10.0 CONCLUSIONS

This work has stressed the importance of determining metal speciation and its use in investigating the interactions between metals and microorganisms. Two instrumental methods have been used to examine these phenomena in an *in situ* manner: analytical electron microscopy (AEM) and X-ray absorption spectroscopy (XAS). A key development in this work has been the application of XAS to observe environmentally relevant samples. Novel methods of data collection, processing, and analysis have been developed, calibrated, and utilized to further the understanding of metal dynamics in the sedimentary systems. Of particular importance is the method of spectral deconvolution utilized to probe environmental samples with a combination of different metal species. This method also utilizes the experimental errors from the data collection and carries these errors through each step of the data reduction to calculate the final error of the deconvolution. Performing traditional sequential extractions also validated these methods. The results showed that the speciation results from both methods, the sequential extractions and XAS deconvolution, closely matched each other. Further tests of the XAS results must be performed, planned in the near future, in order to further validate the XAS technique.

The microscopic and spectroscopic methods were used in parallel to characterize the zinc speciation in the sediments of Lake DePue, a small, backwater lake of the Illinois River. The processes are difficult to interpret since the levels of the lake change dramatically throughout the year, but many important trends and observations can be discerned that hint at the processes occurring. X-ray diffraction data of sediments with large zinc concentrations showed the presence of mostly quartz with calcite and dolomite peaks. This suggests that the zinc present in these samples is mostly amorphous, as any crystalline phases should be noticed at these zinc levels. Results by AEM showed that zinc was associated with several different morphology types in the most contaminated areas. However, a common theme among the different morphologies was that many of the zinc-rich particles appeared to be templated by biological surfaces.

Zinc is most predominant in the sediments at the location where a drainage creek enters the Lake DePue (site C_1). At this site, the zinc is relatively labile, present primarily as carbonates and weakly adsorbed zinc. The presence of a significant fraction of weakly sorbed zinc is not surprising, due to the large concentrations of aqueous zinc in the water. AEM visualizations of the particles present in the sediments showed that zinc was present at some degree on nearly all the particles that were observed. Carbonate zinc is present likely due to the mineralization of organic matter creating increases in the CO_2 concentration. There is also likely to be a fair amount of direct carbonate precipitation from the water column at this location. Given the alkalinity, pH, and zinc concentration in the creek, zinc carbonate is supersaturated by a factor of 10 to 100. Major phases of zinc at this location also include zinc-iron phosphate globules observed directly through AEM visualizations. These globules are intimately associated with various biological features, such as algal cells. Several of the globules were also observed to be encapsulated in a membrane-like structure. Data suggest that the globules are formed along a gradient of zinc concentration, probably along the bed of the creek. This is due to the fact that among a sampling of the particles, there is a distinct anti-correlation between the major cations present, iron and zinc. However, close examination of the particles show that their ratios within a single particle remain constant. This suggests that the different particles were formed in various locations with varying zinc concentrations before sedimentation. The constant concentration profiles rejects the hypothesis that the zinc particles were formed through cation substitution processes, where the zinc would predominate at the rims of the particles. Zinc sulfide is also present in the sediments of the creek, and increases with depth. It is likely that there is sulfide production through microbial processes at most depths in the sediment and that the HS^- produced is quickly scavenged by the large concentration of metals.

The speciation of zinc was also examined at the outlet of the creek into the lake. Due to the lower concentrations of metals, zinc is not as ubiquitous on the sediment particles as it was in the sediments of the creek. The site is deltaic, and shows cycles of zinc speciation patterns that match the sedimentation cycles in Lake DePue. These patterns are evidenced by the changes in the zinc-phosphate species in the sediment that vary from nearly absent to around 15% of the zinc with a period of approximately 5 cm. The phosphate fraction results from transport of the zinc-phosphate globules from the creek to the lake. These globules were observed directly by AEM in both the water column and sediments at the creek outlet sampling site. Carbonate species of zinc were also prevalent, present as nearly half of the zinc in the sediment throughout the sediment core.

