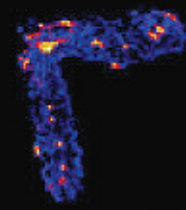


Shining Light on Metals in the Environment



David H. McNear Jr., Ryan Tappero,
and Donald L. Sparks¹

Elucidating the speciation of heavy metals in the environment is paramount to understanding their potential mobility and bioavailability. Cutting-edge synchrotron-based techniques such as microfocused X-ray absorption fine-structure (XAFS) and X-ray fluorescence (XRF) spectroscopy and microtomography have revolutionized the way metal reactions and processes in natural systems are studied. In this article, we apply these intense-light tools to decipher metal forms (species) and associations in contaminated soils and metal-hyperaccumulating plants.

KEYWORDS: X-ray absorption fine-structure spectroscopy (XAFS), metal-hyperaccumulator plants, metal speciation

INTRODUCTION

The contamination of surface and subsurface environments with heavy metals is a problem worldwide. To predict the mobility, bioavailability, and ultimately the impact of toxic metals on plants, animals, and humans, it is imperative to understand metal–soil interactions at a fundamental level. Metals can be introduced into soil and water environments from both natural (as reaction products via the dissolution of metal-bearing minerals) and anthropogenic (biosolids, mine wastes, dredged materials, fly ash, and atmospheric deposits) sources (Adriano 2001; Roberts et al. 2003).

The toxicity of heavy metals in ecosystems depends not only on the total metal concentration in solution, but more significantly on the chemical form or speciation of the metal. Accordingly, quantitative metal speciation and its variation with time is a prerequisite for long-term risk assessment. Speciation encompasses both the chemical and physical (spatial distribution) form an element takes in a geochemical setting. A detailed definition of speciation includes the following components: (1) the identity of the element; (2) the oxidation state of the element; (3) the associations and complexes to solids and dissolved species (surface complexes, metal–ligand bonds, surface precipitates); and (4) the molecular geometry and coordination environment of the element (Brown et al. 1999; Roberts et al. 2003). The greater the number of parameters that can be identified, the more precisely one can predict the potential risk of metal toxicity to organisms.

Geoscientists have often used total metal concentrations or chemical extraction techniques (e.g. sequential extraction methods) to provide some insight into metal “speciation” and bioavailability. However, total concentration measurements do not take into account that not all of the metal is labile or available for uptake by plants and other organisms. Sequential extraction methods attempt to overcome this ambiguity by employing multiple chemical extractants, each

progressively more aggressive, to remove specific physically or chemically sorbed and/or occluded metal ions (e.g. exchangeable, associated with carbonates, oxides, etc.). However, the accuracy of the metal–solid phase associations derived from sequential extractions is strongly dependent on the type of extracting reagent used and thus prone to misinterpretation (Tessier et al. 1979; Roberts et al. 2003).

SYNCHROTRON RADIATION

The use of intense light to investigate metal reactions and processes and to determine the speciation of metals in natural systems such as soils has revolutionized the geosciences. Light, or in this case electromagnetic radiation, consists of longer wavelength, lower energy forms of radiation such as radio- or microwaves, and shorter wavelength, higher energy forms such as ultraviolet, X-rays, and gamma rays (Fig. 1). Electromagnetic radiation has both particle (photon) and wave properties such that light at a particular wavelength corresponds to a particular scale of detection (O’Day 1999). Therefore, light employed to “see” an object must have a wavelength equal to or less than the object’s size. The wavelength of light is inversely proportional to the energy, and therefore, in order to observe objects at the atomic or molecular scales, higher energy or more intense light is required. Intense light, ranging from <1 eV (infrared) to >10⁶ eV (hard X-rays), can be produced at a synchrotron facility. Synchrotron radiation is created when electrons (or positrons) are accelerated to near the speed of light in a circular path using electromagnets. As they travel along this path, they emit (tangentially) high-energy photons in a wide fan. These photons are focused using precision magnets called wigglers and undulators and are then directed towards an experimental station or “beamline” where additional optics are used to select the desired wavelength and further focus the beam. X-ray absorption spectroscopy experiments are performed at these synchrotron beamlines.

