Synchrotron-based X-Ray Approaches for Examining Toxic Trace Metal(loid)s in Soil–Plant Systems

Peter M Kopittke, Peng Wang,* Enzo Lombi, Erica Donner

Abstract
Elevated levels of trace metal(loid)s reduce plant growth, both in soils contaminated by industrial activities and in acid agricultural soils. Although the adverse effects of trace metal(loid)s have long been recognized, there remains much unknown both about their behavior in soils, their toxicity to plants, and the mechanisms that plants use to tolerate elevated concentrations. Synchrotron-based approaches are being utilized increasingly in soil–plant systems to examine toxic metal(loid)s. In the present review, brief consideration is given to the theory of synchrotron radiation. Thereafter, we review the use of synchrotron-based approaches for the examination of various trace metal(loid)s in soil–plant systems, including aluminum, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, and cadmium. Within the context of this review, X-ray absorption spectroscopy (XAS) and X-ray fluorescence microscopy (μ-XRF) are of particular interest. These techniques can provide in situ analyses of the distribution and speciation of metal(loid)s in soil–plant systems. The information presented here serves not only to understand the behavior of trace metals in soil–plant systems, but also to provide examples of the potential applications of synchrotron radiation that can be used to advantage in other studies.

Core Ideas
- Synchrotron analyses allow in situ analyses of metal(loid)s in soils and plants.
- This review provides a brief introduction to the theory of synchrotron radiation.
- The distribution and speciation of metal(loid)s in soils and plants is reviewed.

TRACE metals and metalloids, hereafter referred to as metal(loid)s, are natural components of the geosphere, hydrosphere, biosphere, and atmosphere, but their presence at elevated concentrations can result in toxicity. The importance of elevated trace metals in the environment has long been recognized (e.g., Veitch, 1904). Elevated levels of a range of trace metal(loid)s occur in sites contaminated by mining, industry, and transport. Similarly, acid soils, in which soluble aluminum (Al) and manganese (Mn) are increased, comprise ~3.95 billion ha of the global ice-free land or ~40% of the world’s arable land (von Uexküll and Mutert, 1995; Eswaran et al., 1997). These degraded lands may be improved by liming of acid agricultural soils or by burying and landfiling of polluted soil, but the cost of remediation is prohibitive in many cases. As a result, there is a continued interest in understanding the toxic effects of metal(loid)s in the soil–plant continuum.

Investigation of metal(loid) toxicity requires a multidisciplinary and multitechnique approach. Although many complementary techniques are suitable for the examination of metal(loid)s in soil–plant systems, it is not the intention of this review to compare this multitude of approaches. Rather, the focus of the present review is to consider synchrotron-based approaches, these being some of the few techniques that allow for in situ measurements of metal(loid) distribution and speciation with minimal sample preparation. Of particular interest here is synchrotron-based X-ray fluorescence microscopy (μ-XRF) and X-ray absorption spectroscopy (XAS). While other synchrotron-based approaches may also potentially be useful, μ-XRF and XAS are the most commonly used and versatile techniques for metal(loid)s. The information presented here complements that given in previously published reviews that have considered the use of synchrotron-based approaches for investigating both soils (Schulze and Bertsch, 1995; Singh and Grafe, 2010) and plants (Lombi and Susini, 2009; Punshon et al., 2009; Donner et al., 2012; Sarret et al., 2013; Zhao et al., 2014; Vijayan et al., 2015).

Copyright © American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. 5585 Guilford Rd., Madison, WI 53711 USA. All rights reserved.

doi:10.2134/jeq2016.09.0361
This is an open access article distributed under the terms of the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
Received 24 Sept. 2016.
Accepted 12 Dec. 2016.
*Corresponding author (p.wang3@uq.edu.au).

Abbreviations: Eh, redox potential; EXAFS, extended X-ray absorption fine structure; LEXRF, low-energy X-ray fluorescence; μ-XRF, X-ray fluorescence microscopy; NOM, natural organic matter; XANES, X-ray absorption near edge spectroscopy; XAS, X-ray absorption spectroscopy; XRD, X-ray diffraction; XRF, X-ray fluorescence.

PM. Kopittke and P. Wang, The Univ. of Queensland, School of Agriculture and Food Sciences, St. Lucia, QLD 4072, Australia; P. Wang, Nanjing Agricultural Univ., College of Resources and Environmental Sciences, Nanjing, Jiangsu 210095, China; E. Lombi and E. Donner, Univ. of South Australia, Future Industries Institute, Mawson Lakes, SA 5095, Australia. Assigned to Associate Editor Ganga Hettiarachchi.
Synchrotron Radiation

The theory of synchrotron-based approaches is reviewed to advantage elsewhere (e.g., Bilderback et al., 2005), so only a brief introduction is given here as relevant for the investigation of trace metal(loid)s in soils and plants.

Introduction to Synchrotron Radiation

A synchrotron typically consists of a linear accelerator, a booster ring, and a storage ring (Fig. 1). Synchrotron light sources typically use electrons, although it is also possible to use protons (protons are 1840 times heavier than electrons and hence require a higher energy to produce the same electromagnetic radiation as an electron beam; Bilderback et al., 2005). The electrons are generated by an electron gun, with the electrons generated in bunches, often nanoseconds apart. A linear accelerator is then used to accelerate the electron beam, with the electrons already approaching the speed of light by the time they exit the linear accelerator. Next, the electrons often enter a booster ring, which increases their energy even further before injection into the storage ring. The storage ring is near circular in shape and is designed to allow the electron beam to circulate for as long as possible. Within the storage ring, when electrons are made to follow a curved trajectory, electromagnetic radiation is emitted and the electron loses energy. Thus, the presence of bending magnets and insertion devices (wigglers and undulators) within the storage ring results in the production of electromagnetic radiation (photons) that are released in the forward direction (i.e., at a tangent to the storage ring) (Fig. 1). Depending on the nature of its production (including the device used: bending magnet, wiggler, or undulator), the electromagnetic radiation ranges from infrared to hard X-rays (Fig. 1). It is this electromagnetic radiation (synchrotron light) that is used in experimental “end stations” that are located around the storage ring. The number of end stations at a synchrotron depends on the number of bending magnets and insertion devices, with the end stations differing not only in the energy of the electromagnetic radiation used, but also in the techniques utilized. The electromagnetic radiation produced at synchrotrons is similar to that produced using bench-top sources (e.g., a benchtop infrared microscope).

Fig. 1. (a) A typical synchrotron, consisting of (1) an electron gun, (2) linear accelerator, (3) booster ring, (4) storage ring, and (5) beamline end-station; (b) an undulator (insertion device) in which (1) magnets are used to accelerate (bend) (2) an electron beam to produce (3) synchrotron light; (c) the electromagnetic spectrum, showing the range produced at typical synchrotron facilities.
However, there are several important differences, with synchrotron radiation being (i) extremely bright, with the flux being several orders of magnitude higher; (ii) tuneable; and (iii) highly polarized, being linear, circular, or elliptical.

