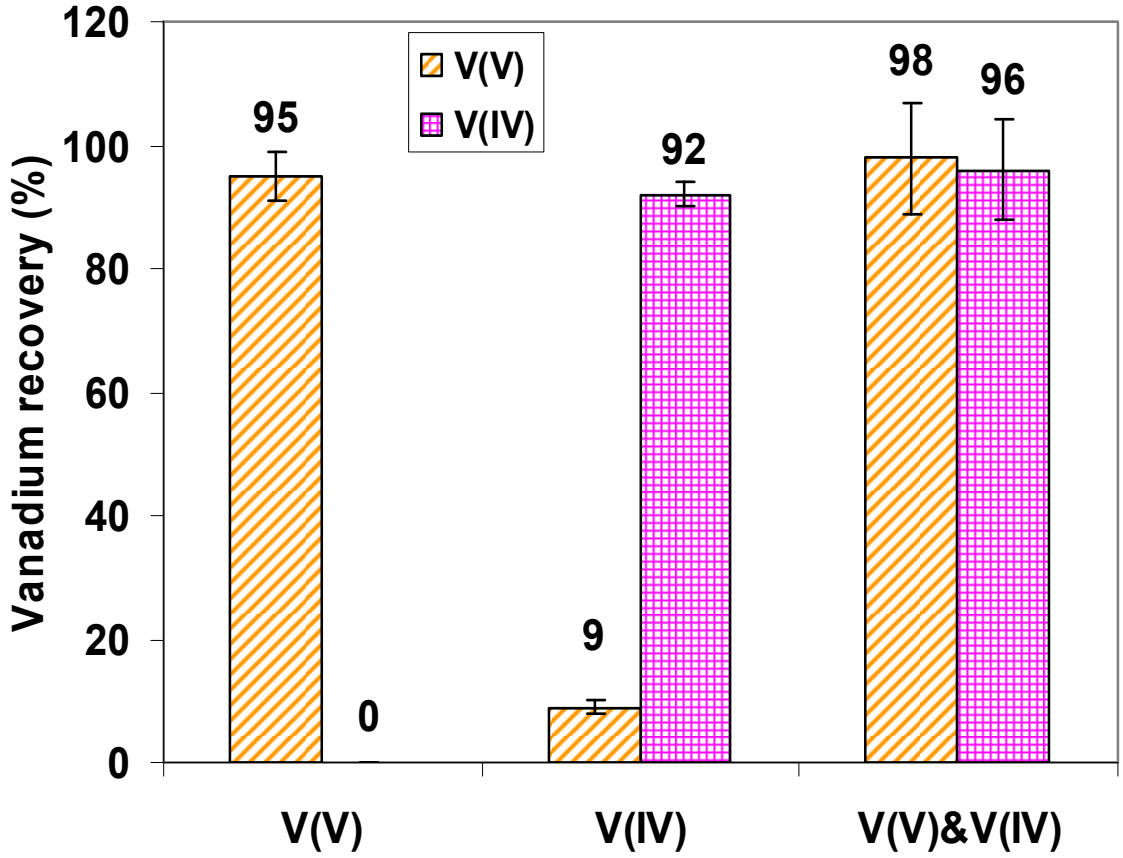

Figure 3: Vanadium recoveries of V (V) only, V (IV) only and both V (V) and V (IV) in synthetic seawater samples (All in 40 nM; Error bars are standard deviation, n=4).

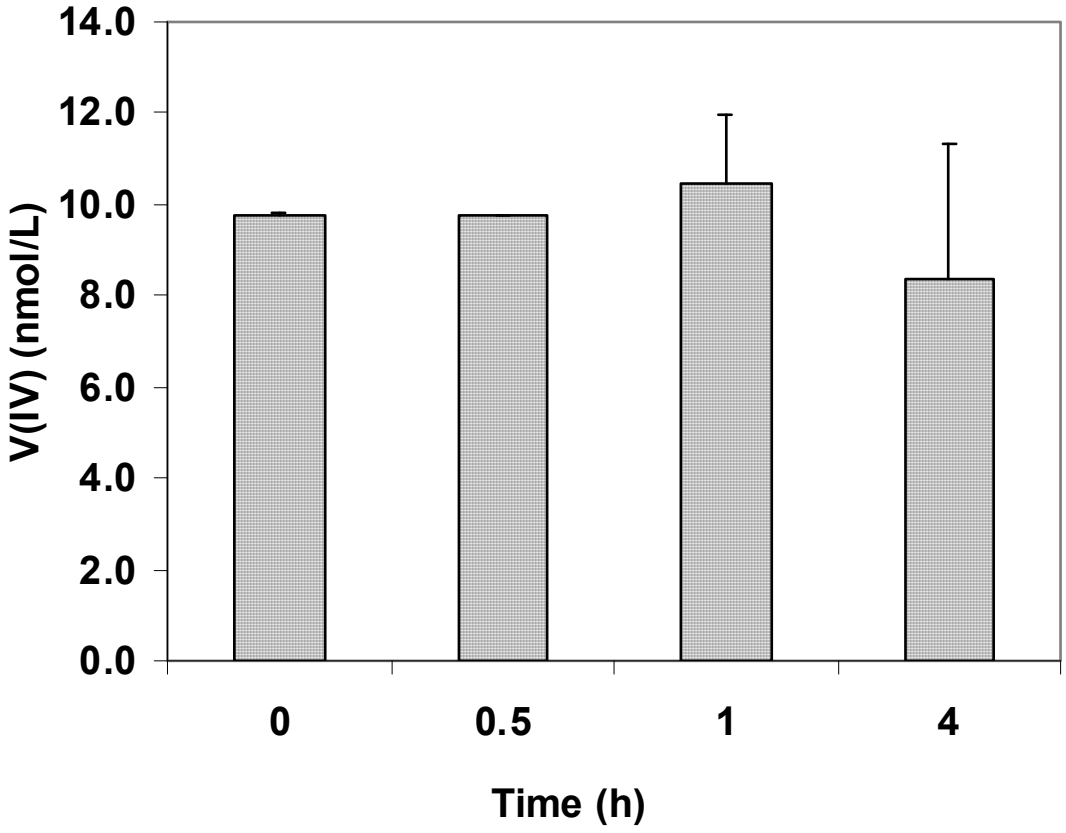

Figure 4: Changes in V (IV) concentrations with time after adjusting the sample pH to 4.5 (Error bars are standard deviation, n=2; The samples consisted of bottom waters collected at the head of Peconic River Estuary with dissolved oxygen of ~10 µM and pH=7.5).

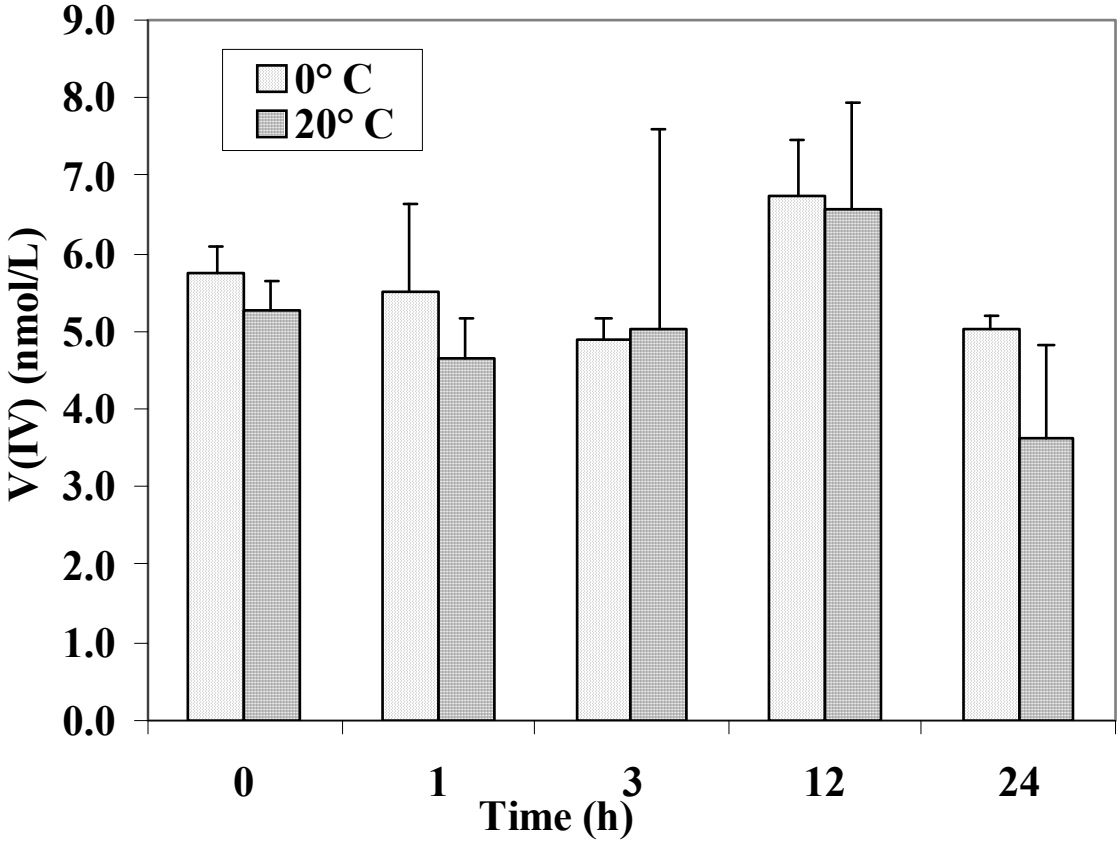

Figure 5: Changes in V (IV) concentrations in water samples under incubation at 0 and 20^{o} C for up to 24 h (Error bars are standard deviation, n=2; The samples were bottom waters collected at the head of Peconic River Estuary with DO levels of ~10 µM and pH=~7.5).

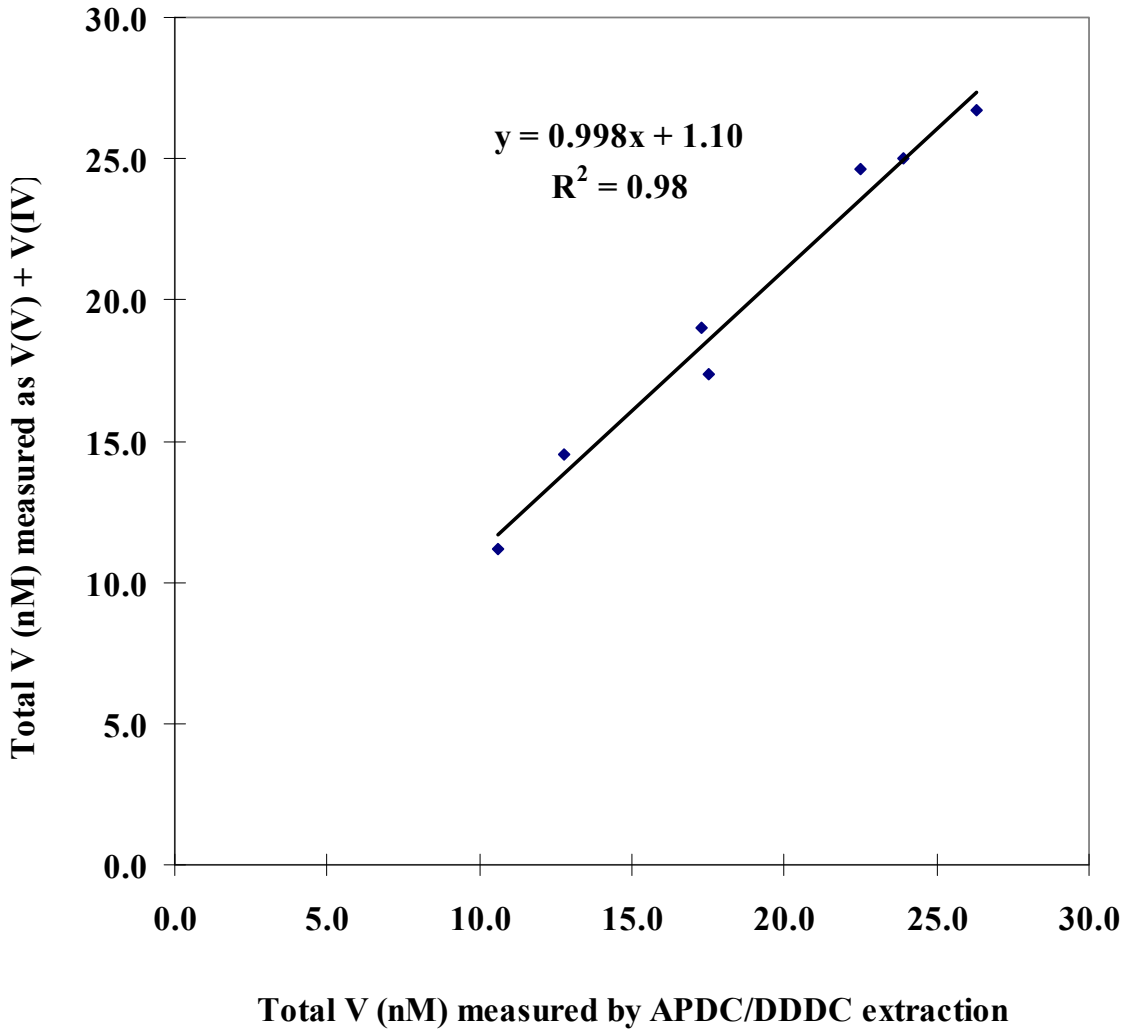

Figure 6: Comparison of total vanadium measured by the two protocols: solid phase extraction and APDC/DDDC extraction (Water samples were collected in Long Island Sound during April and September 2005).

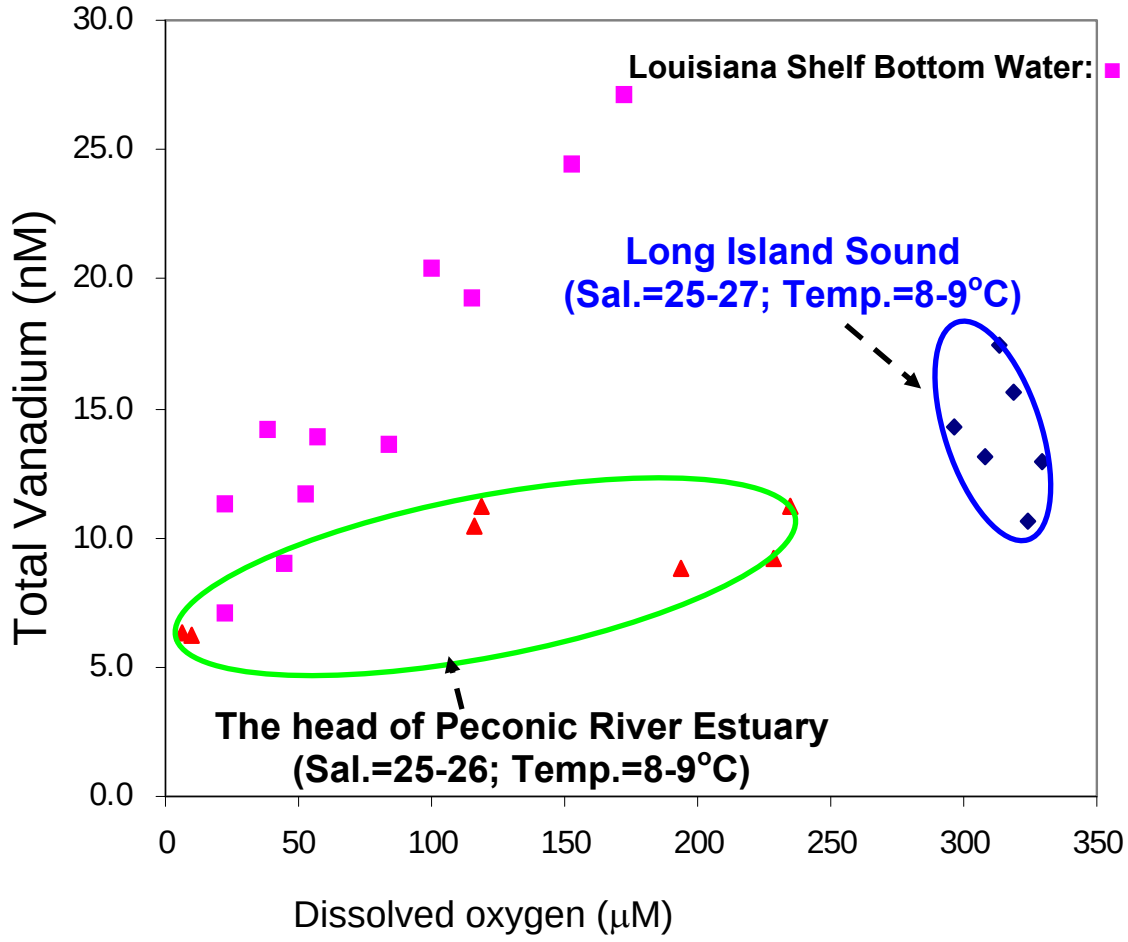

Figure 7: Total vanadium versus dissolved oxygen in coastal waters (April 2005, ◆— the head of Peconic River Estuary; ▲—Long Island Sound; ■--Louisiana Shelf Bottom waters from Shiller et al., 1998 at salinity 35).

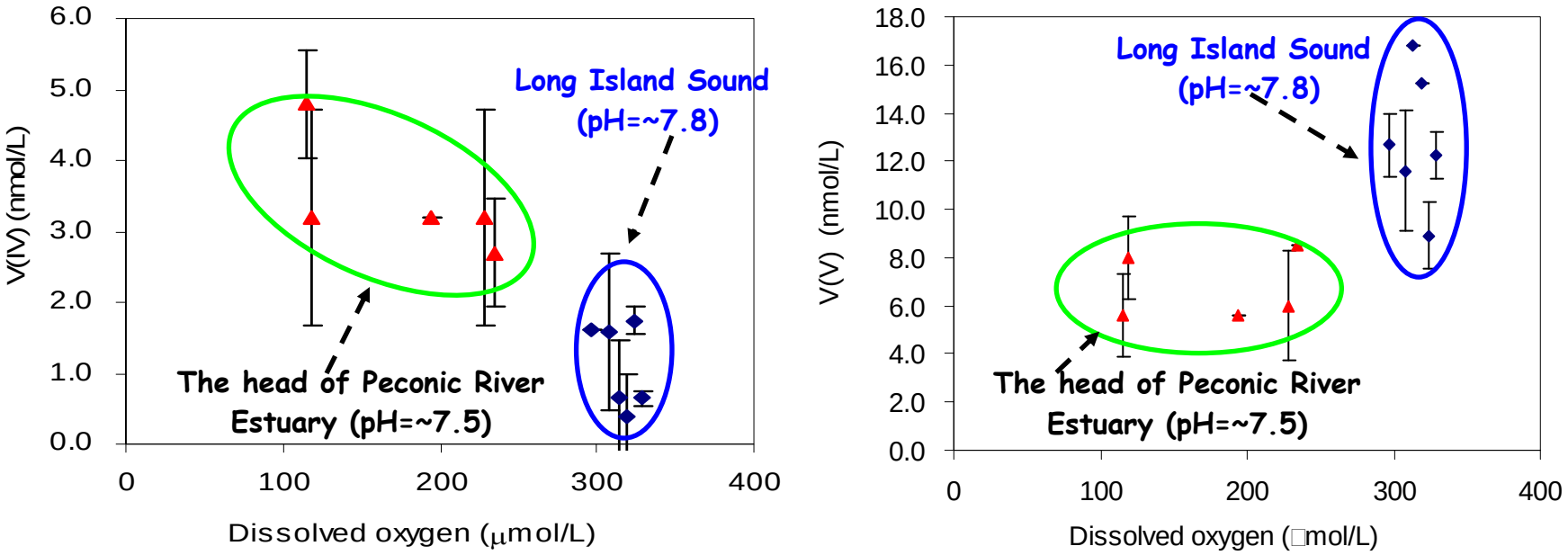

Figure 8: Dissolved V (IV) (left) and V (V) (right) concentrations versus dissolved oxygen in coastal waters (April 2005, ▲—at the head of Peconic Bay; ♦—in Long Island Sound).

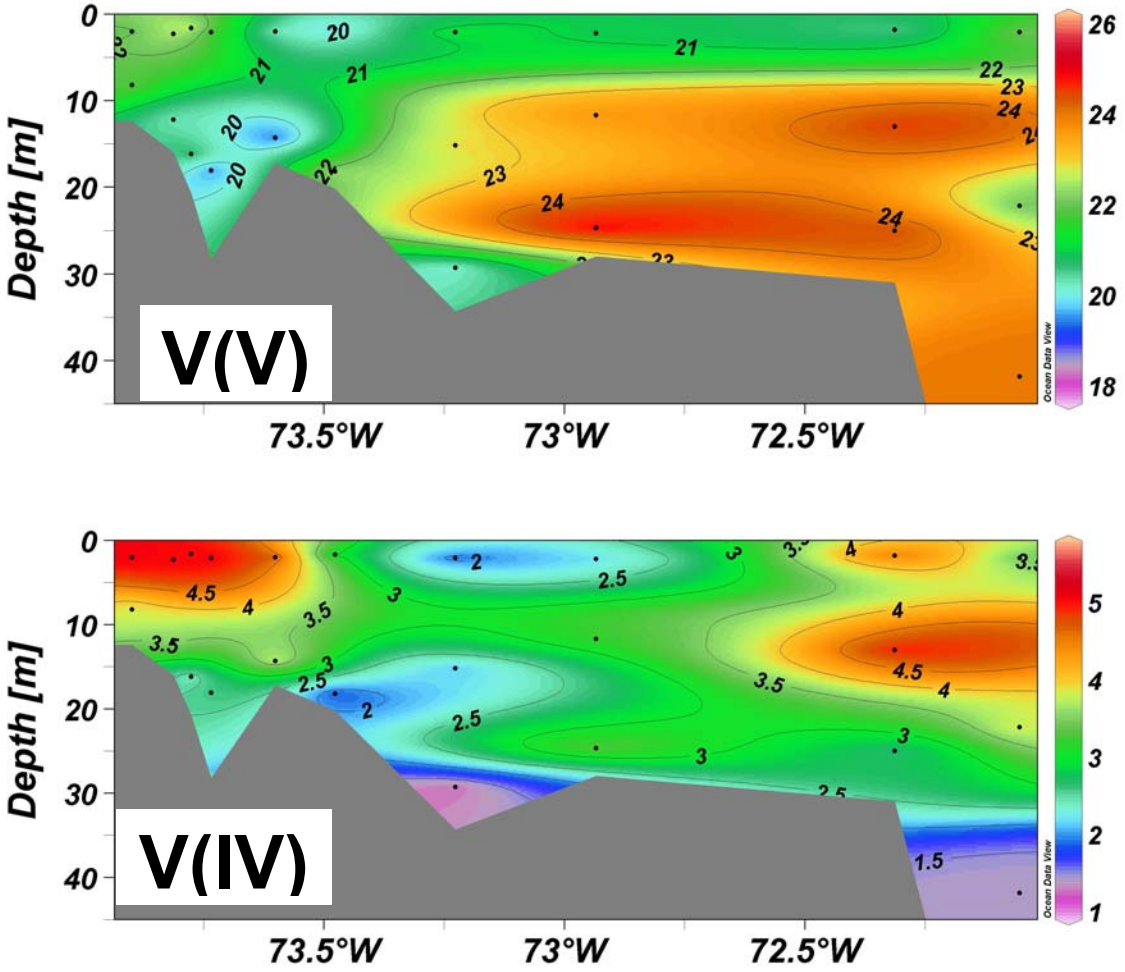

Figure 9: Distribution of V(V) and V(IV) along a transect from the East River to eastern Long Island Sound (September 2005; all units are nM).