X-RAY ABSORPTION SPECTROSCOPY

One of the most widely used synchrotron-based spectroscopic techniques in the geosciences is X-ray absorption spectroscopy (XAS). Its use has revolutionized, and will continue to do so, our understanding of metal and other contaminant reactions and processes in natural systems such as soils and sediments. XAS can be used to study most elements in crystalline or non-crystalline solid, liquid, or gaseous states over a concentration range of a few mg L⁻¹ to the pure element. It can be used to probe structural sites in

¹ Environmental Soil Chemistry Research Group
Department of Plant and Soil Sciences, 152 Townsend Hall
University of Delaware, Newark, DE 19717-1303, USA
Corresponding author: dlsparks@udel.edu

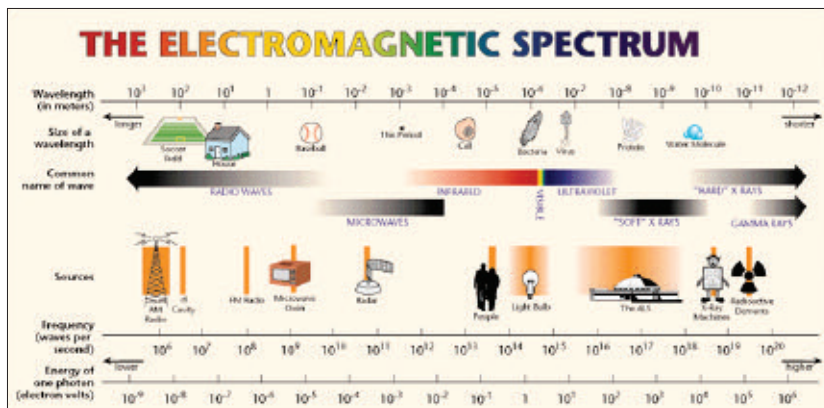


FIGURE 1 The electromagnetic spectrum

crystals and glasses, and at the mineral–water interface. XAS is an in situ technique, which means one can study reactions in the presence of water with minimal sample preparation. This is a major advantage over analytical techniques that require a sample to be dried or subjected to ultrahigh vacuum or both.

XAS, in general, is an element-specific, bulk-spectroscopic method that yields information about the average local structural and compositional environment of an absorbing atom. It “sees” only the two or three closest shells of neighboring atoms around the absorbing atom, within a distance up to 0.6 nm (i.e. short-range order), due to the short distance an electron travels in most substances before hitting another atom. XAS can be used to ascertain important chemical information, such as the oxidation state, information on next-nearest neighbors, bond distances, and coordination numbers. An XAS experiment records a spectrum of the modulation of the absorption coefficient as a function of photon energy. The experiment consists of exposing a sample to an incident monochromatic beam of synchrotron X-rays, which is scanned over a range of energies below and above the absorption edge (K, L, M) of the element of interest. When X-rays interact with matter, a number of processes can occur: X-ray scattering production of optical photons, production of photoelectrons and Auger electrons, production of fluorescence X-ray photons, and positron–electron pair production.

The energy region extending from just below to about 50 eV above the absorption edge is the XANES (X-ray absorption near-edge structure) portion of the spectrum. Fingerprint information (e.g. oxidation states) can be gleaned from this region of the XAS spectrum. The energy region from 50 to 1000 eV above the absorption edge is the EXAFS (extended X-ray absorption fine-structure) portion of the spectrum, which contains the critical information required to determine the local coordination environment (bond distances, coordination numbers, etc.) of the element of interest. The fine structure refers to the small oscillations in the extended portion of the XAS spectra caused by the interaction between outgoing photoelectrons and those backscattered off atoms neighboring the central absorbing atom. In order to extract information from this region, mathematical procedures must be performed to isolate and amplify these subtle oscillations. The spectrum is first converted from electron volts to photoelectron wave numbers (k , in \AA^{-1}) based on a specified value for E_0 , the threshold energy for X-ray absorption. The oscillatory fine structure data is then isolated by subtracting a background function representing one atomic X-ray absorption event. The resulting function $[\chi(k)]$ is often weighted, most commonly by

the square or cube of the wave number $[\chi(k) \cdot k^2$ or $\chi(k) \cdot k^3]$, to amplify the dampened oscillations occurring at higher k . Transformation of these data using Fourier filtering methods can then be performed to separate the contributions from the different atoms surrounding the absorbing atom, resulting in a radial structure function (RSF) with peaks representative of the location and distance of the neighboring atoms. The RSF or the k -weighted χ function can be modeled using non-linear least squares fitting to determine the structural parameters for the element in the material. However, in heterogeneous systems where more than one species is present, it is often difficult to isolate the individual contributions from neighboring atoms (i.e. mixed coordination shells), and thus alternative methods of data analysis

(discussed below) are required. Additional detail on XAS methodology, sample preparation, and data analyses can be found in a number of excellent sources (Fendorf et al. 1994; Schulze and Bertsch 1995; O’Day 1999; Bertsch and Hunter 2001; Brown and Sturchio 2002).