Regardless of the approach being used, it is necessary to consider whether the X-ray beam is damaging the sample during analysis, as reviewed elsewhere (Lombi et al., 2011b; Terzano et al., 2013b). For example, bulk XAS analyses were found to rapidly change speciation of selenium (Se) by causing photoreduction from Se(VI) to Se(IV) (Wang et al., 2013b). Thus, care needs to be taken to ensure that the findings are valid, with any potential beam damage explicitly investigated.

XRF and XAS

For the study of trace metal(loids) in soils and plants, X-rays (both hard and soft) are of interest. In particular, X-ray absorption and fluorescence are useful due to the photoelectric effect. To describe this effect, consider a sample consisting of a particular elemental atom placed in front of an X-ray photon beam that is steadily increased in energy. Once the photon energy is increased to the binding energy of the core electrons, there is a sharp increase in absorption of the incoming photons and an associated emission of photoelectrons (this energy is referred to as the "absorption edge"). As a consequence, an electron from a higher energy fills the vacancy left by the emission of the photoelectron, this resulting in the release of fluorescence, the energy of which corresponds to the difference between the two electron levels. Thus, X-ray-based approaches characterize either the absorption of incident photons or the resulting fluorescence. The precise photon energy at which emission of photoelectrons occurs varies with the element (Fig. 2).

Using this photoelectric effect, there are two broad approaches of interest, X-ray fluorescence (XRF) and XAS. X-ray fluorescence has been utilized as a laboratory-based approach for many years to examine the elemental composition of bulk samples. Similarly, XRF microscopy (i.e., \(\mu\)-XRF) allows elemental mapping and is possible with a variety of techniques, including energy-dispersive X-ray spectroscopy (EDS) coupled with electron microscopy. However, synchrotron-based \(\mu\)-XRF offers the advantage of improved sensitivity (higher flux) and tuneability.

For \(\mu\)-XRF, the X-ray beam is focused to a small spot size (often \(\leq 5 \mu m\), with some beamlines now approaching \(10 \mu m\)) and the sample "scanned" to obtain an elemental map with a resolution approximately equal to the spot size. In this approach, the incident X-rays are generally of comparatively high energy, such that atoms of all elements with a lower characteristic energy (Fig. 2, 3, and 4) are excited and release photoelectrons. Thus, using this approach, multi-element maps can be obtained comparatively easily.

The second approach is XAS, which allows information to be obtained, normally from bulk samples, on the nature of the bonding environment (i.e., elemental speciation). In this approach, the energy of the incident photon is progressively increased in extremely small increments. As the absorption edge is reached, the absorption (and associated fluorescence) rapidly increases, as characteristic for that element (Fig. 3 and 4). Importantly, the exact position (energy) of the edge, together with the spectral features, typically differ depending on the oxidation state of that element, as well as the bonding environment (for instance the electronegativity of the associated ligand) (Fig. 3 and 4). Thus, XAS can be utilized to examine the oxidation state and binding of trace elements. The XAS spectrum is normally divided into two components, with X-ray absorption near edge spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS). For XANES, the spectrum extends from the pre-edge to \(~50 eV\) above the absorption edge, with the EXAFS spectrum extending beyond this point.

While \(\mu\)-XRF and XAS are the two main approaches of interest, the boundary between the two approaches is not always clear. For example, although \(\mu\)-XRF beamlines are typically used for mapping elemental distribution, because the X-rays are focused to a small spot size, it is also often possible to do XAS analyses at specific points of interest (i.e., \(\mu\)-XAS). Specifically, the small X-ray beam is positioned on the point of interest (typically identified after elemental mapping of the sample), and the energy of the incident photons is progressively increased over the absorption edge to obtain an XAS spectrum. This approach is quite commonly used and not only has the advantage of being able to relate speciation at various points of interest to elemental distribution, but spectra from \(\mu\)-XAS analyses can potentially be easier to interpret, as they often contain only one or two species (in contrast, bulk analyses are typically a mixture of several species).

The recent development of fluorescence-XANES imaging also demonstrates the complementarity between \(\mu\)-XRF and XAS. In XANES imaging, laterally resolved speciation (XANES) maps are obtained by scanning the same area (map) repeatedly at multiple X-ray energies extending above and below the absorption edge (Etzschmann et al., 2014; Kopitke et al., 2014). This approach, in theory, allows for extraction of XANES spectra from every pixel of the map. This differs from the typical \(\mu\)-XAS approach where an elemental map is obtained before individual XANES spectra are collected from limited points of interest. Approaches such as XANES imaging are becoming possible thanks to the recent development of fast XRF detectors that now allow for on-the-fly scanning. For example, the Maia detector at the XFM beamline of the Australian Synchrotron routinely enables transit times per pixel \(<1 ms\), thereby allowing for rapid collection of megapixel images (Paterson et al., 2011). These fast XRF detectors will also open opportunities to repeatedly scan living plants to enable time-resolved studies of trace...
Fig. 3. In X-ray fluorescence microscopy ($\mu$-XRF), the X-rays are focused to a small spot size and the sample “scanned,” with elemental maps obtained for elements with a lower characteristic energy. In X-ray absorption spectroscopy (XAS), the energy of the incident X-rays is increased incrementally across the absorption edge, with the resultant spectrum providing information on speciation. An example is given here for (i) soil As-contaminated particles examined using $\mu$-XRF (examining distribution of As, Ca, and Fe) with the averaged $\mu$-XRF spectrum also shown, and (ii) a comparison of bulk XAS spectra for As(V) and As(III).
metal(loid)s without causing significant beam damage. For instance, in the case of XANES imaging, if a transit time per pixel of 2 ms is used, collection of 100 energy image stacks will translate in a cumulative irradiation of only 200 ms per pixel. This is, at the very least, three orders of magnitude smaller than in conventional µ-XAS.

Finally, it is useful to briefly consider synchrotron-based XRF microtomography. In this approach, intact samples are scanned repeatedly while being rotated. From the resulting two-dimensional sinograms, it is possible to reconstruct a virtual cross-section of elemental distribution (Terzano et al., 2008; Kopittke et al., 2012). Due to the longer scan times required, care must be taken not to cause experimental artifacts (Lombi and Susini, 2009). Although previously used only for samples of low water content (such as seeds), fast XRF detectors now also allow analyses of highly hydrated samples, such as roots (Lombi et al., 2011a).

XRD

The focus of the present review is on XRF and XAS. However, it is useful to briefly examine synchrotron-based X-ray diffraction (XRD) and micro X-ray diffraction analysis (µ-XRD). These approaches are markedly similar to the laboratory-based equivalents, and thus the theory is not discussed in detail. However, compared with laboratory-based systems, synchrotron-based systems have a higher flux, a well-defined wavelength, and better collimation. As a result, synchrotron-based XRD systems offer better resolution of diffraction peaks and better sensitivity (Lombi and Susini, 2009), which is particularly useful for the identification of trace metal(loid)s in soils (Terzano et al., 2007).