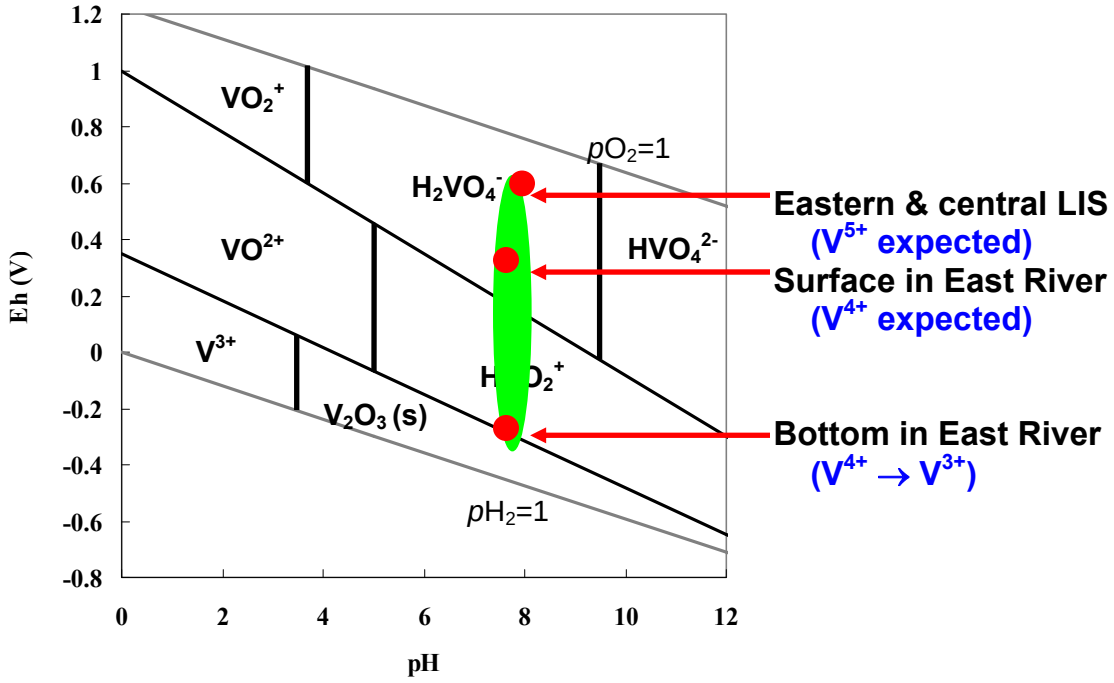

Figure 10: Eh-pH diagram of vanadium in natural environment (The pH-Eh calculated for waters in LIS are also shown; Assuming activity for dissolved V=10^{-7} M; modified from Peacock and Sherman, 2004; V(IV) forms are from Pope et al., 1980).

CHAPTER THREE: A NEW METHOD FOR THE QUANTIFICATION OF DIFFERENT REDOX-SPECIES OF MOLYBDENUM (V AND VI) IN SEAWATER

ABSTRACT

A new method for the direct determination of reduced and oxidized Mo species, (Mo (V) and Mo (VI)), in seawater has been developed using an ion exchange procedure. The method includes complexation of Mo (V) with tartrate, solid phase extraction of the Mo (V)-tartrate complex by a XAD 7 resin, followed by elution with acidic acetone. The eluted Mo (V) was quantified by graphite furnace atomic absorption spectrometry. The detection limit of this protocol was on the order of 0.2 nM. The analytical precision is 10% of ~10 nM. This new method was successfully applied to the determination of Mo (V) and Mo (VI) in bottom waters at the head of Peconic River Estuary. Concentrations of Mo (V) in these aquatic environments ranged from 0 nM to ~16 nM, accounting for 0% ~ 15% of the total dissolved Mo pool.

INTRODUCTION

Molybdenum (Mo) is the most abundant transition trace metal in the ocean, and displays a conservative behavior with depth (about 107 nM) (Collier, 1985; Morris, 1975), although slight depletion in Mo concentrations are observed in surface waters (Adelson et al., 2001; Yamazaki and Gohda, 1990; Tuit and Ravizza, 2003). In contrast to open ocean waters, a sharp depletion in Mo levels occurs in deep waters at the Black Sea and Cariaco Basin (Bertine, 1972; Emerson and Huested, 1984). Reduction of Mo is expected to occur in those reducing waters, although the actual amount of reduced Mo

has never been reported. Molybdenum (VI) is generally assumed to be the dominant oxidation state of Mo in oxic natural waters, while Mo (V) exists in reducing environments (Bertine, 1972; Brookins, 1988).

Molybdenum is an essential trace element for plants, animals and micro-organisms (e.g., Bortels, 1930), as Mo forms part of the active sites of different metalloenzymes that execute key transformations in the biogeochemical cycles of nitrogen, sulfur and carbon (e.g., Mendel 2005). For example, Mo is part of several cofactors required for nitrogen-fixation and nitrate reductase (e.g., Fogg and Wolfe, 1954; Mendel, 2005), which catalyzes the reduction of N_2 and nitrate to bioavailable N in the ocean (e.g., Fogg and Wolfe, 1954; Mendel, 2005). In fact, more than 60 enzymes have been identified with Mo cofactors (listed in Appendix I: Table 2).

Lack of Mo severely limits the growth of phytoplankton (e.g., Howarth and Cole, 1985), although high levels of this element are toxic (e.g., Turnlund, 2002). Bacteria and algae can acquire Mo as molybdate using different surface transporters, siderophores and the sulfate and phosphate uptake systems (Heuwinkel et al., 1992; Marschner, 1995; Liamas et al., 2000; Pau and Lawson, 2002; Liermann et al., 2005). However, molybdate is not biologically active until this Mo species is subject to a series of reactions and incorporation into different enzymes (e.g., Mendel, 2005). Electron paramagnetic resonance has confirmed the existences of Mo (V) in biological samples (e.g., Godfrey et al., 1984; Kaul et al., 1985; Sears et al., 1995; Canne et al., 1997), suggesting that this reduced form of Mo is the most bioavailable in aquatic environments (e.g., Howarth and

Cole, 1985; Howarth et al., 1998). Therefore, as reported for other trace metals, the bioavailability of Mo is more dependent on different Mo species than on the total pool.

Although many studies have reported levels of total Mo concentrations in different aquatic systems (e.g., Collier, 1985; Emerson and Huested, 1991), none of them has reported levels of the different redox species of this element in those environments. The limited work published on Mo speciation (e.g., Sugio et al., 1988; Ahmed and Haque, 2002) reported Mo (V) concentrations in the order of several mM, an order of magnitude higher than total concentrations found in natural waters (e.g., 5 nM in river and 107 nM in seawater, Morford and Emerson, 1999). Hence, these methods are only suitable for analyzing samples with high concentrations of Mo such as mine effluents (Sugio et al., 1988), but inapplicable for quantifying the extremely low levels of Mo (V) expected in marine systems.

In this study, a method based on solid-phase extraction was developed to measure the Mo speciation (V and VI) in seawater. The analytical protocol was thoroughly investigated, and then applied to filtered surface and bottom water at the head of the Peconic River Estuary in Long Island, New York.

MATERIALS AND METHODS

a) Method description

The analytical protocol established for separating Mo (V) and Mo (VI) in natural waters is shown in Figure 1. In this protocol, a 60 ml water sample was collected and the pH adjusted to 7.0 with a phosphate buffer (pH=7.0, 0.25 M KH_2PO_4 and 0.25 M Na_2HPO_4) and a small amount of perchloric acid when necessary. The Mo (V) in the pH-adjusted sample was complexed with tartrate (0.5 ml of 10% tartrate solution) and extracted with 2.0 g of pretreated XAD-7 resin packed in a poly-prep column at a flow rate of 2 ml/min (Figure 1). Because this resin is selective only for Mo (V)-tartrate complex, the water sample was collected after passing through the resin for the subsequent analysis of Mo (VI). Mo (V)-tartrate was then eluted with 40 ml (8 elutions of 5 ml each) 1 N acidic acetone (10% of concentrated nitric acid in acetone, v/v). These eluents containing Mo (V) were dried on a hot plate, redissolved with 5 ml 0.1 N nitric acid and quantified by GF-AAS.

The eluents containing Mo (VI) were reduced to Mo (V) by adding the reducing agent (0.5 ml 10% $SnCl_2$) and then analyzed as the protocol described for Mo (V). The total Mo concentration was obtained by adding both Mo (V) and Mo (VI). The detection limit of this protocol was on the order of 0.2 nM from both species of Mo. The analytical precision was ~ 10% for samples with 10 nM Mo.

b) Preparations of standard solutions for Mo speciation recovery experiments

Mo (VI) stock solutions were prepared by dissolving 184.0 mg ammonium molybdate tetrahydrate in 100 ml Milli-Q water, and subsequently standardized by GF-AAS. Mo (V) stock solutions were prepared by dissolving 284.7 mg molybdenum (V) chloride in 100 ml 0.1 N perchloric acid. Standard solutions of Mo (VI) and Mo (V) were prepared by diluting stock solution in synthetic seawater (degassed for at least 1 h with nitrogen gas) to obtain the following Mo species concentrations: 100 nM Mo (VI) only; 100 nM Mo (V) only; and 100 nM of both Mo (V) and Mo (VI). It should be noted that, in contrast to previous studies where several mM of Mo were used (e.g., Sugio et al., 1992), the 100 nM level used in this analytical protocol reflects the natural concentrations of Mo found in seawater.

Tartrate solution was prepared by dissolving 10 g potassium sodium tartrate tetrahydrate in 100 ml Milli-Q water. The phosphate buffer was prepared by dissolving potassium dehydrogenate phosphate and disodium hydrogen phosphate in Milli-Q water to a final concentration of 0.25 M of each solute. The pH of the buffer solution was adjusted to pH 7 with 10 N NaOH. All of the chemicals used were analytical reagent grade or the highest purity available (e.g., HPLC-grade absolute acetone).

c) Considerations for the use of tartrate as a ligand for Mo (V)

Mo (V) is not thermodynamically favored under oxic conditions. However Malthouse et al. (1980, 1981) and Yamanouchi et al. (1977) reported the existence of Mo

(V) in biological samples as a dominant species. The exact form of Mo (V) in natural waters is not well understood. Bertine (1972) suggested that Mo (V) may exist in natural waters as Mo_3O_8 (a mixture of Mo (V) and Mo (VI)). However, Mo_3O_8 usually exists as crystals (Titley and Anthony, 1961). Szilágyi (1967) suggested that MoO_2^+ occurred when Mo (VI) was reduced by humic acids. The structure of this Mo (V) compound has been confirmed by measuring the distance of the Mo-O complex (Spivak and Dori, 1975; Stiefel, 1987). In addition, Mo (V) has a strong tendency to dimerise (Bard et al., 1985). Therefore, as previously reported (e.g., Coughlan, 1980; Modec and Brenčič, 2002; 2004), Mo (V) most likely exists as $Mo_2O_4^{2+}$ in natural waters.

$Mo_2O_4^{2+}$ can be easily complexed with many organic ligands (e.g., EDTA and oxalate; Bard et al., 1985; Modec et al., 2004). Those ligands usually yield either polymeric materials or discrete entities (Modec et al., 2002; 2004). During the development of this analytical protocol, we found that Mo (VI) was also involved in complexation of Mo (V) with oxalate and EDTA (results not shown). Therefore, Mo (V) could not be completely separated from Mo (VI). Compare to oxalate, the dicarboxylic acid tartrate has fewer functional groups (Modec et al., 2002; 2004) suitable only for the bonding of Mo (V). Ahmed and Haque (2002) further reported that tartrate can form complexes only with Mo (V) and not Mo (VI). Therefore, tartrate was used as the complexing ligand to selectively extract Mo (V) from water samples.

d) XAD resin preparation

By yielding higher enrichment factors, greater efficiency and handling simplicity (Soylak et al., 1997; 2001), solid phase extractions have some advantages over other techniques used to isolate Mo species at high concentrations (e.g., spectrophotometric and colorimetry). Furthermore, the XAD resin used in this analytical protocol is of high purity and has good sorption properties including porosity, durability, uniform pore distribution, high surface area (e.g., Soylak et al., 1997; 2001; Tewari and Singh, 2001). Therefore, amberlite XAD-copolymers have been widely used for preconcentrating trace metal ions (e.g., King and Fritz, 1985; Soylak et al., 1997; Tunceli and Türker, 2004; Soylak et al., 2001). In those applications, the trace metal ions were first converted into metal chelates or inorganic complexes, then absorbed on the XAD resin, and finally eluted with different eluting agents (e.g., Soylak et al., 2001). Because of its hydrophilic surface and intermediate polarity with a weak cation-exchange capacity, amberlite XAD-7 (as polyacrylic acid ester polymers), was used to isolate Mo (V) in this study.

The XAD-7 resin was first preconditioned and purified to eliminate trace metal ions and other contaminants by the method of Soylak et al. (2001). The XAD-7 resin was washed with methanol, Milli-Q water, 1 N nitric acid in acetone, Milli-Q water, 1 N NaOH, Milli-Q water and finally acetone. A preconcentration system was set up using a peristaltic pump with rotor heads (Cole-Parmer, Masterflex, Model 7553-12, 1-100 rpm), poly-prep columns and Teflon tubing that extended from the head of the columns into the bottom of a 60 ml polycarbonate bottle reservoirs (Figure 1).

e) Graphite Furnace Atomic Absorption Spectrometry setup

Graphite Furnace Atomic Absorption Spectrometry (GFAAS) offers the necessary sensitivity for Mo measurements (Johnson et al., 1973; Morrice et al., 1989). In this study, Mo was determined by GF-AAS using a Perkin-Elmer spectrometer (AAnalyst-800) with a longitudinal Zeeman-effect background correction system, a transversely-heated graphite atomizer (THGA), and an auto-sampler (Perkin-Elmer, AS-800). The hollow cathode lamp (Perkin-Elmer, PN: N305-0186) for Mo is operated at 15 mA, with a slit of 0.7 nm and a 313.3 nm wavelength. The volume pipetted into the graphite tube was 20 μl, for samples and calibration solutions, and high-purity argon (UHD grade) was used as the purge gas. The adjusted thermal program used in GF-AAS was the following: drying at 130^{o} C for 30 seconds, pyrolyzing at 1500^{o} C for 20 seconds, atomizing at 2450^{o} C for 5 seconds, and cleaning at 2500^{o} C for 5 seconds. The gas flow rate was set at 250 ml/min.

f) Stability of Mo (V)

In order to establish whether changes in Mo speciation occurs during the solid-phase extraction, the stability of Mo (V) at pH=7.0 needed to be determined. For this objective, a bottom water sample (temp.=22^{o} C, salinity=14, pH=~7.5, and dissolved oxygen (DO) = ~ 10 μM) was collected at the head of the Peconic River estuary. The water samples (250 ml, two replicates) were adjusted pH=7.0 immediately with

perchloric acid, and incubated in the lab at room temperature (20^{o} C). Mo (V) was then determined at different time intervals (up to 4 h from collection) following the method described above.

The stability of naturally occurring Mo (V) was also assessed to determine whether biological activity occurring inside the sample bottles could affect Mo speciation during the handling and operation. Water samples (1 L) were collected also from the head of Peconic River Estuary (same hydrological condition as above) and incubated at 20^{0} and at 0^{0} C (two replicates). Mo (V) concentrations were measured according to the protocol described above at different time intervals over 24 h.

g) Determination of Mo speciation in natural water samples

Surface and bottom water samples at the head of Peconic River Estuary were collected for Mo speciation (Figure 2). Bottom water samples were collected weekly from June to October 2006, and surface water samples were collected in September 2007 during the summer anoxia at the head of the Peconic River Estuary (Breuer et al., 1999). Bottom water samples were collected with a peristaltic pump through Teflon tubing extended on a plastic pole and lowered to the depth of 0.2 m above the bottom. Surface water samples were collected from 0.2 m below the surface. Dissolved samples for Mo speciation were obtained by filtration through a trace metal clean, polypropylene capsule filter (0.2 μm). All sampling material used in this study was prepared using trace metal clean techniques (Flegal et al., 1991).

Water samples for Mo (V) and Mo (VI) speciation were immediately processed in situ for Mo (V), and analyzed in the laboratory within a week for Mo (VI). The eluents were stored at -10^{o} C in a freezer until analyzed by GF-AAS. Salinity, pH, temperature and dissolved oxygen in all the water samples were also determined using established protocols.

RESULTS AND DISCUSSION

a) Recovery experiments of Mo (V) and Mo (VI) addition in synthetic seawater

In order to establish the accuracy of the Mo speciation technique, a series of recovery experiments were conducted with spiked Mo (V) and Mo (VI) concentrations typical of oceanic waters (Figure 3). The recovery of the Mo species was 101% for Mo (V) and 93% for Mo (VI) when added together and 94% (Mo (V)) and 108% (Mo (VI)) when added alone (Figure 3). These are excellent recoveries and showed that Mo (V) ions could be analytically isolated from Mo (VI). Naturally occurring Mo (V) species are expected to be more stable than the free Mo (V) ions due to complexation with organic ligands.

Since there is no certified reference material for Mo species available yet, the currently available certified reference seawater, CASS-4, was analyzed for Mo (V) and total Mo. The CASS-4 seawater was collected in 1988 from coastal waters off Halifax, Canada and acidified for more than 18 years (pH ~ 1.6) (NRC, 1999). CASS-4 has a

certified total Mo concentration of 91.5 ± 9.0 nM. The total Mo concentration obtained using the speciation method was 86.5 ± 3.1 nM; no Mo (V) was detected in the SRM. The reason of the dominance of Mo (VI) species detected under those acidic conditions is still unclear.

Spike experiments were also conducted using natural seawater collected from the Atlantic Ocean about 5 miles off Southampton, NY (Figure 4). The speciation protocol showed that the water sample contained only Mo (VI) with a concentration of 116.7 nM. After spiking the water sample with 117.0 nM of only Mo (V), 84% of the added Mo (V) (101.2 nM) was recovered (Figure 4). The amount of Mo (VI) present in the sample also increased slightly to 124 nM, consistent with the fact that free Mo (V) ions are easily oxidized to Mo (VI) (oxidation half-time of ~30 min; Sugio et al., 1992).

b) Stability of Mo (V)

The short- term stability study showed that the levels of Mo (V) in the water sample collected in the Peconics changed rapidly after collection with a pH adjusted to 7.0 (Figure 5). The results suggest that at pH of 7.0 the reduced form of Mo can almost double within an hour, indicating that the speciation analysis has to be carried out within the first 15 minutes of the water collection. Furthermore, the similar results were obtained in the different temperature incubation experiments (0^0 and 20^0 C) (Figure 6). Mo (V) may be produced biologically (e.g., Szilágyi, 1967; Lloyd, 2003). The small temperature dependence, however, suggests that biological effects may not be direct, that

is, that whatever is acting as the reductant is present in the water (e.g., humic substances, Szilágyi, 1967; Bertine, 1972), Regardless of the mechanism of reaction, separation of Mo (V) and Mo (VI) should be conducted out in situ as soon as possible.

c) Mo (V) and Mo (VI) concentrations in waters collected at the head of Peconic River Estuary

The new method was first applied to measure Mo speciation in surface and bottom waters collected at the head of Peconic River Estuary during summer 2006 when anoxia occurred. The speciation of both, Mo (V) and Mo (VI) were investigated in bottom waters (DO = 0 ~ 10 μM, which are considered suboxic (<10 μM O_2) or anoxic (<1 μM O_2) as defined by Tyson and Pearson, 1991) and in surface oxic waters (DO=150~180 μM).

Figure 7 showed that total dissolved Mo (as Mo (V) + Mo (VI)) at the head of Peconic River Estuary increased with increasing salinity. The total dissolved Mo ranged from 2.5 nM in the river water to 120 nM in bottom seawater. The lowest levels of total dissolved Mo (2.5-4.0 nM) were measured in the low salinity (0-5) waters in the upper Peconic River. The Mo concentrations are slightly lower than the values reported for the Chao Phraya River (3.2-4.9 nM, Dalai et al., 2005), and the average world river water (5.0 nM, Martin and Meyback, 1979). The levels of total Mo in surface oxic water were lower (10-15 nM) than those measured in bottom anoxic or suboxic waters (100-120 nM) (Figures 7 and 8). Total Mo measured in bottom anoxic or suboxic waters showed a non-

conservative excess with respect to conservative mixing (Figure 7). In contrast, total dissolved Mo in surface waters showed a non-conservative removal. Depletion of total dissolved Mo in surface waters has been attributed to phytoplankton and bacteria uptake and/or particle adsorption (e.g., Brumsack and Gieskes, 1983; Yamazaki and Gohda, 1990; Falkowski et al., 1983; Howarth and Colllier, 1985; Paerl et al., 1987; Tuit and Ravizza, 2003). In oxic water, Mn oxides serve as the most effective scavenger for Mo (Wedepohl, 1978; Shimmield and Price, 1986). On the other hand, in anoxic or suboxic waters, desorption of Mo from river-borne particles may contribute to the non-conservative excess of Mo (Jones, 1974; Dalai et al., 2005; Morford et al., 2005; Dellwig et al., 2007).

