Spectroscopic Speciation to Understand Bioavailability and Remediation

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INTRODUCTION

In the context of conducting research to understand the role that metal contaminants play in bioavailability and resulting remediation to eliminate biological availability, there are many tools available to decipher pieces of the total puzzle. In some instances, the human eye can clearly identify the impact of metals at a contaminated site. From this standpoint, researchers can collect representative samples for macroscopic and microscopic laboratory examinations as well as develop field plots to investigate the characteristics responsible for the negative impact of metals. Soil samples taken to the laboratory can undergo an array of analyses including batch reactors to examine kinetics and thermodynamics; chemical extractions to determine total metal content, operationally defined speciation (sequential extractions), or bioaccessibility (to mimic human bioavailability); and instrument analysis to identify mineralogy and other fundamental properties of the sample. It is at this point where most evaluations are completed and it is determined that characteristics such as metal concentration, soil pH, iron oxide concentration, and soil organic matter are indicative of the dose-response paradigm. However, aside from knowing the total metal concentration, little is often known or determined with regard to the chemistry of the metal. This information is often inferred from the soil properties – which can be a valid approach – but often involves inconclusive guesswork through the use of sequential extraction procedures or broad based modeling efforts. To gain a more complete picture of the chemistry of metals, one must employs techniques that definitively determine the speciation (the true chemical form and physical coordination) of contaminants. There are several excellent spectroscopic methods capable of determining metal speciation but the most authoritative approach involves the application of synchrotron methods such as X-ray absorption spectroscopy (XAS). X-ray absorption spectroscopy distinguishes the oxidation state, coordination environment, interatomic bond distances, and identity of nearest-neighboring elements relative to a specific metal of concern. Information gleamed from XAS experiments provides an in-situ look at the current chemical form of a metal and can be used to predict the long-term fate of the metal and its potential bioavailability based on known solubility products. The impact of metal speciation on risk assessment has gained much attention over the past couple decades and will continue to grow in acceptance as an important part in our understanding of metal bioavailability and remediation.

X-RAY ABSORPTION SPECTROSCOPY

X-ray absorption spectroscopy (XAS) refers to the interaction of how X-rays are absorbed by an atom at energies near and above the core-level binding energies of that particular atom. XAS is the modulation of an atom’s X-ray absorption probability due to the chemical and physical state of the atom. XAS spectra are especially sensitive to the formal oxidation state, coordination chemistry, and the interatomic distances, coordination number and species of the atoms in the surrounding proximity of the selected element of interest. As a result, XAS provides a practical and simple way to determine the chemical state and local atomic structure for a selected atomic species.

XAS can be used in a wide variety of systems and bulk physical environments. Since XAS is an atomic probe, nearly all substances can be studied. All elements have core shell electrons. Crystallinity is not a factor for XAS measurements making analysis of noncrytalline material, disordered compounds, and solutions feasible and attractive. XAS is capable of detection sensitivities of a few parts per million. An important aspect from an environmental perspective is that XAS is an *in-situ* spectroscopy allowing for the investigation of samples in their natural state.

A typical XAS spectra is broken into 2 regimes (Figure 1):

XANES - X-ray Absorption Near-Edge Spectroscopy
EXAFS - Extended X-ray Absorption Fine-Structure

which contain related, but slightly different information about an element's local coordination and chemical state. X-rays are absorbed by all matter through the photoelectric effect: An x-ray is absorbed by an atom, promoting a core-level electron (K, L, or M shell) out of the atom and into a continuum. The atom is left in an excited state with an empty electronic level (a core hole). The electron ejected from the atom is called the photoelectron. When X-rays are absorbed by the photoelectric effect, the excited core-hole will relax back to a "ground state" of the atom. A higher level core electron drops into the core hole, and a fluorescent X-ray or Auger electron is emitted.

