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Using zone plates for X-ray microimaging and microspectroscopy in environmental science

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Understanding the transport and ultimate fate of environmental contaminants is of fundamental importance for developing effective remediation strategies and determining the risk associated with the contaminants. Focusing X-rays by using recently developed zone plates allows determination of the spatial distribution and chemical speciation of contaminants at the micron and submicron length scales. This ability is essential for studying the microscopic physical, geological, chemical, and biological interfaces that play a crucial role in determining contaminant fate and mobility. The following is an overview of some current problems in environmental science that are being addressed with synchrotron-based X-ray microimaging and microspectroscopy.

Keywords: zone plates, environmental science, X-ray microbeams, X-ray imaging, biogeochemistry

Introduction

Chemical contamination of soil and groundwater is a universal problem of immense complexity and great global concern. Sources of contamination include past and present agricultural and industrial activities, operations at national defense sites, and mining and manufacturing processes. Chemical contaminants include heavy metals (such as Pb, Cr, As, Zn, Cu, Cd, Ba, Ni, and Hg), radionuclides (such as U, Pu, Sr, Cs, Co, and Tc), and potentially hazardous anions such as selenate. An understanding of contaminant mobility is essential for the development, application, evaluation, and selection of remediation and sequestration technologies. This understanding requires molecular-level information about the chemical speciation of the contaminant.

A current focal point of molecular environmental science involves the pathways, products, and kinetics of chemical reactions of contaminant species with inorganic and organic compounds, plants, and organisms in the environment. These reactions often occur at aqueous solution-solid interfaces and can have many different results. The contaminant can be precipitated from the solution on to the solid interface, transformed into a different species, incorporated into a solid phase, or released from the solid surface into the solution. Such interfacial reactions play a very important role in the transport and dispersal of toxic species in soils and natural waters. Therefore, discovering what is occurring at these interfacial surfaces is key in understanding the bioavailability of many contaminants. Despite this importance, these surfaces and the associated chemical reactions are not well understood. Consequently, little is known about the mechanisms by which plants, fungi, and microorganisms determine the speciation, forms, reaction rates, and distribution of contaminants in soils and groundwater.

Interfaces of environmental importance may involve microbes, plants and their roots, fungi, groundwater, and soil constituents such as minerals and organic debris; such heterogeneity makes the study of interfaces very difficult. Because environmental samples are almost always hydrated, the use of high-energy X-rays to penetrate the water in a sample is very useful. It also is valuable to probe both sides of the interface to elucidate transformations that result in the movement of the contaminant across the interface. Thus, it is imperative to use the smallest possible probe so that the homogeneous regions on either side of the interface can be analyzed selectively. These requirements make the use of micron and submicron X-ray beams advantageous.

X-ray fluorescence (XRF) microscopy offers significant advantages over other techniques for determining the spatial distribution of trace elements in environmental samples. For example, fluorescence signal-to-background ratios are 10 to 10^5 times larger for excitation by X-rays than for excitation by charged particles. Thus, although charged-particle microprobes can provide significantly better spatial resolution than X-rays, the elemental sensitivity of the former (10-100 ppm) is much worse than that achieved by X-ray microprobes (Forslind, Malmquist, and Pallan, 1991). Another advantage of an X-ray microprobe is that, for a given sensitivity, the radiation dose delivered to a sample is typically 10^{-3} to 10^{-5} times less than the dose from a charged-particle microbeam (Sparks, 1980). In addition, X-ray absorption microspectroscopy makes it far easier to obtain chemical state information about a particular substance.

Third-generation X-ray sources like the Advanced Photon Source (APS), where our experiments were performed, provide an increase in brilliance of approximately three orders of magnitude compared to second-generation synchrotron X-ray sources. In addition, advances in microfabrication technologies have produced X-ray phase zone plates (Lai *et al.*, 1992) with spatial resolution better than 0.2 μm and focusing efficiency better than 33%. The combination of the increased brilliance of X-ray beams from the APS and improved zone plate fabrication technology provides unique capabilities in X-ray microscopy and spectromicroscopy.

The role of mycorrhizal fungi in contaminant transport

Photosynthetic organisms are one of the principal entry points for heavy metals and radionuclides into the food chain (Rauser, 1990). The way plants respond to high concentrations of heavy metals and radionuclides has a significant effect on the transport dynamics of these contaminants through the topsoil and the root zone and plays a large role in determining the ultimate ecological effects of the contamination.

