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The bioinorganic chemistry of the ancient ocean: the co-evolution of cyanobacterial metal requirements and biogeochemical cycles at the Archean–Proterozoic boundary?

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Dedicated in honor of Professor Fraústo da Silva

Abstract

Recent evidence from the sulfur isotopic record indicates a transition ~2.5 billion years ago from an ocean chemistry first dominated by iron and then by sulfide. It has been hypothesized that the selection of metal centers in metalloenzymes has been influenced by the availability of metals through geological time, in particular as a result of large differences in the solubility of metals-sulfides. In this study, we examine the trace metal requirements and sensitivities of marine cyanobacteria and use recent stability constants to model the abundance and chemical speciation of metals across this chemical transition ~2.5 billion years ago. Two major results are reported here: (1) the marine cyanobacterial species studied thus far show trace metal preferences and sensitivities that are consistent with their evolution in a sulfidic marine environment, and (2) in an ancient ocean dominated by high fluxes and concentrations of iron, the relative availability of trace metals would have been similar to that of a sulfidic system—Fe > Mn, Ni, Co ≫ Cd, Zn, Cu—as a result of the formation of dissolved sulfide complexes. Thus, the formation of strong aqueous metal-sulfide complexes was likely as important as the precipitation of minerals in influencing the selection of metals in biology. These results suggest that marine biogeochemical cycles and marine bioinorganic chemistry have co-evolved, and that the evidence for this co-evolution has been preserved in the physiology and genomes of modern descendants of the early cyanobacteria.

Keywords: Metal-sulfide complexes; Cyanobacteria; Archean ocean; Cobalt; Zinc; Cadmium

1. Introduction

The notion that there has been a biological selection of elements in biomolecules, as presented in the works of da Silva and Williams, is beginning to have a significant influence on the fields of geochemistry and marine bioinorganic chemistry. This special issue of ICA dedicated to da Silva is an ideal opportunity to discuss how the chemistry of the ancient Earth might have influenced the evolution of metal containing biomolecules. Gaining insight to the chemistry of the Earth during the Archean and the Proterozoic is difficult due to a lack of well-preserved samples from those eras. Yet, an excellent source of information may be available that has been overlooked: the genetically coded biochemical utilization of and physiological responses to trace elements in extant phytoplankton. There are several reasons to consider the genomes of organisms as feasible reservoirs of geologic and bioinorganic information. First, enzyme evolution appears to be relatively slow: many enzymes appear to have been invented only once or a few times in Earth history. Furthermore, improvements in the activity of enzymes, e.g. Rubisco, have taken millions of years of evolution to occur [1]. This implies that any signal encoded in the enzyme may be well preserved on geologic time scales. Second, the selection of an element in the active site of an enzyme is fundamentally dependent on the presence of that

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element in the local environment of the evolving organism, as discussed by da Silva and Williams:

> One may therefore say ... that in its selection of chemical elements Nature responded to abundance and availability following a principle of ‘the economical utilization of resources’, i.e. choosing those elements less costly in terms of energy required for uptake, *given the function for which they are required* ... While it would appear that the solvent for life has always been water, other conditions, such as those of temperature and the dominant chemical species in the atmosphere, have changed with time. Life, while itself producing many of these changes, has evolved with the changes. ... We must note too that the changes in the atmosphere and the consequent changes in the sea and in the earth’s surface over millennia have had a considerable effect especially on rare element distribution and uptake. Some of the elements found in biology were selected a long time ago, in very different circumstances. (da Silva and Williams, 1991, p. 9–10) [2]

This article aims to explore the notion of a selection process for chemical elements in biology based on the ancient geochemistry of the Earth. da Silva and Williams have postulated that, because of their higher solubilities in the presence of sulfide, Ni, Co, and Fe were more abundant and Cu and Zn were scarcer in the Archean than in the modern ocean. Recent geochemical advances in sulfur isotopes now provide a rough history of sulfide chemistry on Earth [3]; here, we utilize this information to model the trace metal chemistry of the ancient oceans and compare it to new and accumulating literature evidence for unique metal requirements and sensitivities in modern cyanobacteria descendant from microbes that dominated the early oceans.

Recent studies of sulfur isotopes have suggested that the oceans may have been dominated by sulfides throughout much of Earth’s history, and that the gradual oxygenation of the deepwaters by oxygenic photoautotrophs (cyanobacteria) in surface waters may have taken hundreds of millions of years to accomplish. As a result, the Proterozoic (2.5–0.5 billion years ago) was likely a time when the atmosphere and a thin layer of the surface ocean were oxygenated, but the deepwaters were sulfidic. This hypothesis [3,4] is a departure from previous ideas of the entire oceans becoming oxic relatively soon after the rise of oxygenic photosynthesis. This is also a departure from the presumption of da Silva and Williams that the earlier, Archean, oceans were sulfidic [5]. Canfield [3] and his predecessors [6] argue that prior to 2.5 billion years ago there would have been little sulfide in the oceans due to high ferrous iron concentrations and precipitation of pyrite. Only after oxygenation of the atmosphere, and the resultant increased weathering of continental sources of sulfur, would sulfate and sulfide (through bacterial sulfate reduction) have accumulated in the oceans. Hence, a major point by Canfield is that the Archean ocean would have been dominated by ferrous iron followed by a Proterozoic ocean that was dominated by sulfide chemistry. There is a contradiction between the views of da Silva and Williams who consider that the early prokaryotes have metal requirements that are consistent with a sulfidic environment, and of the geochemists’ view of the Archean ocean where oceanic sulfide concentrations were initially low and did not rise until the Proterozoic, ~2.5 billion years ago. Here, we show that the cyanobacteria do in fact have numerous biochemical and physiological characteristics consistent with their evolution in a sulfidic ocean, but that metal-sulfide complexation caused the relative trace metal availabilities in the Archean ocean to be similar to that of the Proterozoic despite large differences in iron and sulfide concentrations.