Determining the Distribution and Speciation of Trace Metal(loid)s in Soils and Plants

Given the ability of synchrotron-based XAS and µ-XRF to provide in situ information on metal(loid) distribution and speciation in soils and plants (including in hydrated plant tissues) with minimal sample preparation, these approaches are being used increasingly to understand their behavior in soils, their toxicity to plants, and the mechanisms that plants use to tolerate elevated concentrations. Hereafter, we discuss how these synchrotron-based approaches have provided important information for a range of metal(loid)s.
exhaustive, we have focused on the most commonly examined metal(loid)s. The toxicity of engineered nanoparticles is not discussed, but recent comprehensive reviews have been published elsewhere (Capaldi Arruda et al., 2015). Furthermore, distinction is made between hyperaccumulators and non-accumulating plant species, given that their response to elevated levels of metal(loid)s often differs markedly.

**Aluminum**

Aluminum, at ~8%, is the most common metallic element in soils (Sposito, 2008). As soils acidify, soil minerals dissolve, releasing Al into the soil solution. Indeed, soluble Al is the most common factor limiting the growth of plants on acidic soils, which comprise ~3.95 billion ha of the global ice-free land or ~40% of the world’s arable land (von Uexküll and Mutert, 1995; Eswaran et al., 1997). It is estimated that in Australia, for example, ~50% of the agricultural land (i.e., 50 million ha) is impacted on soil acidity, costing AU$1.5 billion yr⁻¹ in lost productivity (NLWRA, 2002; Hajkowicz and Young, 2005).

Unlike the remaining trace metals discussed in this review, the examination of Al requires a soft X-ray beamline due to the low energy of the Al K-edge, 1560 eV (Fig. 2). As a result, analyses are conducted in a vacuum, which for the analysis of plants, requires dehydration of the sample. Surprisingly few studies have used synchrotron-based approaches to examine the distribution or speciation of Al in soils and plants. Doyle et al. (1999) used K-edge XANES to examine the speciation of Al on the thin surface coatings of quartz and feldspar grains in a loess soil (these Al-containing coatings being important because of their sorption reactions, which influence ion behavior and availability), with the authors finding that the Al coating on soil particles was gibbsite-like. In a similar manner, Ildefonse et al. (1994) used K-edge XANES to examine the coordination of Al in imogolite and allophane.

While the focus of studies examining Al in soils tend to be investigations of Al as a structural component (including its effects on ion sorption), for studies of plants, the focus is on its toxicity due to its increased solubility in acid soils. For the Al accumulator tea [Camellia sinensis (L.) Kuntze], Tolrà et al. (2011) examined distribution of Al in leaves using low-energy XRF (LEXRF). These authors found that Al accumulated mainly within the cell walls of the leaf epidermal cells, with almost no Al detectable in the symplast—this finding suggesting that accumulation in the apoplasm represents a tolerance mechanism in tea plants. Similarly, Kopitke et al., (2015) used LEXRF and found that Al accumulated almost exclusively in the roots of soybean [Glycine max (L.) Merr.] exposed to Al for as little as 30 min. These authors identified the primary mechanism by which Al rapidly reduces root elongation, demonstrating that the binding of Al to the cell wall in roots of soybean causes an inhibition of the loosening of the cell wall as required for anisotropic cell elongation and root growth.

**Chromium**

Chromium (Cr) is used in various industrial processes, including for stainless steel and chromate plating, in pigments, and as a wood preservative (chromated copper arsenic, CCA). It has a range of oxidation states, with Cr(III) and Cr(VI) being the main forms. Given its higher mobility, Cr(VI) is generally considered more toxic than Cr(III), with the remediation of contaminated soils often involving the transformation of Cr(VI) to Cr(III). In most soils, Cr(III) tends to be the dominant form, with concentrations of this form substantially higher than for Cr(VI). For example, using bulk EXAFS, Landrot et al. (2012) examined a soil contaminated by leather tanning industry and reported that Cr(III) was the dominant form, with only low levels of Cr(VI). Furthermore, these authors used μ-XRF to examine the distribution of Cr within the soil, finding that much of the Cr was present as Cr hotspots, with some of these hotspots being associated with high concentrations of iron (Fe [presumably Fe hydridoxides]). Studying an industrially contaminated soil, Terzano et al. (2007) reported that Cr was present largely as chromite. In a similar manner, Szczechowski et al. (1997) examined contaminated soils from the United States and found that <10% was present as Cr(VI) in most samples, while Hopp et al. (2008) examined a CCA contaminated soil and reported that Cr was present as Cr(III), either in a recalcitrant phase (such as residual CCA-treated wood) or in a mixed Fe(III)/Cr(III) solid phase. This reduction of Cr(VI) to Cr(III), such as during the remediation of a contaminated soil, can occur due to the influence of organic matter. Jardine et al. (1999), for example, examined a contaminated waste storage area and reported that, while both Cr(VI) and Cr(III) were present, the rate at which Cr(VI) was reduced to Cr(III) increased in soils with higher levels of organic matter. In a similar manner, Lee et al. (2006) examined Cr(VI)-spiked soils and found that the addition of compost resulted in the reduction from Cr(VI) to Cr(III), with a concomitant decrease in Cr availability. In contrast with this organic-matter-induced reduction of Cr(VI) to Cr(III), some soil components have been found to promote the oxidative conversion of Cr(III) to Cr(VI). For example, Fandeur et al. (2009) used bulk XANES to demonstrate that, while Cr(VI) only accounted for 20% of the total Cr in the soil, it was present exclusively in association with Mn-oxides, thereby suggesting that Mn-oxides have a role in oxidizing Cr(III) to Cr(VI).

Regardless of whether it is present as Cr(III) or Cr(VI) in the soil solution, Cr accumulates almost exclusively as Cr(III) within plant roots, this being demonstrated in both soil-based systems and nutrient solutions, including for Indian mustard [Brassica juncea (L.) Czern.] (Bluskov et al., 2005), subterranean clover (Trifolium brachycalyximus L.) (Howe et al., 2003), Silene vulgaris (Moench) Garcke (Pradas del Real et al., 2014), Protopis L. spp. (Aldrich et al., 2003), Convolvulus arvensis L. (Montes-Holguin et al., 2006), and Parkinsonia aculeate (Zhao et al., 2009). The first study to demonstrate this conversion to Cr(III) using bulk XANES was Zayed et al. (1998a), who examined a wide range of vegetable crops. Whether the transformation from Cr(VI) to Cr(III) occurs within the rhizosphere or rapidly on uptake by the root is unclear (Pradas del Real et al., 2014), but regardless, concentrations of Cr(VI) in root tissues are often sufficiently low that they are not easily detectable using synchrotron-based approaches. Within root tissues, this Cr(III) is often complexed with simple organic acids (Howe et al., 2003; Bluskov et al., 2005; Zhao et al., 2009), although Pradas del Real et al. (2014) found that Cr accumulated in the apoplasm of the root apical tissues.

Upon its translocation to the shoots, Cr(III) remains the dominant form, often remaining complexed by simple organic acids (Howe et al., 2003; Bluskov et al., 2005; Zhao et al., 2009).
Within the leaves, Cr tends to accumulate in the leaf margins, as shown in Subterranean clover (Howe et al., 2003) and Silene vulgaris (Pradas del Real et al., 2014). Furthermore, Pradas del Real et al. (2014) used μ-XRF to identify that concentrations of Cr were highest in the mesophyll cells (both palisade and spongy) of the leaf tip. These authors hypothesized that the accumulation of Cr in the apoplast is a tolerance mechanism.