Total dissolved Mo concentrations showed an inverse relationship with water pH (Figure 8). In general, total dissolved Mo increased from 20-120 nM with decreasing pH (7.5 to 7.0) in bottom waters (Figure 8). Between water pH of 7.5 and 8.3 measured in surface waters, the levels of dissolved Mo were lower and less variable (< 20 nM). Mo (V) was also low at the head of Peconic River Estuary, ranging from 0 to 16 nM, accounting for as much as 15% of the total dissolved Mo in some samples (Figure 9). Mo (V) concentrations were higher in waters with low pH, and lower in waters with high pH (Figure 9). In general, Mo (V) increased from 2 to 16 nM with decreasing pH (from 7.5 to 7.0). In surface waters, the levels of Mo (V) were also very low (< 2 nM) and apparently independent of water pH, although the low Mo concentrations compromise any strong conclusion regarding pH dependence. Because total Mo concentrations were

low in surface waters of relatively low salinity, Mo (V) accounted for a high proportion of the total dissolved Mo pool (as high as 30% in some samples). The results suggested that these Mo (V) cations tended to remain in surface waters enriched with river-borne positively charged particles.

The source of the Mo (V) species is still unclear, although this could be the result of reduction in anoxic bottom waters due to the existences of many reductants (e.g., Fe^{2+}, S^0 and S^{2-}) (e.g., O'Sullivan et al., 1991; Lloyd, 2003) and/or diffusion from reducing sediments.

d) Thermodynamic considerations for the presence of Mo (V) in coastal waters

Mo (VI) could possibly be reduced by many inorganic reducing agents with lower Eh values (e.g., Fe^{2+} and sulfide) (e.g., Turner et al., 1981; Bard et al., 1985). These inorganic agents have been detected in natural waters (e.g., Fe^{2+}, O'Sullivan et al., 1991; S^0, Lloyd, 2003). It has been reported that molybdate is reduced by polysulfide to Mo (V) under anoxic conditions (Draganjac et al., 1982; Pan et al., 1983), and even Mo (IV) as molybdite MoS_2 under strongly sulfidic conditions (Crusius et al., 1996; Vorlicek and Helz, 2002). In addition, molybdate could also be reduced to Mo (V) by organic acids under anoxic conditions (e.g., Bertine, 1972; Sugio et al., 1988).

Theoretically, both pH and Eh strongly dictate the speciation of Mo in natural waters: Mo (VI) generally dominates under oxic conditions (pH=~8.0, and Eh > 0.6 V), while reduced Mo (V) dominates in anoxic environments (Figure 10; Brookins, 1988). In

this Eh-pH diagram, anoxic is simply defined as the condition when Fe reduction occurred (pH =7.2-7.6 and Eh= ~0.0 V) (Brookins, 1988). Although we don't have Eh data for the Peconic River Estuary, based on the levels of oxygen (0~10 μM), it is reasonable to assume suboxic or anoxic conditions with a large range of Eh values such as 0.0~0.6 V for bottom waters and oxic conditions with Eh >0.6 V for surface waters. Combined with the water pH (range: 7.0-7.5 in bottom waters and 7.5-8.3 for surface waters), it is possible to use the Eh-pH diagram to establish the presence of the different Mo redox-species at the head of the Peconics during sampling (Figure 10). These analyses showed that, consistent with our field results, Mo (VI) is the predominant species of Mo in surface oxic waters and Mo (V) could exists in bottom suboxic or anoxic waters (Figure 10). These thermodynamic calculations do not explain the presence of Mo (V) in surface waters. However, the head of the Peconics is a shallow environment strongly influenced by diffusion from reducing sediments where Mo (VI) is reduced to Mo (V). The boundary of the Eh value for the Mo (VI)/(V) couple (Figure 10) could be raised from 0 to 0.48 V(Loach, 1970) if the Mo is complexed with organic ligands as expected in natural waters (Szilágyi, 1967; Bertine, 1972; Zhou et al., 2005). Although Mo (V) could be absorbed onto solid particles, this sorption process is relatively slow, ranging from months to years (Bertine, 1972). Therefore once formed, naturally occurring dissolved Mo (V) could be metastable in natural waters for a long time (Bertine, 1972).

SUMMARY

A new method for the direct determination of reduced and oxidized forms of Mo in seawater was developed. The protocol is based on the complexation of Mo (V) with tartrate under neutral conditions, extraction of this complex with an XAD 7 resin, elution with acidic acetone, and quantification by GFAAS. The detection limit of this protocol was on the order of 0.2 nM for both species. The analytical precision was ~10%. The method was successfully applied for the determination of Mo (V) and Mo (VI) in surface and bottom waters at the head of Peconic River Estuary. Consistent with our thermodynamic calculations, the existence of Mo (V) was confirmed in natural waters. Concentrations of Mo (V) ranged from 0 nM to ~15 nM, accounting for 0% ~ 15% of the total dissolved Mo pool in bottom suboxic or anoxic waters. Total Mo (Mo (V) + Mo (VI)) ranged from 20-120 nM with a non-conservative excess in bottom water with respect to conservative mixing. Our results also showed that Mo (V) contributed to this excess. Although the source of this reduced Mo is still unknown, benthic remobilization from reducing sediments is the most probable source.

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TABLE CAPTION

Table 1: Optimized graphite furnace temperature program for the determination of molybdenum.

Step	Temperature (^{o}C)	Ramp time (s)	Hold time (s)	Gas flow (ml/min)
Dry 1	110	1	30	250
Dry 2	130	15	30	250
Pyrolysis	1500	10	20	250
Atomization	2450	0	5	0
Cleanout	2500	1	5	250

LIST OF FIGURES

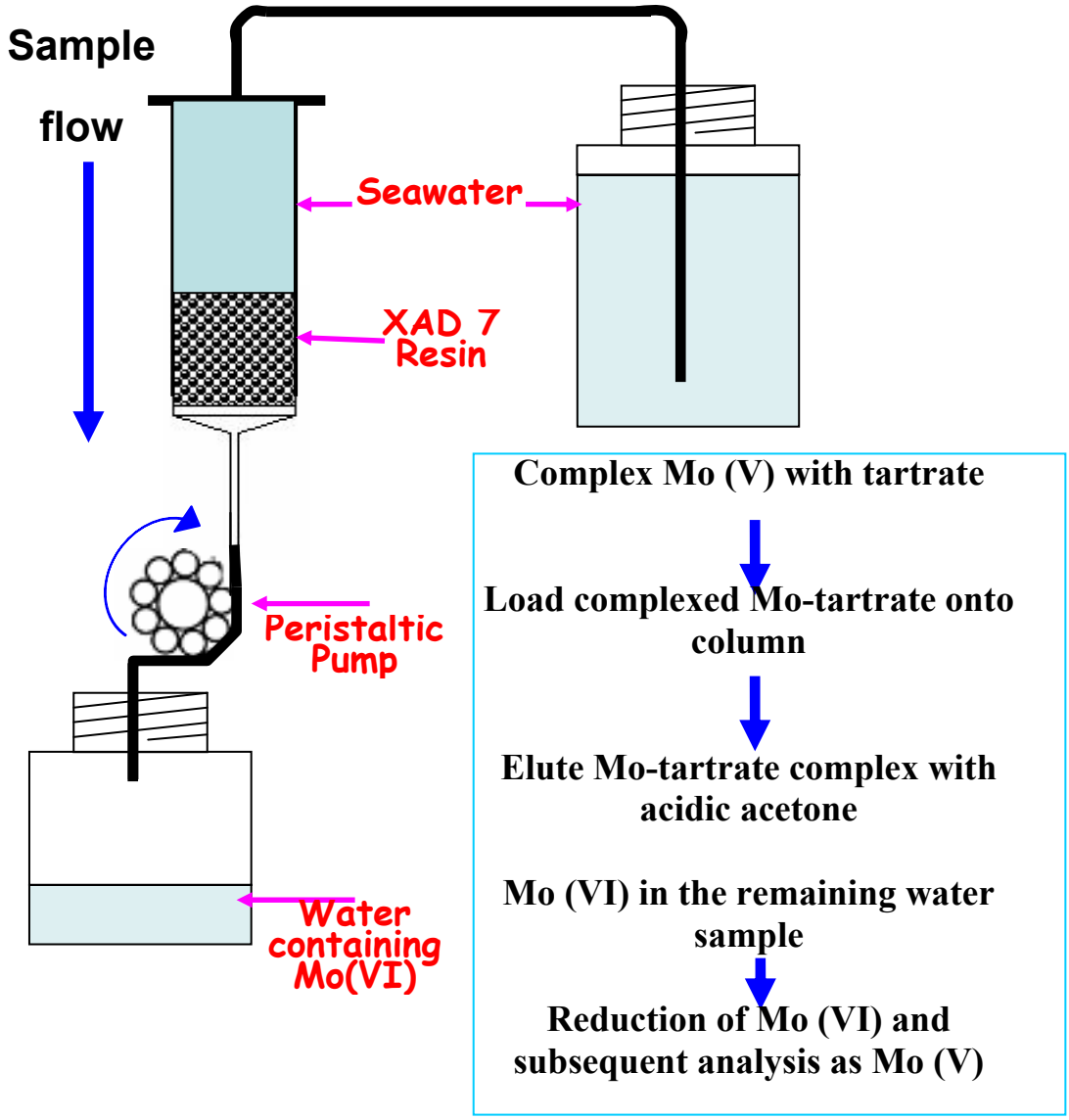

Figure 1: Schematic of the analytical protocol for separating Mo (V) from Mo (VI) in natural waters.

Figure 2: Sampling locations in this study.

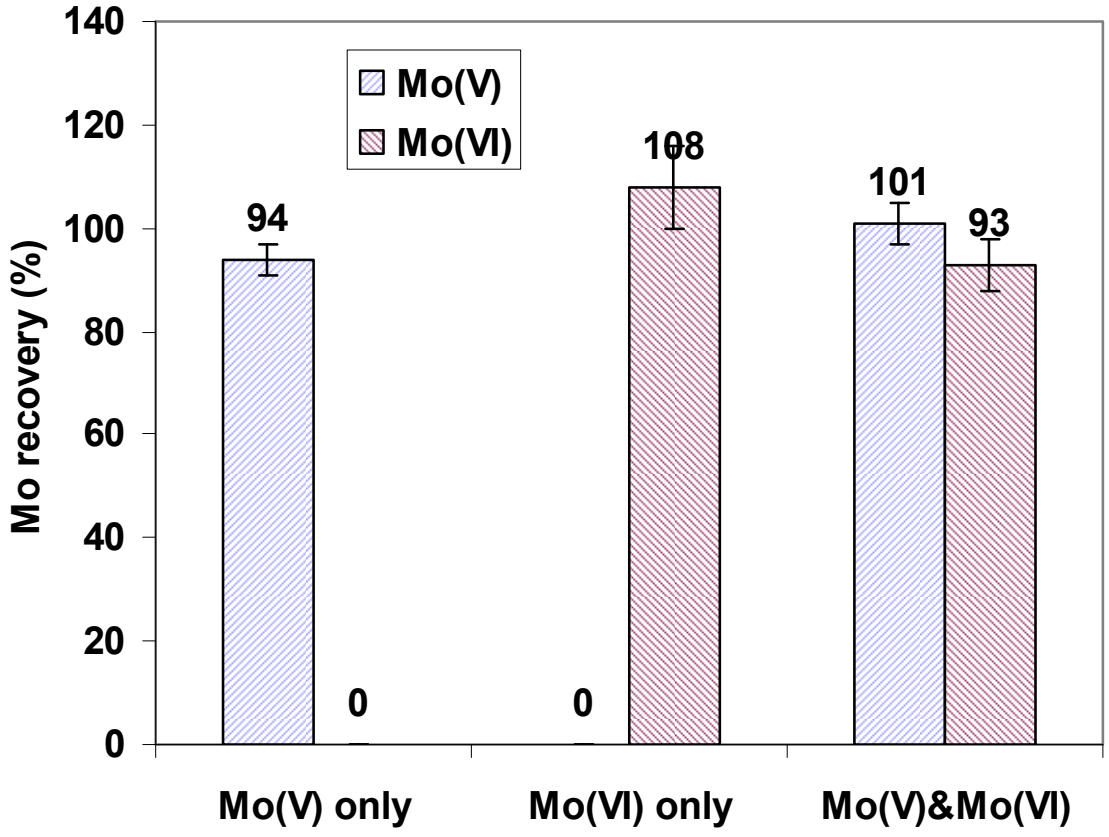

Figure 3: Molybdenum recoveries of Mo (V) only, Mo (VI) only and both Mo (V) and (VI) in synthetic seawater (All additions = 100 nM; Error bars are standard deviation, n=4).

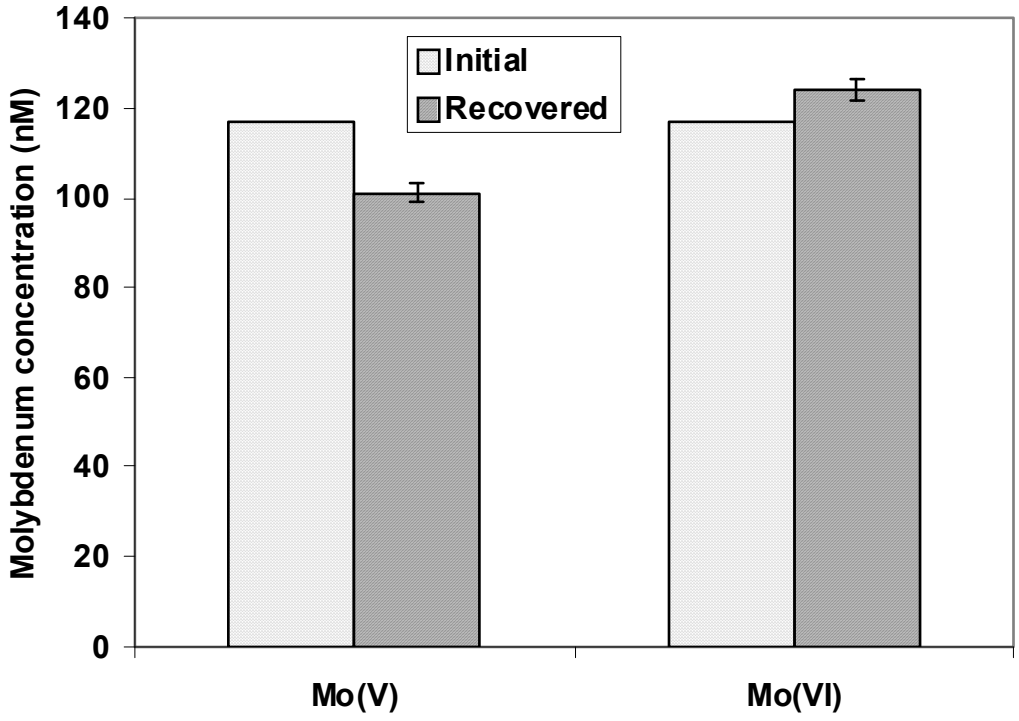

Figure 4: Recovery of Mo (V) in spiked seawater samples (The water sample was collected in the Atlantic Ocean, ~5 miles offshore of Southampton, NY; The initial Mo is essentially all Mo (VI) with a concentration of 116.7 nM as the initial bar Mo (VI); The initial bar Mo (V) represents the Mo (V) added to the seawater samples. The other bars are the recoveries of the spikes).

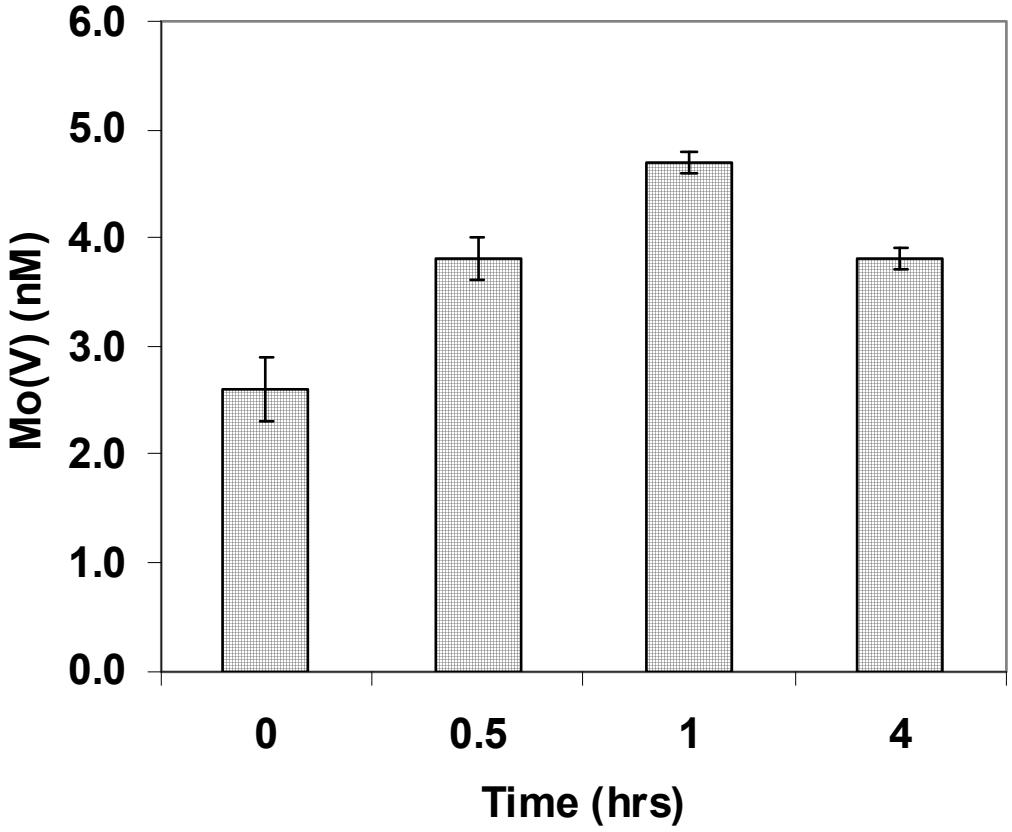

Figure 5: Changes in Mo (V) concentrations with time after adjusting the sample pH to 7.0 (Error bars are standard deviation, n=2. The samples were bottom waters collected at the head of Peconic River Estuary in September 2007 with dissolved oxygen levels ~10 µM and pH=7.5).

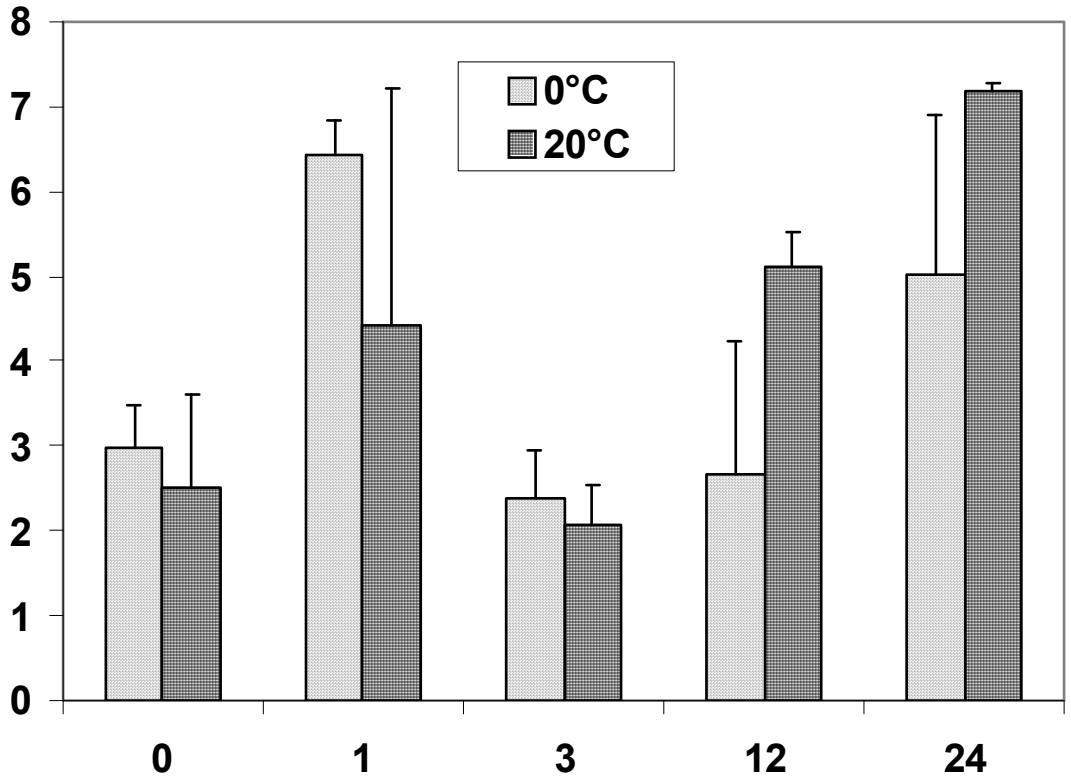

Figure 6: Changes in Mo (V) concentrations in water samples incubated at 0 and 20^{o} C for up to 24 h (Error bars are standard deviation, n=2; The samples were bottom waters collected at the head of Peconic River Estuary with DO levels of ~10 µM and pH=~7.5).

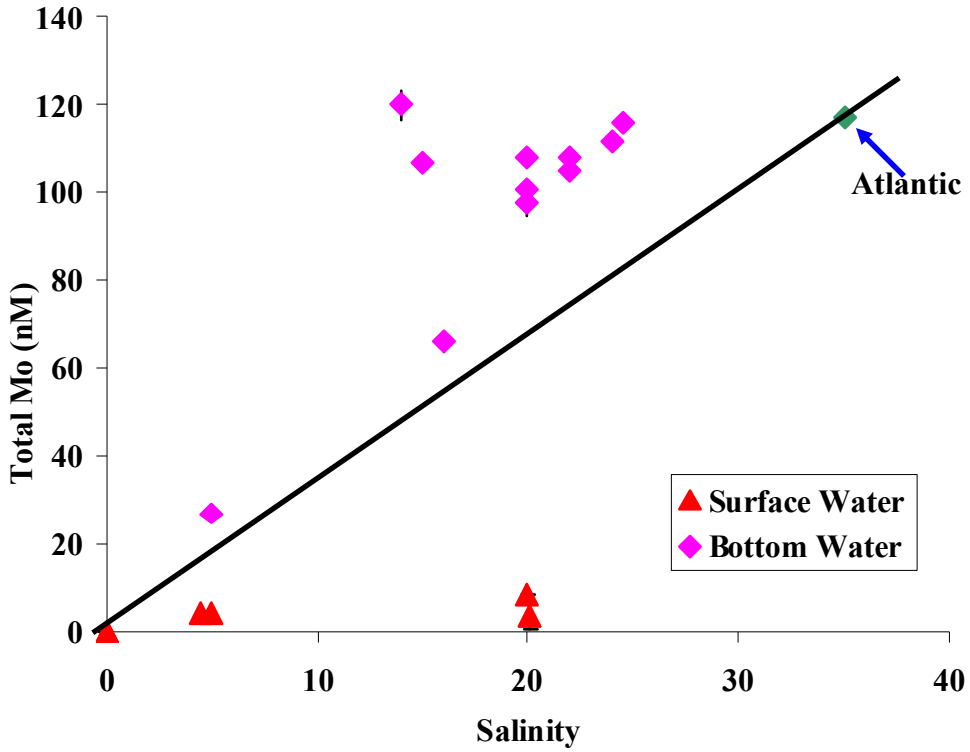

Figure 7: Total dissolved Mo concentrations (as Mo (V) + Mo (VI)) versus salinity at the head of Peconic River Estuary (The Atlantic seawater sample was collected from ~5 miles offshore of Southampton, NY).