Evidence suggests that a fundamental role for one type of mycorrhizal fungi, arbuscular mycorrhizal fungi, is that their hyphae bridge the annular space within soil, producing a physical connection between the root surface and surrounding soil particles (Marschner, 1995; Miller, 1987). In creating such bridges, the hyphae increase the effective surface area of the root and decrease resistance to water flow to the root surface by allowing closer contact with the soil particles.

We have used hard X-ray phase zone plates to investigate the fungus-root symbiotic relationship, specifically studying the spatial distribution of many of the 3*d* transition metals in a fungal-infected plant root. The zone plate used in these microscopy experiments produced a focused beam of cross section 1 μm × 3 μm, with 4×10^{10} photons/s/0.02% bandwidth. The zone plate had an effective focal length of 52.5 cm at 10.5 keV and an effective spot size of 1 μm (vertical) × 3 μm (horizontal). The samples were mounted on a computer-controlled XYZ stage at 20 or 45 degrees to the incident beam, producing a footprint up to 4.2 μm on the sample in the sample's horizontal dimension. The XRF radiation intensities were monitored by using an energy-dispersive, single-element, solid-state detector.

Figure 1 shows the spatial distribution of Mn in a wet root-fungus sample. Maps of Mn, as well as Fe, Zn, S, P, Ni, K, Cu, and Ca, were obtained by scanning the sample in 5-μm steps through the focused monochromatic X-ray beam (10.5 keV), then integrating the selected Kα fluorescence for 3 seconds per point. The total data collection time was approximately 4 hours, and the elemental sensitivity was approximately 500 μg/L. Comparison of Figure 1 with optical micrographs indicated that the Mn tends to be most concentrated in areas typically exhibiting the highest concentrations of fungal mass, suggesting that the fungus plays a role in metal transport to the plant.

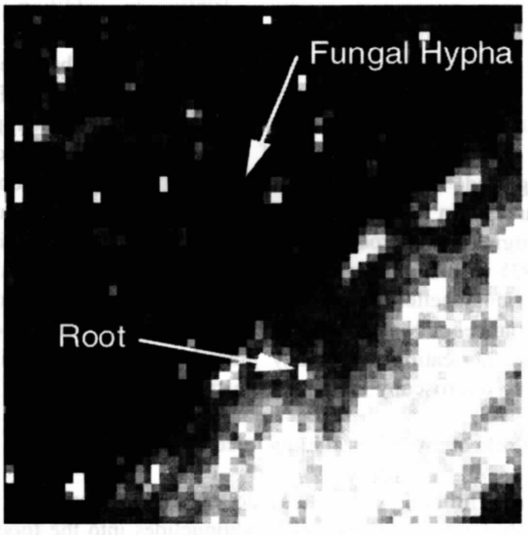

Figure 1.
Spatial distribution of Mn for a hydrated *Plantago* plant root infected by the mycorrhizal fungus *Glomus mosseae*. Elemental distribution was determined from the characteristic Kα fluorescence intensity produced by a focused 10.5-keV X-ray beam.

Correlation of XRF intensity with elemental concentration within the root-fungus system has not been performed to provide quantitative information on elemental concentrations. Doing this accurately will require well-calibrated standards with similar elemental concentrations that are embedded in matrices with densities similar to that of the plant-fungus system. Such well-characterized standards are difficult to create because local densities (contained within cubic micron volumes) of the root-fungus system can vary and are not always well known. One approximation that can be made is that the average density is constant throughout the hydrated root-fungus system. This approximation may introduce errors of up to a factor of two in the final determination of elemental concentrations. In addition, because the depictions of elemental concentrations shown here are two-dimensional projections of three dimensional objects, the intensities of the fluorescence radiation, and thus the elemental concentrations, are not normalized to the thickness of the object. These problems are often less significant with charged-particle probes; however, their shorter mean free path length, compared to that of X-rays, reduces the effectiveness of charged-particle probes for investigating realistic hydrated environmental samples.

Figure 2 shows a representative step-height normalized Mn K-edge XANES spectrum obtained in the hyphae, stele, and cortex regions of the sample as well as a $MnCO_3$ standard representing Mn2+. Comparison of these data indicates that in all cases the Mn is in the more soluble, bioavailable +2 valence state.