Manganese

Manganese is essential for plant growth, but concentrations often increase to toxic levels in acid or waterlogged soils high in Mn minerals. The three most common oxidation states of Mn are Mn(II), Mn(III), and Mn(IV), forming various oxides, carbonates, silicates, sulfates, and phosphates. Given that the solubility of Mn(II) is substantially higher than Mn(III) or Mn(IV), the oxidation state is important in influencing concentrations of soluble Mn (Lindsay, 1979).

For well-aerated soils, the majority of Mn is typically present as either Mn(III) or Mn(IV), with overall concentrations of Mn(II) generally low. For example, in surface soils from Indiana (USA), Mn(IV) was found to account for the majority of the Mn, with Mn(II) accounting for 26 to 44% (Schulze et al., 1995). Similar results have also been reported by Guest et al. (2002), with Mn(II) accounting for 0 to 10% of the total Mn in oxidized soil. However, it is known that pH is important in influencing the oxidation state of Mn in these well-aerated soils, with acidic conditions tending to favor Mn(II). This was shown elegantly by Negra et al. (2005) who examined soils from Vermont (USA) and reported that the proportion of Mn as Mn(II) increased linearly with decreasing pH (the pH values examined ranged from 4.4 to 7.1). Similarly, Hernandez-Soriano et al. (2012) found that, at low pH (4.7), most Mn was present as Mn(II), but that for soils with pH > 6, Mn(III) and Mn(IV) dominated. Not only does pH influence the oxidation state of Mn, but it is also well known that waterlogging is important, with anaerobic conditions favoring the formation of Mn(II). For example, in the surface soils from Indiana in which Mn(II) initially accounted for only 26 to 44% (Schulze et al., 1995), saturation and reduction resulted in complete conversion to Mn(II). Similarly, Hernandez-Soriano et al. (2012) found that waterlogging reduced Mn(III) and Mn(IV) and increased solubility, and Guest et al. (2002) found that, while Mn(II) accounted for 0 to 10% in oxidized soils, it accounted for 91 to 100% in flooded reduced soils.

Uptake of Mn from the soil solution occurs as Mn$^{2+}$ (due to its higher solubility compared with Mn(III) and Mn(IV)), with uptake depending on the Mn concentration in the soil solution (Hernandez-Soriano et al., 2012). Using μ-XRF, Kopittke et al. (2013) demonstrated that uptake of Mn is rapid in roots of the model plant, cowpea [Vigna unguiculata (L.) Walp.], with Mn observed to be accumulating in the root cap and mucigel within 5 min of exposure. This Mn that accumulated within the root tissues was present as Mn(II), being associated with citrate or malate (Kopittke et al., 2013; Blamey et al., 2015). Although Mn can accumulate to high concentrations in root tissues, Mn exerts its toxic effects in the shoots (not the roots), and thus most research has focused on the aboveground tissues. Of particular interest, Blamey et al. (2015) studied four plant species differing markedly in their tolerance to Mn in the root environment and found that tolerance was unrelated to Mn concentrations or speciation in roots (Mn(II) dominating in all plant species), nor was it related to bulk concentrations of Mn in shoot tissues. Rather, Blamey et al. (2015) demonstrated that tolerance was associated with effective sinks for Mn in leaves, preventing Mn accumulation in the cytoplasm and apoplastic. The data suggest that non-accumulating plant species lack effective sinks and that the Mn accumulates in the apoplast, where it oxidizes to Mn(III) and forms necrotic lesions (Blamey et al., 2015). In contrast, Mn-accumulating plant species (including hyperaccumulators) are able to sequester excess Mn in effective sinks, such as in trichomes or vacuoles. Indeed, sequestration of Mn in trichomes has been shown to be important in a range of plant species, including in sunflower (Helianthus annuus L.) (Blamey et al., 2015), Alyssum murale Waldst. & Kit. and Alyssum corymbum Duby (Broadhurst et al., 2009; McNear and Kupper, 2014), Arabidopsis halleri L. and Arabidopsis lyrata L. (Sarret et al., 2009), Brassica juncea (Freeman et al., 2006b), and cucumber (Cucumis sativus L.) (Tomasi et al., 2014). Within trichomes, Mn has been found to accumulate as both Mn(II) (Broadhurst et al., 2009) and Mn(III) (Blamey et al., 2015). In a similar manner, accumulation of Mn(II) in vacuoles has also been shown to be important for Mn tolerance, including in white lupin (Lupinus albus L.), where the Mn(II) is associated with malate or citrate (Blamey et al., 2015). Indeed, complexation of Mn with simple organic ligands has been shown to be an important mechanism of tolerance in hyperaccumulators (Xu et al., 2008; Fernando et al., 2010).

Cobalt

Cobalt (Co) is present at naturally high concentrations in soils formed from ultramafic minerals, although elevated concentrations can also form as a result of human activities. The most common oxidation states are Co(II), Co(III), and Co(IV), with the latter being the least common of these three forms. It has been suggested that Mn oxides play an important role in the oxidation of Co(II) to Co(III). For example, examining sediment, the importance of Mn oxides was confirmed by Kay et al. (2001), who reported that Co(II) was oxidized to Co(III) and incorporated into Mn oxides, with only 10 to 20% remaining as Co(II). Similarly, using Co(II)-spiked soils from Europe and Australia, Beak et al. (2011) found that the distribution of Co was not homogenous, but rather, it was closely associated with the Mn- and Fe-oxide fractions as either Co(II) or as mixed Co(II) and Co(III). Furthermore, these authors reported that Co in close proximity to roots of rice (Oryza sativa L.) redistributed to a Co-precipitate.

In plants, it has been reported that, on uptake by roots of Alyssum murale, Co tended to accumulate in the rhizodermis and outer cortex (Tappero et al., 2007). In wheat (Triticum aestivum L.) and tomato (Solanum lycopersicum L.), the Co that accumulated in the roots remained as Co(II), having been complexed by carboxylate-containing organic acids (Collins et al., 2010). Upon its movement to the shoots, Co speciation remained similar to that in the roots (Collins et al., 2010), with Co accumulating as Co(II) near the leaf tips and margins (which is in contrast to nickel [Ni]) (Tappero et al., 2007).

Nickel

Concentrations of Ni are often two orders of magnitude higher in soils formed from ultramafic (serpentine) minerals than in soils formed from other parent materials (Kabata-Pendias,
Within soils, Ni is typically associated either with primary minerals, organic matter, or (hydr)oxide surfaces. For example, McNear et al. (2007) used µ-XRF to examine the distribution of Ni in a soil enriched from the aerial deposition of Ni from a refinery. These authors found that much of the Ni was present as discrete particles of NiO (from the refinery), with these dominating the Ni forms. However, these authors also examined the diffuse Ni (i.e., non-hotspot) areas and found that Ni- and Al-layered double hydroxide phases were present (leading to a reduction in Ni mobility), while for soils high in organic matter, much of the diffuse Ni was bound to organic matter. In a soil containing naturally high levels of Ni, Levard et al. (2009) reported that ~75% of the Ni was bound to short-range ordered aluminosilicates. Finally, Mamindy-Pajany et al. (2014) used µ-XRF and µ-XANES to examine a spiked agricultural soil to which biosolids were applied, with Ni found to accumulate in well-defined hotspots. Using µ-XANES, these authors estimated that 72% of the Ni was associated with organic matter, 19% as Ni(OH)$_2$, and 9% was associated with Fe-oxides.