Over the past 15 years, major advances have been made in elucidating metal speciation and sorption mechanisms at the mineral–water interface. Studies using bulk XAFS have provided a plethora of information about metal sorption on model systems (e.g. clay minerals, metal oxides and hydroxides) including details on structure, stoichiometry, attachment geometry (inner- versus outer-sphere, monodentate versus bidentate or tridentate), the presence of multinuclear complexes and precipitate phases, and the presence of ternary surface complexes (when complexing ligands are present in solution). Many of these studies are reported in comprehensive reviews (Brown and Parks 2001; Brown and Sturchio 2002).

There are a number of disadvantages in using bulk XAFS to determine the speciation of metals in heterogeneous systems such as soils. Soils are complex, containing an array of inorganic and organic components including humic substances, clay minerals, metal oxides and hydroxides, macro- and micropores, and microorganisms (all intimately associated with each other). In such systems, some microenvironments contain isolated phases in higher concentrations relative to the average in the total (bulk) matrix. For example, the oxides, minerals, and microorganisms in the rhizosphere (i.e. soil adjacent to plant roots) have quite a different chemical environment compared to the bulk soil. Often these phases may be very reactive and of significance in the partitioning of metals, but may be overlooked using other analytical techniques that measure the average of all phases. Standard bulk XAFS techniques probe an area of several square millimeters and provide information on the average local chemical environment. Thus, where more than one type of surface species is present, bulk XAFS will detect only the primary (or average) type of surface product/species in the bulk sample (i.e. sums over all geometric configurations of the target atom). However, the most reactive sites in soils have particle sizes in the micrometer range, and metal speciation may vary over regions of a few $100 \mu\text{m}^2$. Since multiple species exist in soils, atomic shells will overlap, and it is difficult to ascertain the precise metal speciation with bulk XAFS. Minor metal-bearing phases, even though they may constitute the most reactive or significant species, may not be successfully detected with bulk analyses.

However, third-generation synchrotron light sources with higher flux and higher brightness X-rays, together with state-of-the-art X-ray detectors and better beamline optics, produce microfocused beams for spectromicroscopy and

imaging studies. These developments provide a means for probing element speciation and associations in heterogeneous materials such as soils and plants. The following examples demonstrate how microfocused synchrotron-based spectroscopic techniques are being used to determine metal speciation and distribution in soils enriched by nickel refinery fallout and in specialized metal-accumulating plants.

μ-XAFS AND μ-SXRF INVESTIGATIONS OF METALS IN SOILS

Many studies in the past ten years have focused on defining the conditions favorable for transition metal (e.g. Cr, Ni, Co, and Zn) surface precipitate formation under laboratory conditions utilizing model sorbents (e.g. clay minerals, metal oxides and hydroxides), fixed metal concentrations, and a range of pH values (Charlet and Manceau 1994; O'Day et al. 1994; Scheidegger et al. 1997; Ford and Sparks 2000). These studies demonstrate that surface metal precipitates can form at concentrations below monolayer coverage and at pH values undersaturated according to the thermodynamic solubility product of the metal hydroxide precipitate (Sparks 2002). As these precipitates "age," metal release is greatly reduced. Precipitation could represent a significant mechanism for long-term metal sequestration in natural systems disturbed by anthropogenic metal inputs.

We used synchrotron-based microfocused X-ray absorption fine structure and X-ray fluorescence spectroscopy (μ-XAFS and μ-SXRF, respectively) to examine the occurrence of surface precipitates in natural soils. The material we studied was a mineral soil enriched with ~6000 mg kg⁻¹ of aerielly deposited nickel from an historical nickel refinery.