The role of bacteria in contaminant transport

Although microorganisms are known to participate in a wide range of metal oxidation-reduction reactions, the effect of microbiological activity on geochemical speciation is not well understood. A true understanding of bacterially catalyzed molecular transformation of inorganic contaminants is fundamental to risk assessment and the evaluation of bioremediation processes. The focus of our work in this area is the interactions among trace metal contaminants, mineral

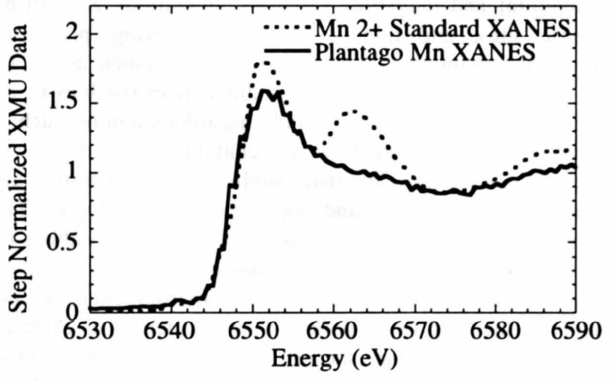

Figure 2.
Mn K-edge XANES spectrum obtained with a 1 μm by 3 μm focal spot aligned to the stele in the central portion of the image shown in Figure 1 and Mn XANES spectrum from $MnCO_3$ standard. Comparison of the spectra indicated that the majority of the Mn is in the +2 oxidation state.

surfaces, and bacterially produced extracellular material at the microbe-metal contaminant-geosurface interface.

The objectives of our studies are (1) to determine the spatial distribution and chemical speciation of metals near bacteria-geosurface interfaces and (2) to use this information to identify the interactions occurring near these interfaces among the metals, mineral surfaces, and bacterially produced extracellular materials under a variety of conditions. To accomplish these objectives, we are using X-ray microbeams to investigate the spatial distribution of metals in single hydrated bacterial cells adhered to Kapton film, with the goal of progressing to spectromicroscopy studies of metals at the bacteria-geosurface interface.

We have used hard X-ray phase zone plates to investigate the spatial distribution of 3*d* elements in a single hydrated *Pseudomonas fluorescens* bacterium adhered to a Kapton film. The zone plate used in these microscopy experiments produced a focused beam of cross section 0.15 μm^2 and had an effective focal length of 12.5 cm at 10.0 keV. The X-ray beam passed through a 10-μm order-sorting aperture, and the focus was adjusted to be on the sample. The samples were mounted on a piezo that in turn was mounted on a computer-controlled XYZ stage at 5 degrees to the incident beam, thus negligibly affecting the X-ray footprint on the sample in the horizontal dimension. Figure 3 shows the spatial distribution of Cu in a hydrated *Pseudomonas fluorescens* bacterium adhered to a Kapton film at ambient temperature. The dimensions of the image are 6 μm × 13.8 μm. The map of the elements was obtained by scanning the sample in 0.15-μm steps through the focused monochromatic X-ray beam (10.0 keV) and integrating the selected Kα fluorescence for 5 sec per point. The total data collection time was approximately 8 hours. Although these results demonstrate the utility of imaging hydrated bacteria at ambient temperature, for spectromicroscopy studies a cryostat will be required to freeze the samples in order to reduce the effects of radiation damage.

Figure 3.
Spatial distribution of Cu in a hydrated *Pseudomonas fluorescens* bacterium adhered to Kapton film, as determined from the Kα fluorescence intensities produced by a 0.15 μm^2, 10.0 keV X-ray beam.

Summary

We have demonstrated the utility of X-ray microbeams, particularly those produced by hard X-ray phase zone plates, for investigating a variety of environmental systems. Specifically, we have illustrated (1) the use of 1- to 5-μm hard X-ray beams for determining the spatial distribution of metals in fungal-infected plant roots and (2) the use of submicron hard X-ray beams (0.15 μm) for determining the spatial distribution of metals in a hydrated bacterium adhered to Kapton film. The further development of these techniques for such applications promises to provide unique opportunities in the field of environmental research.

Acknowledgments

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