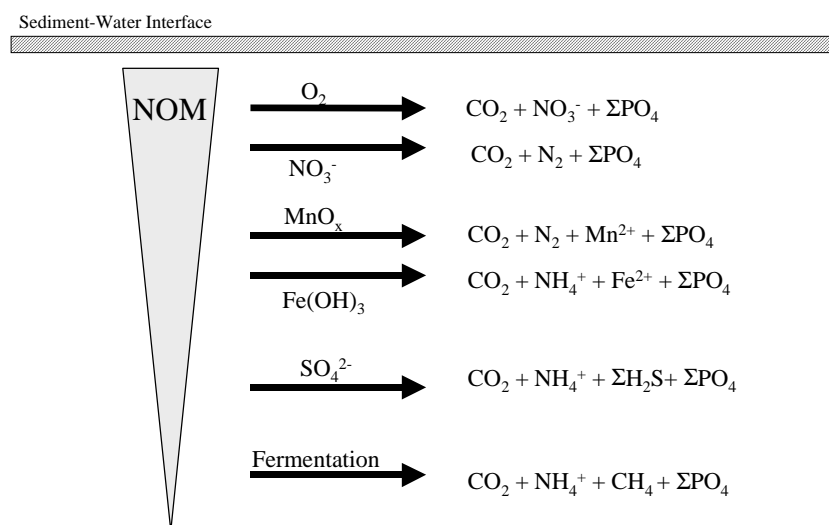


## 2.0 BACKGROUND

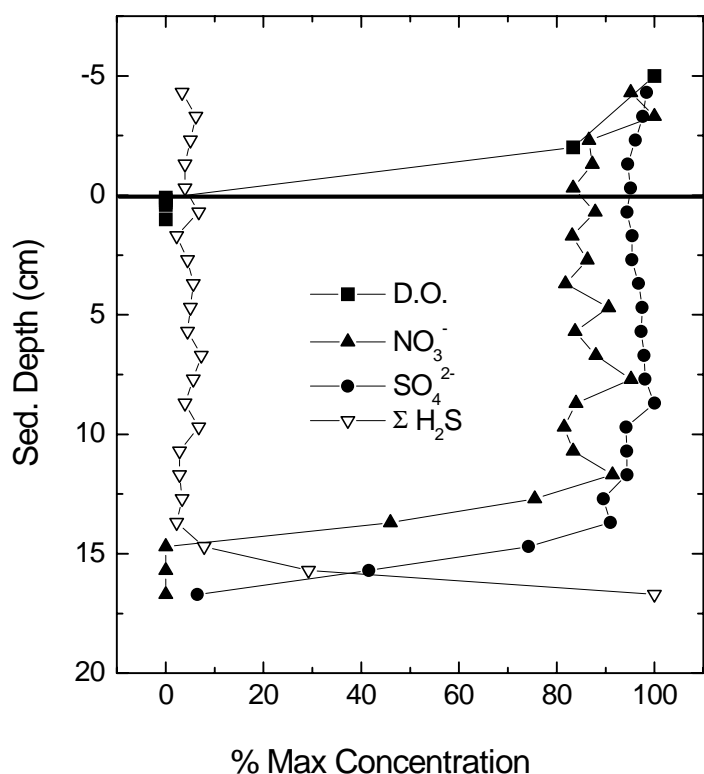
### 2.1 BIOGEOCHEMICAL PROCESSES IN FRESHWATER SEDIMENTS

Sediments are the ultimate repository of aquatic particles derived from either biogenic or detrital pathways. Once these particles are deposited in the sediments, extensive chemical, microbial, and physical transformations occur. These transformations are driven by the biological degradation of natural organic matter (NOM). The mineralization of NOM is the key driving process that affects changes in the chemical composition of the sediments and their associated porewaters during diagenesis (Berner, 1980). Diagenetic reactions are mediated in part by various bacterial communities (Fenchel and Blackburn, 1979), and usually follow a sequence of degradation reactions



**Figure 2.1:** Sequence of early diagenetic reactions occurring in surficial sediments. Based from Lovley, 1991.

as function of depth as shown in Figure 2.1. This sequence corresponds to the utilization of electron acceptors (*e.g.*,  $O_2$ ,  $NO_3^-$ ,  $Mn^{IV}$ ,  $Fe^{III}$ , and  $SO_4^{2-}$ ) according to a decrease in the yield of energy of the reactions (Froelich *et. al.*, 1979). These reactions and transformations lead to the release of primary metabolic products, such as  $CO_2$ ,  $CH_4$ ,  $NO_3^-$ ,  $NH_3$ ,  $H_2S$ , and  $PO_4^{3-}$ , into the porewaters (Gaillard, 1993). In addition, reduced metal species such as  $Mn^{2+}$  and  $Fe^{2+}$  are mobilized (Berner, 1980). Some of these reduced species diffuse toward the surface and react further with oxidizing species, such as  $O_2$ , or react with sulfides to form insoluble metal-sulfide precipitates.