2. Methods

In the past decade, there have been several studies using sensitive electrochemical techniques to directly determine the stability constants of metal-sulfide complexes using competitive ligand exchange [7–10]. Previous efforts focused on determining the solubility of metal-sulfides and then modeling the possible stoichiometries of metal-sulfide complexes. In this study, geochemical modeling was carried out using the software MINEQL+ with stability constants from the direct electrochemical methods for metal-sulfide species (Table 1) and the NIST critical stability constant database for other chemical species [11,12]. Metal-sulfide stability constants used for these calculations are shown in bold in Table 1. Computations were conducted using standard modern seawater parameters of: 0.545 M Na^+, 0.468 M Cl^-, 0.0102 M Ca^{2+}, 0.0532 M Mg^{2+}, and variable metal and sulfide concentrations. Initial model conditions have equimolar amounts (10 nM) of Mn, Co, Ni, Cd, Cu(I), Cu(II) and Zn to allow comparisons among metals, and are not intended to reflect estimates of absolute concentrations. A dissolved inorganic carbon (DIC) concentration of 5 mM was used, as calculated from ~20-fold higher atmospheric CO_2 than present values [13,14]. pH was set at 7.5 based on Canfield, slightly lower than the modern pH of about 8.1 [3]. While the DIC and pH values could each be the subject of a separate study to better constrain their ancient values, the calculations and conclusions of this study are not particularly sensitive to these parameters. While the modern ocean is known to have significant concentrations of organic metal ligands with very high

Table 1
Stability constants of metal-sulfide complexes and solubility of sulfides

Equations modeled:	Log *K*	Reference
Aqueous stability constants (seawater ionic strength)		
$Fe^{2+} + HS^- \Leftrightarrow FeHS^+$	**5.3**, 5.07, 6.1	[7–9]
$Fe^{2+} + 2HS^- \Leftrightarrow FeHS_2$	n.f., n.f., n.f.	[7–9]
$Co^{2+} + HS^- \Leftrightarrow CoHS^+$	**5.3**, 4.68, 6.8	[7–9]
$Co^{2+} + 2HS^- \Leftrightarrow CoHS_2$	n.f., n.f., 10.4	[7–9]
$Zn^{2+} + S^{2-} \Leftrightarrow ZnS$	**6.63**	[7]
$Zn^{2+} + HS^- \Leftrightarrow ZnHS^+$	**6.0**, n.f., 7.4	[7,8,10]
$Zn^{2+} + 2HS^- \Leftrightarrow Zn(HS)_2$	**13.7**	[8]
$2Zn^{2+} 3S^{2-} \Leftrightarrow Zn_2S_3^{2-}$	41.09, 13.83	[7](*K* and K_{cond})
$Cd^{2+} + HS^- \Leftrightarrow CdHS^+$	6.3, **8.4**, 8.0	[8–10]
$Cd^{2+} 2HS^- \Leftrightarrow Cd(HS)_2$	12.7, **15.5**	[8,9]
$Cu^{2+} + S^{2-} \Leftrightarrow CuS$	5.98	[7]
$Cu^{2+} + HS^- \Leftrightarrow CuHS^+$	**7.0**, n.f., 12.9	[7–9]
$Cu^{2+} + 2HS^- \Leftrightarrow Cu(HS)_2$	**13.0**, 19.4	[8,9]
$2Cu^{2+} + 3S^{2-} \Leftrightarrow Cu_2S_3^{2-}$	38.29, 11.68	[7](*K* and K_{cond})
$Cu^+ + HS^- \Leftrightarrow CuHS$	6.8, **11.8**[a], 16.67	[8,9]
$Cu^+ + 2HS^- \Leftrightarrow Cu(HS)_2^-$	12.6, **17.6**[a], 23.17	[8,9]
$Ni^{2+} HS^- \Leftrightarrow NiHS^+$	**5.3**, 4.97, 5.1	[7–9]
$Ni^{2+} 2HS^- \Leftrightarrow Ni(HS)_2$	n.f., 10.8	[8,9]
$Mn^{2+} + HS^- \Leftrightarrow MnHS^+$	6.7, **4.76**, 4.5	[7–9]
$Mn^{2+} + 2HS^- \Leftrightarrow Mn(HS)_2$	n.f., 9.9	[8,9]
Solubility products (25 °C, $I = 0$)		
FeS	−**4.2**	[61]
$CoS_{(s)}$ α	−**7.44**, −4.6	[61,69]
$CoS_{(s)}$ β	−**11.07**	[61]
Sphaelerite (ZnS)	−**10.93**	[61]
Wurtzite	−**8.95**	[61]
$CdS_{(s)}$ (greenockite)	−**14.36**	[62]
Covellite (CuS)	−**22.3**	[61]
Millerite (NiS)	−**5.6**	[61]
MnS (pink)	**3.34**	[61]

Bold indicates value used in calculations; n.f., not found.
[a] corrected for strong Cu^+ chloro complexes.

stability constants [15–18], organic metal ligands are ignored in these calculations since we have no evidence of their existence in an ancient ocean. Ionic strength corrections were made using the Davies equation in MINEQL+ for constants not measured at seawater salinity. Results are plotted as free ion concentrations $[M^{n+}]$ and metal-sulfide species. Inorganic metal complexes such as chloro and hydroxyl complexes are included in calculations, but their abundances are low relative to that of metal-sulfide complexes.

Simplifying assumptions were made mostly regarding uncertainties in redox chemistry and biological processes. Changes in the redox state of metals are known to be microbially mediated and/or to require the presence of strong oxidants such as O_2 or reactive oxygen species (ROS). Biology or kinetics can cause the trace element chemistry to deviate somewhat from thermodynamic equilibrium; hence, modeling them accurately is problematic. In particular, sulfide is known to be able to reduce Cu(II) to Cu(I); hence, both species are included here to show the range of possibilities. Ferrous iron only tends to exist transiently in the modern ocean as a result of rapid oxidation rates with oxygen and ROS, yet the Archean ocean would have had significant ferrous iron because of limited oxygen abundance [6]. Co and Mn are also modeled as Co(II) and Mn(II), their favored oxidation states in reducing aqueous environments. It should be pointed out that Fe, Co, and Mn are all known to be involved in microbial oxidation and microbial reduction processes at modern O_2–H_2S interfaces. The precise date of the evolution of these microbial processes is uncertain, yet their influence would be significant only near the surface ocean in the Canfield model of a reducing deep ocean.

Culture studies were carried out using cleanly collected Atlantic Gulf Stream water and axenic *Synechococcus* WH8102 cultures. Seawater medium was prepared under trace metal clean conditions using 11.7 μM EDTA as a metal-ion buffer as described previously [19] with the exception of 50 μM nitrate replacing ammonia and urea amendments. Nitrate and phosphate solutions were run through methanol extracted Chelex-100 (Bio-Rad) columns to remove contaminants [20]. Cultures were grown in 250 ml polycarbonate bottles cleaned with citranox and 10% HCl prior to use. Growth rates were determined by flow cytometry (FACScan, Becton Dickenson) using 1.0 μm diameter fluorescent beads and 1:10–1:50 dilutions of cells into filtered seawater. Metal concentrations are plotted as divalent cation concentrations as set by equilibrium with the metal-ion buffer [19].