Despite their importance, surprisingly few studies have examined the distribution of Ni in non-accumulating plant species. Using µ-XRF to study the annual rings in woody tissue of black willow (Salix nigra Marshall) grown in a contaminated soil, Punshon et al. (2003, 2005) found that Ni was conservatively located in the annual rings, thereby demonstrating a sudden onset and cessation of uptake together with a lack of post-growth mobility. Analysis of the localized Ni-enriched regions in xylem vessels within these woody tissues showed that the Ni was present as Ni(II), with the spectra showing similarities to those of Ni-pectic acid complexes, Ni-histidine, and NiSO$_4$. In another non-accumulator, cowpea, Kopittke et al. (2011) used µ-XRF to examine the distribution of Ni in fresh (hydrated) roots, finding that Ni concentrations were high in both the cortex and the meristem, while concentrations in the stele were comparatively low. Kramer et al. (2000) compared a non-accumulator (Thlaspi arvense L.) with a hyperaccumulator (as discussed below) and found that, for the leaves of the non-accumulator, Ni was complexed by glutamine (39%), histidine (36%), and citrate (25%). Finally, Tomasi et al. (2009) examined leaves of tomato and found that Ni was present mainly in the primary and secondary veins.

In contrast with non-accumulators, organic acids appear to play an important role in the sequestration and storage of excess Ni. First giving consideration to the roots, Tapper et al. (2007) used microtomography to examine Ni distribution in roots of the hyperaccumulator Alyssum murale and reported that Ni concentrations were highest near the meristem, while at a distance of 6 mm from the apex, the Ni tended to accumulate in the rhizodermis. Within these roots of Alyssum murale, it has been found that Ni is complexed with simple organic acids (citrate and malate)—this is also observed in roots of Leptopax emarginata (Boiss.) O.E. Schulz and Thlaspi caerulescens Presl & Presl (Montargès-Pelletier et al., 2008). From the roots, Ni is translocated to the shoots through the xylem, with Ni in the xylem sap reported to be complexed by histidine (Kramer et al., 1996; McNear et al., 2010). However, it is the accumulation of elevated levels of Ni in the leaves that are of particular interest, with tolerance of hyperaccumulators to excess Ni seeming to result from the ability to sequester Ni within the vacuoles, where it is complexed by simple organic acids. For example, Kramer et al. (2000) compared Thlaspi goesingense Halácsy with the non-accumulator Thlaspi arvense. For the accumulator, much of the Ni accumulated within the cell wall, 98% of the remaining Ni was complexed with citrate, presumably within the vacuole. In contrast, for the non-accumulator (Thlaspi arvense), Ni was complexed with glutamine, histidine, and citrate, indicating that a higher proportion of Ni accumulates in the cytoplasm of this species. Indeed, the importance of simple organic acids in hyperaccumulators has also been identified in Alyssum murale (Montargès-Pelletier et al., 2008; McNear et al., 2010), as well as in Leptopax emarginata and Thlaspi caerulescens (Montargès-Pelletier et al., 2008). Interestingly, Ni and Mn have been found to be strongly spatially correlated in Alyssum murale and Alyssum corsicum, suggesting that Ni hyperaccumulation potentially results from a Mn-handling system (Broadhurst et al., 2009).

**Copper**

Elevated levels of copper (Cu) can result from contamination by mines and smelters, as well as the widespread use of Cu-containing fungicides or from municipal wastes. It is known that Cu binds strongly to sulfur (S), with its principal minerals being chalcopryrite (CuFeS$_2$), bornite (Cu$_3$FeS$_4$), chalcocite (Cu$_2$S), and covellite (CuS) (Kabata-Pendias, 2011). Copper is found in the environment as three oxidation states, Cu(0), Cu(I), and Cu(II), with the latter being the most common.

In soils, data from in situ XANES analyses indicate that in well-aerated systems, Cu tends to bind strongly to organic matter. For example, in two vineyard soils from France, Jacobson et al. (2007) reported that Cu was commonly present in hotspots within the soil matrix (i.e., its distribution was highly heterogeneous), with the hotspots being associated with organic matter, rather than clay minerals or Fe (hydr)oxides. Similarly, in six Cu-contaminated soils (three spiked, three “naturally” contaminated), Strawn and Baker (2009) found that the Cu hotspots were not associated with calcium (Ca) carbonates, Fe oxides, or Cu sulfates. Instead, results from bulk analyses indicated that much of the Cu was associated with organic matter. Similar results were also found by Strawn and Baker (2008) in calcareous agricultural soils. In mine tailings with very low concentrations of organic matter, Cu was present as Cu(II) and was generally associated with Fe oxides (Yang et al., 2014). In flooded (anaerobic) soils, it has been reported that at least some of the Cu is present as Cu(I), such as in paddy soils (Lin et al., 2010). In a similar manner, Weber et al. (2009) found that, in aerobic soils, 95% of Cu was present as Cu(II) complexed to organic matter, but after 16 d of flooding, the Cu was converted to Cu(I) sulfide precipitates. Although these studies on bulk soils are important, it has been found that the speciation of Cu can change within the rhizosphere. For example, in the study of Lin et al. (2010) examining flooded soils, due to its higher redox potential (Eh), the proportion present as Cu(I) in the rhizosphere decreased due to oxidation to Cu(II). Manseau et al. (2008) reported the formation of Cu nanoparticles in the rhizosphere of wetland plants.
Zinc contamination of soils can result from mining and smelting activities or from the application of Zn-enriched municipal wastes, with Zn commonly present as Zn(II). For soils containing anthropogenically elevated levels of Zn (such as those contaminated from smelters), the Zn is often found to be in the form of outer-sphere Zn, Zn-illite, Zn-kaolinite, and humic-acid-Zn. Upon translocation to the shoots, the speciation of Cu seems to vary between species, with either O- or S-containing ligands of importance. In the hyperaccumulating water plant Crassula helmsii A. Berger, Cu was found to be associated almost exclusively with O-containing ligands, such as organic acids, rather than S-containing ligands (Kupper et al., 2009). As for the root tissues, Polette et al. (2000) reported small amounts of Cu as Cu(I) in shoot tissues of tomato and oat, with both O- and S-containing ligands. Similarly, after displacement of apoplastically bound Cu, Ryan et al. (2013) reported that S-coordinated Cu(I) species dominated in tomato and oat. Finally, Mijovilovich et al. (2009) examined Thlaspi caerulescens, a hyperaccumulator for cadmium (Cd) and zinc (Zn) but not Cu, and reported that Cu was bound with S-containing ligands, possibly metallothioneins.