**Figure 2.2:** Vertical porewater profile from Lake DePue sediment. Maximum concentrations of species are: D.O. = 120  $\mu M$ ;  $NO_3^-$  = 250  $\mu M$ ;  $SO_4^{2-}$  = 675  $\mu M$ ,  $\Sigma H_2S$  = 8  $\mu M$ . Horizontal line indicated the sediment-water interface. Note the rapid depletion of  $O_2$  in the sediment. The lag between oxygen consumption and other electron acceptors is not typical of most lake systems.

These major chemical changes, coupled with physical processes such as sediment mixing by benthic fauna and molecular and ionic diffusion, result in the formation of sediment layers having differing redox properties. Each layer reflects the differences in the chemical composition of the interstitial water and bulk sediments as a result of the succession of oxidants used. Figure 2.2 shows porewater profiles from anoxic Lake DePue sediments that exemplifies the results of these phenomena. As seen in the profile, the penetration depth of oxygen in the sediment is very shallow. This is due to the high loading of NOM into the sediment that overwhelms the flux of oxygen (Rabouille and Gaillard, 1991a). Since microbial respiration is typically very active in these upper sediments, the oxygen is typically entirely consumed in the first several millimeters of many freshwater sediments, leaving the bulk of the sediment system anoxic. Thus the study of anaerobic systems is important in understanding metal cycling dynamics in sediments.

Extensive modeling efforts in past literature have attempted to understand the roles of these processes in the geochemical cycle and to quantify the rates of release and diffusion of these species in sediment interstitial waters (Berner, 1964; Gaillard *et. al.*, 1986; Middelburg, 1989; Rabouille and Gaillard, 1991b; Gaillard, 1993; Van Cappellen and Gaillard, 1996; Boudreau, 1997; Hunter *et. al.*, 1998). These studies show the importance of microbial driven processes in the composition and chemistry of surficial sediments.

## 2.2 METAL CYCLING IN LAKE SEDIMENTS

Although metals are not degradable, the degradation processes mentioned in the previous section drive many of the processes that control their fate. In sedimentary systems, their fate is controlled by the rates of their burial, their potential to be remobilized, and their rates of transformation.

Burial and preservation of metals in sediments is determined by the formation of solid phases. The partitioning of metals to these solid phases means that the sediments act as an ultimate repository for metals and represent a concentrated reservoir of metals in freshwater environments (Forstner, 1990). Examples of important solids phases in the freshwater environment are reactive iron and manganese oxides, carbonates, sulfides, and phosphates (Stumm and Morgan, 1996). Most of these authigenic minerals are either directly or indirectly derived from microbial metabolism. For instance, the mineralization of NOM at all depths releases  $\text{CO}_2$ , which increases the carbonate species in the porewaters (Gaillard, 1993). Thus the degradation of organics may lead to the precipitation of carbonate minerals (Gaillard *et. al.*, 1987), as many metals form carbonates relatively easily (Martell and Smith, 1974-1989), even at neutral pH. In a similar manner, sulfide produced diagenetically at depth in the sediment may also lead to the sequestration of metals in the form of sulfide minerals. This has been shown to a major sink for trace metals in anoxic sediments (Gaillard *et. al.*, 1986), particularly in the form of iron sulfide scavengers (Elderfield *et. al.*, 1979). However, most of these types of studies have been performed in marine environments (Berner, 1967; Lord and Church,

1983; Berner, 1984), which have high concentrations of sulfate (28.2 mM) compared to the average freshwater system (typically < 1 mM) (Stumm and Morgan, 1996). Thus, the importance of sulfides in freshwater systems is generally considered to be weak, as compared to its dominance in marine systems. This may be particularly true in a highly contaminated system, where the dissolved metal concentrations exceed the total sulfide concentrations. In these cases, the metals act as the scavengers of sulfide, effectively eliminating sulfide from the porewater. Sulfide minerals and precipitates will still exist, but other solid phases will dominate the particulate metal speciation.