3. Results and discussion

The notion that metal abundances in the environment have exerted an evolutionary selection pressure on the ‘choice’ of metals within metalloenzymes has seldom been discussed at length in the literature. Here, we present and review evidence and models that are consistent with this idea. First, we discuss how the cyanobacteria have numerous characteristics that are consistent with their evolution in a sulfidic ocean. Second, we present geochemical modeling results that examine the trace metal speciation in light of recent geochemical evidence about ancient sulfide chemistry. Third, we discuss some aspects of how biochemical and molecular evolution are consistent with these scenarios of Earth history.

3.1. Evidence consistent with the evolution of marine cyanobacteria in a sulfidic ocean

The cyanobacteria are believed to be responsible for supplying the oxygen to the surface ocean and eventually oxygenating the atmosphere. They evolved perhaps as early as 3.2 billion years ago, but at least 2.75 billion years ago, earlier than the transition period to a sulfidic ocean described by sulfur isotopic measurements. Numerous trace metal physiological studies have been carried out on marine phytoplankton, which can be used to examine their metal preferences. Furthermore, marine photoautotrophs may be the ideal organisms to study trace metal limitation physiology: unlike most cultivation media with yeast extract or agarose that are difficult if not impossible to clean for low metal work, marine phytoplankton growth media contain only seawater and inorganic nutrients (N, P, metals). Furthermore, utilizing clean-room technology, clean seawater collection techniques, synthetic metal chelators (EDTA), and iminodiacetic acid resins (Chelex-100), exceptionally low trace metal media concentrations have been achieved [22], allowing two decades of detailed trace metal physiological studies on marine phytoplankton. The application of EDTA as a metal-ion buffer that sets precise free metal-ion concentrations, $[M^{n+}]$ or more precisely aquo-ions, $[M^{n+}(H_2O)_n]$, has been critical in allowing differentiation between metal-ion uptake through cation transporters and acquisition of metals in organic complexes [19,21–25]. In this literature there is accumulating evidence that the marine cyanobacteria have physiological and biochemical characteristics that are significantly different from that of eukaryotic phytoplankton. The principal cyanobacteria studied thus far are the ubiquitous *Synechococcus* and *Prochlorococcus*. Though relatively recently discovered [26,27], these organisms are major contributors to primary production, perhaps accounting for ~25 and ~80% of gross primary production in the subtropical gyres of the Atlantic and Pacific, respectively [28,29]. Phylogenetic analysis shows that these microbes are closely related to each other [30]. da Silva and Williams have postulated that Fe(II), Co, Ni, and Mn, would be relatively abundant compared to Cu, Cd, and Zn in an early sulfidic ocean, as supported by our modeling results (see below). Culture studies carried out under stringent trace metal clean conditions show the cyanobacteria to have physiological and biochemical characteristics that are remarkably consistent with this idea of metal requirements that evolved in a sulfidic environment (Table 2). In the following paragraphs, we describe these requirements from both new data on Cd interactions with *Synechococcus* and from the literature for Co, Zn, Cu, Cd, and Fe.

da Silva and Williams discuss how cobalt and nickel requirements are likely “remnants from early life” [2]. For cobalt, this discussion is based on vitamin B_{12}, the cobalt containing molecule that has been postulated to have evolved 2.7–3.5 billion of years ago based on its synthesis without an oxidative step [31,32] and its redundancy in function with respect to more recently evolved enzymes. *Synechococcus* and *Prochlorococcus* have measurable absolute requirements for cobalt but not for zinc (Fig. 1(A)) [19,33]. This is in sharp contrast to many eukaryotic algae studied in equivalent growth medium, which show high zinc requirements and no cobalt requirement in the presence of zinc [34,35]. In addition, some eukaryotic phytoplankton have the ability to substitute cobalt for their biochemical use of zinc in vivo in the enzyme carbonic anhydrase [36], whereas this cobalt–zinc substitution has not been observed in the cyanobacteria [19,33]. The absolute requirement for cobalt in the cyanoibacteria may be related to the need for cobalt in vitamin B_{12} (cobalamin). The B_{12} synthesis pathway is present in the genomes of the marine cyanobacteria sequenced thus far, but has not been found in the genome of the first Eukaryotic phytoplankter to be sequenced, *Thalassiosira pseudonana*. However, estimates of cobalt and cobalamin content of cyanobacterial cells suggest that there is far more cobalt than stoichiometrically needed to account for all of the cobalamin molecules in *Synechococcus* [19,37], implying that there may be additional uses of cobalt in the cyanobacteria. One hypothesis is that the γ-carbonic anhydrase in *Synechococcus* might be a cobalt enzyme: molecular clock calculations estimate this gene to be billions of years old, and its metal center has not yet been determined [38].

Table 2

Observed physiological characteristics of marine prokaryotes	Reference
Absolute cobalt requirement	[19,33]
Little to no measurable zinc requirement	[19,33]
Extreme cadmium sensitivity and high copper sensitivity	This work, [39–42]
Manganese and nickel super-oxide dismutases	[a]
Large populations of *Prochlorococcus* in subsurface suboxic waters	[50]
Few zinc finger DNA binding motifs	[a]
Low metal tolerance of hydrothermal Archaea in the absence of sulfide	[48]

[a] *Synechococcus* and *Trichodesmium* genome sequences (http://www.jgi.doe.gov/JGI_microbial/html/).

The marine cyanobacteria have also been observed to be sensitive to Cu [39,40] and very sensitive to Cd toxicity (Fig. 1(B)) [41,42]. Both of these metals exist at exceedingly low concentrations in the modeled early oceans (see below), but exist today at concentrations that are toxic to the cyanobacteria in many environments [43]. It is now believed that the production of strong organic copper binding ligands by *Synechococcus* serves as a means of detoxifying surface waters with otherwise toxic concentrations of copper [39,44,45]. There is currently insufficient cadmium speciation field data to determine if naturally occurring organic cadmium ligands provide a similar detoxicification mechanism. The cyanobacterial sensitivity to cadmium at the picomolar level is in contrast to the importance of