A typical μ-XAFS experiment (FIG. 2) begins by first collecting X-ray fluorescence elemental maps from which metal correlations can be observed. From these maps, regions of interest (i.e. hotspots) are selected and further probed using the microfocused X-ray beam. Analysis of XAFS spectra collected from multicomponent systems cannot rely on traditional fitting procedures in which the spectra are broken down into individual atomic shells. Therefore, to determine the species present within a mixed system, a dataset of spectra from multiple spots throughout a sample are analyzed statistically using principal component analysis (PCA). The PCA technique determines if the data set can be described as weighted sums of a smaller number of components, which would be the case if each spot in the dataset is comprised of a smaller number of distinct compounds. Target transformation (TT) is then used to identify the components by taking a spectrum of a known reference compound and mathematically removing from the spectrum anything that does not look like the principal components identified by PCA. If minimal information has to be

removed from the known reference spectrum, then one can conclude it is most likely present in the sample. After the contributing standard phases are identified, linear least squares fitting (LLSF) is used to determine the amount (%) of each standard species within the individual sample spectra making up the dataset. The accuracy of this fitting approach is dependent upon the data quality, the completeness of the standards data set, and the range over which the data were fit. Additional and more thorough discussions on μ-XAFS applications and methods of data collection and analysis for contaminants in heterogeneous systems can be found in Bertsch and Hunter (2001) and Manceau et al. (2002).

Metal refining produces particles of varying composition and morphology, depending on the process (e.g. electrolytic or anode) and the type of ore (lateritic or sulfidic). Prior to adequate emission standards, these particles were released into the environment (Höflich et al. 2000). Our soil samples contain mainly nickel-bearing particles with spherical morphology (FIG. 3A, white arrow in FIG. 3B) ranging from <5 μm up to ~50 μm in diameter. μ-XAFS spectra were collected (data not shown) on these particles, and data analysis indicated that they were composed primarily of NiO (bunsenite). The μ-SXRF map indicated little correlation of Ni with Fe and Mn. Nickel is present either as discrete NiO particles (e.g. arrow, FIG. 3B) or as a diffuse "coating" on soil aggregates.

To determine the speciation of nickel within the diffuse "coatings," μ-XAFS spectra were collected at several locations throughout the SXRF map (spots 1-3, FIG. 3B). These spectra represent a small subset of the total spectra collected throughout the soil and are used here as an example of the utility of these techniques (McNear et al. in review). Principal component analysis revealed that our spectral dataset was best represented by two or three components, which were identified using target transformation as NiO, Ni(OH)₂, and Ni/Al layer double hydroxide (LDH). Using LLSF we determined the proportion of each of these species present within spots 1-3 (FIG. 3c). Spots 1 and 3, which correspond to diffuse areas of Ni on the edge of the soil aggregate, were comprised of roughly 70-90% Ni/Al LDH