Oxides of manganese and iron have been reported to be efficient metal scavengers in the environment due to their high surface reactivities (Balistrieri and Murray, 1982; O'Day *et al.*, 1988; Davis and Kent, 1990; Manceau *et al.*, 1992; Charlet and Macaeau, 1993). Often these mineral phases are formed authigenically in sediments by the diffusion of reduced metal ions into the oxic layer of the sediments. At this interface, they become oxidized and can form a peak of particulate, metal oxides (Wehrli *et al.*, 1995). Trace metals present at these horizons can be effectively removed from solution by adsorption to the oxide surface or by co-entrainment into the oxide matrix. This has been shown to occur at a variety of oxic-anoxic interfaces, in both water columns (Lienemann *et al.*, 1997; Taillefert *et al.*, 2000) and sediments (Wehrli *et al.*, 1995). However, these oxides are also very important in the remobilization of trace metals as well. As discussed above, the hydrous oxides act as trace metal scavengers in the both the water column and sediments. These particles become buried in sediments and eventually reach the anoxic zone of the sediments where they are reduced by both sulfide-mediated

pathways (Buridge and Nealson, 1986) and direct microbial reduction (Ehrlich, 1986). Again, it is important to note that both of these processes are driven by microbial activity in the sediment. Once the oxide is reduced and dissolved, the metals incorporated into the oxide matrix or adsorbed to the surface will be released into the porewaters (Markwiese and Colberg, 2000). Thus, these oxides play an important role in the cycling of metals in the sediments and water column.

Organic particles that are present in aquatic systems (*i.e.*, humics, polysaccharides, microorganisms) have also been reported to mediate the cycling of both essential and toxic trace metals (Kavanaugh and Leckie, 1980; Anderson and Rubin, 1981; Baccini, 1984; Solomons and Förstner, 1984; Buffle, 1988). Several studies have studied the effect of humic acids and dissolved organic matter on the mobility and speciation of metals. Humics are an ill-defined matrix of organic compounds, consisting of a diversity of functional groups, such as carboxylic, hydroxyl, amino, and phenolic groups (Bryan *et al.*, 1997). Molecular weights range from a few hundred to 10,000 daltons (Rashid and King, 1969). Because of the complex and polydisperse nature of humic substances, characterizing their interactions with metals is a difficult task (Altmann and Buffle, 1988). Metal binding has been shown to alter the structure of organics by consolidating the molecule, thus reducing the humic-metal complex into colloidal particles (Davies *et al.*, 1997). This may be an important process in the removal of metals from solution into the particulate phase in high organic content systems. Additionally, several studies have shown that organic humic substances control the speciation of dissolved metals. This has been found to be true for trace concentrations of Pb in an uncontaminated,

meromictic lake (Taillefert and Gaillard, 1999; Taillefert *et. al.*, 2000) as well as for Pb, Cu, Ni, and Zn in polluted lakes (Nriagu and Gaillard, 1984). Humic compounds have also been found to act as electron shuttles in the reduction of metal oxides of iron and manganese. *Geobacter metallireducens* has been found to have the ability to directly donate electrons to organic compounds, such as humics, which are then shuttled to iron and manganese oxides (Lovley and Phillips, 1988; Lovley *et. al.*, 1996). This shuttling restores the humics to their oxidized form, thus acting as catalysts. This has an impact in the cycling of iron and manganese as well as any trace contaminant metals that are sorbed to the surface. Finally, experiments have shown that the mobilization of some metals directly correlates with the dissolved organic matter (DOM) content. Percolates from soils that had accumulated heavy metals showed that the concentrations of Cr, Hg, Cu, and As all correlated well with the amount of DOM (Kalbitz and Wennricj, 1998). Thus, the humics had the effect of complexing and solubilizing metals and increasing their mobility through the soil.

In addition to dissolved organic ligands in the system, studies have shown that particulate organic matter (POM) is also important in metal speciation. Particulate organics can complex metals directly, in a similar manner to DOM, as well as serve as nucleation site for hydrous ferric oxides (Taillefert *et. al.*, 2000). Much of the POM found in the natural system is also remnants of microbial activity, such as extracellular polymeric substances (EPS) and microbial polysaccharides. The next section discusses the importance of the interactions of microbial processes and byproducts with metals.

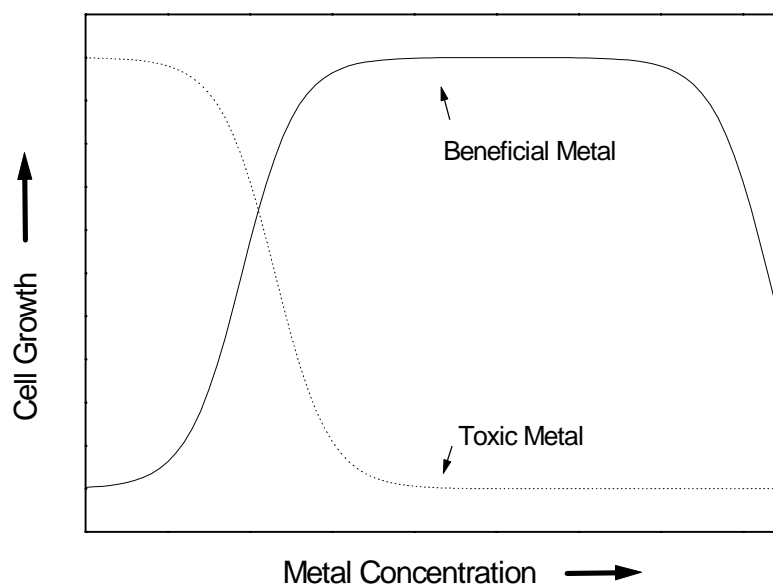
### 2.3 MICROBIAL INTERACTIONS WITH METALS