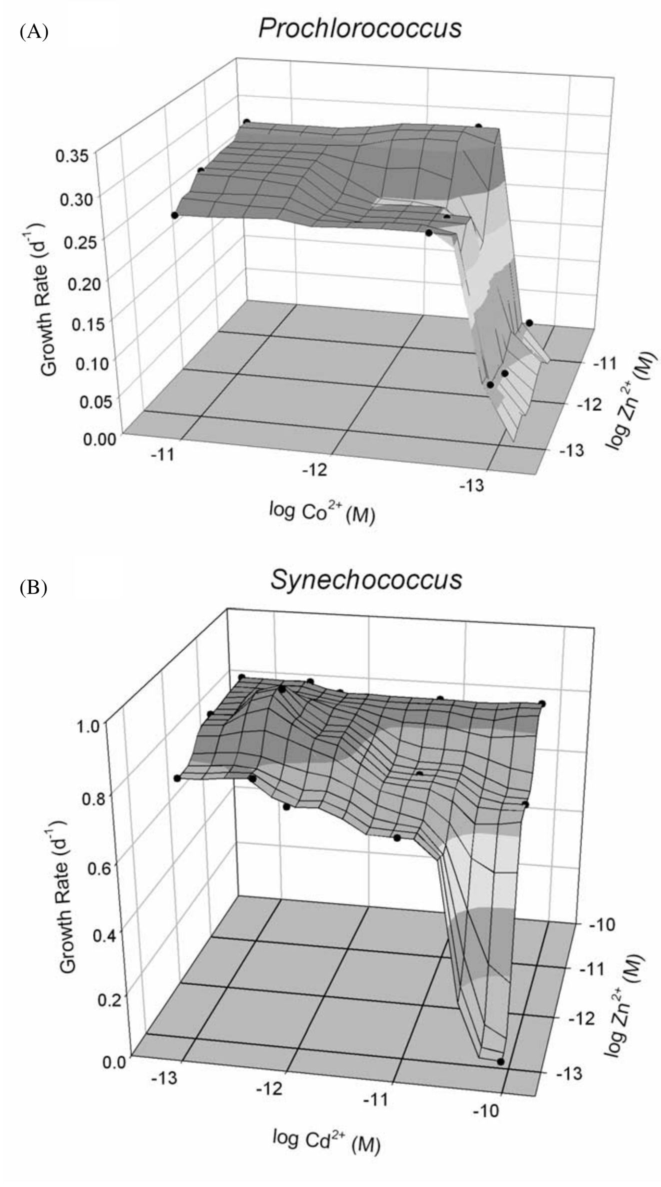

Fig. 1. Physiological culture experiments with *Prochlorococcus* and *Synechococcus* showed (A) an absolute requirement for cobalt that cannot be substituted for by zinc, and very low sensitivity to zinc limitation. (data from [19]) and (B) high sensitivity to Cd toxicity, low sensitivity to zinc limitation, and alleviation of cadmium toxicity by increasing zinc concentrations (this work).

cadmium as a micronutrient in eukaryotic phytoplankton at equivalent environmental concentrations. For example, the diatom *Thalassiosira weissflogii* has been shown to use cadmium in a novel carbonic anhydrase, the first known biochemical function for cadmium [46].

An intriguing question based on this study is the extent to which zinc enzymes occur in the cyanobacteria, and the prokaryotes in general. da Silva and Williams have discussed the proliferation of zinc enzymes later in evolution, especially in eukaryotic cells [2]. While it is now known that zinc containing proteins are very abundant in eukaryotic cells (e.g. ~3% of human proteins are putative zinc proteins) the zinc finger proteins that make up the bulk of such estimates are much scarcer in prokaryotes [47]. While the cyanobacteria will take up zinc in copious amounts if it is available (as is true for most divalent cations), growth limitation by zinc has been observed to occur at much lower zinc concentrations than for eukaryotic algae (Fig. 1(A)). With the scarcity of zinc in the early oceans, one can wonder if early cyanobacteria utilized less zinc due to its scarcity, made use of alternate metal centers, or obtained zinc enzymes more recently through lateral gene transfer. The first of these three explanations is supported by data available at this time: the zinc-sulfide complexes that would have dominated zinc speciation are much stronger than cobalt-bisulfide complexes, which may explain the absolute cobalt requirement but small zinc requirement observed in Fig. 1(A). Furthermore, recent studies suggest that metal-sulfide complexes are not as biologically available to microbes as the aquated ion and other inorganic complexes [48], likely due to the high stability constants of the complexes. While Cd and Cu are calculated to be very scarce in the ancient oceans, ZnS complexes appear to be quite soluble relative to their mineral phases. Thus, despite the scarcity of Zn^{2+} relative to Co^{2+} due to sulfide complexation, the use of zinc in some important enzymes such as polymerases may reflect the availability of a small amount of zinc to early microbes, either as low free zinc concentrations or via some type of zinc-sulfide uptake mechanism.

Iron requirements of the cyanobacteria are also thought to be higher than those of eukaryotic phytoplankton due to their higher PSI/PSII ratios [49], although there is as yet limited experimental work on the oceanic and non-diazotrophic marine cyanobacteria. *Prochlorococcus* has been observed to be present at very high cell densities in the low-light oxygen minimum zone of the Arabian Sea and the Eastern Equatorial Pacific [50]. While *Prochlorococcus* and *Synechococcus* are typically found in well-oxygenated surface waters, the presence of large populations of *Prochlorococcus* in these low-light suboxic zones is intriguing and suggests no difficulty in surviving under low O_2 conditions in which sulfide may be present.

3.2. Geochemical modeling

There are many uncertainties involved in modeling the chemistry of the early ocean; even basic parameters and elemental fluxes are difficult to approximate. Nevertheless, we can make basic assumptions that should envelope the range of possibilities. Furthermore, as will become evident in the calculations, the difference in concentration between many chemical species is so great that knowing initial concentrations to within a few orders of magnitude is likely to be sufficient to generally describe the salient chemical features of the system.

We begin by showing the influence of increasing sulfide concentration on selected trace elements, taking into account both solubility products and stability constants of metal-sulfide complexes (Table 1; Fig. 2). The modern ocean contains sub-nanomolar concentrations of sulfide [51], despite high concentrations of sulfate, while anoxic waters can have concentrations up to ~0.1 mM. For the purposes of comparison, initial model conditions have equimolar amounts (10 nM) of Mn, Co, Ni, Cd, Cu(I), Cu(II) and Zn. Thus, modeled metal concentrations should be considered relative to other metals, not as estimates of accurate absolute values. High total Fe(II) concentrations are used here (50 μM) to reflect expected high values in the early ocean. With increasing sulfide, aquated ion concentrations (M^{n+}) decrease immediately when the total sulfide concentration exceeds the concentrations of Zn, Cd, and Cu, but two orders of magnitude additional sulfide is required before Co, Mn and Ni free ion concentrations decrease significantly (panels A and C). Panels B and D of Fig. 2 show the strong effect of sulfide complexation and the low solubility of Cu, Cd, and Zn relative to Co, Ni, and Mn. While these overall trends are somewhat evident from looking at solubility constant data, the metal-sulfide speciation calculations are particularly interesting when considered in the context of recent evidence that metal-sulfide complexes are significantly less available to microbes than the aquated ion and other inorganic complexes [48]. As a result, the potential disparity in metal bioavailability between these two groups of metals can occur at very low sulfide concentrations even before precipitation occurs (e.g., $[Zn^{2+}]$ and $[Cd^{2+}]$ are greatly decreased before precipitation, as indicated by the plateau in $ZnS_{(aq)}$ and Cd-sulfide concentrations). Iron is also quite soluble as $FeHS^+$ complexes and thus has higher concentrations relative to the modern ocean, where Fe is rapidly oxidized to Fe(III) and forms hydrolysis species