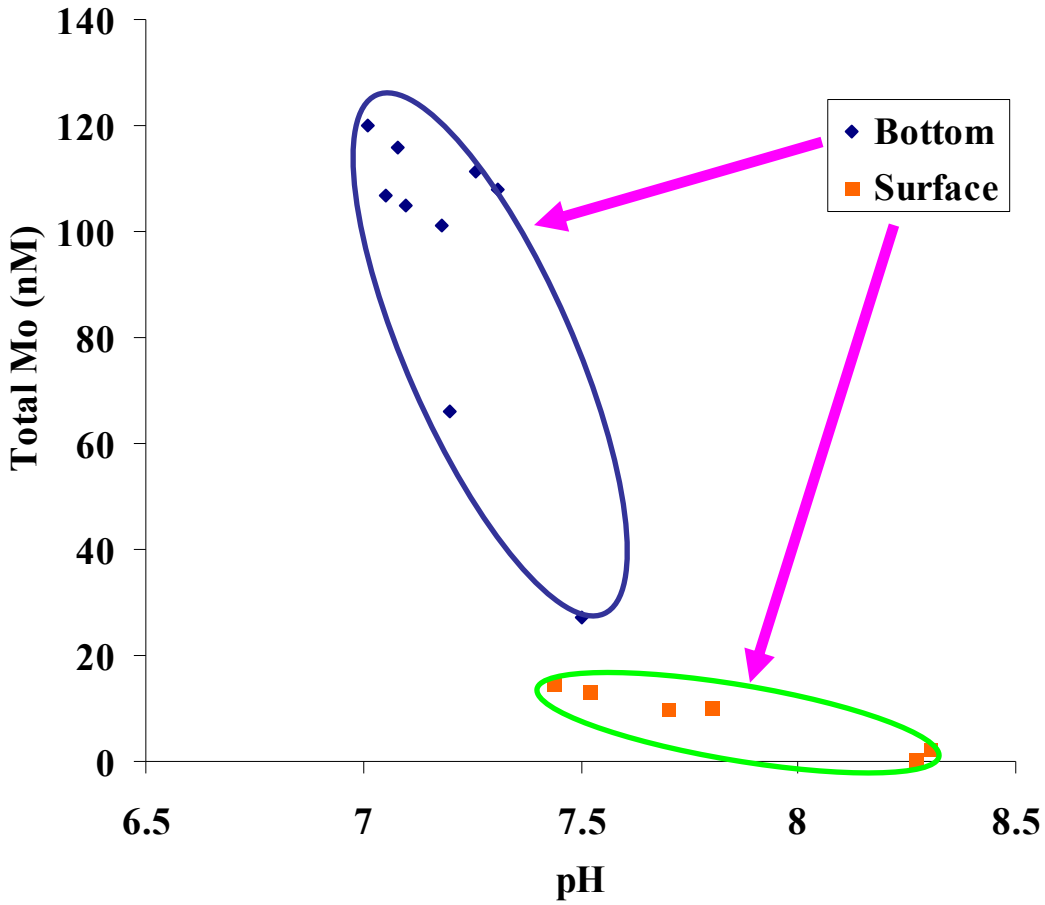

Figure 8: Total Mo concentrations of Mo (as Mo (V) + Mo (VI)) versus water pH in surface and bottom waters collected at the head of Peconic River Estuary.

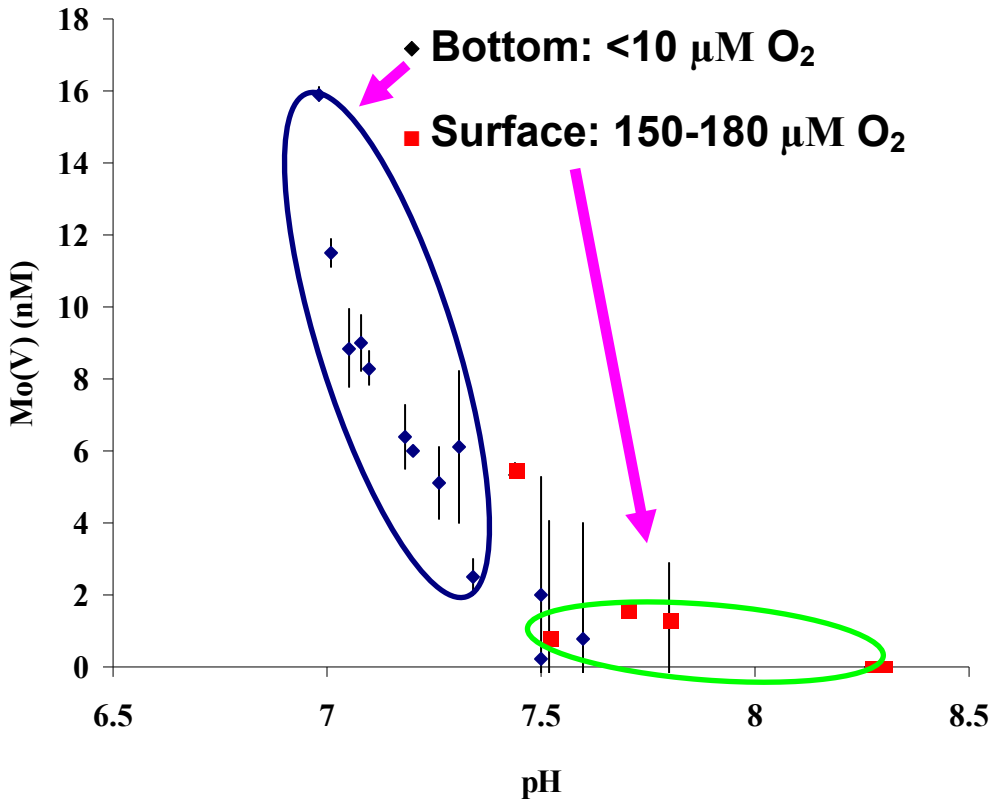

Figure 9: Mo (V) concentrations versus water pH in surface and bottom waters at the head of Peconic River Estuary.

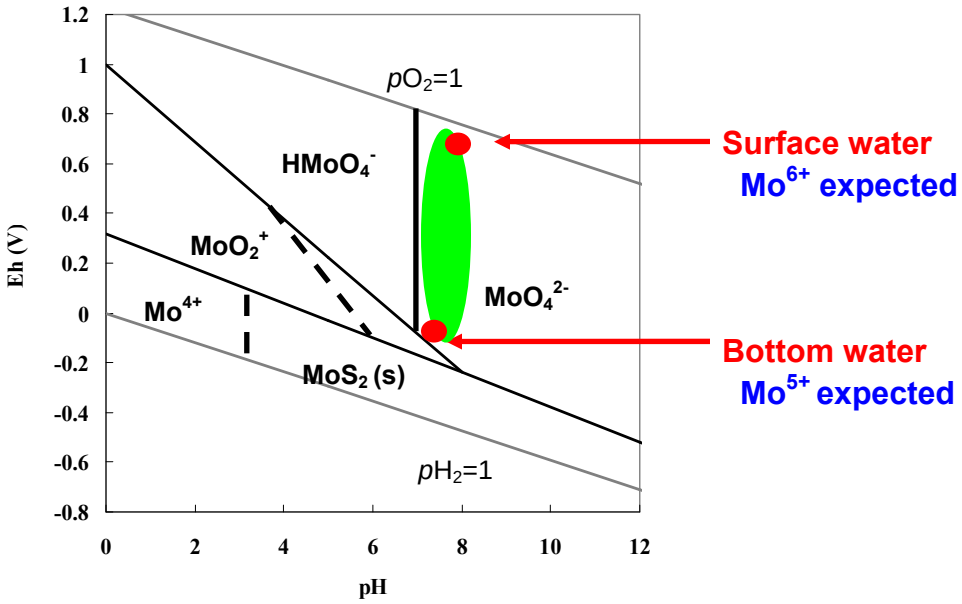

Figure 10: Eh-pH diagram of Mo in natural environments (The pH-Eh calculated for surface and bottom waters at the head of Peconic River Estuary are also shown; Assuming an activity of dissolved Mo=10^{-7} M, and sulfide=10^{-3} M; modified from Brookins, 1988).

CHAPTER FOUR: SPECIATION, AND GEOCHEMICAL PROCESS STUDIES OF MOLYBDENUM IN SEDIMENT POREWATER, FLAX POND, LONG ISLAND

ABSTRACT

For several decades, many studies have investigated Mo biogeochemical cycling and the use of Mo as a paleoceanographic indicator for low oxygen conditions. The use of Mo as a proxy for anoxia is based on the fact that molybdate is reduced to Mo (V) and Mo (IV), and may be precipitated as MoS_2 under reducing conditions. Although molybdenum is very abundant in the oxic oceans in the form of oxygenated Mo (VI): molybdate MoO_4^{2-}, the bioavailability of this element is more dependent on particular redox species than the total pool. Mo (V), as the less abundant form of Mo, is expected to be more bioavailable than Mo (VI). The study of biogeochemical cycling of this element, however, has been hindered by a lack of knowledge of its chemical speciation.

Based on the new method developed in this dissertation (Chapter Three), we measured reduced Mo, Mo (V) in porewater at Flax Pond, and studied the behavior of Mo with other redox sensitive elements, Fe, Mn, and S. Vertical profiles showed high concentrations of Mo, especially Mo (V), in porewater under nonsulfidic or low sulfide conditions at Flax Pond. Lower total Mo and little Mo (V) were founds under highly sulfidic conditions. Anoxic incubation experiments using surface sediments showed that Mo was mobilized and Mo (V) increased with time when sulfide was low. However, when sulfide built up, Mo (V) was eliminated, probably as a result of further reduction to Mo (IV) and precipitation as solid MoS_2. Mo (VI) became the dominant dissolved species presumably because Mo (VI) continued to be desorbed from carrier phases, e.g., Mn, Fe oxides and organic particles, and a portion was stabilized in solution as MoS_4^{2-}. During oxic incubation of reduced sediments from depth (4-5 cm), a large amount of Mo was released as Mo (VI). In this case, Mo (VI) was the only thermodynamically stable

form under oxic conditions. Mo (V) and Mo (IV) were expected to be formed as intermediates during the oxidation although they were undetectable during the experiments presumably due to rapid transformation to Mo (VI).

INTRODUCTION

Molybdenum has an extensive redox chemistry with oxidation states of +II to +VI. Only Mo (VI) and Mo (V) are soluble and thus available for biological systems (e.g., Mendel, 2005). Mo (VI), as in molybdate MoO_4^{2-}, is generally assumed to be the dominant oxidation state of Mo in oxic natural waters, while Mo (V) is expected to exist in reducing waters (Bertine, 1972; Brookins, 1988). Mo is the most abundant transition metal in the ocean, and displays a conservative behavior under oxic conditions (about 107 nM) (Morris, 1975; Collier, 1985; Prange and Kremling, 1985), with a slight depletion in surface water (Yamazaki and Gohda, 1990; Adelson et al., 2001; Tuit and Ravizza, 2003).

Bertine (1972) and Emerson and Huested (1991) showed that in the Black Sea and Cariaco Basin, 30 to 70% of molybdenum was depleted in strongly reducing deep water compared to surface water. Molybdenum is enriched in highly sulfidic sediments, with a concentration as high as 140 μg/g in the Black Sea and Saanich Inlet compared to the crustal average of about 1.1 μg/g (Berrang and Grill, 1974; Colodner et al., 1995; Morford et al., 1999). In the open ocean, Mo enrichments are highest in regions with oxygen-depleted bottom waters and high organic carbon flux with highly sulfidic settings (e.g., Zheng et al., 2000; Nameroff et al., 2002; Algeo and Maynard, 2004), both of which result from ocean anoxic events. In waters with low sulfide, Mo can also be

scavenged by Fe, Mn oxides, humic material, or by organosulfur ligands (e.g., Zheng et al., 2000).

Based on these findings, Mo has been widely used as a paleoceanographic proxy of oceanic anoxic events in sediment cores (Emerson and Huested, 1991; Dean et al., 1999; Nijenhuis et al., 1998; Nameroff et al., 2002). Under highly sulfidic conditions, molybdate is expected to be reduced to Mo (V) or Mo (IV) and may precipitate as MoS_2 (Nameroff et al., 2002; Vorlicek and Helz, 2002; Vorlicek et al., 2004; Algeo and Maynard, 2004). Nameroff et al. (2002) further verified that direct Mo-sulfide precipitation occurs at above 100 μM H_2S. Depending upon pH and sulfide concentrations, molybdenum as Mo (VI) may also exist as various thiomolybdate ions under sulfidic conditions, which may further experience scavenging (Erickson and Helz, 2000; Adelson et al., 2001). It is still unclear which one (scavenging or precipitation) is the dominant process contributing to Mo accumulation in sediments. Reduced Mo, Mo (V), is expected to be involved in these removal processes although to this point there has been no direct quantification.

In oxic sediments, Mo concentrations can also be elevated as a result of Mo adsorption onto Mn oxyhydroxides (Bertine and Turekian, 1973). Under suboxic conditions, carrier phases such as Mn, Fe oxides and organic matter can be reduced and adsorbed Mo is released to porewater. As a result, suboxic sediments contain only lithogenic concentrations of Mo (e.g., only ~0.6 μg/g for terrigenous sediments derived from eastern Vancouver Island; Morford et al., 2005). Under highly sulfidic conditions as mentioned previously, Mo (VI) is reduced to Mo (IV) and precipitates (Chaillou et al., 2002). Reduced sediments may contain as much as 140 μg/g Mo (e.g., Bertine and

Turekian, 1973; Koide et al., 1986; Francois, 1988; Emerson and Huested, 1991; Crusius et al., 1996; Zheng et al., 2000; Adelson et al., 2001).

This study, for the first time, uses the analytical methods developed to speciate Mo (Chapter 3) to study the dynamics of Mo speciation in sediment porewater at Flax Pond (a back-barrier salt marsh environment in eastern Long Island, NY), to examine the early diagenetic behavior of Mo, and to infer redox changes of Mo in response to different redox conditions by conducting manipulative laboratory experiments. Mo is shown to have dynamic diagenetic behavior characterized by rapid mobilization during reduction or oxidation of carrier phases, dynamic speciation changes in solution, and reprecipitation in response to different redox conditions.

MATERIALS AND METHODS

a) Sediment pore water sample collections

Sediment cores were collected at intertidal sites in Flax Pond, Long Island during low tide, May to June 2007. Flax Pond is a back-barrier salt marsh environment and the study location is shown in Figure 1. The salinity of overlying bottom water in Flax Pond was ~28, and temperature was 21^{o} C, pH=7.6 and DO was 50 μM for water close to surface sediments. Organic inputs to the sediments in Flax Pond include marsh detritus, terrigenous matter, and plankton debris. The sediments were anoxic below the upper few millimeters (Jacobson et al., 1987). Sediment samples were collected with a hand-held acrylic box corer (165 cm^2 cross-sectional area). Sediment samples were then extruded at 1 cm intervals into 250 ml clean polypropylene bottles under nitrogen, and then pore water was separated by centrifugation at 4000 rpm for 10 min. Supernatant samples were

filtered through 0.2 μm Nuclepore membrane filters in plastic holders, and separated for different chemical redox species of Mo, Mo (V) and Mo (VI), immediately and analyzed via GFAAS within a week according to the newly developed method in Chapter 3. Filtered pore water was also collected in Teflon bottles, and analyzed for dissolved sulfide, Fe, and Mn by standard spectrophotometric methods (Cline, 1969; Stookey, 1970; Goto et al., 1962). All sampling materials used in this study were prepared using trace metal clean techniques (Flegal et al., 1991) and all of the separation procedures were carried out inside a nitrogen-filled glove bag (Bray et al., 1973; Troup et al., 1974).

b) General description of the method for separating Mo (V) and Mo (VI)

Water samples were adjusted to pH=7.0 with phosphate buffer (0.25 M KH_2PO_4, and 0.25 M Na_2HPO_4). Mo (V) was selectively complexed with tartrate solution (10% tartrate sodium) under neutral conditions, and then the Mo (V)-tartrate complexes were removed by passing solutions through poly-prep columns with 2.0 gram Amberlite XAD 7 resins. Mo (V) was eluted off the column with acidic acetone (0.1N), and analyzed by Graphite Furnace Atomic Absorption Spectrometry. Mo (VI) was reduced by 10% stannous chloride solution, and analyzed according to the above steps as Mo (V). The total Mo was obtained by summing Mo (V) and Mo (VI). All of the chemicals used were of analytical reagent grade or the highest purity available. Milli-Q water (18.2 MΩ, Millipore), HPLC-grade absolute acetone was used throughout.

c) Sediment incubation experiments

Anoxic incubation of surface sediments:

Sediment incubations are often used in biogeochemical studies to reveal behavior during aerobic and/or anaerobic processes (e.g., Aller and Yingst, 1980; Aller and Mackin, 1989; Elsgaard and Jorgensen, 1992; Hansen et al., 2000). In this study, anoxic incubation of initially oxic surface sediments was done by homogenizing surface sediments (collected at depth of 0-1 cm in Flax Pond during low tide) and distributing portions into a set of incubation bottles (200 ml wide-mouth HDPE). These bottles were sampled serially with time (e.g., Aller and Yingst, 1980; Kristensen et al., 1999). The anoxic incubation set-up is shown schematically in Figure 2. All sediment filled bottles were sealed with no air inside. All sample bottles were buried in anoxic sediment to maintain the anoxic conditions. The time series were sampled as follows: 0 h, 3 h, 12 h, 24 h, 2 d, 3 d, 4 d, 6 d, and 7 d. Once a bottle was taken out of the incubation container, it was centrifuged at 4000 rpm for 10 min, and supernatant filtered using 0.2 μm filters. Pore water was processed immediately and analyzed for sulfide, Mn, and Fe in the laboratory within one week. All the sample handling and operation was under nitrogen in a glove bag. All bottles, filters and sampling apparatus were maintained under nitrogen for at least 12 h ahead of experiments (Bray et al., 1973; Troup et al., 1974).

Oxidation of reduced sediments:

Subsurface, reduced sediments (4-5 cm) were taken from a muddy sediment core, and a proportion of the sediments (219.5 g) were placed into a 2 L plastic beaker. 2000 ml of seawater was added into the beaker, and the seawater was aerated and stirred

continuously. The schematic of the oxidation experimental setup was shown in Figure 3. 100 ml water samples were removed at 0 h, 3 h, 12 h, 24 h, 2 d, 3 d, 4 d, 6 d, and 7 d; The water sample was immediately filtered through a 0.2 μm filter, and measured for S, Mn, Fe, Mo (V) and Mo (VI). All bottles, filters and sampling apparatus were maintained under nitrogen for at least 12 h ahead of experiments. All sampling materials used in this study were prepared using trace metal clean techniques (Flegal et al., 1991).

RESULTS AND DISCUSSION

a) Early diagenesis of Mo in sediment pore waters at Flax Pond

Vertical profiles of total Mo in porewater at two sediment sites are shown in Figure 4. Distributions of total Mo behave quite differently at these two sites. The concentration of total Mo (100~150 nM) was relatively low with little variation vertically in muddy site 1, while total Mo increased sharply with increasing depth, from ~100 nM within the surface layer (0-3 cm) to as high as 1200 nM at depth (below 4 cm) in sandy site 2. For comparison, Figure 4 shows a vertical profile of total Mo in sediment porewater in Australia (Sinclair, 2004), which also had a very high value of total Mo in deep layers (similar to sandy site 2). Sinclair (2004) suspected that the high total Mo was related to reductive remobilization of Mo from carrier phases, e.g., Mn and Fe oxides.

Mo speciation was also measured in porewater at these two sediment sites, and the results are shown in Figure 5. Mo (VI) was the dominant species (100-120 nM), with little Mo (V) at muddy sediment site 1, and the speciation and concentration of Mo didn't vary with depth at this site. Mo behaves quite differently at sandy sediment site 2: Both speciation and concentration varied with depth. Similar to muddy sediment site 1, low

Mo (VI) and little Mo (V) was detected within the surface layer (0-3 cm). In contrast, very high concentrations of Mo (V) and also Mo (VI) were found in deep layers (below 4 cm) at sandy sediment site 2. The results showed that significant redox reactions of Mo (Mo (VI) was reduced to Mo (V)) occurred in deep layers at sandy sediment site 2.

Vertical profiles of Fe and Mn were investigated in porewater at these two sediment sites (Figure 6). Both dissolved Fe and Mn showed peaks within surface layers (0-3 cm): the Fe peak (4 μM for site 1 and 16 μM for site 2) occurred within 1 cm at surface, while the Mn peak (13 μM) occurred at the depth of 3-4 cm for both sites. Distributions of dissolved Mn in deep layers were very similar for both sites, but dissolved Fe was much lower at muddy sediment site 1 than at sandy sediment site 2, especially in deep layers (below 4 cm). Lower values of Fe at site 1 suggested a more extensive precipitation of Fe, likely as FeS.

Vertical profiles of sulfides at both sites are shown in Figure 7. Surprisingly, sulfides behaved quite differently at these two sites. In muddy sediment 1, sulfide increased sharply with increasing depth, from 0 at surface to as high as 2100 μM at 7 cm depth, indicative of a highly sulfidic environment (as defined here with sulfide concentration of greater than 100 μM) in deep porewater in muddy sediment 1. As shown in Figure 7, Mo (V) was low in muddy sediment 1. On the other hand, sulfide was relatively much lower in sandy sediment site 2, with a peak (240 μM) occurring at depth of 2-3 cm, then disappeared in deep layers (below 4 cm), indicating a nonsulfidic or low sulfide environment (as defined here with sulfide concentration of less than 100 μM) in deep sediments. As shown in Figure 7, Mo (V) was very high in deep layers (below 4 cm) in sandy sediment site 2.