As it is established that microbes drive the processes that cycle and transform metals in sediments, it is important to examine closely the interactions between metals and microbes. These interactions play a particularly important role in understanding the fate of metals in the environment, yet little is known about metal-microbe interactions such as the microbial control of metal speciation. The literature suggests that microbial populations may play important roles in metal speciation in a variety of environments. Copper complexing ligands have been found to form in sediments (Skrabal *et. al.*, 1997) and certain bacteria produce extracellular copper binding ligands (Schreiber *et. al.*, 1990; Croot *et. al.*, 1999). It is thought that some of these ligands may actually control the speciation of copper in the ocean and point to an active biological cycling of copper (Gordon, 1998). In addition, bacteria can also cause the precipitation of metal minerals either inside the cell or at the cell membrane (Beveridge and Doyle, 1989). For example, deposits of micrometer sized spherical aggregates of zinc sulfide have been shown to be formed within bacterial biofilms in flooded mine tunnels (Labrenz *et. al.*, 2000). However, it is still widely unknown to what extent sediment microorganisms actually control the speciation of metals in sediments.

It is well documented that microbes interact with metals in a variety of ways (Mills, 1997). First, many metals are essential elements for life, as they serve critical functions in many proteins and biochemical reactions, *e.g.*, Cu, Zn, Fe, Ni, Mn, and Co (Theil and Raymond, 1994). For example, zinc is crucial for the structural integrity of “zinc finger”



proteins responsible for DNA transcription (Mills, 1989). Manganese is responsible for the redox chemistry at the active site of photosynthetic reaction centers as well as in some superoxide dismutases in bacteria (Madigan *et. al.*, 1997). Copper and iron also participate in a similar manner in a host of biological functions, such as the binding of dioxygen in hemoglobin, electron transfer in cytochromes, and redox centers in superoxide dismutases (Theil and Raymond, 1994). Cobalt is found in vitamin B<sub>12</sub> (Sennett *et. al.*, 1981) and nickel is a key metal in various hydrogenases and S-methyl CoM reductase, which is the catalyst of methane production in all methanogenic bacteria (Theil and Raymond, 1994). These transition metal nutrients, normally available at nanomolar concentrations in the environment, may also be toxic at elevated concentrations (Lippard and J.M., 1994). Second, other transition metals, which serve no



**Figure 2.3:** Representation of the concentration dependence of beneficial/nutrient metals and toxic metals on cell growth. Note that at high concentrations, even beneficial metals become toxic. Adapted from Lippard and Berg, 1994.

known biological function (e.g., Hg, and Pb), are toxic, in particular when they bioaccumulate. The effects of metal ions on cell growth are illustrated in Figure 2.3, which shows a representation of the concentration dependence of beneficial nutrient metals and toxic metals.

Third, some metals serve a role either as electron donors or electron acceptors (e.g.  $\text{Fe}^{\text{II}}$  or  $\text{Fe}^{\text{III}}$  respectively) in energy generating reactions. Dissimilatory metal reduction has been recognized as a significant process (Myers and Nealson, 1988; Lovley, 1991; Nealson and Myers, 1992; Davison, 1993; Nealson and Saffarini, 1994) in sediments and may play an important indirect role in heavy metal speciation. For instance, the reduction of iron or manganese oxides may serve to remobilize co-precipitated or sorbed metals (Francis and Dodge, 1990). Finally, many metal mineral phases, such as carbonates, oxides, phosphates, and sulfides, can nucleate at the surface of bacterial cell walls (Doyle, 1989; Ferris, 1989; Ferris *et. al.*, 1989).

As a consequence of the integral relationship with metals, microbes have developed a variety of both uptake and resistance mechanisms. Some of these mechanisms may alter metal speciation or prevent metals from reaching toxic concentrations inside the cell. Basic resistance mechanisms include valence state changes (serving to immobilize or volatilize the metal), ion selective expulsion, bioaccumulation, and mineral precipitation.