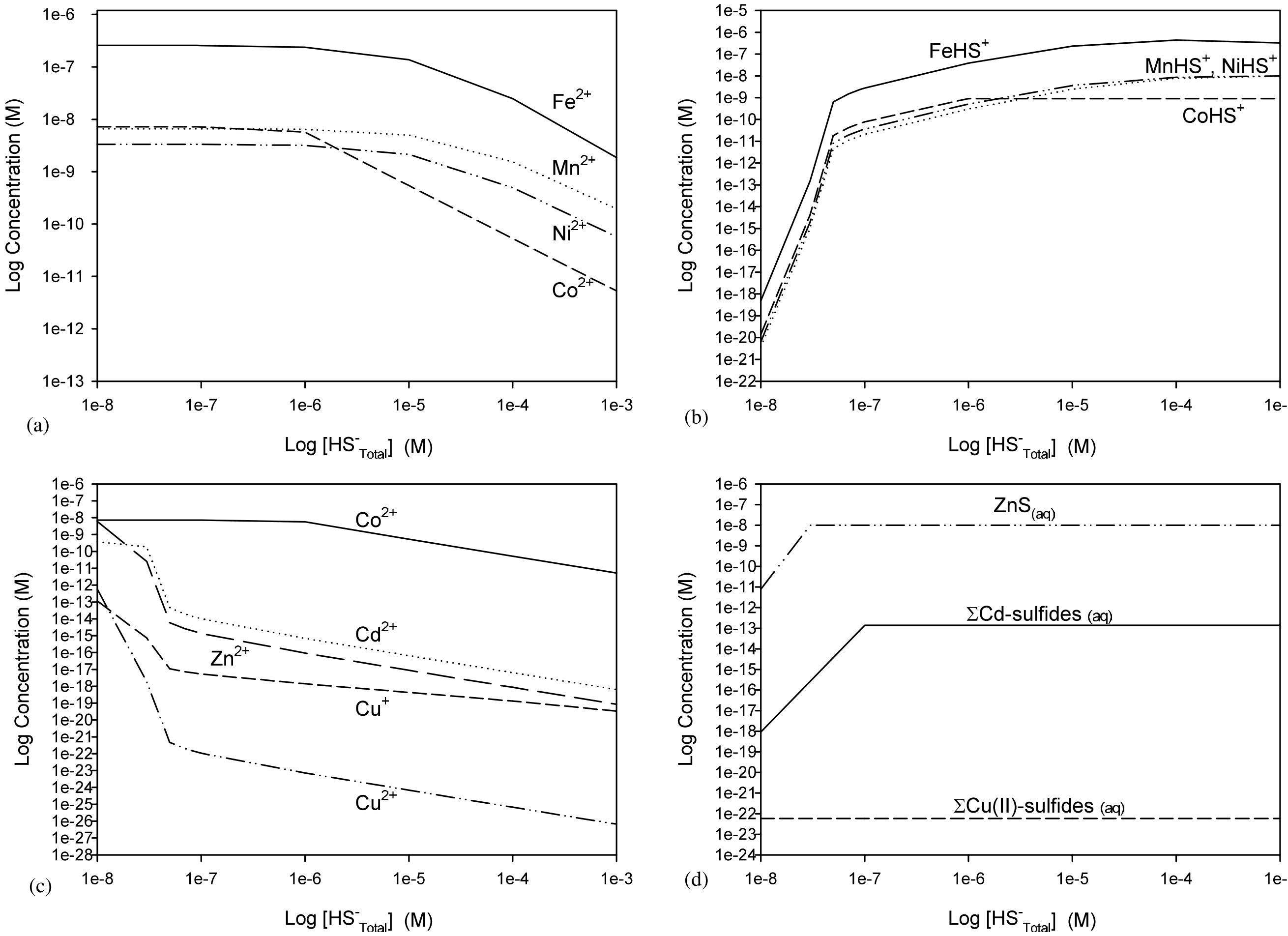

Fig. 2. Model output showing metal-sulfide complex and free metal-ion concentrations under increasing sulfide concentrations. With increasing sulfide concentrations free ion concentrations decrease significantly due to complex formation and/or precipitation. Once sulfide concentrations exceed total metal concentrations (10 nM for all metals but Fe), Mn, Ni, and Fe have concentrations several orders of magnitude higher than Zn, Cd and Cu (modeled as both Cu(I) and Cu(II), see text for explanation). Iron precipitates initially as amorphous $FeS_{(s)}$ followed by precipitation with carbonate as siderite once sulfide concentrations drop significantly.

with very low solubilities [52]. Thus, as expected, the high concentrations of sulfide in the Proterozoic are predicted to have resulted in relatively high free metal concentrations of Co, Mn, Ni, and Fe, while making Zn, Cd, and Cu quite scarce.