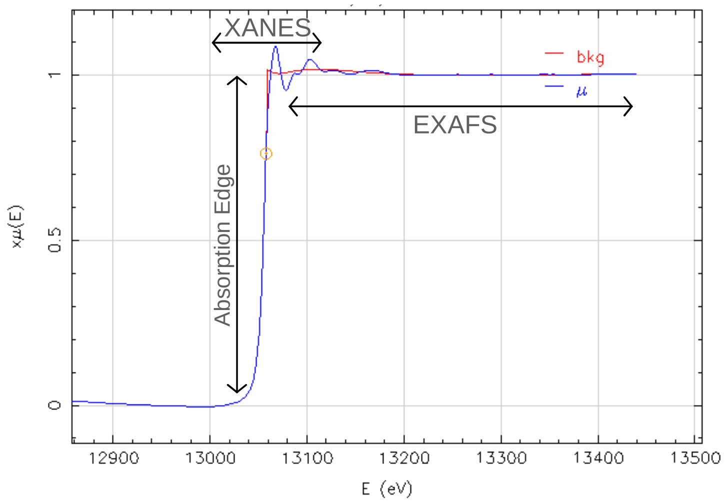

Figure 1. Location of XANES and EXAFS regions of an XAS spectrum. The characteristic energy required to excite core level electrons is unique to each element and is known as the absorption edge or edge step.

The intensity of an X-ray beam as it passes through a material of thickness, t, is given by the absorption coefficient, μ:

$$I = I_0 e^{-\mu t}$$

where I_0 is the X-ray intensity hitting the material, and I is the intensity transmitted through the material. The absorption coefficient depends strongly on X-ray energy, E, and atomic number, Z, and on the density,   , and atomic mass, A:

$$\mu \approx (\quad Z^4)/(AE^3)$$

In addition, μ has sharp absorption edges (Figure 1) corresponding to the characteristic core level energies of the atom. The energies of the K-edge absorption edges go roughly as $E_K \sim Z^2$. All elements with Z>18 have either a K-, or L-edge energies between 3 and 35 keV, which can be accessed at many synchrotron sources.

X-RAY ABSORPTION SPECTROSCOPY DATA COLLECTION

Due to its need for high energy X-rays, XAS data collection can only be accomplished with a synchrotron radiation source. There are approximately 25 synchrotron facilities world-wide with eight such laboratories within the United States. Figure 2 shows the Advanced Photon Source (APS) synchrotron radiation research facility at Argonne National Laboratory in the southeastern suburbs of Chicago, IL. Applying components of Figure 2B to explain structures in Figure 2A, the operation of the APS synchrotron facility entails (A) production of electrons in a linear accelerator which are deposited into (B) the booster/injector ring to bring the electron packets near the speed of light. The electron beam is then sent to the storage ring (C) from which beamlines (D) as either insertion devices, ID, or bending magnets, BM, are constructed for experimental research (E). In-line with the research beamlines are monochromaters that tune the electron beam to selected energies via Bragg diffraction and must be capable of energy resolutions of ~ 1 eV at 10 keV.