The last site to be examined was a site that was located in a relatively uncontaminated section of Lake DePue. Concentrations of zinc in the sediment were much lower, with most of the sediment core at background levels of zinc. The upper 1 cm of the core had slightly elevated levels of zinc as a result of fresh sedimentation. Since the concentrations of zinc were low, spectral deconvolution was more difficult. However, speciation calculations show that the bulk of the zinc present was in the form of zinc sulfides. This is supported by the AEM observations, which showed that the only individual particles showing detectable zinc were poorly crystalline zinc sulfide particles. The poor crystallinity suggests that the particles were formed as authigenic precipitates rather than alteration of transported particles. The lack of zinc-phosphate globules in this sediment also shows that these particles generally are not transported this far into the lake. This is also supported by the plume transport study, which showed that the zinc concentration in the water column drops off drastically away from the convergence of the creek into Lake DePue.

The CS-XAS technique developed within this work was also instrumental in examining the speciation of zinc and cadmium in microorganisms isolated from Lake DePue. Examination of zinc binding to bacterial surfaces showed that the amount of zinc bound and the coordination groups responsible for binding are highly dependent on pH. These results also showed that a wide variety of coordination environments exist among the bacteria isolated. The amount of zinc in the microbes indicated that processes other than surface absorption were present, as the signal from these microbes was much larger than that of absorption alone. Zinc coordination in the cells varied from entirely sulfur bound to entirely oxygen bound with some organisms having mixed coordinations of both sulfur and oxygen. Examination of the XANES region of the spectrum showed that the zinc coordination was asymmetrical and not an inorganic form of zinc sulfide. Additionally, the second coordination shell of the oxygen-coordinated microbes suggested that the zinc was bound to phosphate groups. Cadmium exposed organisms showed a much smaller diversity of coordination environments. All of the organisms examined appeared to bind cadmium in a bidentate, binuclear, phosphate complex that is likely to be surface binding to cell wall surfaces. This may be a result of the lower tolerance of the organisms to cadmium, as the metal binds mainly to the cell wall, as opposed to being internalized. This observation suggests that there is no specific binding of cadmium in these isolates. Thus, the internalization and sequestration of zinc into a bound and presumably non-toxic form may confer a higher resistance to zinc than cadmium, which may only use a cadmium ion export system for resistance.

The study of the Lake DePue system proved the utility of joint CS-XAS and AEM examinations. The benefits of this combination of techniques were shown in the investigation of the speciation of this contaminated, aquatic system. Observations on the individual particle scale as well as the molecular scale showed the trends in speciation and elucidated transport effects as zinc enters the lake. These studies also showed the varied coordination environments of microorganisms isolated from the lake sediments.

Several interferences still exist for the CS-XAS data collection in its current experimental set-up. These include the presence of high background fluorescence from the sample matrix and concentration limitations. Upgrading the detector system to a solid-state detector and using filters to isolate the X-rays of interest can address some of these problems. These ideas will be implemented during future validations of the CS-XAS technique.

The CS-XAS methods developed rely on obtaining the experimental spectra of appropriate standards for the environmental sample of interest. This presents a major limitation for the widespread implementation of the technique over varied natural systems. Careful forethought must be applied to obtain a complete basis set of probable standards. This is an example where AEM as well as careful geochemical intuition can provide critical assistance. Again, it is important to stress that no one method should be relied on entirely when making assessments of chemical systems. It is always critical to collect data using a variety of techniques and checking to see that all of these methods point to a common conclusion.

Keeping these issues in mind, the CS-XAS method has many advantages for its use in environmental settings. The foremost being that it is a direct probe of the coordination environment rather than a combination of operationally defined phases. It also provides a degree of *in situ* measurement, so long as the sediments are isolated in a way that minimizes chemical reaction. Finally, the application of the CS allows the errors in the measurements to be quantified at every step of the data reduction. These properties make CS-XAS an important tool for studying metal speciation for the environmental chemist.