The critical problem in reconciling this notion of cyanobacterial evolution in a sulfidic ocean with recent geochemical data is the influence of a high ferrous iron–low sulfide ocean on trace metal chemistry in the Archean ocean, prior to 2.5 billion years ago. Upon consideration of iron inputs with bacterial sulfate reduction rates, previous researchers suggested that very high fluxes of iron to the oceans in the Archean would have stripped sulfide from the system via pyrite formation and precipitation resulting in a ferrous iron dominated ocean [3,6]. This raises a problem, how can the cyanobacteria have requirements and sensitivities that are consistent with their evolution in a sulfidic ocean, when geochemical data show they actually evolved in a ferrous ocean thought to have low sulfide? This conceptualized Archean ocean is modeled here with increasing ferrous iron to simulate the effect of high iron fluxes on sulfide. The resultant effects on trace metal chemistry are shown in Fig. 3. An initial sulfide concentration of 0.05 mM, which is low relative to many modern anoxic environments, was choosen to provide a worse-case-scenario for possible sulfide depletion. While additions of ferrous iron to millimolar levels depletes bisulfide to ~50 nM (panel A), the total dissolved sulfide does not fall below 15 μM as a result of the formation of the $FeHS^+_{(aq)}$ complex which becomes the dominant sulfide species (panel B). Because the equations for the solubility product and iron-bisulfide complexes (Eqs. (1) and (2)) are coupled via their dependence on Fe^{2+} and HS^- concentrations, a maximum $FeHS^+$ concentration is set by pH when $FeS_{(s)}$ is saturated (Eq. (3)):

$$K_{sp} = \frac{[Fe^{2+}][HS^-]}{[H^+]} \quad (1)$$

$$K = \frac{[FeHS^+]}{[Fe^{2+}][HS^-]} \quad (2)$$

$$[FeHS^+] = K_{sp}K[H^+] \quad (3)$$

This maximal $FeHS^+$ value then becomes the predominant form of sulfide in the system, effectively buffering total sulfide as free HS^- drops with increasing total ferrous iron loads ($[FeHS^+] = 10^{-6.4}$ M, Fig. 3(A and B)). This maximal $FeHS^+$ then becomes critical to the speciation of trace elements at lower concentrations: those with weak metal-bisulfide complexes (Co, Ni, Mn) dissociate and become dominated by weaker inorganic ligands such as chloro complexes (panels C and D). In contrast, those with strong metal-bisulfide or sulfide complexes remain predominantly bound by sulfides (panels E and F), resulting in much lower free metal concentrations than Co, Ni, and Mn despite the large decrease in HS^- concentrations. Once iron concentrations are high enough siderite begins to precipitate. While high iron fluxes drastically reduce the concentrations of bisulfide in the Archean relative to the Proterozoic, the result is actually a ‘‘ferro-sulfidic’’Archean ocean (rather than a ferrous Archean ocean) with sufficient residual sulfide concentrations to make Fe, Ni, Co and Mn more available than Cu, Zn and Cd (Fig. 4). The sulfur isotope studies predict a marine sulfate inventory in the Archean that is less than or equal to 5% of modern concentrations (≤ 0.2 mM) [3]. Assuming significant conversion to sulfide via bacterial sulfate reduction (a microbial process known to have predated the evolution of oxygenic photosynthesis in the Archean [53]), this lower sulfate inventory should have been capable of supplying the necessary sulfide to create the trace metal conditions described above. This ferro-sulfidic concept is consistent with the sulfur isotopic data: The lower marine sulfate inventories in the Archean ocean would have resulted in a complete reduction to sulfide by bacterial sulfate reduction and minimized sulfur fractionation effects. When sulfate concentrations became high enough that the sulfate reservoir was no longer continually exhausted by bacterial sulfate reduction, fractionation signals would then have been preserved in the geologic record. Because the trace elements of interest tend to be in the nanomolar concentration range, having only 5% of the sulfate inventory in the Archean and its complete conversion to sulfide would still have provided the micromolar quantities of sulfide needed to control the trace metal speciation. Thus, a ferro-sulfidic Archean would have resulted in a biological selection for trace metals that is similar to that of a sulfidic system. Future work should verify this equilibrium-based ancient ocean by utilizing a kinetic approach with varying iron and sulfide fluxes and non-equilibrium processes such as particle scavenging.

3.3. *Trace metal chemistry in modern anoxic basins*

Despite the prevalence of oxygen in the modern atmosphere, several large anoxic marine basins persist due to slow circulation and/or high biological productivity. It is useful to compare our modeling calculations with these modern anoxic ocean basins since they likely have commonalities with ancient ocean chemistry and to examine how well a thermodynamic equilibrium model describes the chemistry of the system despite all the complications resulting from kinetic, biological, or physical processes. While these modern environments have high sulfide concentrations in deepwaters, they are capped by a lens of oxygen-rich waters at equilibrium with the atmosphere. Early efforts at reconciling trace