Zinc contamination of soils can result from mining and smelting activities or from the application of Zn-enriched municipal wastes, with Zn commonly present as Zn(II). For soils containing anthropogenically elevated levels of Zn (such as those contaminated from smelters), the Zn is often found to be in the form emitted from the source. For example, Scheinost et al. (2002) studied soils near a smelter in the United States and found that, in the topsoil, the Zn was present as smelter-emitted minerals (franklinite and sphalerite). This contrasted with the subsoil, in which the dominant forms were outer-sphere Zn, in addition to Zn incorporated in phyllosilicates. In a similar manner, Nachtegaal et al. (2005) reported that much of the Zn in a contaminated soil from Belgium was smelter related, although appreciable Zn was also incorporated into newly formed Zn precipitates (also see Roberts et al., 2002; Van Damme et al., 2010). However, the speciation of Zn in contaminated soils also depends on organic matter content, with increasing levels of organic matter increasing Zn binding. For example, after spiking soils with soluble Zn, Fan et al. (2016) found that the main forms were outer-sphere Zn, Zn-illite, Zn-kaolinite, and humic acid-Zn, with the proportion of humic acid-Zn increasing with increasing soil organic matter content. Indeed, examining the organic horizon (37% carbon [C]) of a soil in close proximity to a smelter, Sarret et al. (2004) found that while Zn primary minerals (franklinite, sphalerite, and willemite) accounted for ~15% of total Zn, most Zn was associated with organic matter or as outer-sphere complexes. Seemingly in contrast to these findings, however, Terzano et al. (2008) found that the addition of compost (at a rate equivalent to 60 Mg ha$^{-1}$) to a Zn-contaminated soil did not significantly influence Zn speciation or availability. Finally, Khaokaew et al. (2012) studied the effect of Eh (flooding) in contaminated paddy soils, with the authors reporting that there was almost no change in Zn speciation regardless of the flooding or draining period, with Zn present as Zn-layered double hydroxides and Zn-phyllolysilicates.

Upon growth of plants in soils containing elevated levels of Zn, uptake occurs as Zn$^{2+}$ from the soil solution. We are aware of only three studies that have investigated Zn non-hyperaccumulators, with complexation by phosphorus (P)-containing ligands seeming to be important in these species. In roots, Zn tends to accumulate as Zn-phytate in roots of Eruca vesicaria (L.) Cav., cowpea, and Arabidopsis lyrata (Terzano et al., 2002; Terzano et al., 2008; Kopittke et al., 2011). In Eruca vesicaria, this Zn accumulated outside the root endodermis with some Zn translocated in the xylem as Zn-citrate (Terzano et al., 2008), while in roots of cowpea, Zn accumulated mostly in the meristematic region (Kopittke et al., 2011; Wang et al., 2013a). Upon translocation to the shoots, ~50% of the Zn precipitated as Zn-phosphates, with the remaining Zn complexed by cysteine and histidine (Terzano et al., 2008) (also see Sarret et al., 2002).

In contrast to non-accumulating species, hyperaccumulators tend to have efficient mechanisms for storing excess Zn, particularly in the leaf tissues, where complexation with organic acids seems to be important (compared with the formation of Zn-phosphate in non-accumulating species). For example, it has been reported that in Nicotiana caerulescens (Thlaspi caerulescens), Zn in the roots is complexed with histidine before transport as Zn$^{2+}$ and sequestration in the leaves through complexation with organic acids (Salt et al., 1999; Monsant et al., 2011). Although increased supply of NO$_3^-$ increased Zn uptake in this species, this could not be attributed to changes in speciation of Zn within plant tissues (Monsant et al., 2011). Confirming the importance of complexation with organic acids, Sarret et al. (2009) found that the tolerance of Arabidopsis thaliana × Arabidopsis lyrata progeny to Zn was related to the proportion of Zn complexed to organic acids. Furthermore, these authors found a negative correlation between the vein:tissue fluorescence ratio and Zn accumulation, indicating a higher xylem unloading in leaves of stronger accumulators. The importance of organic acids in leaves of Arabidopsis thaliana has also been demonstrated by Sarret et al. (2002).

In addition to their complexation with P-containing compounds (non-accumulators) or organic acids (hyperaccumulators), it has also been reported that Zn accumulates at the base of trichomes in a manner similar to that described earlier for Mn and Ni. This has been reported for Zn in both non-accumulators such as Arabidopsis lyrata (Sarret et al., 2009) and tobacco (Nicotiana tabacum L.) (Sarret et al., 2006; Straczek et al., 2008), as well as in hyperaccumulators such as Arabidopsis thaliana (Sarret et al., 2002; Fukuda et al., 2008). Interestingly, for the trichomes of tobacco, which are glandular, Zn-substituted calcite precipitates at the head of the trichomes as an apparent mechanism of detoxification (Sarret et al., 2006; Straczek et al., 2008).

Arsenic

Groundwaters in different areas of the world contain elevated levels of arsenic, especially in the Indian subcontinent. These
high-arsenic (As) waters are of concern not only due to their direct ingestion, but also because of their irrigation for crop production. Indeed, an estimated 0.9 to 1.36 Gg of As is brought onto arable land annually (Ali, 2003), representing a health concern in regions with high grain consumption, particularly rice (Carey et al., 2012). Soils may also be contaminated through other processes, including mining and smelting. The most common oxidation states are As(III) and As(V).

In well-aerated soils, As is dominated by As(V), with most of this As(V) being adsorbed to Fe-(hydr)oxides. For example, using μ-XRF to investigate an aerobic soil from a contaminated rice paddy in West Bengal (India), Kramar et al. (2016) found that As was present in hotspots within soil aggregates and was mainly associated with Fe-(oxy)hydrxides. Acton et al. (2005) examined a soil in the United Kingdom contaminated by mining and processing, with As(V) being the dominant species, most likely associated with hydrous oxides of Fe and adsorbed on Al (hydr)oxides. Similar results have also been found for soils historically contaminated from the manufacture of pesticides and herbicides (Cancès et al., 2005, 2008; Niazi et al., 2011) and in an As-containing acid sulfate soil (Strawn et al., 2002). While As(V) dominates in aerobic soils, decreases in Eh result in the increased formation of As(III). In a peat soil, Langner et al. (2013) found the formation of realgar and arsenopyrite in this strongly reducing environment, with As(III)-NOM (natural organic matter) complexes formed due to sorption of As(III) to NOM. Similarly, in a paddy soil from Japan that had been contaminated from mining activities, Yamaguchi et al. (2011) found that flooding decreased Eh and caused an increase in As(III), with this form accounting for up to 80% of total As. Importantly, this decrease in Eh and formation of As(III) resulted in an increase in soluble As concentrations. Furthermore, these authors found that microbial activity was necessary for the reduction of As(V) to As(III), even when the Eh reached the required value.

Arsenic distribution and speciation can change markedly within the rhizosphere, particularly within anaerobic soils. Of particular interest, it has been shown that the release of O₂ into the rhizosphere of waterlogged soils can result in formation of an Fe-rich plaque (ferrihydrite) surrounding the root system, with the concomitant oxidation of As(III) to As(V), which then adsorbs strongly to the Fe-plaque (Liu et al., 2006; Seyffert et al., 2010; Frommer et al., 2011). However, whether the Fe-plaque acts as a sink for As (thereby decreasing its uptake) or whether it increases uptake of As by accumulating As adjacent to the root surface remains unclear.