Based on these observations, it is clear that under highly sulfidic conditions, Mo (V) was of minor importance and Mo (VI) dominated, but at low levels, while under nonsulfidic or low sulfide conditions, Mo (V) was very high and total Mo was also very high. Therefore, we proposed an hypothesis for Mo diagenetic behavior under low and high sulfide conditions as shown in Figure 8. Mo (VI) was remobilized from carrier phases, e.g., Fe and Mn oxides, when these carrier phases were reduced. With decreasing Eh, Mo (VI) was first reduced to Mo (V). Under highly sulfidic conditions, Mo (V) was reduced to Mo (IV) and precipitated as solid MoS_2, and therefore little Mo (V) was detected. It appeared that Mo (VI) continued to be desorbed from carrier phases and a portion remained in solution. Under nonsulfidic or low sulfide conditions, Mo (V) couldn't be further reduced to Mo (IV) due to the lack of sulfide. Instead, Mo (V) accumulated, and high levels of Mo (V) were built up. Mo (VI) was also high apparently due to the redox equilibrium of Mo (VI)/(V), and the continuous desorption of Mo (VI) from carrier phases. In nonsulfidic or low sulfide sediments, reduced phases of Mo such as MoS_2 formed under sulfidic conditions may be subject to reoxidiation to Mo (V) and Mo (VI). Summarizing: there are two major possibilities for the Mo (V) sources at Flax Pond: one is from reductive release of surface oxic sediment carrier phases, e.g., Mn and Fe oxides, enriched with Mo (VI), during reduction of Mo (VI), and the other is release from sulfidic sediments during oxidation of MoS_2, or other labile reduced compounds. Both processes are expected to occur in our study areas and are likely dominant in different regions of the sediment. These hypotheses were examined using manipulative laboratory experiments described subsequently.

b) Experimental evaluation of early diagenetic reactions of Mo

Anoxic incubation of surface sediments indicated that Mo (VI) was successively reduced to Mo (V) and lost from solution during the build-up of H_2S. Loss of Mo (V) can be attributed in part to reduction to Mo (IV) and precipitation as MoS_2 (Figures 9 and 10). During the anoxic incubation of surface sediments, sulfide concentration increased significantly (Figure 9). Initially sulfide was low within 24 h (<100 μM, defined as low sulfide) and increased slightly with time. However, after 24 hrs sulfide increased rapidly to levels of 2 mM at 2 d and to as high as ~10 mM at 7 d (>100 μM, defined as highly sulfidic).

Concentrations and speciation of Mo changed dynamically with time as shown in Figure 10. Total Mo (the sum of Mo (V) and Mo (VI)) was about 80 nM at the beginning (0 h), and increased with time, reaching a peak concentration of 180 nM at 24 h. Total Mo gradually decreased as a result of net precipitation process until the end of the experiment (7 d), reaching a level of 100 nM. Speciation of Mo also changed dynamically with time (Figure 10). At the beginning (0 h), only Mo (VI) was detected in the porewater. Mo (V) increased from 0 nM until 160 nM at 24 h. Subsequently, Mo (V) decreased with time to 0 nM at 4 d, and Mo (VI) gradually became dominant. These progressive temporal patterns in speciation and overall concentrations largely mirror the depth dependent patterns found at muddy site 1.

We infer that Mo (V) was reduced to Mo (IV) and solid MoS_2 under highly sulfidic conditions. Due to the extremely low value of $K_{sp}=10^{-43}$ (calculated from data in Garrels and Christ, 1965), MoS_2 will be supersaturated assuming concentrations of Mo (IV) = 1 nM and sulfide = 100 μM. Therefore MoS_2 can precipitate once Mo (V) is

reduced to Mo (IV) in sediment porewater at Flax Pond. In addition, disproportionation of Mo (V) may occur (e.g., Ueyama et al., 1990; Barral et al., 1972; Matsuda et al., 1979; Thompson et al., 1993; Lee et al., 1990) under highly sulfidic conditions, which could contribute to the formation of both Mo (IV) and Mo (VI). We therefore hypothesize that under highly sulfidic conditions Mo (VI) continues to be released during reductive decomposition of carrier phases. As sulfide progressively builds up, a portion of the Mo (VI) is reduced to Mo (V). Once formed Mo (V) may be further reduced to Mo (IV) and precipitated as MoS_2, decreasing the total concentration of Mo in solution. Mo (V) may also disproportionate to Mo (IV) and Mo (VI), the latter precipitating from solution, again as MoS_2. The eventual dominance of dissolved Mo (VI) in highly sulfidic solution presumably results from a balance between continued production from carrier phases, formation from Mo (V) during disporportionation, and partial stabilization in the form of thiomolybdates as total sulfide builds up. Mo (VI) is known to form a series of thiomolybdates represented as: MoS_4^{2-} (e.g., Helz et al., 1996; Erickson et al., 2000). Erickson et al. (2000) reported that MoS_4^{2-} was very stable once formed, and MoS_4^{2-} (at concentrations of 1 mM) may remain conserved over 60 d. Studies from Bostick et al. (2003) showed that MoS_4^{2-} was readily bound onto pyrite only at pH<6. Almost no scavenging of MoS_4^{2-} onto pyrite occurred at high pH of 8.5 (Vorlicek et al., 2004). Therefore adsorption should not be strong and Mo (VI) likely exists as MoS_4^{2-} in highly sulfidic porewater (pH of 7.0-7.5) at Flax Pond.

During the oxidation of highly sulfidic sediments, Mo (VI) was low at the start (0 h) with a value of 80 nM, and gradually increased with time, reaching a level of 500 nM at 2 d, and a value of 1500 nM by 7 d (Figure 11). Fe, Mn, sulfide and Mo (V) were

undetectable during the entire experiment period. This oxidation experiment verified that a large amount of Mo can be released during reoxidation processes. Transient intermediates Mo (IV) and Mo (V) must exist when MoS_2 is oxidized; however, Mo (V) was not detected in experiments. We presume that Mo (V) was quickly oxidized to stable MoO_4^{2-} under oxic conditions.

c) Implications of early diagenesis of Mo in geochemical studies

Based on this study, the Mo cycle in Flax Pond was summarized in a conceptual model as shown in Figure 12. Tidal flow from the LI Sound contributed the major source of Mo as Mo (VI) to the Flax Pond. Mo (VI) may be scavenged onto carrier phases (Mn and Fe oxides, and organic particles) and accumulated in surface sediments. In sediments, carrier phases (Mn and Fe oxides, and organic particles) enriched with Mo (VI) was reduced first during suboxic diagenesis, and then Mo (VI) was released and reduced to Mo (V) under nonsulfidic or low sulfide conditions. Mo (V) may be further reduced and precipitated as MoS_2 in highly sulfidic sediments. On the other hand, MoS_2 may also be reoxidized if exposed to O_2 during bioturbation or sediment reworking. Consequently, there are two major possibilities for the sources of Mo (V) at Flax Pond (Figure 12) as described earlier: reductive release from surface oxic sediments, by reduction of Mo (VI); oxidative release from deep sulfidic sediments, by oxidation of MoS_2. Formation of Mo (V) as an intermediate during oxidative release is inferred, although only Mo (VI) was found as the stable product of oxygenation in experimental incubations.

Mo excess (the difference above the conservative mixing line) in bottom suboxic waters has been reported widely (e.g., Dalai et al., 2005; Fox and Doner, 2003; Dellwig et al., 2007). Generally Mo is associated with carrier phases such as Mn, Fe oxides and organic particles under oxic waters (e.g., Berrang and Grill, 1974; Zheng et al., 2000). Mn and Fe-oxides undergo reduction during suboxic diagenesis and Mo associated with these carrier phases is released. Mo content in sediments under suboxic conditions is relatively low, e.g., ~1.6 μg/g Mo in the Chao Phraya River Estuary, Thailand (Dalai et al., 2005), which is much lower than in oxic sediments (~ 8 μg/g Mo, Morford and Emerson, 1999) and in anoxic sediments (~50 μg/g Mo, Morford and Emerson, 1999) Lower content of Mo in suboxic sediments has also been reported widely (e.g., Elbaz-Poulichet et al., 1997; 2004; Dellwig et al., 2007; Nameroff e al., 2002). All the above researches suggested that Mo was released from carrier phases into porewater and even diffused into overlying bottom water during suboxic diagenesis.

Therefore during suboxic diagenesis, assuming the original Mo content is 8 μg/g (e.g., Morford and Emerson, 1999), 50% Mo is released under suboxic conditions (e.g., Dalai et al., 2005), and the sediment porosity is 80% (v/v) and the density of particles is 2.5 g/cm^3. The contribution of Mo desorption to porewater would be as high as ~24 mM. Apparently upward diffusion accounted for Mo excess occurred in overlying bottom waters at head of Peconic River Estuary (Chapter Three in this dissertation) and in the Chao Phraya River Estuary, Thailand (Dalai et al., 2005), while downward diffusion provides the source of Mo in deep sediment porewater at Flax Pond (as summarized in Figure 8). Fox and Doner (2003) reported that total Mo was 8-16 μmol/L in porewater at a salt marsh with *Spartina*. Similar to our results in nonsulfidic or low sulfide conditions,

Sinclair (2004) also found as high as 2-3 μM Mo in sediment porewater in Australia (in Figure 4).

The marine geochemistry of Mo is characterized by its removal in sulfidic basins (Shaw et al., 1990; Colodner et al., 1995). In anoxic sediments (likely with nonsulfidic or low sulfide settings), Mo accumulated with a content of 50 μg/g Mo (e.g., Emerson and Huested, 1991; Morford and Emerson, 1999; Algeo and Maynard, 2004). Based on this research, Mo reduction occurred and Mo (VI) was reduced Mo (V) under nonsulfidic or low sulfide (<100 μmol/L H_2S) conditions. Under low sulfide conditions, Mo (VI), likely MoS_4^{2-}, was also partly scavenged by carrier phases such as pyrite (e.g., Helz et al., 1996). These processes have been verified in our manipulative incubation experiments as a net loss of Mo (VI) and build-up of Mo (V) under low sulfide conditions (Figure 10). Nameroff et al. (2002) reported that direct precipitation as Mo sulfide occurred under highly sulfidic conditions (>100 μmol/L H_2S). Our results indicated that Mo (V) was further reduced to Mo (IV) and precipitated as MoS_2, and a portion of Mo (VI), likely MoS_4^{2-}, still remained in solutions. In enclosed basins, such as the Black Sea and the Saanich Inlet, concentrations of Mo in sediments can be as high as 140 μg/g respectively (Berrang and Grill, 1974; Emerson and Huested, 1991). Algeo and Maynard (2003) reported black shales formed under highly sulfidic settings have a much higher accumulation of Mo in sediments than those formed with anoxic settings. In sediments formed during anoxic events (likely with highly sulfidic settings), Mo content could be as high as 400 μg/g (Nijenhuis et al., 1998).

Once an anoxic event has ceased and reoxygenation occurs, highly Mo enriched sediments reexposed to oxic overlying seawater, can release Mo (VI) back into overlying

seawater, as our oxidation experiments showed (Figure 11). It suggested that besides Mo desorption from carrier phases, high Mo in nonsulfidic or low sulfide environments, e.g., sandy sediments (Figure 5) may also result from oxidative release during oxidation of reduced sediments enriched with MoS_2. No Mo (V) was observed under well-oxygenated conditions in the oxidation experiments (Figure 11) apparently because Mo (V) was quickly oxidized to stable Mo (VI). Under somewhat less intense oxygenation, Mo (V) might be observed as a more stable intermediate, e.g., under nonsulfidic or low sulfide conditions.

In paleoceanographic studies, Mo accumulation has been used to estimate the extent of anoxic events. For example, an anoxic event occurred in the Mediterranean Sea during the early Pliocene Epoch (~4 Ma before present) (Figure 13, Nijenhuis et al., 1998), based on Mo accumulation, given a 7.5 cm thick layer, this anoxic event lasted about 6 thousand years. However, such a simple estimation ignores the possibility of reoxygenation and diffusive loss. If reoxygenation is considered, the extent of the anoxic event could be much higher than predicted. Therefore, paleoceanographic studies based on Mo accumulation in sediments have potentially underestimated the extent of anoxic events due to the benthic remobilization of this trace element if re-oxygenation occurs.

Finally we proposed sulfide as a geochemical switch, in which Mo transforms from a soluble form as Mo (V) to a solid form as MoS_2. Nameroff et al. (2002) reported that direct Mo sulfide precipitation occurs above 100 μM sulfide. The transition point of sulfide was therefore defined when sulfate reduction occurred with sulfide of ~ 100 μM, which has also been verified in our field investigation in porewater at Flax Pond and manipulative laboratory experiments on reduction of surface oxic sediments.

In our model, we divided Mo reduction into two stages: 1) Mo (VI) to Mo (V) under nonsulfidic or low sulfide conditions, and 1) Mo (V) to Mo (IV) under highly sulfidic conditions. Under the first stage, Mo (VI) desorbed from carrier phases (organic matter, Fe and Mn oxides) was reduced to soluble Mo (V). Under the second stage, Mo (V) was further reduced to solid Mo (IV). Correspondingly, Mo was little accumulated in sediments under nonsulfidic or low sulfide conditions (Algeo and Maynard, 2004), in which Mo was more likely removed by a weaker process: the scavenging of Mo (V) onto organic particles. In contrast, Mo was highly accumulated in sediments under highly sulfidic settings (e.g., Nameroff et al., 2002; Algeo and Maynard, 2004), in which Mo was more likely removed by a more intense process: precipitation as MoS_2. Somewhat paradoxically, under highly sulfidic conditions, a portion of dissolved Mo is also stabilized as Mo (VI) in the form of MoS_4^{2-}.

CONCLUSIONS

Mo concentrations and speciation change dynamically in porewater at Flax pond in response to redox conditions. Total Mo was relatively low under highly sulfidic sediments with Mo (VI) dominating and little Mo (V), while total Mo was very high under nonsulfidic or low sulfide conditions with high Mo (V). High levels of Mo (V) under nonsulfidic or low sulfide sediments may be from reductive release from surface oxic sediments (reduction of MoO_4^{2-}) without substantial loss as MoS_2. Oxidative release of Mo from deep highly sulfidic sediments may occur during exposure to O_2 (oxidation of MoS_2).

Mo is rapidly mobilized and re-precipitated during early diagenesis of sediments. Reduction of surface oxic sediments showed that total Mo was mobilized and Mo (V) increased in contrast to Mo (VI) with decreasing Eh under nonsulfidic or low sulfide conditions, while Mo (V) was further reduced to Mo (IV) and precipitated as MoS_2 under highly sulfidic conditions. Mo (VI) continued to be desorbed from carrier phases, e.g., Mn, Fe oxides and organic particles, and a portion was apparently stabilized by sulfide by forming soluble thiomolybdate, MoS_4^{2-}. The precipitation of MoS_2, rather than nonspecific scavenging, is likely the dominant process of Mo accumulation in sediment under highly sulfidic conditions.

Mo may be released as transient dissolved intermediates, such as Mo (V) but is quickly converted to Mo (VI) and not analytically detectable during the oxidation of particulate carrier phases (Fe-sulfides and Mo sulfides in deep sediments). In contrast, Mo (V) appears to be a stable intermediate during progressive reduction under nonsulfidic or low sulfide conditions.

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FIGURE CAPTIONS

Figure 1: Sampling location in the Long Island.

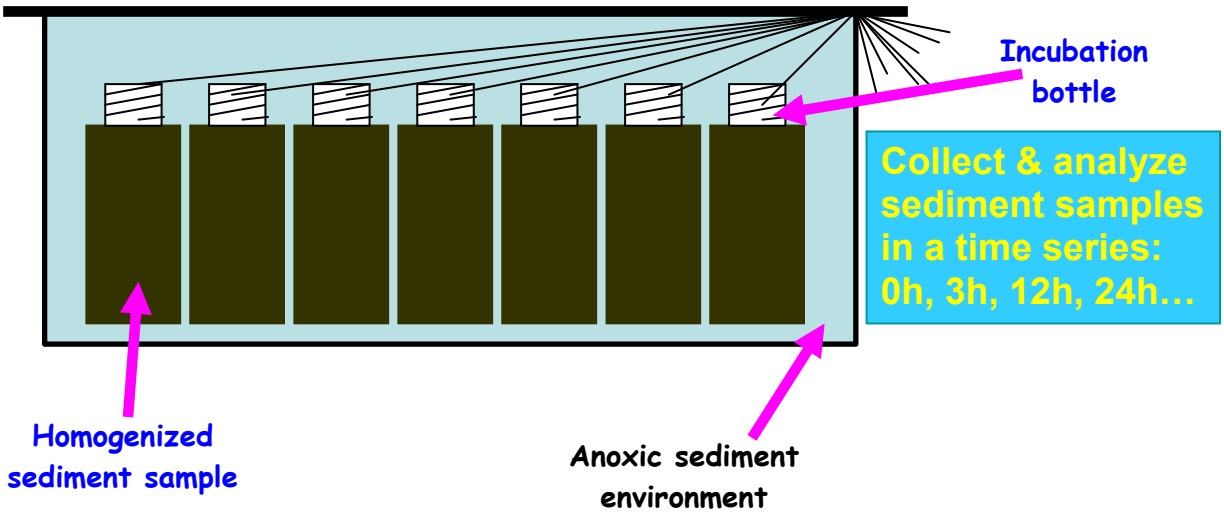

Figure 2: Schematics of anoxic incubation of surface oxic sediments taken from Flax Pond.

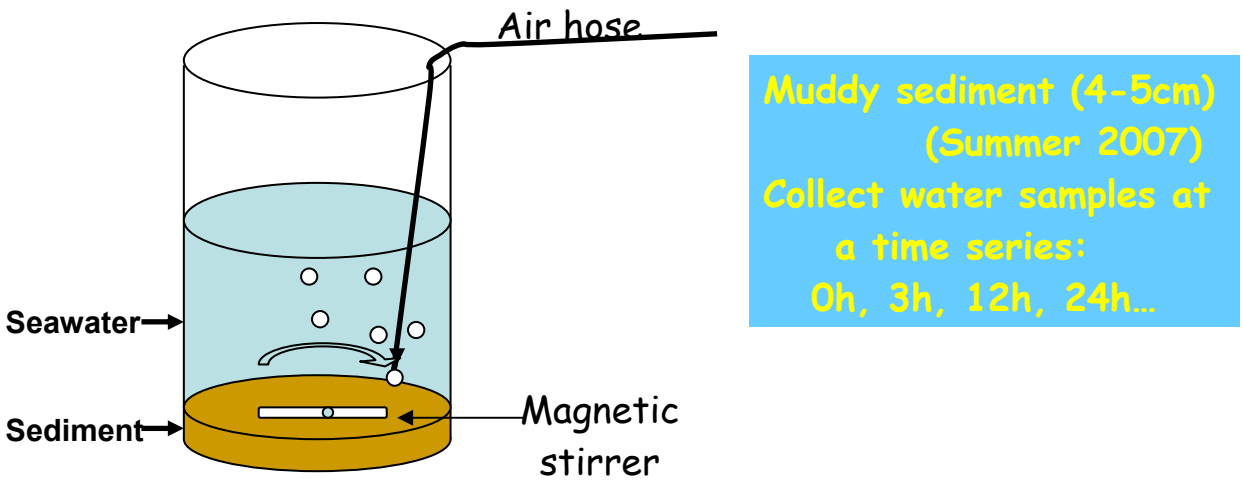

Figure 3: Schematics of oxic incubation of deep reduced sediments taken from Flax Pond.

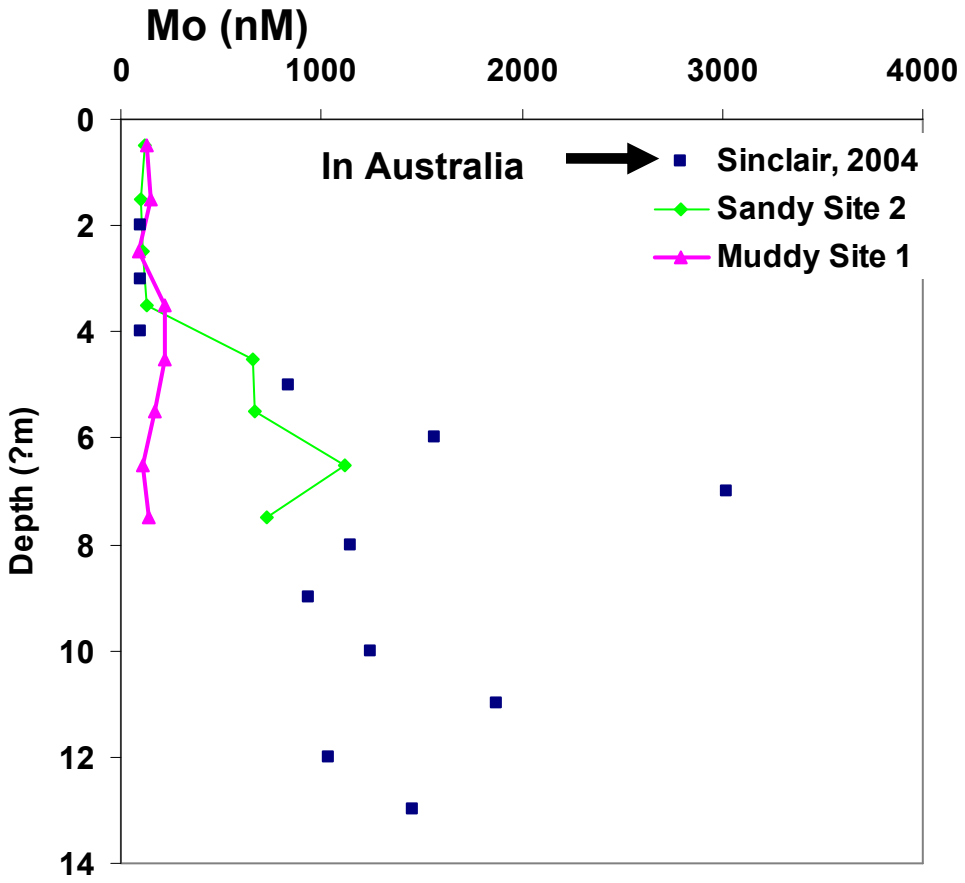

Figure 4: Vertical profiles of total Mo in sediment porewater at Flax Pond and in a sediment core from Australia (Sinclair, 2004) (The depth unit is meters for data from Sinclair, 2004, rather than cm in this research).