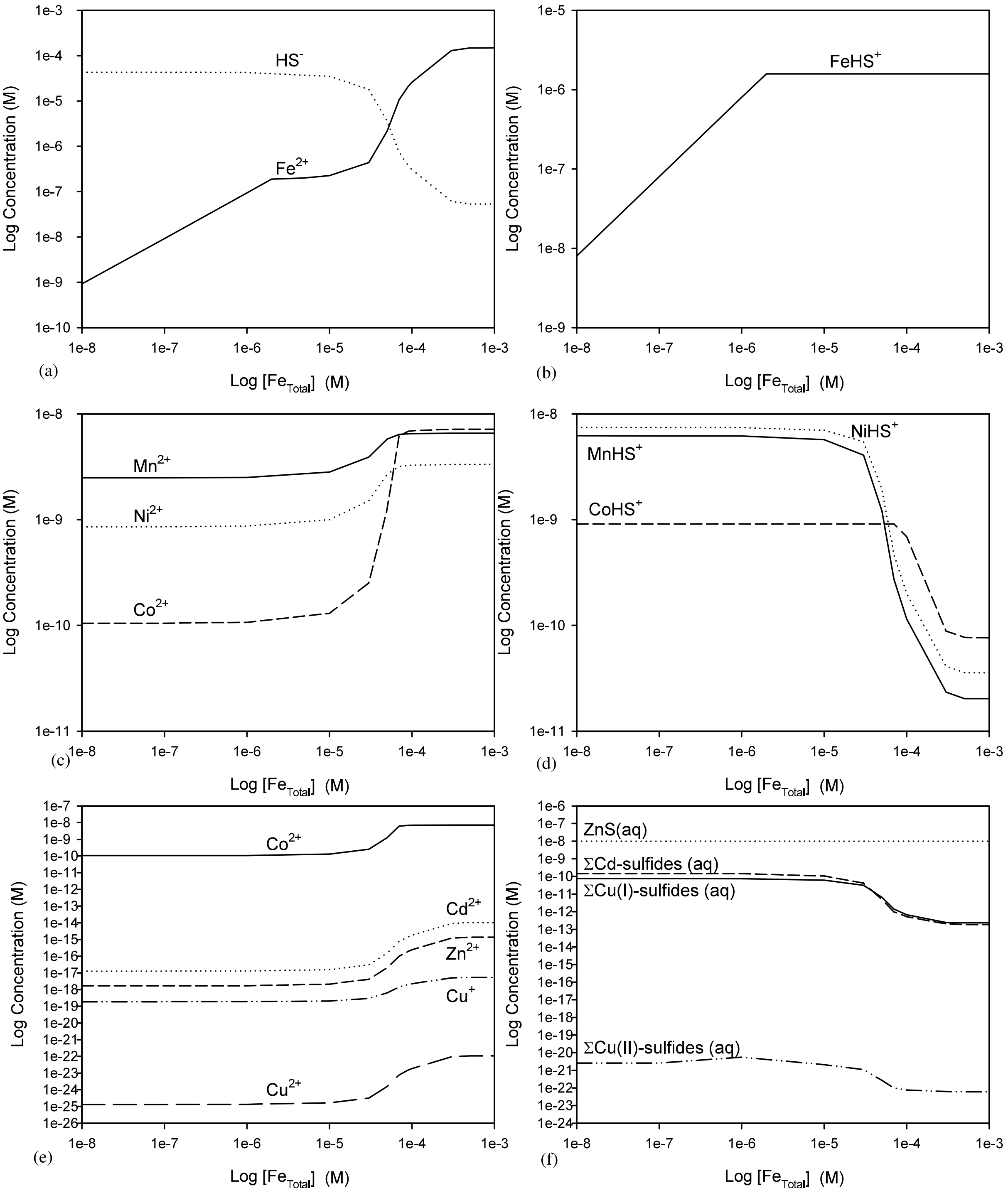

Fig. 3. Model output examining the influence of increasing ferrous iron on metal speciation and sulfide abundance. As ferrous iron surpasses the total sulfide concentration (50 μM) it begins to precipitate significant quantities of $FeS_{(s)}$ (amorphous pyrite) resulting in decreases in total sulfide as indicated by [HS^-]. As $FeS_{(s)}$ precipitates, $FeHS^+_{(aq)}$ remains constant while HS^- continues to decrease (until siderite begins to precipitate at very high ferrous iron levels). The chemical speciation of Co, Ni and Mn changes from sulfide complexes to much weaker inorganic complexes, while stronger metal-sulfide complexes involving Cd, Zn, and Cu(I) and Cu(II) continue to dominate the speciation of those metals. These results show that even low concentrations of sulfide in a ferrous iron dominated ocean result in a similar pattern of trace metal abundances as observed in a sulfidic ocean.

element abundances and thermodynamic predictions in a sulfidic fjord were limited by analytical concerns (samples close to or below detection limits) and by the availability of metal-sulfide stability constant data at the time [54–56]. In addition, measuring the abundance of trace elements in sulfidic waters is difficult as a result of

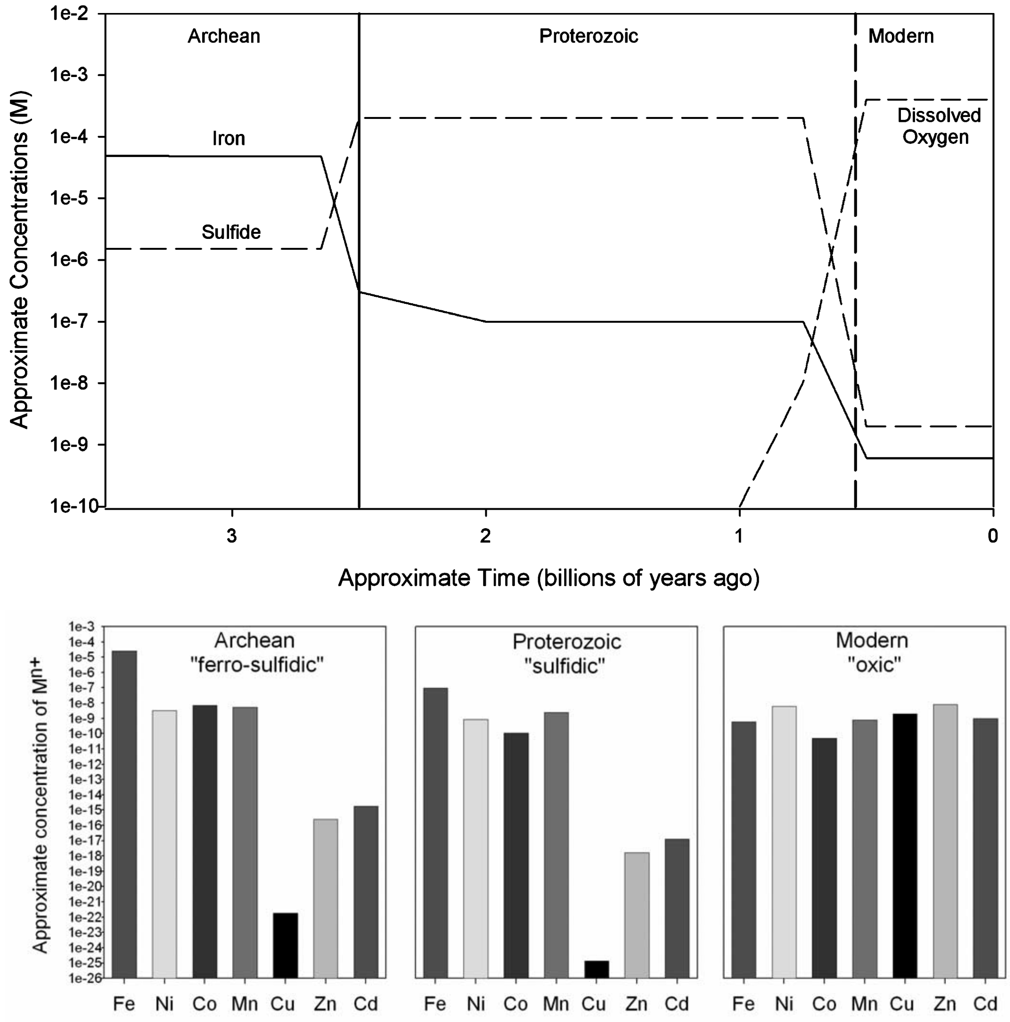

Fig. 4. Approximate relative differences in metal concentrations through major ocean chemistry changes. Top panel shows three major conceptualized ocean chemistries: ferrous iron dominated in the Archean, sulfide dominated in the Proterozoic, and oxygen (O_2) dominated in the modern ocean. Oxygen refers to deepwater oxygen; atmospheric oxygen is thought to have increased long before the oceans. Archean and Proterozoic free metal estimates are taken from 0.1 mM and 1 μM total ferrous iron calculations, respectively (Fig. 3). Modern concentrations represent actual measured values [51,65–68].

the possibility of oxidation and precipitation of the samples [57], and only recently have methods been developed to measure metal-sulfide species in the natural environment [10,58].