In plants, uptake of As(V) occurs via the phosphate pathway, while As(III) is assimilated via the silicic acid transport system (Asher and Reay, 1979; Ma et al., 2008). Regardless of whether As(V) or As(III) is supplied in the rooting medium, it is As(III) that is commonly observed within the root tissues, thereby indicating the efficient reduction of As(V) to As(III) in planta. For example, using fluorescence XANES imaging to examine roots of cowpea, Kopittke et al. (2014) found that As(V) was rapidly reduced to As(III) within the root, with As(V) only identified within the rhizodermis. However, this As(III) is generally rapidly complexed, with Kopittke et al. (2014) reporting that there was no uncomplexed As(III) found in root tissue due to the efficient formation of the As(III)-thiol complex (also see Pickering et al. 2000; Kopittke et al., 2012). This rapid complexation of As(III) reduces translocation to the aboveground portions, as it is the inorganic forms [mainly As(III)] that are present in the xylem and phloem (Pickering et al., 2000). This movement in the phloem is particularly important for the accumulation of As within rice grains, with As present mainly in inorganic forms [As(III)] and as methylated As (particularly dimethylarsinic acid) (Meharg et al., 2008; Lombi et al., 2009).

Several studies have also investigated the As hyperaccumulator, Pteris vittata L., which can accumulate As at a concentration of up to 23,000 mg kg⁻¹ (Ma et al., 2001). As observed for the non-hyperaccumulators (above), even when supplied with As(V) in the rooting medium, As within the tissues of Pteris vittata accumulates as As(III) (Hokura et al., 2006a). Lombi et al. (2002) found that ~96% of the As that accumulated within this hyperaccumulator was present in the fronds, with ~75% of this being inorganic As(III) (Webb et al., 2003). Furthermore, Pickering et al. (2006) found that the As is excluded from the cell walls, rhizoids, and reproductive areas, with the As in the gametophytes compartmentalized in the vacuole.

Selenium

Selenium is an essential micronutrient in animals, with cereals and nuts being the main sources of dietary Se. It is commonly found in four oxidation states: Se(-II), Se(II), Se(IV), and Se(VI), with Se(-II) dominating in organic Se compounds. However, in alkaline and well-oxidized soils, it is known that Se(VI) dominates, while in well-drained soils at acidic to neutral pH values, Se(IV) is the main form. However, few studies have used synchrotron-based approaches for the examination of Se in soils. Strawn et al. (2002) examined an acid sulfate soil material at pH 4 and found that Se(VI) was the dominant form. On a reclaimed mine site in the United States, Ryser et al. (2006) found that much of the Se was still present as the primary Se-bearing minerals, although the Se was progressively oxidizing to also form Se(IV) and Se(VI). Factors that influence Eh (either in the bulk soil or in the rhizosphere) will influence Se speciation. For example, it was found that the reduction of Se(VI) was higher in the regions of higher microbial activity adjacent to decomposing roots (Sutton et al., 1995).

In contrast with many of the other metal(loid)s examined here, a comparatively large amount of research has investigated Se in non-accumulating plants, presumably due to the importance of Se in animal nutrition. The uptake of selenite tends to be slower than for selenate, resulting in lower concentrations of Se in root tissues of selenite-exposed plants than selenate-exposed plants. Furthermore, for selenite, the Se is rapidly converted to organic forms. For example, Wang et al. (2013b) reported that 100% of the Se within roots of cowpea exposed to selenite was present as organoselenium, with similar findings in a range of other plant species (Zayed et al., 1998b; Cruz-Jímenez et al., 2005; Bulska et al., 2006). Indeed, in roots of wheat and rice, Wang et al. (2015) used fluorescence XANES imaging to provide laterally resolved data on Se speciation and found that, even in the rhizodermis, only a small proportion of Se was present as uncomplexed selenite, despite this tissue being exposed directly to the selenite in the external solution. Although Se in selenate-exposed plants is also converted to organoselenium, it must first be reduced to selenite, with this reduction from selenite to selenate being the rate-limiting intermediate step. This is evidenced by the observation that, for selenate-exposed roots,
much of the Se typically remains as the uncomplexed selenate, and although some Se is present as organoselenium, the tissue concentrations of the intermediate Se(IV) are often undetectable (de Souza et al., 1998; Zayed et al., 1998b; Cruz-Jimenez et al., 2005; Bulska et al., 2006; Wang et al., 2013b). Accordingly, Pilon-Smits et al. (1999) found that ATP sulfurylase not only mediates selenate reduction, but that it is also the rate-limiting step. Unsurprisingly, increased Se concentrations in leaves of selenate-exposed plants led to increased Se distribution in the leaf tissues as organoselenium in selenate-exposed plants, and although organoselenium often dominates in leaves of selenate-exposed plants, the leaf tissues may also contain appreciable quantities of uncomplexed Se(VI) (Zayed et al., 1998b; Bulska et al., 2006; Wang et al., 2015). Similarly, organoselenium has been shown to be the dominant form in seed, including in Brassica and in rice, with Se primarily located in the cotyledons and roots of seed embryos (Li et al., 2010; Sun et al., 2010).

In hyperaccumulators, Se behavior appears to be somewhat similar to that discussed above for non-accumulators. Indeed, El Mehdayi et al. (2012) compared two hyperaccumulators [Astragalus bisulcatus (Hook.) A. Gray and Stanleya pinnata (Pursh) Britton] with two nonaccumulators (Astragalus drummondii Douglas ex Hook. and Stanleya elata M.E. Jones) grown on seleniferous and non-seleniferous soils, and in all instances, the majority of Se accumulated as organoselenium. Similarly, in leaves of Stanleya pinnata, Se was present almost exclusively as MeSeCys (Freeman et al., 2006a). In the hyperaccumulator Astragalus bisulcatus, Se was present mainly as organic (C-Se-C) compounds in roots, stems, and leaves (Valdez Barillas et al., 2012). An important difference in Se speciation between hyperaccumulators and non-accumulators is potentially the form of organoselenium present (e.g., LeDuc et al., 2004), although XAS techniques often lack the sensitivity to differentiate between such forms. While speciation is potentially similar in hyperaccumulators and non-accumulators, the distribution of Se appears to differ (Freeman et al., 2006b). In the leaves of the hyperaccumulator Astragalus bisulcatus, Se was found to accumulate largely within trichomes (Freeman et al., 2006b). In contrast with these hyperaccumulators, within leaves of both Brassica juncea and Arabidopsis thaliana (L.) Heynh, Se was observed to accumulate within the vascular tissues and mesophyll cells (Freeman et al., 2006b). In flowers of the hyperaccumulator Stanleya pinnata, Quinn et al. (2011) found that Se accumulated in the ovules and pollen, while in the non-hyperaccumulator Brassica juncea, Se accumulated comparatively evenly throughout the organ.

Cadmium

The anthropogenic release of Cd into the environment is a substantial environmental problem, occurring through metal refining, disposal of mine wastes or municipal wastes, or through the use of P fertilizers in agricultural lands. Like Zn (which is also in Group 12 of the periodic table), Cd is most commonly found as Cd(II), with Cd and Zn also having similar ionic structures, electronegativities, and chemical properties (Kabata-Pendias, 2011). Unlike many other metal(loid)s, Cd contamination is generally of concern due to its potential to accumulate to levels in plant tissues that are toxic to animals but not to the plant itself.