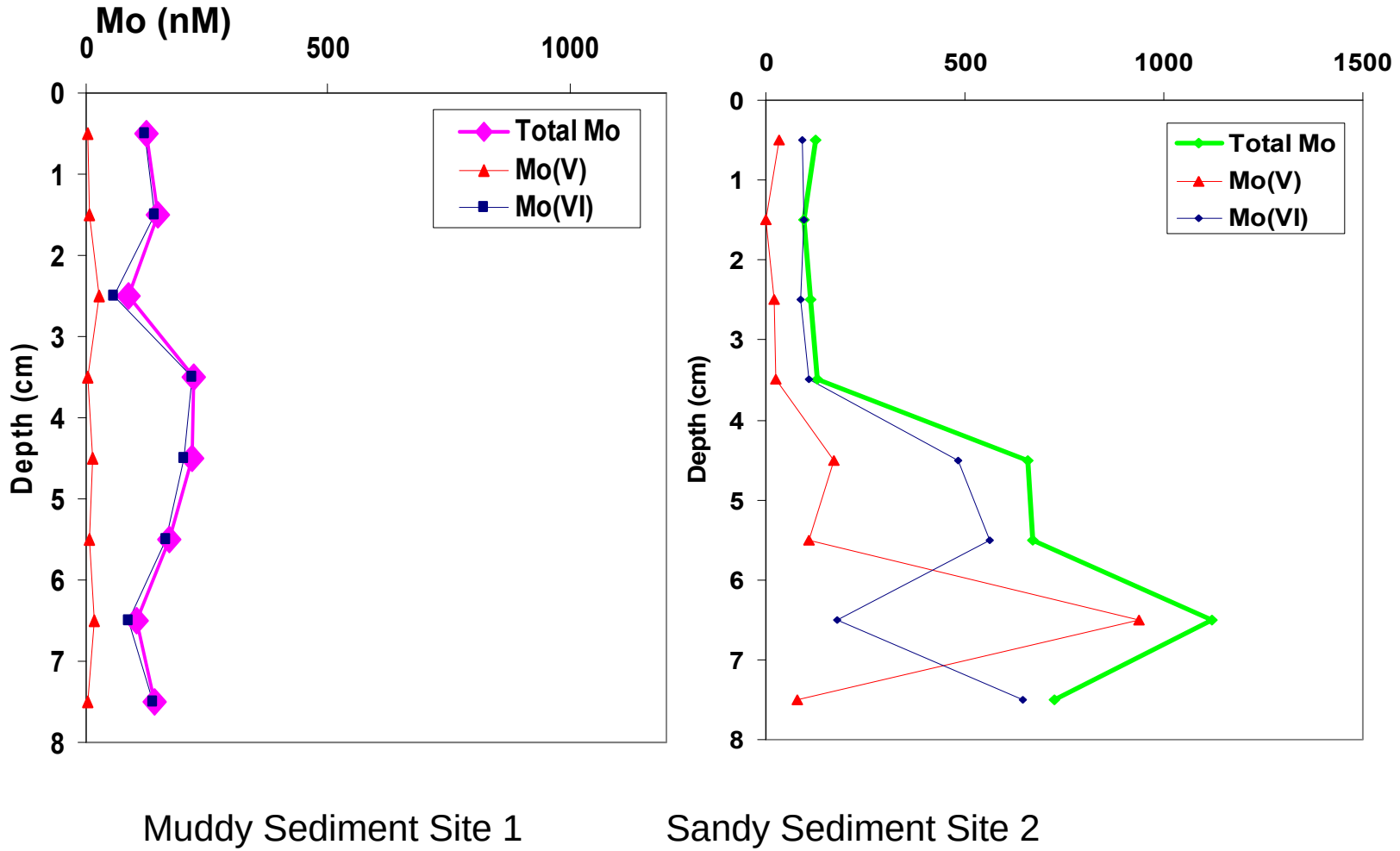

Figure 5: Vertical profiles of Mo (V), Mo (VI) and total Mo in sediment porewater at Flax Pond.

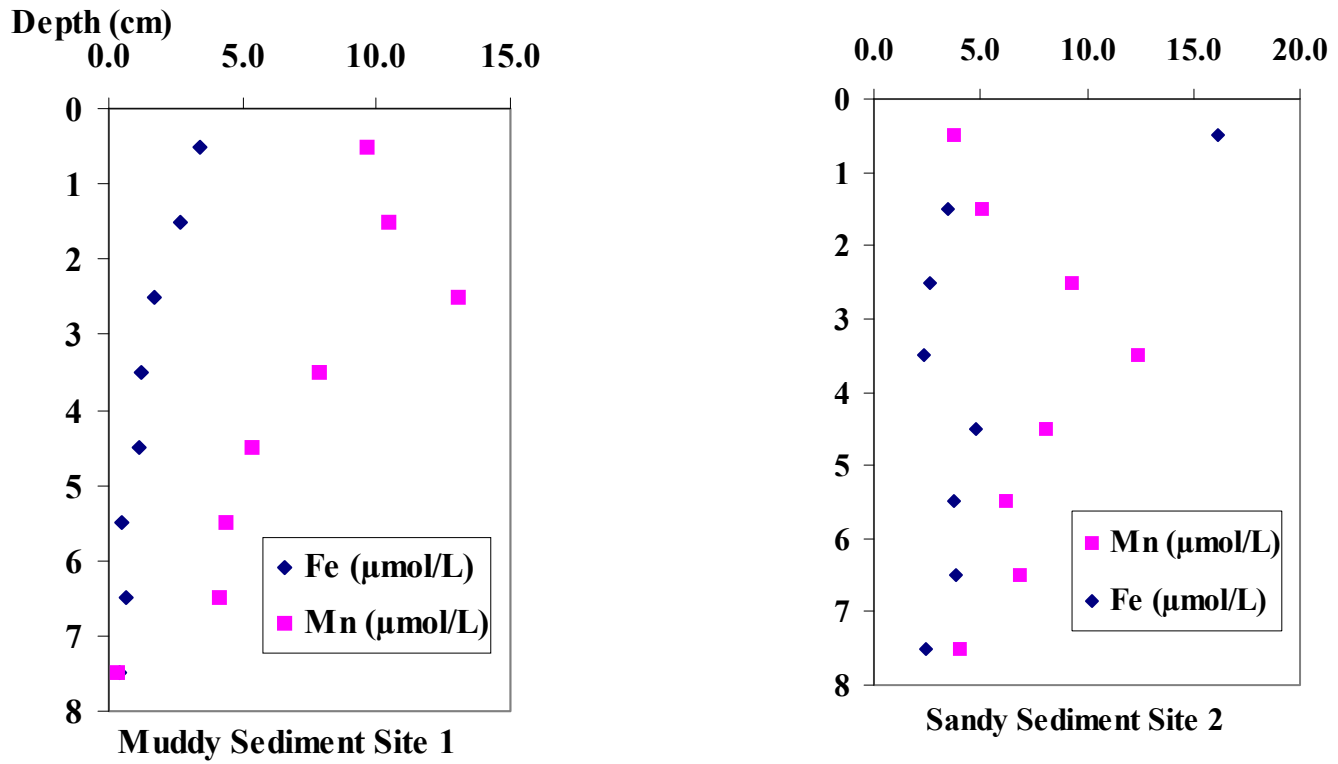

Figure 6: Vertical profiles of dissolved Fe and Mo in sediment porewater at Flax Pond.

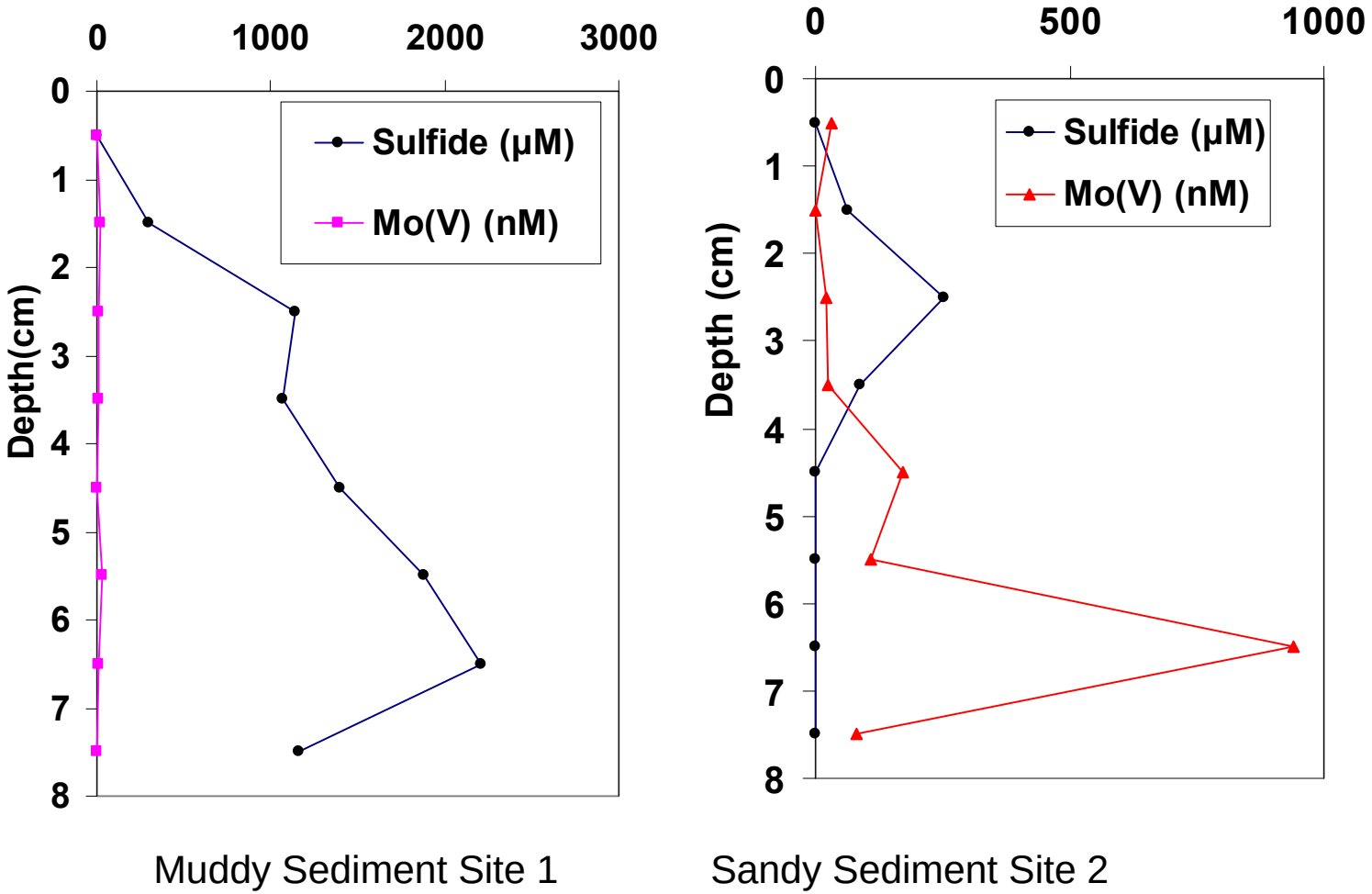

Figure 7: Vertical profiles of dissolved sulfide and Mo(V) in sediment porewater at Flax Pond.

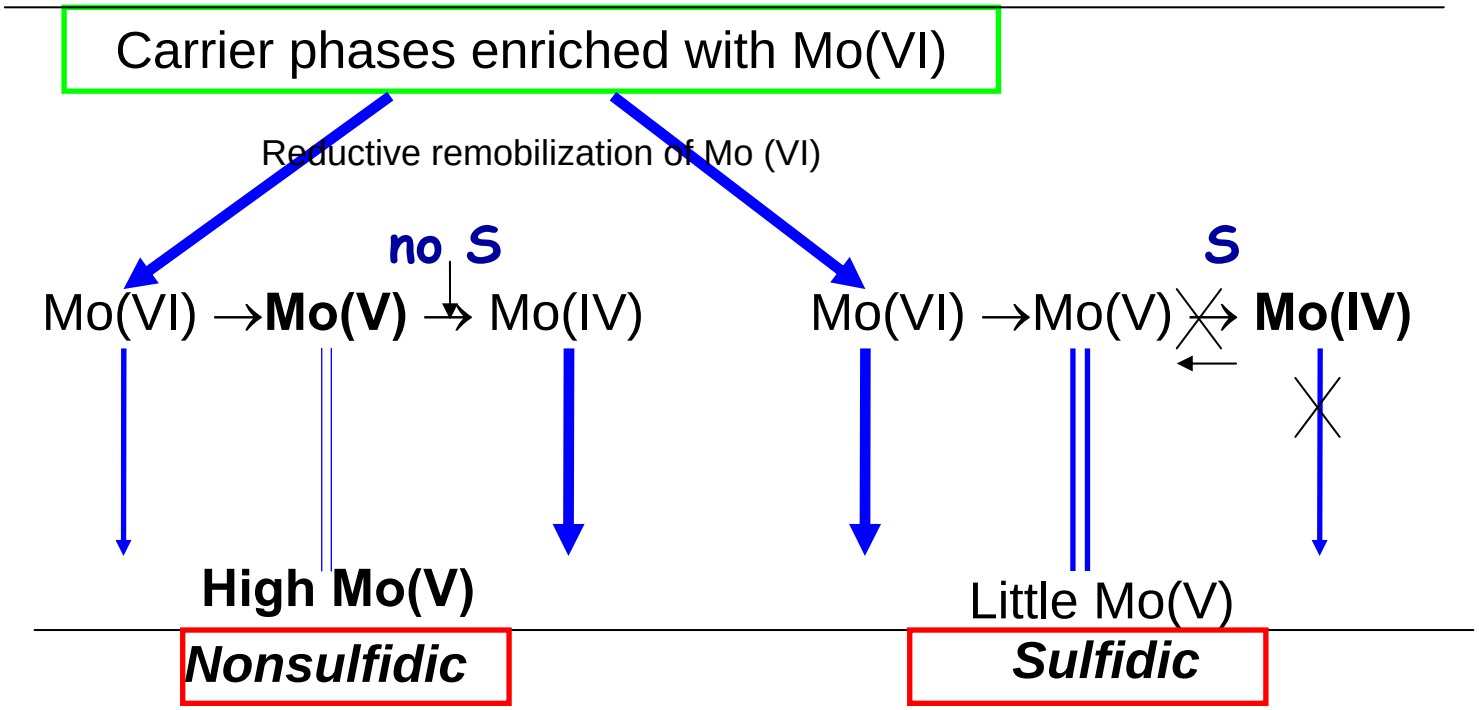

Figure 8: Possible mechanism of Mo (V) formation under sulfidic versus nonsulfidic or low sulfide conditions (Carrier phases: Fe, Mn oxides and organic particles).

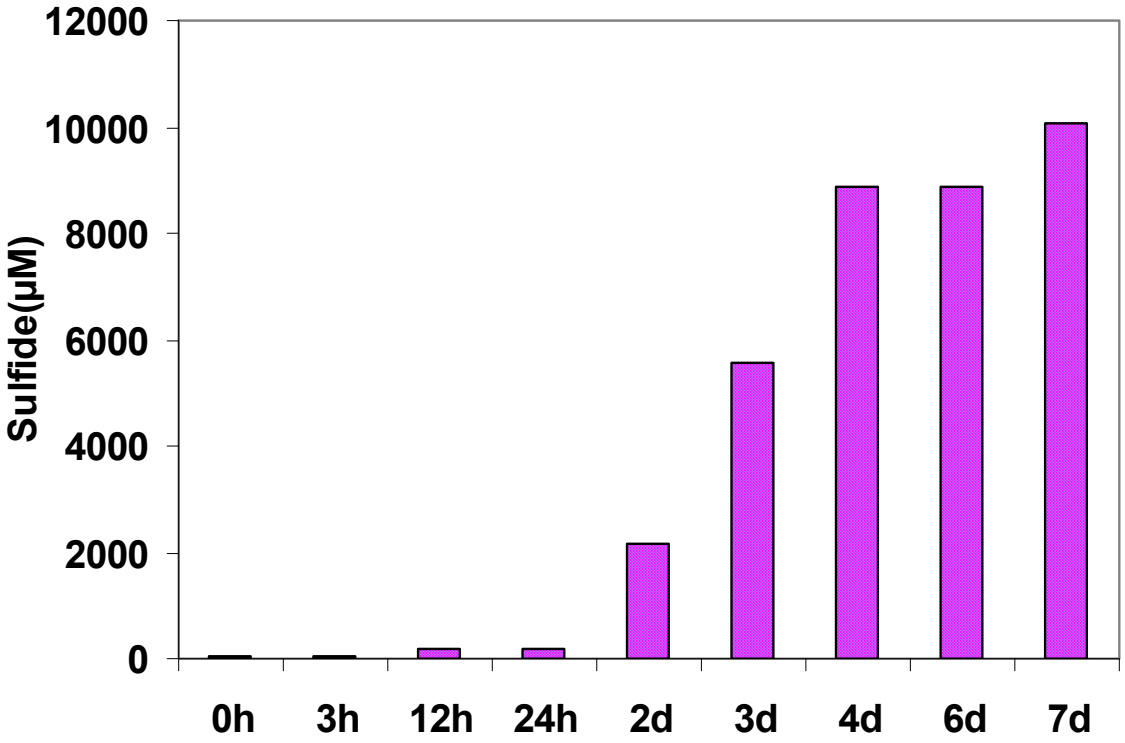

Figure 9: Variation of sulfide with time during anoxic incubation of surface sediments (The sediments were collected at depth of 0-1 cm in Flax Pond).

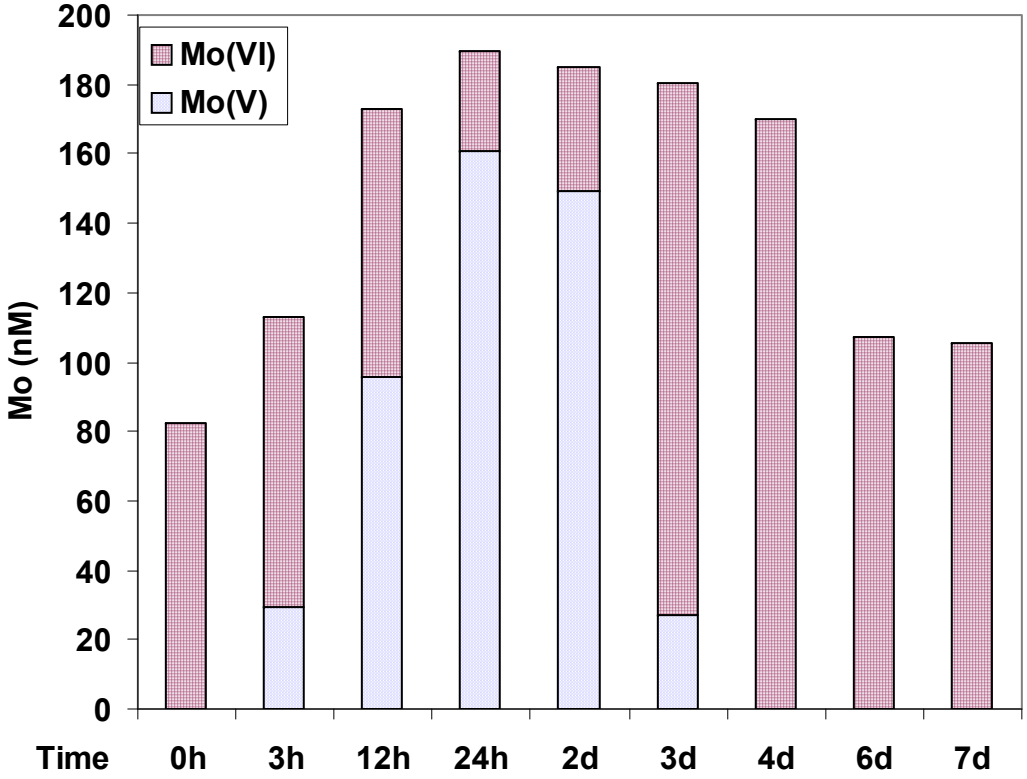

Figure 10: Variation of Mo (V), Mo (VI) with time during anoxic incubation of surface sediments (The sediments were collected at depth of 0-1 cm in Flax Pond).

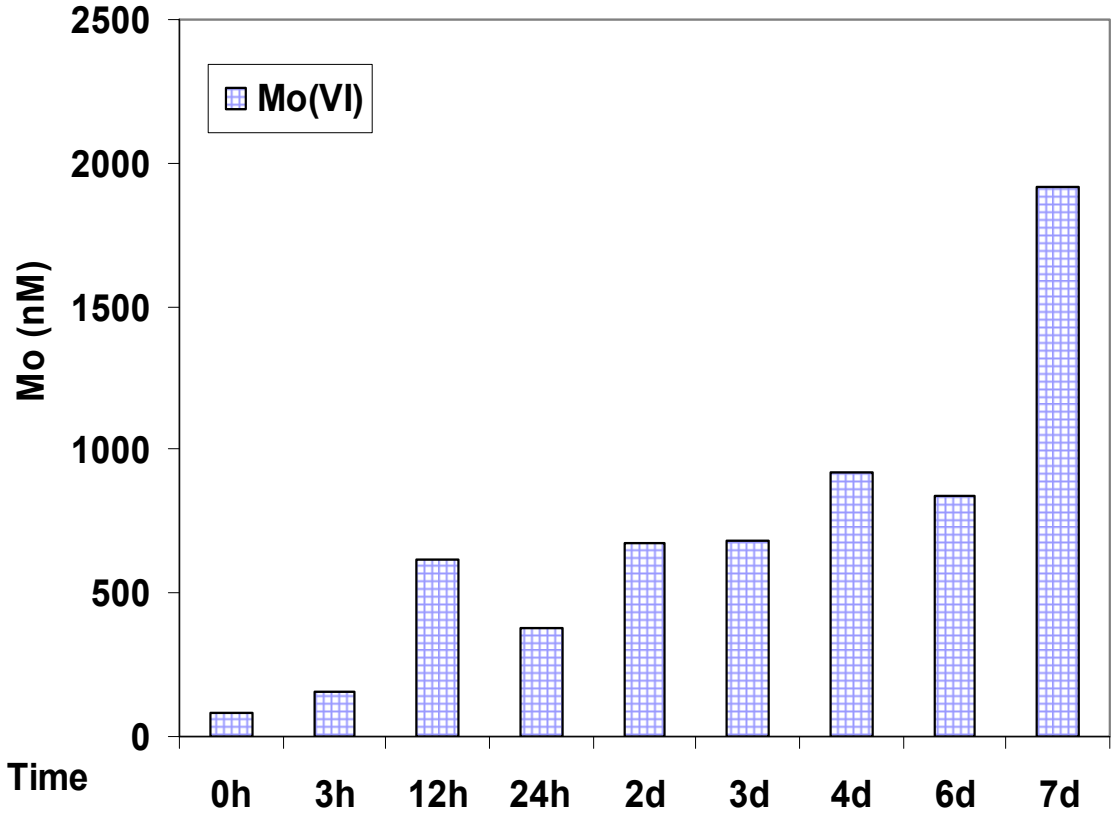

Figure 11: Variation of dissolved Mo (VI) with time during oxidation of reduced sediments (The sediments were collected at depth of 4-5 cm in Flax Pond; Fe, Mn, Sulfide, Mo (V) were not detected).