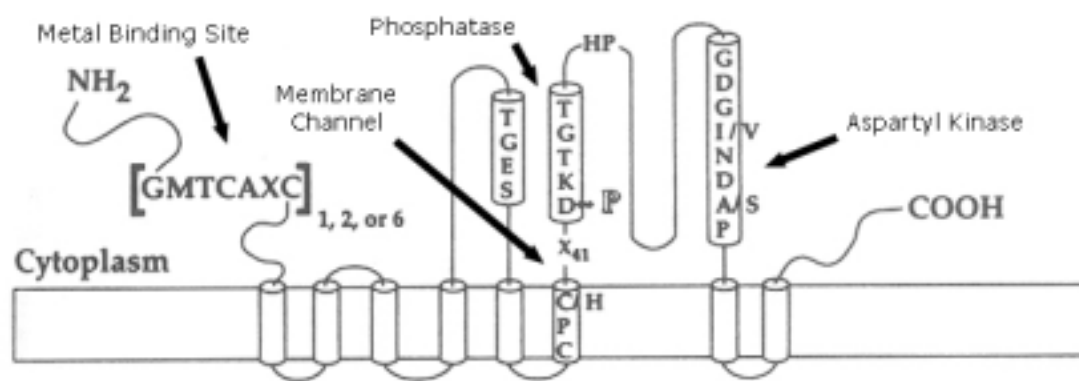
Bioaccumulation includes the binding of metal ions to proteins, peptides, polysaccharides, or other ligands inside the cell. Strong heavy metal binding to the cell

walls, common for many microorganisms (Matthews *et. al.*, 1979; Doyle, 1989) can also be included in this category. Many species of bacteria produce polysaccharides external to the cell. This layer of organic material can provide a sheath of protection from metal ions by binding them and preventing the metals from ever reaching the internals of the cell. Exopolysaccharides produced by an *Acinetobacter* species were found to be capable of protecting against metal ions such as  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  (Pirog, 1997).

Proteins within the cells may also provide a mechanism for isolating metal ions and conferring a degree of resistance. Metallothioneins are a particularly well-characterized family of metal binding proteins. The metallothioneins are simple, cysteine rich, small molecule proteins that are found in many organisms, from single cell eukaryotes to plants and animals, and even in some cyanobacteria (Kagi and Kojima, 1987). These proteins have a high affinity for metal ions in Groups 11 and 12 (most commonly  $\text{Cd}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{Hg}^{2+}$ , and  $\text{Zn}^{2+}$ ) and complex 7 to 18 metal ions forming one or two metal-thiolate clusters (Stillman, 1995). Given their unusual structure, lability, and induction by a variety of agents, metallothioneins are thought to play an important role in metal homeostasis, detoxification, and transfer (Kagi and Kojima, 1987; Kagi, 1991).

Ion-selective transport systems participate in both the uptake of essential ions and the efflux of toxic metals (Silver *et. al.*, 1993). Many resistances to toxic metals are encoded on plasmids (Ji and Silver, 1995), including resistances to  $\text{Ag}^+$ ,  $\text{AsO}_4^{3-}$ ,  $\text{Cd}^{2+}$ ,  $\text{CrO}_4^{2-}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Sb}^{3+}$ , and  $\text{Zn}^{2+}$ . Although the most commonly encountered type of plasmid encoded resistance function is the efflux pump it is reasonable to assume that other

plasmid encoded resistance mechanisms exist, since the resistance mechanisms displayed in pure cultures may not necessarily reflect those occurring naturally in the environment. The efflux pump relies on an energy dependent P-type ATPase, which exports metals that have entered the cell via transport systems, used for the uptake of required trace metals. For example, the CadA (see Figure 2.4) system widely found in gram-positive bacteria is attributed to conferring resistance to both  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  (Silver and Phung, 1996). The ATPase contains a metal binding domain consisting of a pair of adjoining cysteine residues and several membrane spanning regions thought to be

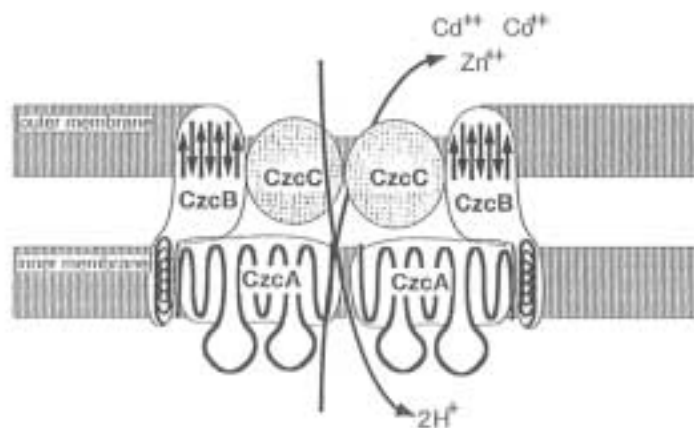


**Figure 2.4:** Heavy metal cation ( $\text{Cd}^{2+}$ ) P-type ATPase. The predicted motifs as shown on the figure show the cation-binding, phosphatase, membrane channel, and aspartyl kinase regions. From Silver, 1987.

involved in the translocation of the metal. The two other intracellular domains are involved in the phosphorylation process (Silver, 1997).

