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Review

Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy

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Contents

Abstract

Although traditional technologies for cleaning contaminated soils and waters have proven to be efficient, they are usually expensive, labor intensive, and in the case of soil, they produce severe disturbance. More recently, the use of plants in metal extraction (phytoremediation) has appeared as a promising alternative in the removal of heavy metal excess from soil and water. Phytoremediation of polluted waters, is based on the cultivation of aquatic plants such as *Eichhornia crassipes* and *Azolla filiculoides* Lam., which have demonstrated a high capability to absorb cadmium (Cd), copper (Cu), nickel (Ni), and zinc (Zn) from aqueous solutions. Studies have demonstrated that terrestrial plants such as *Brassica juncea*, *Salsola kali*, and *Prosopis* spp. cultivated in hydroponics and agar are able to uptake significant amounts of heavy metals, which indicates their possible utilization in phytoremediation processes. On the other hand, a wide variety of plants have demonstrated the ability to grow and uptake heavy metals from severely polluted sites. Several species of *Thlaspi*, *B. juncea*, *Salix* spp., and *Populus* spp., among others, have been already tested in pilot projects or are currently in commercial application in phytoremediation projects. Recently, researchers have realized that phytoextraction can also be used for the recovery of precious metals such as gold, silver, platinum, and palladium, which indicates the wide possibilities of the phytoremediation technology with regards to mining. X-ray absorption spectroscopy (XAS) consists of two complimentary techniques X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) which provide invaluable chemical information. XAS has been used to investigate a number of different elements within inorganic chemical systems. However, more recently, it has been applied to investigate metal interactions within biosystems. XAS has provided important information on the coordination chemistry of metals and toxic element interactions with phytoremediation systems. XAS has provided information in terms

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of the coordination environment of metals absorbed by plants, their atomic geometry, and the bioreduction of metals within phytoremediation systems. In addition, XAS has provided information about the production of gold and silver nanoparticles by the metal interaction with the plants on phytomining systems.

Keywords: Heavy metal uptake; Phytoextraction; Hyperaccumulator; XAS; EXAFS; XANES; Speciation

1. Introduction

Heavy metal is the generic name given to the group of elements with an atomic density greater than 6 g/cm^3 [1]. While these elements are ubiquitous in the Earth's crust, their concentration and availability in soil and water varies from less than 1000 parts per million (ppm = mg kg^{-1} = mg L^{-1}) to a few parts per billion (ppb = μg kg^{-1} = μg L^{-1}), with the exception of manganese, which is found in soils in concentrations ranging from 20 to 10,000 ppm [1]. Metalliferous soils, however, can contain extremely high amounts of certain elements. For example, as seen in Table 1, the normal range of copper (Cu) in soil is 2–250 mg kg^{-1}, but it has been reported that some metalliferous soils of Zaire can contain up to 50,900 mg kg^{-1} of this element [2]. Similarly, mercury (Hg) is usually found in soil at levels ranging from 0.01 to 0.5 ppm; however, in metalliferous soils, this element can be found at concentrations ranging from 100 to 500 ppm [3], which is 1000–10,000 times higher than concentrations found in plants [4].

The normal concentration of heavy metals in soils, with the exception of metalliferous soils, is harmless to living organisms. However, operations such as mining and energy production, as well as agricultural activities have increased the concentration of those elements in once clean areas [5–7] beyond the necessary critical-concentration. From the limited information available, we can hypothesize that such an increase in heavy metal concentration can represent an increase in availability which, according to Sage (1992), "available means that the living cell-membrane can be passed" [8]. As a consequence, some sites contain enough amounts of essential elements such as Cr, Cu, and Zn to reach toxic levels [8–10]. At the present time, it has been estimated that in the United States alone there are more than 30,000 potential sites for hazardous waste treatment [10]. In addition, 1200 of these sites are on the National Priority List (NPL). Moreover, approximately 720 of the sites on the NPL are contaminated with heavy metals [11]. Both the permanence and mobility of heavy metals in soil are affected by several factors including soil conditions and metal species [12], the solubility in water [13], and the humic substances present in the soil [14–16]. However, the contaminants that are present in soil and air will result in water contamination, which will eventually reach all living organisms of a particular ecosystem [17].

Although current technologies for cleaning contaminated sites such as isolation and containment, mechanical/pyrometallurgical separation, or chemical treatment are efficient, they are usually expensive, labor intensive, and soil disturbing [11]. More recently, the use of plants in metal extraction (phytoremediation) has appeared as a promising alternative in the removal of heavy metal excess from soil and water [11,18,19]. Phytoremediation can be classified based on the contaminant fate or the mechanisms involved [20]. As for the fate, the classification includes degradation, extraction, containment or a combination of these; and as for the mechanisms, phytoremediation can be classified as extraction, concentration in plant tissues, degradation of contaminants, volatilization, immobilization at root level, and finally, erosion and infiltration control [20]. Extraction is called rhizofiltration when the process is accomplished in water-based cultures [10,21]. The processes applied in the removal of heavy metals and other toxic elements include extraction, containment and immobilization, and volatilization. Some plant species volatilize mercury (Hg) and the non-metal selenium (Se) [22,23].