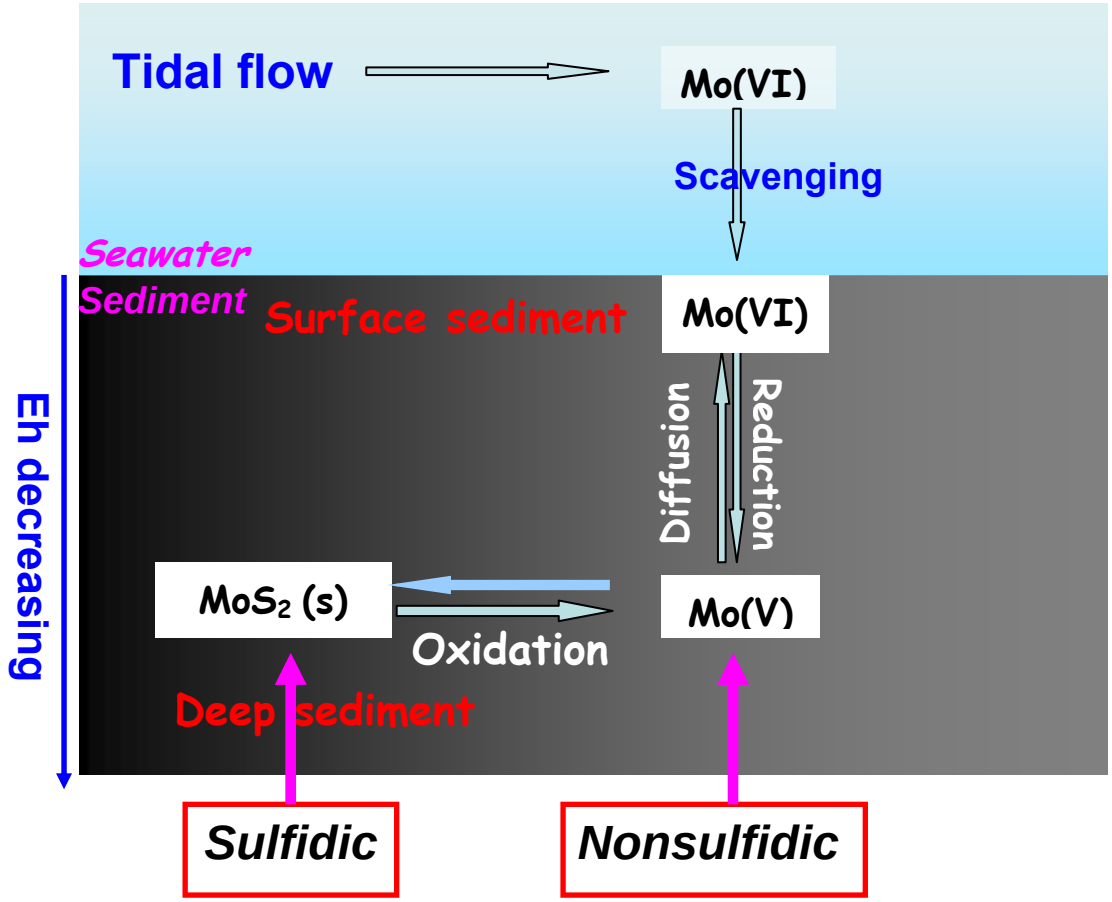

Figure 12: A conceptual model of Mo cycle in Flax Pond (Note: Mo (V) may be from two major sources: oxidative release from deep sediments enriched with MoS_2, and reductive release from surface sediments enriched with Mo (VI)).

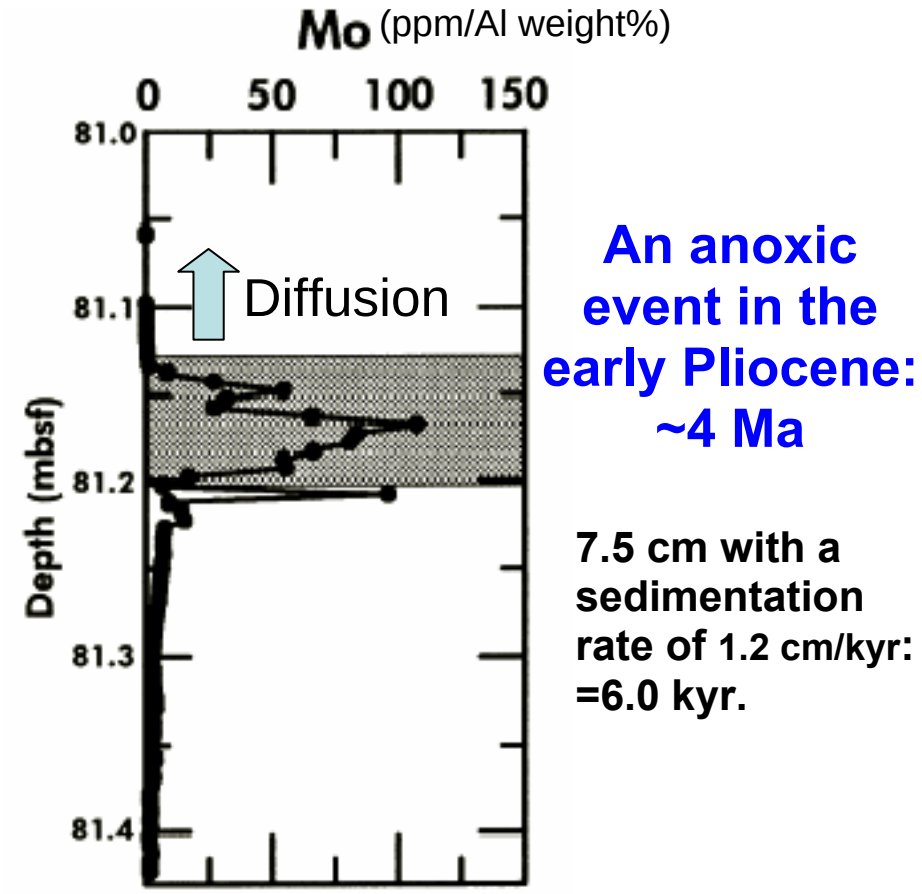

Figure 13: Mo accumulation in a sediment core from the Mediterranean (mbsf: meters below seafloor) (Adapted from Nijenhuis et al., 1998).

CHAPTER FIVE: DISSERTATION SUMMARY

SUMMARY

Mo and V are the most abundant transition metals in the ocean, with Mo concentrations of ~100-120 nM and V of 25-40 nM (e.g., Emerson and Huested, 1991; Morris, 1975). A slight depletion of Mo and V may occur in surface waters mainly because of the algal uptake and possibly particle adsorption. Although abundant, concentrations of Mo and V can be significantly decreased in some cases. For example, Mo and V are very low in river waters. The concentrations in average world river are only 5 nM for Mo and 15 nM for V (Morford and Emerson, 1999). Mo and V are sharply depleted in deep waters in the Black Sea and the Carriaco Trench. In these waters, Mo and V are expected to be reduced to MoS_2 and V_2O_3, and precipitated out of seawater, and enriched in sediments. Because of this, Mo and V accumulation or lack thereof have been used widely as paleoceanographic proxies for bottom water redox conditions (e.g., Nijenhuis et al., 1998).

As an essential trace element for plants, animals and micro-organisms (e.g., Bortels, 1930), molybdenum and vanadium form part of the active sites of metalloenzymes that execute key transformations in the biogeochemical cycles of nitrogen, sulfur, carbon and halides (e.g., Mendel 2005; Redhar, 2003). As far as I know, 73 enzymes have been identified with Mo or V cofactors. Especially both Mo and V form a cofactor in various enzymes for nitrogen fixation and nitrate reduction (e.g., Fogg and Wolfe, 1954; Robson et al., 1986; Mendel, 2005), which catalyzes the reduction of N_2 and nitrate to bioavailable N in the ocean. V-dependent haloperoxidases play a critical role in cycles of halides, which have been widely found in diatom and macroalgae (e.g., Moore et al., 1996; Butler, 1998). By utilizing these enzymes, marine diatoms

could potentially produce various brominated and iodinated volatile compounds. As carriers of chlorine, bromine, and iodine into the atmosphere, these halogenated methanes play an important role in transporting halides to the troposphere, causing the destruction of tropospheric ozone (Barrie et al., 1988; Kritz et al., 1993).

Oxygenated oxyanions (e.g., $H_2VO_4^-$ and MoO_4^{2-}) are the most abundant chemical species of Mo and V in oxic water, and reduced cations, HVO_2^+ and $Mo_2O_4^{2+}$, are expected to dominate in anoxic conditions thermodynamically (e.g., Pope et al., 1980). These expectations have been confirmed in my dissertation research. This research demonstrated that the oxidation states of V and Mo can be differentiated and determined in seawater using a combined ion exchange - AA separation procedure. After developing protocols for separating different species of these two elements, the protocols have been successfully applied to field investigations. Consistent with thermodynamic calculations, reduced forms of Mo and V exist in natural waters and are enhanced under low oxygen conditions.

Mo and V concentrations and speciation change dynamically both seasonally and spatially in estuarine waters in response to redox conditions. Percentages of different vanadium redox species in several typical waters are shown in Figure 1. Generally V speciation varies in different areas in response to different redox conditions. V (IV) accounts for less than 5% of the total in oxic seawater, e.g., central LIS (V_{tot}: 22.0-28.0 nM), and LIS in April 2005 (V_{tot}: 10.0-18.0 nM). In some suboxic conditions, e.g., western LIS (V_{tot}: 20.0-26.0 nM), Peconic River Estuary (V_{tot}: 6.1-11.0 nM), V (IV) may account for 10 ~ 40% of the total as shown in Figure 1. Percentages of different molybdenum species in several typical waters are shown in Figure 2. Mo speciation also

varies in response to redox conditions. Mo (VI) dominated in oxic seawater accounting for >99% of the total (Mo_{tot}: ~120 nM), for example, the Atlantic Ocean. In suboxic or anoxic conditions, e.g., Peconic River Estuary, Mo (V) may account for ~10% of the total (Mo_{tot}: 2.5~120 nM). Mo speciation was very different in sulfidic versus nonsulfidic or low sulfide conditions, Mo (V) accounted for ~5% of the total under highly sulfidic conditions (Mo_{tot}: 60 ~ 120 nM) in porewater at Flax Pond, while ~50% of the total under nonsulfidic or low sulfide conditions (Mo_{tot}: 600 ~1200 nM) in porewater at Flax Pond (Figure 2).

Mo is rapidly mobilized and re-precipitated during early diagenesis of sediments. Mo, and presumably V, are released as transient dissolved intermediates during the reduction and oxidation of particulate carrier phases (Fe, Mn-oxides, organic matter, and Fe-sulfides). The two major possible sources of Mo (V) are as follows: 1) reductive release from surface oxic sediments during burial; 2) oxidative release from deep sulfidic sediments as a result of re-exposure during physical or biological reworking. During anoxic events (with highly sulfidic settings), Mo is first reduced to Mo (V), and further to Mo (IV), which apparently precipitates as MoS_2, and accumulates in sediments.

Based on this dissertation research, a conceptual model was proposed for early diagenetic processes of Mo and V in response to redox changes (Figure 3). Under suboxic conditions, Mo (VI) was released from carrier phases, e.g., Mn and Fe oxides, and organic particles. Under nonsulfidic or low sulfide conditions, Mo (VI) was reduced Mo (V), while desorbed Mo (VI) may also a series of MoS_4^{2-}, which was probably partly scavenged by carrier phases e,g., pyrite (Helz et al., 1996; Vorlicek et al., 2002), therefore contributing a certain extent to the Mo accumulation in sediments under

nonsulfidic or low sulfidic conditions. However under highly sulfidic conditions, our research suggested that Mo (V) was further reduced to solid MoS_2, which quickly precipitated, therefore contributing substantially largely to Mo accumulation in highly sulfidic sediments (e.g., Calvert and Pederson, 1993; Zheng et al., 2000; Nameroff et al., 2002; Algeo and Maynard, 2004). Paradoxically, under highly sulfidic conditions, a portion of mobilized Mo is still retained in solution as Mo (VI), presumably stabilized as thiomolybdates such as MoS_4^{2-}.

As shown in Figure 3, vanadium seems slightly different from Mo. Under suboxic conditions, V (V) was reduced to V (IV), which was likely scavenged by carrier phases, e.g., organic particles (e.g., Emerson and Huested, 1991; Morford and Emerson, 1999). Under strongly reducing conditions (nonsulfidic, low or high sulfide), V (IV) was further reduced to V (III), which was precipitated as V_2O_3 (nonsulfidic) or V_2S_3 (sulfidic) quickly (e.g., Breit and Wanty, 1991; Wanty and Goldhaber, 1992). Although V concentration is lower in seawater than Mo, higher accumulation of V is usually found in reducing sediments than Mo (Morford and Emerson, 1999) likely because of two reasons: 1) the strong scavenging of V (IV) and more precipitation of vanadium as solid V_2O_3 or V_2S_3, and 2) as mentioned above, a portion of mobilized Mo is still retained in solution as Mo (VI), presumably stabilized as thiomolybdates such as MoS_4^{2-}.

From this dissertation research, sulfide may be viewed as a geochemical switch, which triggers the Mo reduction from soluble Mo (V) to solid Mo (IV) but also allows Mo (VI) to remain as MoS_4^{2-} in solution. Based on our research results, the primary differences in Mo behavior occur when sulfide is above 100 μM. Such highly sulfidic conditions are expected during anoxic events, and Mo should be largely removed out of

seawater and precipitated as Mo sulfides in sediments. On the other hand, reoxygenation can release Mo from reduced sediments and diffuse Mo back to seawater. Therefore, paleoceanographic studies based on net Mo accumulation in sediments may potentially underestimate the extent of anoxic events due to the benthic remobilization of this trace element if re-oxygenation occurs.

Both reduced and oxygenated forms of Mo and V, especially reduced forms, are expected to exist in bacteria and phytoplankton (e.g., Kay and Mithchell, 1968; Crans et al., 2004). However, the implications of these reduced forms of Mo and V on biological processes remain unknown.

FUTURE DIRECTIONS

Additional work is required to confirm the existence of reduced Mo and V in other typical suboxic/anoxic waters, e.g., the Black Sea, the Arabian Sea, equatorial Pacific, and anoxic fjords; and possibly the Chesapeake Bay and Saanich Inlet where seasonal anoxia occurs. Further studies are also needed to determine requirements of these two elements in phytoplankton and bacteria, especially how reduced forms of V and Mo are involved in biological uptake processes. Such additional research will greatly further our understanding of the biogeochemical cycles of these two elements in the ocean, and presumably the evolution of life on Earth.

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FIGURE CAPTIONS

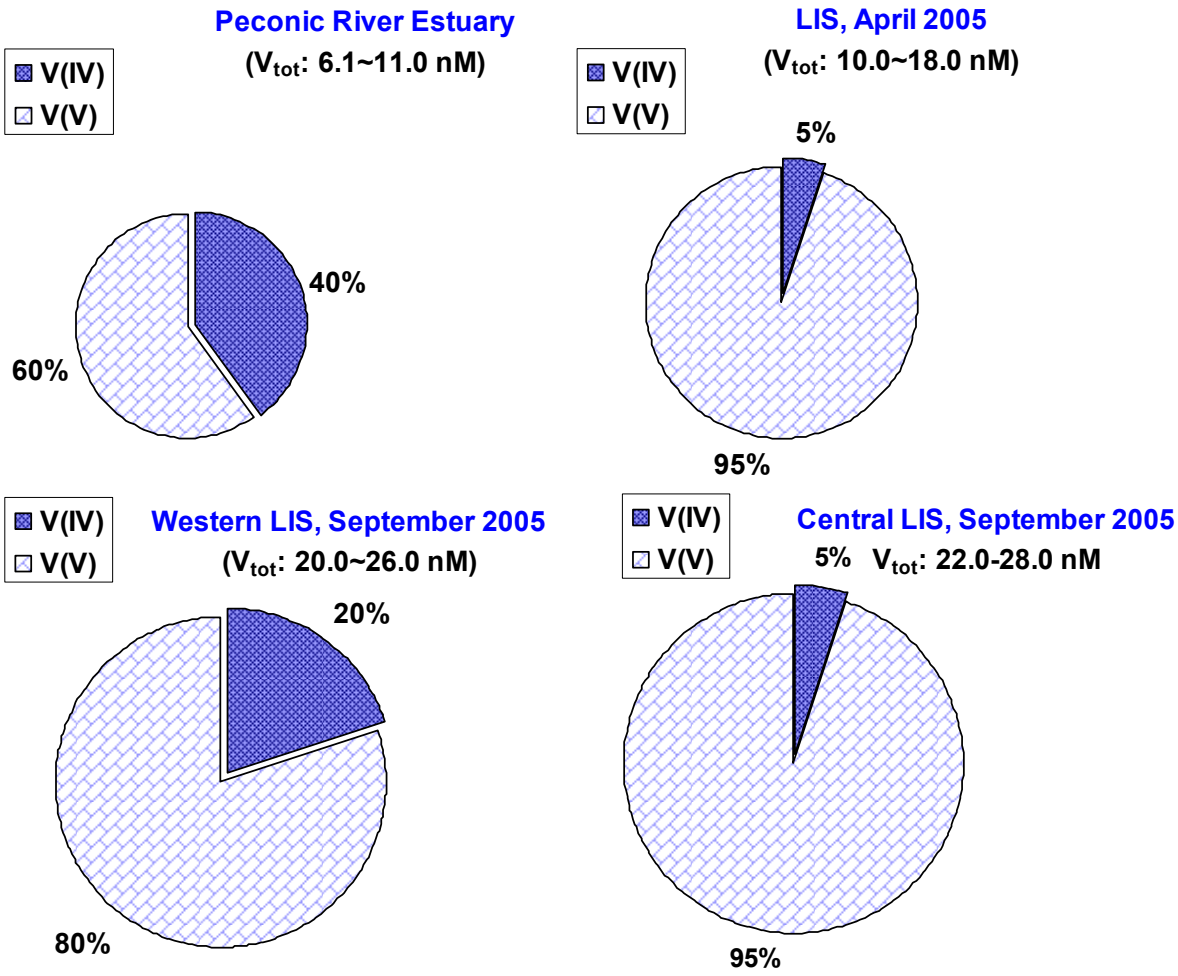

Figure 1: Percentage of different vanadium species in several typical estuarine waters.

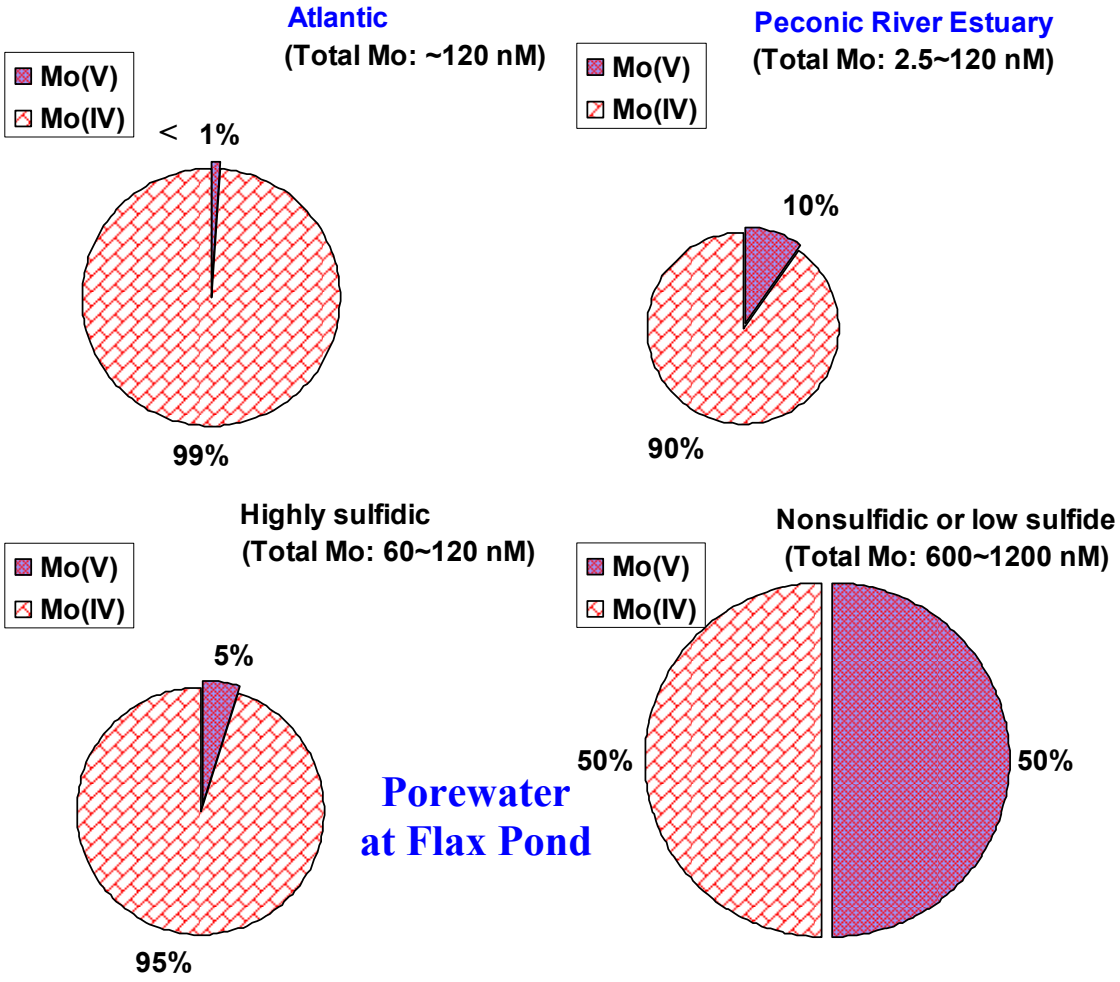

Figure 2: Percentage of different molybdenum species in several typical waters.