Thus in a highly contaminated system, a large amount of energy (an ATP per divalent metal cation (Silver *et. al.*, 1989)) may end up being expended to eliminate toxic metals.

The high use of energy in order to confer resistance may be particularly limiting in anaerobic systems, where the energy yield from substrate utilization is substantially lower than in aerobic systems. In gram-negative bacteria, another efflux pump is commonly found for  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Co}^{2+}$  called Czc (Figure 2.5). This efflux pump functions as a chemiosmotic divalent cation/proton antiporter. The system consists of three proteins, two of which act as channels through the membranes, and the second that is a membrane fusion protein (Silver *et. al.*, 1989).



**Figure 2.5:** Czc model for the cadmium, zinc, and cobalt efflux system. The proton/cation antiporter system consists of the inner membrane (CzcA), outer membrane (CzcC), and "membrane fusion" (CzcB) proteins functioning as a dimer. From Silver and Phung, 1996.

The most important consequence of these types of resistance mechanisms is that they essentially leave the speciation and concentration of the toxic metal unchanged.

Bacteria can be involved in mineral precipitation reactions either directly as catalysts of aqueous chemical reactions (Thompson and Ferris, 1990; Fortin and Beveridge, 1997;

Thompson *et. al.*, 1997), or indirectly as geochemically reactive solids (Mullen *et. al.*, 1989; Fein *et. al.*, 1997). In the first case, metabolic activity of the organism is important in developing supersaturated conditions that allow precipitation to occur (*e.g.* through the production of reactive ligands such as sulfide or carbonate). The local concentrations of these end products of metabolic processes alone can be enough to lower the energy barrier for both homogeneous and heterogeneous nucleation reactions to occur. In the latter case, adsorption of metal ions to reactive sites on bacterial cell surfaces encourages heterogeneous nucleation and precipitation. Mineral precipitates formed from either of these pathways can form on the inside, outside, or even at some distance from the cell. As these processes occur simultaneously in the natural environment, it is very difficult to differentiate them (Fortin *et. al.*, 1997).

A particularly well-studied microbial mechanism to alter metal speciation is the synthesis of low molecular weight ligands. One example of these types of ligands is the liberation of siderophores, which are induced and excreted in iron limiting conditions (Neilands, 1981; Morel *et. al.*, 1991). These non-proteinaceous, soluble, extracellular ligands bind ferric iron with high affinity (stability constant  $\sim 10^{30}$ ) and transport it back to the cell through binding to iron-siderophore selective receptors. However, the iron-selective siderophores also form stable complexes with various other metals, including  $\text{Ga}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Mn}^{2+}$  (stability constants between  $10^{17}$  and  $10^{28}$ ) (Martell and Smith, 1974-1989). As the uptake receptors may not discriminate between the different metal-ligand complexes, these ligands may promote the transport of toxic metals into those cells (Hu and Boyer, 1996). Thus, under some conditions, siderophores may

protect non-synthesizing populations from toxicity, while promoting toxicity to those populations possessing the appropriate uptake mechanisms. The siderophore excretion may also confer resistance organisms by binding the free forms of the metal ion. As an example, a cyanobacteria, *Anabaena sp.*, was protected from copper toxicity when grown under conditions promoting the synthesis of its iron binding siderophore (schizokinen). Protection was conferred apparently because the *Anabaena* transport system does not recognize the cupric complex (Clarke *et. al.*, 1987).

To further illustrate some of these points, a recent study by (Mirimanoff and Wilkinson, 2000) showed the regulation of Zn accumulation in a gram-positive bacterium. The study showed that the uptake by the microorganism was not predicted by the activity of the free  $\text{Zn}^{2+}$  ion in the bulk solution. Uptake kinetics also showed that there was rapid initial adsorption by the cells followed by a slow release of Zn to 25% of its highest value, showing that there was not steady state adsorption or short term linear uptake. They also saw evidence for a low molecular weight (<3 kDa) Zn binding ligand secreted by the bacterium. Additionally, this ligand was produced rapidly (all Zn complexed within 5 minutes) even by cells not exposed to high Zn concentrations previously. Qualitatively, they explained the results by using a combination of mechanisms. At low concentrations of  $\text{Zn}^{2+}$ , resistance mechanisms are shut off or slowed and internalization rates increases in order to allow the necessary nutrient needs. At higher  $\text{Zn}^{2+}$  concentrations, efflux mechanisms and extracellular sequestration by ligands become more important as the surface uptake sites become saturated. In this manner, cellular uptake is regulated by a balance between the normal cellular metabolism and the

resistance mechanisms employed by the organism. These observations underscore the complexity of microbial interactions with metals and the difficulty in predicting the response of the environmental system to metal stress, in particular in complex microbial environments.