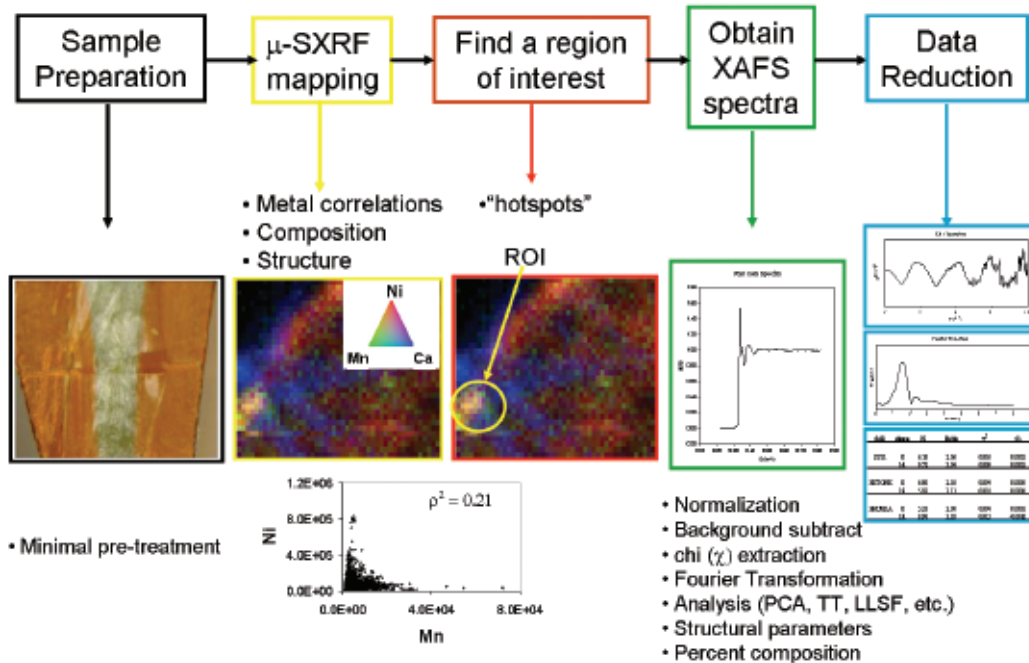


FIGURE 2 Anatomy of a XAFS experiment

precipitates with lesser amounts of NiO and Ni(OH)₂. Spot 2, however, was comprised of equal proportions of both NiO and Ni(OH)₂. The line scan across spot 2 (graph overlay, FIG. 3B) corresponds to a spike in relative Ni concentration and thus most likely indicates a NiO particle.

The persistence of NiO particles in these soils nearly 50 years after their original deposition implies that they are relatively resistant to weathering. However, the identification of Ni(OH)₂ associated with these particles suggests that some dissolution may be occurring. Interestingly, no other refinery-associated particulates—trevorite (NiFe₂O₄), heazlewoodite (Ni₃S₂), godlevskite [(Ni,Fe)₇S₆]—were detected in these soils, possibly as a result of their higher solubility. Therefore, the dissolution and redistribution of those particles may have resulted in an initial “pulse” of Ni into the system during the most active periods of emissions release, while the resistant NiO acts as a source of slow, continued Ni release into the soils. Thus, redistribution of the original refining byproducts deposited in these soils resulted in the incorporation of Ni into surface precipitate phases including a neoformed layer double hydroxide precipitate (Ni/Al LDH). The Ni/Al LDH structure has brucite-like octahedral

layers in which Al³⁺ substitutes for Ni²⁺, creating a net positive charge balanced by hydrated anions in the interlayer space (Sparks 2002).

The impact these precipitates have on the mobility and bioavailability of Ni within these soils is currently under investigation. Included with these investigations is an analysis of how the formation of more stable Ni species influences the metal-removal ability of specialized metal-accumulating plants (hyperaccumulators) proposed as an alternative remediation strategy for this site.

μ-XAFS AND μ-SXRF ANALYSIS OF METALS IN HYPERACCUMULATOR PLANTS

Alternative remediation strategies are necessary when vast areas of land have been contaminated (as in the above example), due in part to the high cost required for traditional remediation techniques (e.g. dig and haul). Phytoremediation is a “green” technology that uses plants to remove contaminants from the environment. Phytoremediation relies on unique plants, capable of accumulating higher than normal metal concentrations (e.g. >1000 ppm for Ni and Co and >10,000 ppm for Zn; Baker 1981) in aerial tissue, to extract metals from contaminated soils.

Cultivating hyperaccumulator plants in metal-rich soils and ashing the harvestable biomass to produce ore (i.e. phytomining) and energy (biofuel) is environmentally sustainable and economically feasible (Chaney et al. 1997). Because most discharges to the environment involve a mixed-waste stream, it is important to consider the influence of co-contaminants on the physiology and biochemistry of hyperaccumulators and ultimately their impact on phytoremediation efficiency for the target metal.

To better understand the mechanisms involved in metal hyperaccumulation and tolerance, it is crucial to know whether accumulated metals are bound by strong (specific) ligands or loosely associated with common organic acids (i.e. speciation) as well as where these metals are stored (i.e. localization or compartmentalization). Synchrotron-based techniques (e.g. μ-SXRF,

μ-XAFS, and μ-tomography) can be used to probe metal localization and speciation in “fresh” hyperaccumulator plant tissues (in vivo) with micrometer resolution. Ultimately, understanding the physiological and biochemical mechanisms underlying metal hyperaccumulation and tolerance will permit optimal metal extraction.