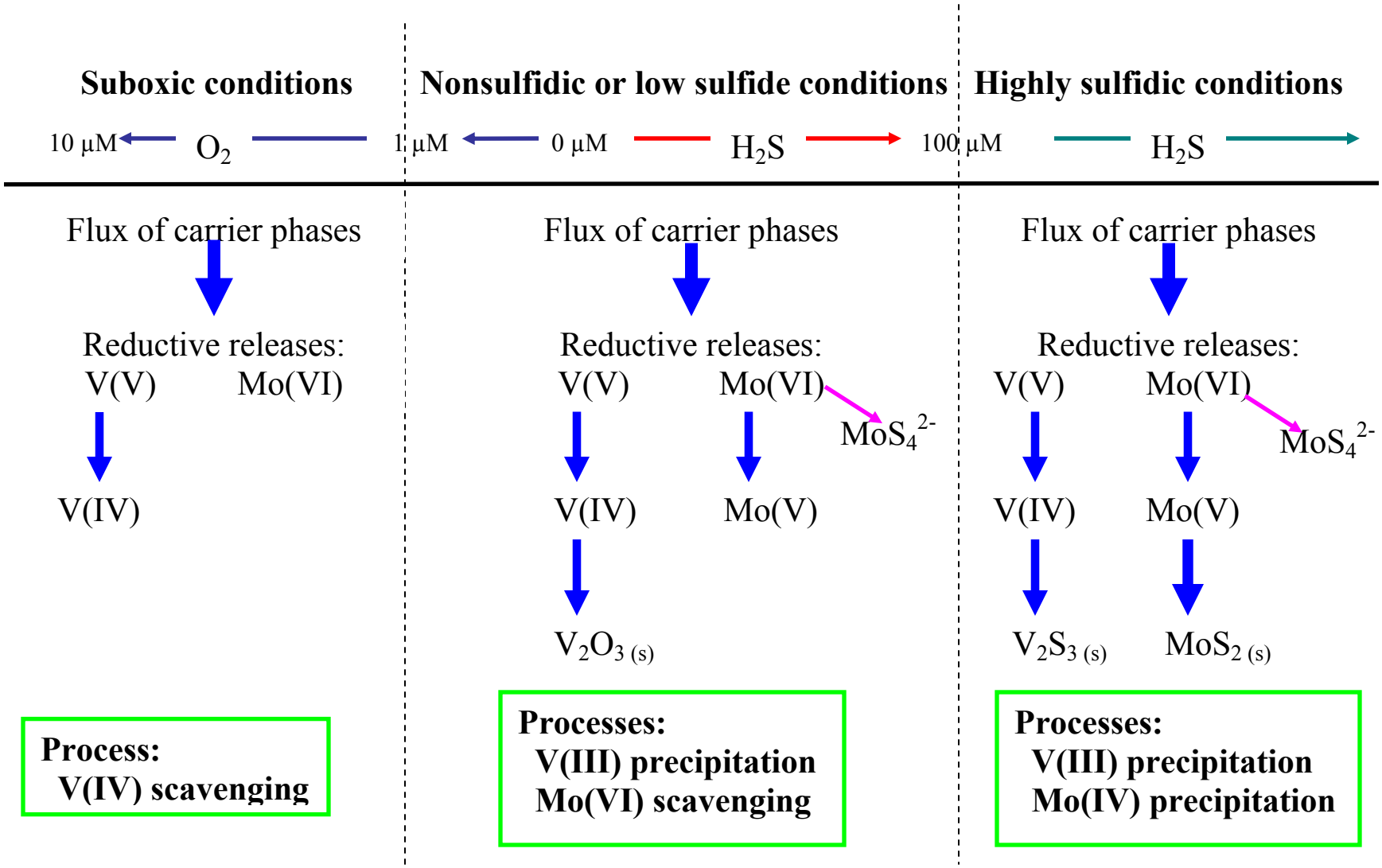

Figure 3: Conceptual models for redox reactions of Mo and V under different redox conditions (Carrier phases: Mn and Fe oxides, organic particles).

APPENDICES

Appendix I: Molybdenum and vanadium containing enzymes

Table 1: Molybdenum containing enzymes.

	Name	Reaction Catalyzed	Ref.
1	Xanthoxin dehydrogenase	Xanthoxin + NAD(+) <=> abscisic aldehyde + NADH	(a)
2	2-oxo-acid reductase	A (2R)-hydroxy-carboxylate + acceptor <=> a 2-oxo-carboxylate + reduced acceptor	(a)
3	Aldehyde oxidase	An aldehyde + H(2)O + O(2) <=> a carboxylic acid + H(2)O(2)	(a)
4	Indole-3-acetaldehyde oxidase	2 indole-3-acetaldehyde + O(2) <=> 2 indole-3-acetate + 2 H(2)O	(a)
5	Pyridoxal oxidase	Pyridoxal + H(2)O + O(2) <=> 4-pyridoxate + H(2)O(2).	(a)
6	Formylmethanofuran dehydrogenase	Formylmethanofuran + H(2)O + acceptor <=> CO(2) + methanofuran + reduced acceptor	(a)
7	Quinoline 2-oxidoreductase	Quinoline + acceptor + H(2)O <=> isoquinolin-1(2H)-one + reduced acceptor	(a)
8	Nitrate reductase (NADH)	Nitrite + NAD(+) + H(2)O <=> nitrate + NADH	(a)
9	Nitrate reductase (NAD(P)H)	Nitrite + NAD(P)(+) + H(2)O <=> nitrate + NAD(P)H	(a)
10	Nitrate reductase (NADPH)	Nitrite + NADP(+) + H(2)O <=> nitrate + NADPH	(a)
11	Trimethylamine-N-oxide reductase (cytochrome c)	Trimethylamine + 2 (ferricytochrome c)-subunit + H(2)O <=> trimethylamine N-oxide + 2 (ferrocytochrome c)-subunit + 2 H(+).	(a)
12	Ferredoxin--nitrate reductase	Nitrite + H(2)O + 2 oxidized ferredoxin <=> nitrate + 2 reduced ferredoxin	(a)
13	Nitrate reductase	Nitrite + acceptor <=> nitrate + reduced acceptor	(a)
14	Hypotaurine dehydrogenase	Hypotaurine + H(2)O + NAD(+) <=> taurine + NADH	(a)
15	Sulfite oxidase	Sulfite + O(2) + H(2)O <=> sulfate + H(2)O(2).	(a)
16	Thiophene-2-carbonyl-CoA monooxygenase	Thiophene-2-carbonyl-CoA + AH(2) + O(2) <=> 5-hydroxythiophene-2-carbonyl-CoA + A + H(2)O	(a)

Table 1: Continued, Molybdenum containing enzymes.

	Name	Reaction Catalyzed	Ref.
17	Xanthine dehydrogenase	Xanthine + NAD(+) + H(2)O <=> urate + NADH	(a)
18	Nitrogenase	8 reduced ferredoxin + 8 H(+) + N(2) + 16 ATP + 16 H(2)O <=> 8 oxidized ferredoxin + H(2) + 2 NH(3) + 16 ADP + 16 phosphate	(a)
19	Nitrogenase (flavodoxin)	6 reduced flavodoxin + 6 H(+) + N(2) + n ATP <=> 6 oxidized flavodoxin + 2 NH(3) + n ADP + n phosphate	(a)
20	Arsenate reductase (glutaredoxin)	Arsenate + glutaredoxin = arsenite + glutaredoxin disulfide	(a)
21	Arsenate reductase (azurin)	Arsenite + H(2)O + azurin(ox) <=> arsenate + azurin(red)	(a)
22	Pyrogallol hydroxytransferase	1,2,3,5-tetrahydroxybenzene + 1,2,3-trihydroxybenzene <=> 1,3,5- trihydroxybenzene + 1,2,3,5-tetrahydroxybenzene	(a)
23	Selenate reductase	Selenite + H(2)O + acceptor <=> selenate + reduced acceptor	(a)
24	Formate oxidase	HCOOH <=> CO(2) + 2H(+) + 2e(-)	(b)
25	CO dehydrogenase	CO + H(2)O <=> CO(2) + 2H(+) + 2 e(-)	(b)
26	Dimethylsulfoxide (DMSO) dehydrogenase	(CH(3))(3)SO + 2H(+) + 2e(-) <=> (CH(3))(3)S + H(2)O	(b)
27	Biotin sulfoxide reductase	Biotin sulfoxide + 2H(+) + 2e(-) <=> biotin + H(2)O	(b)
28	Xanthine oxidase	Xanthine + H(2)O <=> uric acid + 2H(+) + 2e(-)	(b)
29	tetrathionate reductase	(1/2)S(4)O(6)(2-) <=> SO(3)(2-) + S(2-)	(c)
30	chlorate reductase	ClO(3)(-) <=> ClO(2)(-)	(c)
31	Trimethylamine-N-oxide reductase	(CH(3))(3)NO + 2e(-) + 2H(+) <=> (CH(3))(3)N + H(2)O	(c)
32	purine hydroxylase	Xanthine + NAD(+) + H(2)O <=> urate + NADH	(c)
33	Carbon monoxide oxidase (assimilatory)	CO + H(2)O + O(2) <=> CO(2) + H(2)O	(c)
34	nitrate reductase (NADH)	Nitrite + NAD(+) + H(2)O <=> nitrate + NADH	(c)
35	nitrate reductase (NAD(P)H)	Nitrite + NAD(P)(+) + H(2)O <=> nitrate + NAD(P)H	(c)
36	nitrate reductase (NADPH)	Nitrite + NADP(+) + H(2)O <=> nitrate + NADPH	(c)
37	Formate dehydrogenase (NAPH+)	Formate + NADP(+) <=> CO(2) + NADPH	(d)

Table 1: Continued, Molybdenum containing enzymes.

	Name	Reaction Catalyzed	Ref.
38	Carbon monoxide oxidase	CO <=> HCO(3)(-)	(d)
39	Nicotinate hydroxylase	Nicotinate <=> 6-hydroxynicotinate	(d)
40	Mo-protein	Unknown	(d)
41	Isonicotinic acid hydroxylase	No data yet	(e)
42	Nicotinate dehydrogenase	Nicotinate +H(2)O <=> 6-hydroxynicotinate +NADP(+) + NADPH	(e)
43	Nicotine hydroxylase	No data yet	(e)
44	Picolinic acid dehydrogenase	No data yet	(e)
45	Pyrimidine oxidase	No data yet	(e)
46	Isoquinoline oxidoreductase	Isoquinoline <=> 1-oxo-1,2-dihydroquinoline	(e)
47	Quinaldic acid 4-oxidoreductase	Quinaldate + acceptor + H(2)O <=> kynurenate + reduced acceptor	(e)
48	Quinoline oxidoreductase	Quinoline + acceptor + H(2)O <=> isoquinolin-1(2H)-one + reduced	(e)
49	N-Formyl methanofuran dehydrogenase	No data yet	(e)
50	2-Furoyl dehydrogenase	2-furoyl chloride <=> 2-furoyl-leucyl-isoleucyl-glycyl-lysyl-valine	(e)
51	Arsenate reductase (donor)	Arsenate <=> arsenate	(e)
52	Pyrogallol transhydroxylase	1,2,3,5-tetrahydroxybenzene + 1,2,3-trihydroxybenzene <=> 1,3,5-trihydroxybenzene + 1,2,3,5-tetrahydroxybenzene	(e)
53	polysulfide reductase	Polysulfide or S <=> H(2)S	(e)
54	Aldehyde dehydrogenase	Chloroacetaldehyde + NAD(+) + OH(-) <=> chloroacetate +NADH	(e)
55	Aldehyde ferredoxin oxidoreductase	No data yet	(f)
56	Formaldehyde ferredoxin oxidoreductase	No data yet	(f)
58	carboxylic acid reductase	No data yet	(f)
59	hydroxycarboxylate viologen oxidoreductase	No data yet	(f)
60	nitrite oxidoreductase	Ammonia oxidation, or nitrification	(g)
61	4-Hydroxybenzoyl-CoA reductase	No data yet	(h)

Table 1: Continued, Molybdenum containing enzymes.

	Name	Reaction Catalyzed	Ref.
62	Quinoline-4-oxidoreductase	No data yet	(i)
63	6-hydroxynicotinate hydroxylase	6-hydroxynicotinate + H(2)O <=> O(2) + 2,6-dihydroxynicotinate	(i)
64	Pyruvate: ferredoxin oxidoreductase	Pyruvate + coenzyme A + an oxidized ferredoxin <=> acetyl-CoA + CO(2) + a reduced ferredoxin	(i)

Notes:

(a) ExPASy, 2006. Mo containing Enzymes. (http://www.expasy.org/cgi-bin/enzyme-search-ful?Mo) 2006-11-7.
(b) Lippard, S.J., Berg, J.M., Klatt, G., 1994. Principles of bioinorganic Chemistry. University Science Books, 411pp.
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(i) Hille R., 1996. The mononuclear molybdenum enzymes. Chem. Rev., 96: 2757-2816.

Table 2: The list of vanadium containing enzymes.

Name	Reactions catalyzed	Co-factors	Ref.
Vanadium nitrogenase	Dinitrogen to ammonia	V-Fe	(a)
Sulfideperoxidase	Thioethers to sulfoxides	V	(a)
Vanadium nitrate reductase	Nitrate to nitrite	V	(a)
Chloroperoxidase	Oxidize chloride, bromide and iodide to their hypohalous acids	V	(b)
Bromoperoxidase	Oxidize bromide and iodide to their hypohalous acids	V	(c)
Iodoperoxidase	Oxidize iodide to its hypohalous acid	V	(d)
Vanadate-substituted acid phosphatase	Hydrolyse phosphoric acid monoesters into a phosphate ion	V	(e)
Haloperoxidase	Halide to hypohalous acid	V	(a)

Notes:

(a) Rehder D., 2003. Biological and medicinal aspects of vanadium. Inorg. Chem. Comm., 6: 604-617.
(b) Messerschmidt A., Wever R., 1996. X-ray structure of a vanadium-containing enzyme: chloroperoxidase from the fungus *Curvularia inaequalis*. Proc. Natl. Acad. Sci., 93(1): 392-396.
(c) Plat H., Krenn B.E., Wever R., 1987. The bromoperoxidase from the lichen *Xanthoria parietina* is a novel vanadium enzyme. Biochem. J., 248: 277-279.
(d) Colin C., Lebanc C., Wagner E., Delage L., Leize-Wagner E., van Dorsselaer A., Kloareg B., Potin P., 2003. The brown algal kelp *Laminaria digitata* features distinct bromoperoxidase and iodoperoxidase activities. J. Biol. Chem., 278: 23545-23552.
(e) Tanaka N., Dumay V., Liao Q., Lange A.J., Wever R., 2002. Bromoperoxidase activity of vanadate-substituted acid phosphatases from *Shigella flexneri* and *Salmonella enterica ser. Typhimurium*. Eur. J. Biochem., 269: 2162-2167.

Appendix II: Method development for separating different redox species of vanadium

Basic principles

Chelex 100 resin is styrene divinylbenzene copolymers containing paired iminodiacetate ions which act as chelating groups in binding metal ions. The resin acts as a cation exchanger at high pH, while an anion exchanger at low pH. At pH=3~6, the resin may act an exchanger for both anions and cations (Figure 1). Basically, both V(V) as $H_2VO_4^-$ and V(IV) as VO^{2+} may be adsorbed onto chelex 100 resin at pH of 3-6, likely as in the following reactions (R: iminodiacetic groups in chelex 100 resin):

$H_2VO_4^- + H\text{-}R^+\text{-}H \leftrightarrow H_2R\text{-}VO_3 + H_2O$

$VO^{2+} + H\text{-}R^+\text{-}H \leftrightarrow R^+{=}VO + 2H^+$

After completely adsorption of both vanadium species chelex 100 resin only desorbs V(V), but not V(IV) at pH>10,. Therefore only V (V) is separated from the resin using a basic solution of pH>10. V (IV) is thereby eluted off afterwards using an acidic solution of pH<0.8 (Soldi et al., 1996). Therefore, the chelex 100 resin has been previously used for separating different vanadium ions in fresh water with mM levels of V (Soldi et al., 1996; Pyrzyńska and Wierzbicki, 2004), but no studies have been reported yet on water samples with the actual nanomolar concentrations that V exists in seawater.

Method summary

The method is summarized as described in Chapter Two. A 100 ml seawater sample was first adjusted to a pH of 4.5 with concentrated perchloric acid or with an acetate buffer (pH=4.5 in 0.1 M acetic acid and sodium acetate). After the pH adjustment, 10 ml of the water sample was immediately loaded, at a rate of 2 ml/min, onto a poly-prep column with H^+ form Chelex 100 resin (100-200 mesh, Bio-Rad) using a peristaltic pump with Teflon tubing extending from the head of the column to the water sample. Both V (V) and V (IV) species absorbed onto Chelex 100 resin as in $R^-\text{-}V(V)O_2$ and R=V(IV)O (R: iminodiacetic acid groups in chelex 100 resin). After rinsing with 20 ml of the acetate buffer and 20 ml of Milli-Q water, V (V) was eluted with 10 ml of 0.1 M ammonium hydroxide (0.1 M, pH=11.2) (10 elutions of 1 ml each). Following the V (V) elution, V (IV) was eluted with 10 ml (10 elutions of 1 ml each) of 0.2 N perchloric acid (pH=0.8). To avoid any changes in the V speciation during the handling of the samples, the loading and elution protocols were conducted under a nitrogen atmosphere. All the collected eluents for both V (V) and V (IV) analyses were dried on a hot plate and re-dissolved in 2 ml 0.1 N HNO_3. The quantification of both species of V was carried out by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS).

Method optimization

Compared to previous studies (e.g., Soldi et al., 1996; Pyrzyńska and Wierzbicki, 2004), several analytical conditions needed to be optimized to achieve the best separation of V (V) and V (IV) species at the actual nanomolar concentrations that V exists in seawater. Those modifications include sample volume, amount of Chelex 100 resin used in the separation, optimum pH of the water sample to do the chemical separation of both

species, sample loading rate onto the resin, elution volumes required to obtain full extraction, and so on. The different analytical conditions are listed in Table 1 of Chapter Two.

The batch method in Soldi et al. (1996) involved a large volume of water samples (400 ml) and small amount of Chelex 100 resin (0.2 g), leading to an equilibrium time of as long as 2 h (Soldi et al., 1996). After many trials, this method turned out failing in water samples with the actual nanomolar concentrations that V exists in seawater due to the fact that the oxidation half time of added free V (IV) ions in lake water or seawater was only about 7 ~ 15 min (Okamura et al., 2001). All the adsorption and elution are conducted under nitrogen to reduce the chances of oxidation of free V (IV).

In order to decrease the whole handling and operation time, a water volume was decreased to 10 ml instead of 400 ml. Therefore with a loading rate of 2 ml/min, the adsorption will on be ~5 min. 2.0 grams Chelex 100 resin was added inside the column to increase a contact time of V ions with Chelex 100.

As the affinity of metal ions is strongly pH dependent, pH adjustment in water samples is critical for adsorption of both V (V) anion, and V (IV) cation onto the resin (e.g., Pai, 1988). Chen et al. (1993) reported that both vanadium ions was adsorbed best under pH of 3-5, and Soldi et al. (1996) also showed that vanadium ions can be well adsorbed onto Chelex 100 at pH of 4-6. After adjusting pH of water samples to different levels by adding concentrated perchloric acid, the best pH for water samples was in a range of 4-5 in water samples. The affinity of V (V) anions with Chelex 100 will be seriously affected at higher pHs, while affinity of V (IV) cations affected at lower pHs. These results are consistent with previous studies (e.g., Zhang et al., 1985; Chen and Pai, 1991; Chang, 2001). Hence acetate buffer (pH=4.5 in 0.1 M acetic acid and sodium acetate) and a small amount of concentrated perchloric acid (when necessary) were used adjusting water pH. Nitric acid or hydrochloric acid is not good for adjusting pH in water samples due to their strong oxidizing abilities. In addition, acetate buffer was used not only because it will buffer pH in seawater, but also because it complexes and stabilizes dissolved reduced V (IV) (Lyalikova and Yurkova, 1992).

Loading rate of water samples onto Chelex 100 was assessed by controlling the flow rate of the peristaltic pump. A slower flow rate will increase the contact time of vanadium ions with the resin, but it will also comprise the whole loading time. Chen et al. (1993) reported that, a flow rate of 2.0 ~ 4.0 ml/min will ensure a completion adsorption of vanadium ions with 1.0 g Chelex 100. The tests in this research shows that a flow rate of 2.0 ml/min is good enough for a completion adsorption of vanadium ions, and the whole loading time for 10 ml water samples is only about 5 min.

Different pHs of ammonium solutions were used to test the pH effects on elution efficiency of V(V) off the resin. Ammonium was added by different elutions of 1 ml each, and all 1ml elutions were collected separately and measured for V. In this research, 10 ml of ammonium solution (pH=11.2, ~0.1 M ammonium hydroxide), as 10 elutions of 1 ml each, was necessary for a complete desorption of V (V) at the flow rate of 2 ml/min. Lower pH will not recover V(V) completely. Higher pH somehow led to lower V(IV) recovery, and the reason is still unknown. After V(V) was successfully eluted off the resin column, a complete V (IV) desorption was ensured by passing 10 ml 0.2 N perchloric acid of pH=0.8 (10 elutions of 1 ml each) at the flow rate of 2 ml/min.

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FIGURE CATION

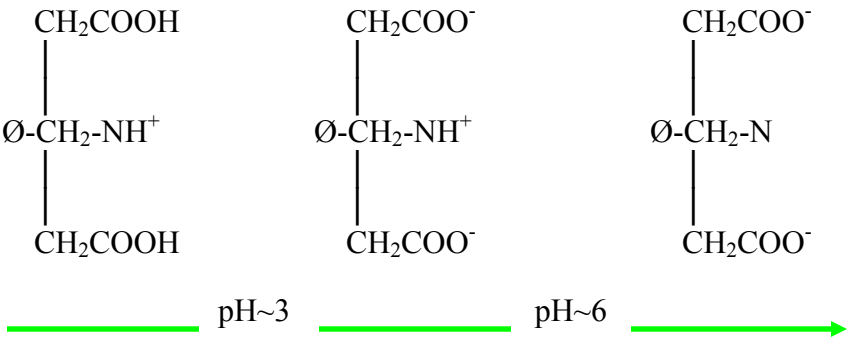

Figure 1: Possible changes in structure of chelex resin in response to pH.