The interaction between metals and microbes is not limited to just microbes affecting the metal speciation in the system. Additionally, the presence of metals in the ecosystem can have an effect on the microbes that exist in the sediments. Due to the toxic nature of some transition elements at elevated concentrations, the microbial diversity, population, and activity can be altered. For this purpose, the speciation of the metal that the bacteria are exposed to is also crucial. Studies have shown that the sulfate reducing activity of *Desulfovibrio desulfuricans* is directly related to the calculated free ion concentrations of Ni and Zn in solution (Poulson *et. al.*, 1997). Additionally, the activity of *Streptococcus mutans* was noted to depend on the speciation of Zn in the media and correlated with the amount of Zn that adsorbed to the cell walls. When complexing agents, such as citrate or EDTA, were added, the inhibitory effects of the zinc were reduced or nearly eliminated. Speciation calculations showed a good correlation of the free  $\text{Zn}^{2+}$  ion to the amount of inhibition (Watson *et. al.*, 1991). Other previous laboratory studies have shown that metal ions, such as copper, have an inhibitory effect on marine bacteria (Sunda and Gillespie, 1979; Zavenhuizen *et. al.*, 1979) and other microorganisms (Chacin and Forester, 1995; Geider, 1999). These studies showed that the adverse reactions are also directly correlated to the free ion concentration of the metal.



Calculations of free ion activity are relatively easy to perform in well-defined laboratory medias, but can present a challenge for those working in more complicated systems, such as the natural environment. Most studies in these types of systems have examined toxicity effects using total metal concentrations. Fabiano, *et. al.* 1994, showed a negative correlation between total cadmium concentrations and total bacterial biomass in marine sediments. The sediments examined were located at the outfall of a mildly contaminated river. The sediments closest to the mouth of the river showed the greatest metal concentrations, and hence the greatest adverse effect on microbe biomass. The cadmium also appeared to have a strong effect on the frequency of dividing cells (Fabiano *et. al.*, 1994). Examination of microbial changes in metal-rich sludge amended soils has also been performed. In this study, the phospholipid fatty acid (PLFA) patterns of the soils were used as a fingerprinting technique to profile the microbial community composition. The results showed that communities that had been exposed to metal contaminated sludges for long periods of time had developed a significant resistance to those metals as compared to the unpolluted control sludges. PLFA patterns changed in all of the metal added treatments, showing a response in the community composition with the addition of metals (Baath *et. al.*, 1998).

The examples in this section show the importance that microbiological processes have on metal speciation as well as the effects that the metal speciation has on the bacterial population. This further stresses the need for *in situ* measurements of metal speciation in the environment. In essence, all of these changes in metal speciation, from those indirectly induced by precipitation and mineral incorporation, to direct microbial

intervention, are driven by microbial activity. Furthermore, this activity is all driven by the energetics of bacterial metabolism of NOM. Thus, metal-microbe interactions are of utmost importance in understanding the fate of metals in the natural environment.

## 2.4 ANALYTICAL CONSIDERATIONS

A major problem that arises in addressing these complicated issues surrounding metal-microbe dynamics, is how does one approach the subject from an analytical viewpoint? In other words, given that these processes are driving the speciation and chemistry of the metals in the sediments, what techniques can one use to examine these chemical processes? Many traditional tools that are used in environmental settings principally examine the bulk chemical properties of the sample. When addressing metal-microbe interactions, many processes occur on the microscale and many chemical and physical microenvironments may exist. By solely examining the macroscale properties, many of the properties of the system may be overlooked. Additionally, the traditional sets of methods used are often based on “wet chemical procedures”. When these procedures are carried out, the original sample is often altered. This leads to the problem that the results from the experiment might not represent the sample as it is present in the natural environment.

To avoid some of these problems, many researchers in the past have studied simplified versions of environmental systems in the laboratory. These are exemplified by studies

such as the adsorption of contaminants on oxide particles (Ainsworth *et. al.*, 1994; Ford *et. al.*, 1997; Lo and Chen, 1997; Karthikeyan *et. al.*, 1999; Khaodhair *et. al.*, 2000) and organic matter interactions (Kinniburgh *et. al.*, 1996; Zhang *et. al.*, 1996; Pinhero *et. al.*, 2000; Ravat *et. al.*, 2000). In these studies, the complexity has been simplified by examining the phases and constituents that are thought to be the major chemical players in the environmental system. While these studies are useful in the understanding of simple systems, the synergies, competitions, and complex interactions of the environment are lost. In particular, the complexity has been shown by the observation that cadmium (Vermeer *et. al.*, 1999) and uranium (Lenhart and Honeyman, 1999) absorption onto hematite and humic acids behaves much differently in a mixed oxide-humic acid system.