Preliminary XAS investigations of the nickel hyperaccumulator *Alyssum murale* exposed to single- or mixed-metal (Ni, Co, and Zn) systems revealed different patterns for Ni and Co localization (FIG. 4A) (Tappero et al. in review b). Nickel was distributed relatively evenly throughout the leaves of *A. murale*, although the Ni fluorescence intensity was slightly elevated in the mid-rib (vein) region, while Co was enriched predominantly near the leaf tips. The tomogram of a leaf

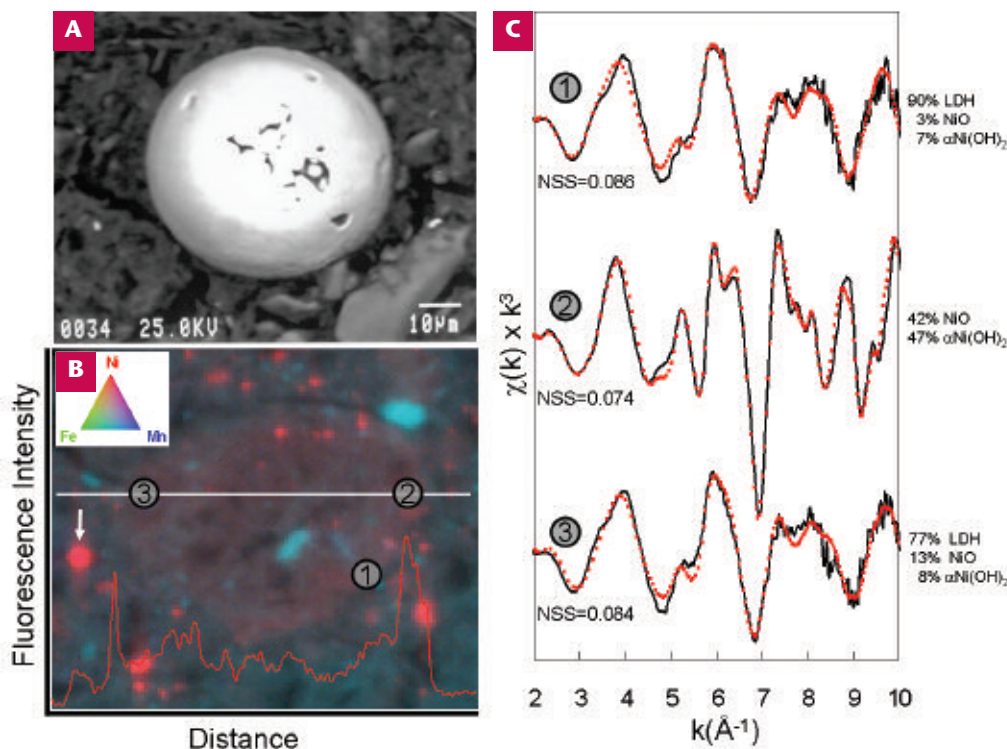


FIGURE 3 (A) Electron micrograph showing a distinct spherical NiO particle. (B) μ-SXRF elemental map showing the distribution and correlation of Ni (red), Fe (green), and Mn (blue) in a resin-embedded thin section of Ni-enriched loam soil from an area adjacent to an historic Ni refinery. Overlain on this figure is the change in Ni fluorescence intensity found along a line (gray = 450 μm) transecting the image. Numbered circles correspond to spots where μ-XAFS spectra were collected. (C) The solid line represents the raw χ data and the red dotted line the best fit obtained with linear least squares fitting using the primary species identified by principle component analysis and target transformation. The amounts (%) of each reference species in the individual sample spectra determined using this approach are displayed to the right of each χ spectra. The normalized sum square (NSS) value was used to assess the goodness of fit, with optimization occurring where the NSS reached a minimum.

cross-section (FIG. 5) shows Ni was present in the veins as well as in the epidermal (surface) tissues but was absent from the photosynthetically active mesophyll, while Co was limited to the leaf veins. Krämer et al. (1996) and Kerkeb and Krämer (2003) have shown that specialized storage compartments (vacuoles) within the epidermal cells are responsible for Ni sequestration and most likely serve as the primary Ni detoxification mechanism for this plant. In addition to epidermal localization, nickel has been observed in the basal compartment of the star-like leaf hairs (stellate trichomes) covering the leaf surface (Broadhurst et al. 2004; McNear et al. 2005). The trichomes of *A. murale* are unicellular and are composed primarily of calcium oxalate and calcite, rendering them quite visible in the Ca channel of the X-ray fluorescence maps (see FIG. 4A). Nickel in the basal compartment of the trichomes is typically co-localized with Mn, but there is no clear consensus as to the role of Mn in Ni sequestration or whether trichomes on the leaf surface of hyperaccumulating plants function as a significant repository for metals.