Dissolved concentrations of trace elements in anoxic waters differ significantly from those of oxic waters, often with high concentrations of Fe, Mn, and Co. These elements tend to reach their highest concentrations just below the O_2–H_2S interface where microbial redox cycling of metal-oxides occurs [59]. Fe and Mn can be found as high as micromolar values and cobalt up to 10 nM. Yet none of the modern anoxic basins appear to have the presumed Archean ocean characteristic of excess ferrous iron relative to sulfide, likely due to the presence of high sulfate concentrations in modern seawater. Dissolved iron values in anoxic basins are similar to the maximal $FeHS^+$ value calculated above (~ 3 μM). Nickel is not significantly affected across the O_2–H_2S interface suggesting it is undersaturated with respect to millerite and reflecting its high solubility in both oxic and anoxic waters, although some co-precipitation or scavenging of nickel has been observed with pyrite formation [60].

Cu, Zn, and Cd tend to decrease across the O_2–H_2S interface suggesting the formation of sulfide precipitates. Ratios of particulate to total (dissolved + particulate) metal concentrations in the anoxic Baltic Sea show that only a small fraction of Mn, Fe, Co, and Ni are present in particulate form (0.018, 2.9, 4.9, and 1.9%, respectively) compared to Cu and Cd (33 and 53%, respectively), consistent with our modeling results [61]. Cu, Cd and Zn have been measured in the dissolved form indicating the presence of metal-sulfide complexes (e.g. 0.5–4 nM Cu, 5–50 nM Zn, and ~ 0.4 nM Cd in the Drammens fjord). These results are somewhat in contrast to the modeling results in Figs. 2 and 3, suggesting that the solubility constants for Cu and Cd are either too high or do not take into account the formation of sulfide clusters. Both of these scenarios are likely the case: Daskalakis and Helz [62] point out in their solubility studies that the use of metastable precipitates instead of crystalline solids can result in a solubility constant that is two orders of magnitude greater than what they report. Furthermore, the presence of Zn sulfide clusters in natural waters has been well documented and could result in higher effective dissolved concentrations than our calculations reflect [58]. Thus, our modeling results seem generally consistent with the current geochemical knowledge of modern anoxic basins with the exception of underestimation of metal-sulfide cluster formation. The metal ions in these clusters would obviously still be strongly bound by sulfide, and would likely be significantly less available to biological uptake as a result [48]. It was likely not only the effective solubility of a metal in sulfidic waters, but also its chemical speciation and resultant bioavailability that determined selection pressures for metal usage in early microbes.

3.4. *Geochemical and evolutionary implications*

The evidence described here showing the cyanobacteria to have trace metal requirements and sensitivities that are consistent with their evolution in a sulfidic ocean presents an apparent paradox with previous interpretations of the sulfur isotope findings (an iron-rich sulfide-poor Archean ocean). It is effectively a timing problem: the cyanobacteria are believed to be the first microbes to evolve oxygenic photosynthesis, and they are thought to be responsible for the increased the atmospheric oxygen concentrations that cause the increased sulfur fluxes from the terrestrial environment to the marine environment via continental weathering [3], eventually leading to the Proterozoic sulfidic ocean. How could the cyanobacteria have a sulfidic ocean heritage if they are the organisms that are responsible for making the oceans sulfidic in the first place? The modeling work presented here resolves this paradox by showing that a modest concentration of sulfide (as would have presumably existed with bacterial sulfate reduction prior to the evolution of oxygenic photosynthesis [53]) in a ferrous dominated ocean is sufficient to make Cu, Zn and Cd significantly less available than Fe, Ni, Co and Mn.

If the trace metal requirements and sensitivities described in the cyanobacteria are in fact vestigial from an ancient ocean, one might wonder why they would persist in a modern ocean. The fact that cyanobacteria not only survive in the modern world, but in fact thrive, often dominating the primary production in their environment, demonstrates that they are successful competitors in the modern ocean. Hence, these vestigial metal requirements do not seem to hinder these modern cyanobacterial descendants. It is often argued that the cyanobacteria have a niche in low nutrient environments where their small size and accompanying high surface to volume ratio gives them an edge over larger eukaryotic phytoplankton. Yet, the cyanobacteria studied thus far, *Synechococcus* and *Prochlorococcus*, also appear to be specialists in producing metal-ligands and/or utilizing them. Siderophores, cobalt-binding ligands, and copper-binding ligands have all been associated with cultures and/or field populations of these cyanobacteria [19,25,44,63]. A plausible explanation for the persistence of these vestigial metal requirements is that the cyanobacteria responded to the gradual oxidation of sulfide and resultant changing trace metal chemistry not by altering their biochemistry, but by evolving the capability for metal ligand synthesis. These organic ligands serve to mimic the aqueous chemistry of the strong metal-sulfides complexes. For example, Fe and Co are quite insoluble in the modern ocean without organic ligands to prevent their oxidized (+3) ions from forming hydrolysis species and precipitating (Fe(III) and Co(III) both have very low solubilities oxic in seawater). But both of these elements are bound by strong organic ligands, which greatly increase their solubility in seawater; moreover, these ligands have been found in deep waters as well as surface waters [17,64]. On the toxic end of the spectrum, copper has been found to be strongly bound to organic ligands in surface waters resulting in free cupric ion concentrations in the picomolar range [65]. Arguably, it might be much easier to evolve an organic ligand synthesis capability for binding metals in seawater than it is to evolve new enzymes with alternative metal centers and equivalent functionalities. For example, nitrogenase has three isoforms utilizing Fe, Fe and Mo, or Fe and V. Despite the crucial importance of this enzyme to marine life, it appears to have only evolved once over billions of years and diverged into three closely related isoforms that maintain their reliance on iron.

4. Conclusions

We have presented the case for the cyanobacteria having trace metal sensitivities that are consistent with their evolution in an ancient sulfidic ocean. We have also calculated the relative abundances of trace metals and their chemical speciation in the Archean and Proterozoic oceans, and shown that both the ferrous iron and sulfide dominated oceans result in the differential availability of trace elements: $Fe > Mn, Ni, Co \gg Cd, Zn, Cu$. The persistence of biochemical characteristics consistent with life in a sulfidic environment in modern cyanobacterial strains is remarkable. It may result from evolving the capability for metal-ligand synthesis, which would result in maintaining some characteristics of ancient sulfidic ocean chemistry. Future work should focus on field studies of metal-sulfide speciation and genomic studies under varying metal conditions to look for novel bioinorganic chemistries and molecular evidence of these vestigial traits.

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