In light of these considerations, new tools need to be developed for probing the chemical nature of these complicated systems. These tools are required to operate on the microscale and molecular levels and have the inherent nature of being nondestructive to the sample. The research herein develops a set of protocols that combines electron microscopy and X-ray absorption spectroscopy in combination and comparison with some traditional wet chemical methods to examine metal speciation. These tools and protocols are tested successfully both in isolated, simplified systems, as well as *in situ* in a complex, contaminated system.

## 2.5 HYPOTHESES

Lake DePue is a zinc-contaminated environment, which provides us with the possibility of studying the interactions between metals and microbes as they occur in a perturbed system. As the sediments are the ultimate repositories of environmental particles and microbial processes control the chemical changes taking place during early diagenesis under anaerobic conditions, the ultimate goal of the project is to test the following central hypothesis:

- Microbial populations in contaminated lake sediments will be responsible for changes in the chemical speciation of metals as a result of the response to metal exposure and stress.

The research contained within this dissertation is based on developing and testing the analytical foundations required to approach this underlying hypothesis. Specifically, the hypotheses addressed by this study are:

- Since the processes that determine metal speciation occur on the microscopic scale, microscale techniques used in tandem, such as analytical electron microscopy and X-ray absorption spectroscopy, will provide a richer detail of speciation than previously achieved.
- Analytical speciation that is derived from the spectral deconvolution of X-ray absorption spectroscopy is superior to that obtained through traditional wet chemical methods. This is particularly true in highly contaminated sedimentary environments.
- Speciation of metals in the sediments of highly contaminated freshwater lakes will be dominated by species other than sulfides (e.g., carbonates) since the relative sulfide concentration is low and the metal concentration is high. This does not mean that metal sulfides will necessarily be absent, but that in the most contaminated regions, they will be present as a smaller proportion of the speciation.

- Microbes isolated from a contaminated system will have a better ability to reduce metal stresses that are imposed upon them. These abilities will likely arise from changes in the chemical speciation of the metal in the media. X-ray absorption spectroscopy will be an ideal tool to examine the coordination of metals on the surface of and interior to the microorganisms.

## 2.6 OBJECTIVES

The primary goal of this research is to develop a set of new protocols that will enable users to examine the speciation of metals on the microscale and in a manner that does not alter the sample during sample preparation or observation. These methods center on the use of analytical electron microscopy (AEM) and X-ray absorption spectroscopy (XAS). Particular emphasis is placed on the development of XAS with the application to examining samples from a complex, natural environment, as this use of XAS is still relatively new in the environmental field. Once these tools are developed and tested, they may be applied to a host of different systems, providing greater insights than the traditional chemical methods used previously.

The largest contribution from this body of work lies in the development of the protocols for XAS data collection and analysis. Specifically, continuous scan XAS (CS-XAS) has been used in order to quantify the experimental error from the data collection and greatly reduce the time involved to collect a spectrum. Through a set of programs developed within this research, the error measured during data collection is propagated through each step of the data reduction. This is then used to perform a spectral decomposition of the sample based upon a database of standard spectra. Again, the errors of the sample and standards are taken into account in this process as well. Thus, with the appropriate basis set, one can determine the phases present in a complex environmental mixture and determine the relative error of each phase based on the

experimental data collection and data reduction processes. The consideration of experimental error in XAS experiments has often been discussed (Krappe and Rossner, 1999; Newville *et. al.*, 1999), but has rarely been implemented in environmental studies. Thus, this work shows how CS-XAS is as an important tool for the determination of metal speciation and develops novel methods for data analysis.

The protocols for sample preparation of environmental particles and colloids for use in AEM are fairly well established (Leppard *et. al.*, 1988; Perret *et. al.*, 1991; He *et. al.*, 1996; Leppard *et. al.*, 1996). These methods use hydrophylic resins to encapsulate the particles to prevent significant alteration of sample morphology during dehydration. This work contributes to the AEM methodologies present by using AEM to evaluate the elemental associations of individual particles. This evaluation has been done to group particles of similar composition using chemometric procedures such as principal component analysis and factor analysis. In addition, the elemental properties of each morphology class can be determined. These observations can lead to important deductions, as the associations of metals change in the various morphology classes in different sedimentary environments.

It is important to realize that no one method should be entirely relied on to reach final conclusions on the behavior of metals in an environmental setting. The weaknesses and biases of one method should always be compensated by exploring the avenues provided by a complementary method. One example of this is the joint utilization of AEM and XAS. AEM provides the power to examine individual particles, but can only provide

information on the morphology and the elemental composition of the particle. One cannot determine what redox state the element is in or in what type of compound the element is a part of. XAS can provide this type of detail, but is at this time still a bulk analysis method, *i.e.*, it gives the average coordination properties of the entire illuminated sample rather than a single particle. Thus these two techniques are well suited to each other, as they give complementary data. By employing this multi-method approach to metal speciation, focusing on the microscopic scale with AEM and coordination environment with XAS, a comprehensive picture of metal speciation can be developed.