Organic matter content is known to be important in controlling Cd speciation in soils. For example, Khosla et al. (2011) examined Cd-Zn co-contaminated alkaline paddy soils, with at least 50% of the Cd associated with organic matter in aerobic soils. However, on flooding, Cd-carbonates became the dominant species, with some Cd also present as Cd-sulfide after prolonged flooding. Interestingly, these authors also used µ-XRF to demonstrate that Cd was more closely associated with Ca than with Zn, despite the co-contamination of the soil. The Cd content of soils is also important in influencing speciation. Furuya et al. (2016) examined paddy soils and found that, in a high-S soil, flooding increased CdS formation (30% after 4 d and 90% after 29 d), whereas in the low-S soil, CdS did not exceed 35%. Subsequent aeration of the soil decreased the CdS, but it did not dissolve completely.

Synchrotron-based studies have also investigated the remediation of Cd-contaminated soils. Hashimoto and Yamaguchi (2013) examined the influence of zerovalent Fe on Cd speciation in a spiked soil at pH 6. These authors found that at least 55% of the Cd was initially associated with soil colloids (kaolinite, ferrihydrite, and humus) rather than as precipitates (hydroxide, carbonate, and sulfate), with the addition of zerovalent Fe decreasing the redox potential and increasing the formation of CdS (similar results have also been reported by Hashimoto et al., 2016). The use of P-containing amendments have also been investigated for the remediation of Cd-contaminated soils, with Siebers et al. (2013) reporting that the addition of either bone char or triple superphosphate to soil resulted in the formation of insoluble Cd-phosphates.

In non-accumulating plant species, Cd is generally complexed with either S- or O-containing ligands. In Arabidopsis thaliana, Is aure et al. (2006) reported that Cd accumulated in the vascular bundles of the roots and was complexed with S-containing ligands. However, in the leaves of this species, the Cd accumulated in trichomes and was complexed with either O or nitrogen (N) ligands. Similarly, Harada et al. (2010) examined willow (Salix spp. L.) and found that Cd accumulated at the tips of the serrations in leaves, with µ-XANES indicating complexation by O ligands. In the non-hyperaccumulator Indian mustard (Brassica juncea), Salt et al. (1995) reported that Cd in the root was complexed with S, while Cd in the xylem sap was complexed with either O or N. Similar findings were reported by Salt et al. (1997).

Complexation of Cd with S- and O-containing ligands is also of importance in hyperaccumulating species. For example, Cheng et al. (2016) examined two hyperaccumulators, Carpobrotus rosulli (Haw.) Schwantes and Solanum nigrum L., using bulk XAS and found that the majority of Cd was complexed with S-containing ligands in all tissues, with the exception of the xylem sap, which contained Cd either as uncomplexed Cd²⁺ or complexed to simple organic acids. Interestingly, although increases in NH₄ + increased accumulation of Cd, they did not change Cd speciation within the plant tissues. In a similar manner, Küber et al. (2004) examined the Cd hyperaccumulator Thlaspi caerulescens using bulk XAS, finding that S-containing ligands are more important in young leaves than in old leaves, with ~35% of Cd bound to S ligands in young leaves (~45–55% O ligands and 10–20%
histidine) compared with 15 to 35% S in mature leaves (and 35–65% O ligands). These authors suggested that Cd is detoxified by sequestration in vacuoles, particularly in mature leaves. Vacuolar sequestration has also been suggested as being important in the hyperaccumulator *Selaginella arenicola*, with Tian et al. (2011) finding that Cd was primarily associated with O ligands and, in particular, malic acid, indicating vacuolar sequestration. Trichomes have also been reported to be important for the sequestration of Cd, including in *Arabidopsis thaliana*, where Cd accumulates in the trichomes as Cd(II) bound to either O or N ligands (Hokura et al., 2006b; Fukuda et al., 2008). In seeds of the hyperaccumulator *Thlaspi praecox* Wolfen, approximately two-thirds of the Cd was complexed with S-containing ligands, with some also associated with P (phytate) (this contrasting to the speciation in roots and shoots, where up to 80% was complexed with O) (Vogel-Mikuš et al., 2010).

**Conclusions and Perspectives**

The present review has demonstrated the utility of synchrotron-based approaches to examine the distribution and speciation of metal(loid)s in soil–plant systems. With the progressive improvement in synchrotron beamlines, the advantages offered by these techniques will continue to grow. Specifically, increasing fluxes and improved detectors and electronics will further decrease acquisition times and provide better resolution. However, especially given that plants (and soils) are hydrated systems, synchrotron-based experiments should explicitly consider whether the analyses are damaging the samples, causing experimental artifacts. As the number of synchrotrons worldwide expands and diversifies, these facilities will become increasingly accessible to a range of scientists. Similarly, the capabilities of laboratory-based X-ray instruments are increasing rapidly, building on the technologies developed at synchrotrons. Indeed, due to the use of improved detectors and optics, together with increasingly brilliant and coherent X-ray sources, these laboratory-based systems are likely to become increasingly useful for the study of trace metal(loid)s in soil–plant systems. Whether using synchrotron- or laboratory-based systems, it is desirable to provide quantitative data. However, soils and plant tissues are highly heterogeneous, being of variable thickness, composition, and density. As a result, full quantification is difficult (Terzano et al., 2013a). Regardless, it is possible to obtain semiquantitative information, as demonstrated using intact roots of tomato and leaves of cucumber (Terzano et al., 2013a).

It is expected that the techniques discussed in the present review will also be beneficial for the study of plant–microbe interactions, including for the study of elemental transformations and sequestration. Although some information is already available in this regard (Valdez Barillas et al., 2012; Lindblom et al., 2013), it is expected that the use of synchrotron-based approaches in combination with microbiome sequencing will lead to new and important insights. Similarly, opportunities exist to study the movement of trace metal(loid)s through the food chain on the consumption of plant tissues by herbivores. Finally, it is comparatively rare that a single experimental approach is able to satisfactorily answer a diverse set of hypotheses. Therefore, it is necessary to consider other experimental approaches that will complement the synchrotron-based techniques considered here, such as nanoscale secondary ion mass spectroscopy (NanoSIMS, which is also able to provide isotopic analyses), laser ablation inductively coupled plasma mass spectrometry (LA-ICP–MS), proton-induced X-ray emission microscopy (micro-PIXE), and electron microscopy.

**Acknowledgments**

We acknowledge funding from the Australian Research Council (ARC) for Future Fellowships for Peter Kopittke (FT120100277) and Erica Donner (FT130101003). The authors acknowledge the assistance of Dr. Gupta and Dr. Grellmann. The support of the XAS and XFM beamlines of the Australian Synchrotron (Clayton, Australia) is acknowledged. Marc Cirera assisted in preparing the illustrations.

**References**


Hokura, Y., A. Kato, N. Suzuki, T. Ohira, M. Otake et al. 2006b. Speciation in roots and shoots, where up to 80% was complexed with O (Vogel-Mikuš et al., 2010).