Alyssum murale hyperaccumulates Ni and Co, but Zn is not translocated to any appreciable extent. In our study, the distribution of zinc in *A. murale* was similar to nickel; however, the mid-rib region of the leaf was more metal enriched in the μ -SXRF map (FIG. 4). The increased signal intensity within the leaf vein region may indicate metal sorption at the boundary (walls) of the plant vascular system, but could possibly be an artifact of sample thickness (thickness effect). In contrast to Ni and Zn, Co preferentially accumulated in *A. murale* at the leaf tips and the margins near tips, a pattern similar to those observed with radio-tracer studies (Gustafson 1956; Kabata-Pendias and Pendias 2000). The observed partitioning of Co may indicate the inability of *A. murale* to detoxify this metal within plant cells. At any point between the root and leaf, Co in the vascular system could be unloaded (from the xylem) and transported to other tissues for subsequent sequestration in plant cells (e.g. vacuoles). However, limited transport out of the xylem fluid (e.g. lack of transporters in aerial tissue) may result in the accumulation of Co at leaf tips and margins. These results are corroborated by the synchrotron computed microtomograms (CMT) in FIG. 5, which show that Co (in contrast to Ni) is limited to the vascular system of the leaf.

The mixing of colors (red light and blue light = cyan) on the tricolor XRF image of an *A. murale* leaf indicated a possible association between Co and Ni in the leaf-tip region (FIG. 4C). The association of Co with other elements can be observed within the spatial context of the sample by generating a line spectrum (FIG. 4D). Inspection of the line spectrum intensity for a segment running up the center of the leaf vein to the leaf tip shows a decrease in the Ni signal and a corresponding increase in the Co signal, which suggests

that Co and Ni are not preferentially co-localized at the leaf tip. Note that the line spectra are sensitive to changes in the Ca signal due to the presence of Ca-rich leaf hairs (stellate trichomes). In a similar fashion, line spectra for other elements can be compared to look for other metal associations.

A. murale plants absorb Co and Ni from solution to a similar extent; however, these plants utilize vastly different mechanisms for metal storage and detoxification. Micro-XAFS was used to investigate the speciation of Co in the leaves of *A. murale* (Tappero et al. in review a). The chi spectra (FIG. 4B, inset) collected from a leaf tip and the mid-leaf region show marked differences. For example, the oscillation occurring at $7-8 \text{ \AA}^{-1}$ is split in the Co spectra collected from the leaf tip, but not in the spectra collected from the mid-leaf region. Likewise, the corresponding Fourier transforms (FIG. 4B) reveal that Co in the middle of the leaf (unlike Co at the tip) lacks any second-shell features indicative of a heavy neighboring atom. This is likely related to complexation with lighter elements (e.g. C in organic acids). The cobalt enrichment observed at the leaf tips is interpreted as a semicrystalline "sap-like" precipitate with some degree of short-range order. Cobalt absorbed by *A. murale* likely follows the transpiration stream until it is excreted from the terminus of the xylem (plant vascular system) and collects (i.e. precipitates) at the leaf tips and/or margins. Additional work is underway to elucidate the speciation and compartmentalization of Co in *A. murale*.