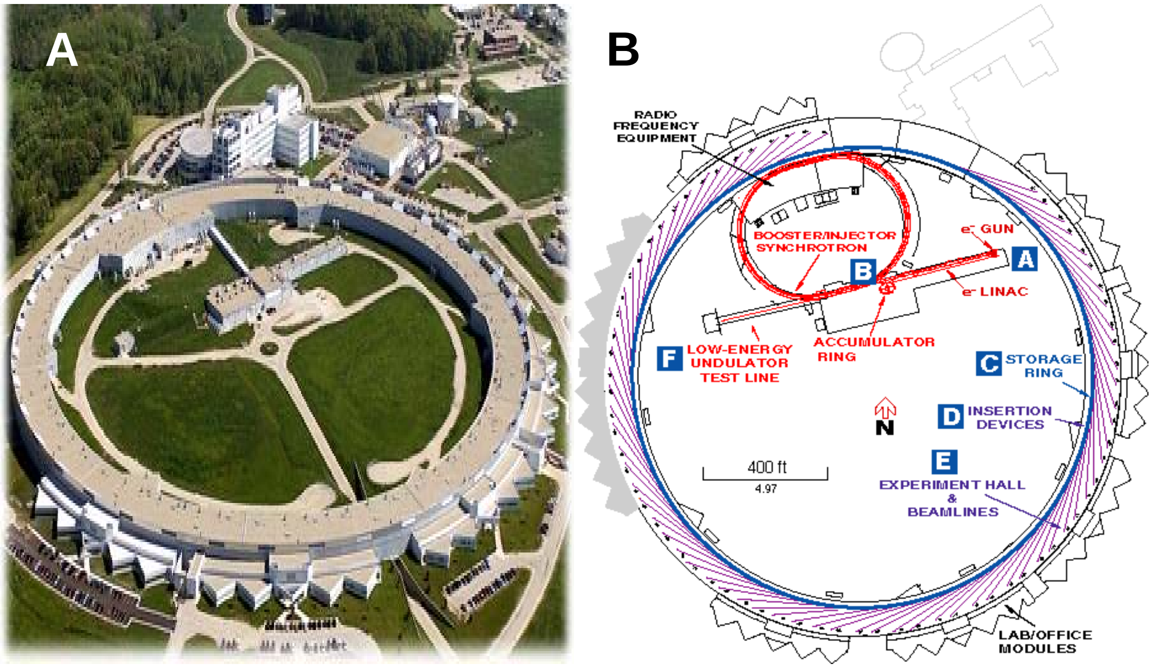

Figure 2. Aerial photo and architectural diagram of the Advanced Photon Source at Argonne National Laboratory, Chicago, IL.

XAS data collection can be divided into two realms: 1) configuration of beamline equipment and 2) sample preparation. XAS measures the energy dependence of the x-ray absorption coefficient, μ(E), at and above the absorption edge of a selected element. μ(E) can be measured in two experimental configuration setups:

Transmission: The absorption is measured directly by measuring what is transmitted through the sample (Figure 3):
$\mu(E)t = \ln(I/I_0)$

Fluorescence: The re-filling the deep core hole, is detected. Typically the fluorescent x-ray is measured (Figure 4).
$\mu(E) \sim I_f /I_0$

Sample preparation can be as crucial as data collection setup when attempting to collect quality data. Although much imagination can be employed for sample preparation, many considerations must be examined to ensure that the integrity of the sample is maintained. For example, with redox sensitive materials such as arsenic, one must be mindful of possible oxidation of arsenite either from oxygen in the air, as a result of materials in the sample holder, and over exposure of the electron beam. Another important issue to evaluate for sample preparation is the adequacy of the beamline one chooses to conduct the experiments. Once an accurate understanding of detector limitations is accomplished for sample preparation, sample mounting should be designed with simplicity in mind.

Sample holders can vary significantly but follow a basic design. Sample holders can be as simple as smearing a solid material on the tacky side of tape and folding the tape back onto itself the secure the sample. Microscopic thin sections are also favorable. Another common sample holder is small block of non-reactive material with a depression or hole lathed into the holder which the sample is added with tape securing the opening. This type of sample holder allows analysis of solids, slurries, and solutions. The thickness and size of openings can be customized. An adaptation of this sample holder in conjunction with a movable sample stage and automation software yields a basic autosampler which permits all types of samples for analysis.

X-RAY ABSORPTION SPECTROSCOPY DATA ANALYSIS

Whether transmission or fluorescence data are collected, the data reduction and analysis are essentially the same. The steps to data analysis are 1) to reduce the raw spectra to k-space (conversion of energy [eV] to wavelength [inverse distance]), 2) Fourier transform the k-space data into R-space (conversion of wavelength to actual distance), and 3) EXAFS data modeling. Background correction of a raw spectrum involves assigning a baseline value of zero to the pre-edge region and a normalized unity value of one to the EXAFS region. The conversion of energy (E) to k-space involves first the identification of the threshold energy, E_o, which is the energy maximum of the edge step. Thereafter, one isolates the EXAFS region in terms of the wave behavior of the photoelectron (k) created in the absorption process. By selecting and emphasizing (k-weighting) only positive k-space, one generates oscillations as a function of the photoelectron wave number also known as the (chi) (k)-function. The oscillations correspond to different near-neighbor coordination shells which, after Fourier transformation, can be described by the EXAFS equation:

$$\chi(k) = \sum_j \frac{N_j f_j(k) e^{-2k^2\sigma_j^2}}{k{R_j}^2} \sin[2kR_j + \delta_j(k)]$$

Where f(k) and (k) are scattering properties of the atoms neighboring the excited atom, N is the number of neighboring atoms, R is the distance to the neighboring atom, and ^{2} is the disorder in the neighbor distance. Though complex in appearance, the EXAFS equation allows one to determine N, R, and ^{2} if the scattering amplitude, f(k), and phase-shift, (k), are known. Since f(k) and (k) depend on the Z of the neighboring atoms, EXAFS is also sensitive to the atomic species of the neighboring atom.