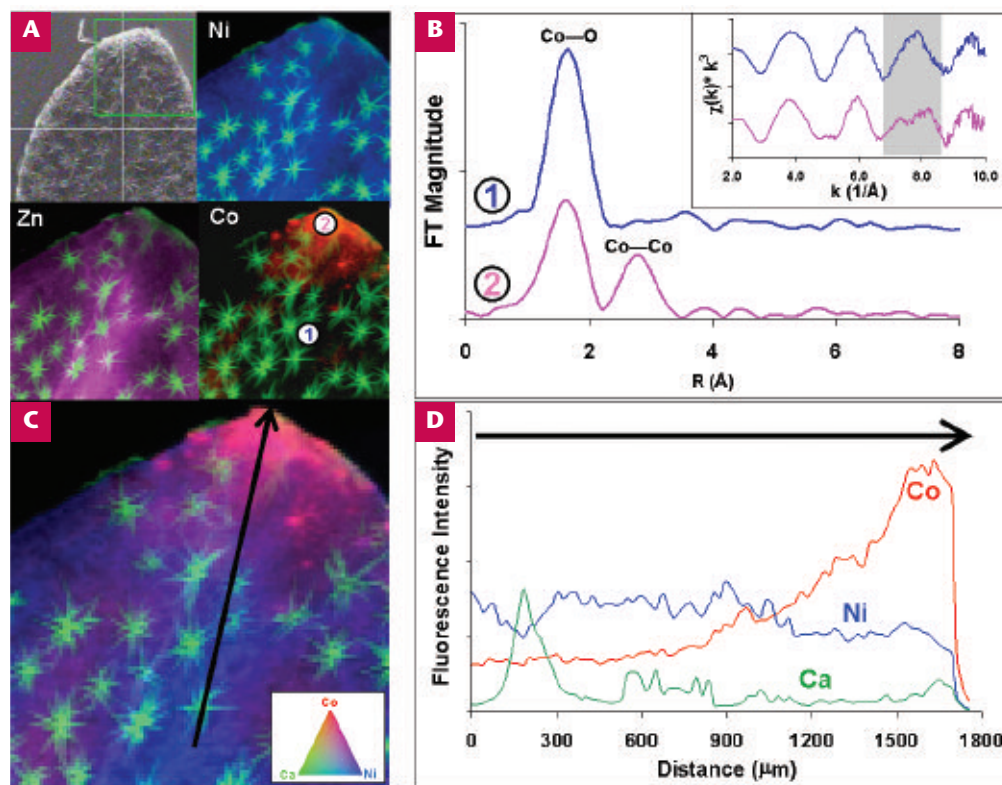


FIGURE 4 (A) μ -SXRF maps (Ni, Co, Zn) and microscope image of a fresh, hydrated leaf from the metal hyperaccumulator *Alyssum murale* (note that the star-shaped trichomes on the leaf surface are depicted in the Ca channel); (B) Co-XAFS k^3 -weighted chi (inset) and the corresponding Fourier transforms for the leaf tip and mid-leaf regions (spots 1 and 2 on the Co μ -SXRF map); (C) tricolor μ -SXRF image of a leaf; and (D) the corresponding line spectra which show the fluorescence intensity of Co, Ni, and Ca present in the leaf following the central vein toward the leaf tip (represented by the black arrow in panel C).

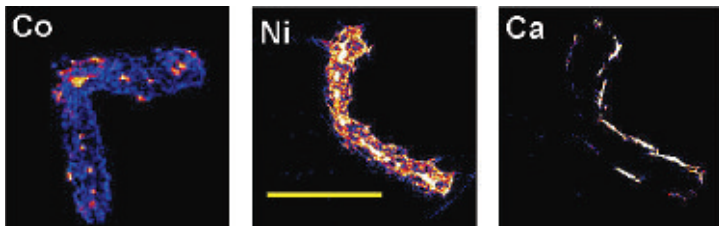


FIGURE 5 Computed fluorescence microtomograms showing the Co, Ni, and Ca compartmentalization in leaves of *Alyssum murale*. Brighter colors represent more fluorescent counts from the target element while darker colors represent fewer counts. Note that the leaf used to collect the Co tomogram came from a plant grown in solutions enriched in Co only. The yellow scale-bar represents ~500 μm .

Future research using synchrotron XAS will shed additional light on the mechanisms of Co accumulation and tolerance in hyperaccumulating plants by identifying the ligands involved in Co transport and sequestration. Synchrotron $\mu\text{-XRF}$ and $\mu\text{-XAS}$, and tomography, are powerful tools for observing metal speciation in heterogeneous systems such as contaminated soils and hyperaccumulating plants.

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SUMMARY

By utilizing microfocused synchrotron-based spectroscopic techniques, it is possible to determine the speciation of metals in soils and plants, where speciation can vary over hundreds of microns. With further advances in X-ray beam optics, beam spot sizes on the order of hundreds of nanometers will be achievable, making possible the exploration of intercellular distribution and speciation of metals in plant tissues as well as interactions of metals at the soil-water interface. In addition, microbeamlines capable of examining the distribution and speciation of lighter elements, such as Si, P, S, and Cl, will significantly enhance our ability to probe elemental associations in natural systems.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Matthew Marcus at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory (beamline 10.3.2) for his assistance with collecting $\mu\text{-XAS}$ data and Drs. Steve Sutton, Matt Newville, and Mark Rivers, at the Advanced Photon Source (APS) at Argonne National Laboratory for help with collecting tomography data. ■