Now that the energy spectrum is converted to k-space, one can Fourier transform the k-space data in R-space (distance). The Fourier transformation is critical to XAFS analysis and is often the area of confusion for novice data analyzers. Since the photoelectron effect causes backscattering in XAFS data collection, a phase shift causes real values of distance to be offset at least 0.5 Å when determining interatomic bond distances in the data modeling techniques. The final step in EXAFS data analysis is modeling which is often the most complex and time consuming aspect of the procedure. The most common method of modeling is to develop a library of fitting paths generated crystallographic parameters found in the literature.

In many instances for environmental samples, the speciation of metals can result in multiple phases. This can make data analysis difficult. For example, EXAFS data analysis is providing information on the average coordination numbers and bond distances for a given shell. When multiple species are present, these parameters are organized into one value that does not represent the complexity of the metal species. The same problem arises when interpreting the coordination environment of metals for XANES data analysis. To overcome this issue, one can apply a statistical fitting procedure that seeks to strip the multiple components of a sample spectrum into individual parts through the assistance of known reference spectra. The two most common methods are linear combination fitting (LCF) and principle component analysis (PCA). LCF analysis of XANES and XAFS spectra (LCF-XANES and LCF-XAFS) is simple to apply to normalized XANES spectrum or the $k^{2 \text{ or } 3}$-weighted -function from EXAFS data reduction. The goal in this procedure is to accumulate enough relevant reference spectra that can explain and represent the unknown environmental sample. Through use of available software, one selects an unknown spectrum to evaluate and multiple known reference spectra to fit against the unknown. By repeating the procedure and removing nonessential reference spectra, one can gain a semi-quantitative analysis of the major metal species present in the unknown sample. Detailed information as collected in EXAFS analysis is not possible but identity of multiple species in the sample is accomplished. For typical environmental samples, LCF-XANES and LCF-XAFS can be a very powerful tool to determine metal speciation when multiple phases are present via a fingerprinting method. This method proves very effective in monitoring contaminated sites to evaluate changes in metal speciation either through in-situ amendments or monitored natural attenuation.

MYTHS ABOUT X-RAY ABSORPTION SPECTROSCOPY

After reading to this point, the average person may step back to think that conducting XAS synchrotron research is very expensive, very complex, and only available to select individuals. These are only a few myths that exist regarding the application of synchrotron research for environmental sciences.

Access to conduct XAS research at synchrotron facilities is accomplished through a competitive general user proposal system. Most facilities have three or four research cycles throughout a year. The proposal system requires the user to identify a specific research question and its impact and to demonstrate how synchrotron research will effectively answer the question. Since most of the United States synchrotron facilities are taxpayer funded, research time, once awarded through the proposal system, is free. The expectation of general users by the synchrotron facilities is to publish the data. Therefore, the direct cost to users is travel, lodging, and meals. If one considers a 4-day trip to the Advanced Photon Source in Chicago, a typical flight of $400 combined with three nights at the Argonne Guest House ($55/night) and four days of meals ($40/day) will cost $725. If one conducts basic XAS research, a sample could be scanned 3 times to collect high-quality data within one hour. To be conservative, one can easily collect data on 20 samples per day resulting in 60 samples analyzed during the fictional 4-day trip with a cost (relative to travel expenses) of about $12/sample. Of course, this number does not account for hourly salary rates or the time that will be involved with full data analysis. However, it is difficult to find such advanced analytical instruments that cost so little on a per sample basis.

The complexity of XAS research lies with the data analysis. Data collection is relatively straightforward and often involves the assistance of beamline scientists. However, beamline scientists are active researchers as well and can be useful colleagues in data analysis as they are seeking to expand their scientific careers via peer-reviewed publications. If that is the intent of new users of XAS, discussion of co-authorship of manuscripts should be discussed early in the collaboration. Another suitable approach is to collaborate with other academic or government researchers, or bring postdoctoral or graduate students into one's group, that have a documented history of success with synchrotron research to utilize their expertise and talents.

There are many ways for a motivated researcher to enter the world of XAS synchrotron research. There is no secret handshake or initiation. Though XAFS measurements can be simple, a complete understanding of XAFS involves a wonderful mixture of modern physics and chemistry and a complete mastery of the data analysis can be somewhat challenging. Though the basic phenomena is well understood, an accurate theoretical treatment is fairly involved and, in some respects still an area of active research.