Table 1
Concentration of heavy metals in soil and plants mg kg^{-1} (ppm) and clean up criteria

Element	Normal range in soil [1]	Critical soil concentration [1]	Normal range in plants [2]	NJDEP non residential clean up criteria [5]	Concentration in metalliferous soils [2]
Cd	0.01–2.0	3–8	0.1–3	100	11–317
Total Cr	5–1500	75–100	0.2–5	NA	47–8,450
Cu	2–250	60–125	5–25	600	52–50,900
Hg	0.01–0.5	0.3–5	0.1–9.5[a]	NA	100–400[b]
Ni	2–750	100	1–10	2400	19–11,260
Pb	2–300	100–400	0.1–5	600	3,870–49,910
Zn	1–900	70–400	2–400	1500	109–70,480

NJDEP: New Jersey Department of Environmental Protection, NA: not available.
[a] [4].
[b] [3].

2. Phytoremediation of heavy metal polluted water

The phytoremediation of polluted water, or rhizofiltration, is a relatively new technology [21]. This process, also referred to as phytofiltration, is based on hydroponically grown plants that have shown to be efficient in removing heavy metals from water [24]. Plants such as *Eichhornia crassipes* (Mart.) have shown potential to concentrate more than 6000 ppm of cadmium and lead in the whole plant, and more than 8000 ppm of copper when grown with 5 ppm of these heavy metals [25]. Another plant, the water fern (*Azolla filiculoides* Lam.), demonstrated the ability to absorb cadmium (Cd), copper (Cu), nickel (Ni), and zinc (Zn). Plants of *A. filiculoides* exposed individually to these heavy metals at concentrations ranging from 8 to 15 ppm, accumulated 10,000, 9000, 9000, and 6500 ppm of Cd, Cu, Ni, and Zn, respectively [26]. As for the accumulation velocity, time dependency studies demonstrated that in *A. filiculoides*, Cd accumulation occurs between 1 and 77 h, indicating that the water cleaning process can be accomplished rapidly [27]. Other aquatic plant species such as *Nelumbo nucifera* Gaertn. and *Nymphaea alba* L. have been tested for their chromium (Cr) uptake capacity from aqueous media [28,29]. In experimental conditions, these plant species have accumulated more than 3000 mg of Cr per kg of tissue. This metal, however, has shown to be very toxic for most plants.

Although the heavy metal accumulation (mg of metal kg^{-1} of tissue) shown by these species is high, the efficiency in absolute terms is low since they present low biomass production and slow-growing roots [21]. In dealing with this inconvenience, researchers have tried to use fast-growing and higher biomass producing plant species. Terrestrial plants such as Indian mustard (*B. juncea*) have been successfully hydroponically cultivated, showing that the absorption of different heavy metals including Zn and Pb can be effectively accomplished [21]. Grasses, spinach, sorghum, and corn are among other plants that have been studied for the removal of heavy metals from water. For example, Colonial bent grass and Kentucky blue grass were found to accumulate more than 150 mg g^{-1} of root dry weight when hydroponically cultivated in a synthetic solution containing 300 mg Pb L^{-1} from $[Pb(NO_3)_2]$ [30]. Studies using aqua-cultured seedlings of *B. juncea* (methodology proposed to be called blastofiltration) demonstrated that it is possible to reduce Cd concentration to 10 ppb in the absence of competing ions [30,31].

Aquatic phytoremediation has also been achieved using the so called wetlands. According to Horne [32] "wetlands are shallow water bodies containing higher plants with at least a 50% aerial cover of submerged or emergent macrophytes or attached algae" [32]. In this environment, plant species such as cattail (*Typha*) and tule (*Scirpus*) have shown the capability to remove a variety of toxic heavy metals including Cu and Zn [32]. Cheng et al. [33] designed a special wetland containing Cd, Cu, Pb, Zn, Al, and Mn at <5 mg L^{-1}. These investigators found that utilizing *Cyperus alternifolius* in the inflow chamber and *Villarsia exaltata* in the outflow chamber, the water was effectively decontaminated after a time period of 150 days. Other studies using wetland plants have shown that *E. crassipes* and *Lemna minor* exposed to Cd contaminated waters are able to accumulate this heavy metal with a bioconcentration factor [metal concentration in plant tissue (mg kg^{-1})/initial metal concentration in the growth medium (mg L^{-1})] of 1225 and 2567, respectively [34]. *Microspora* (a macro-alga) was found able to remove 97% of lead from an aqueous solution containing 39.4 mg L^{-1} of this metal, while *L. minor* (an aquatic plant) removed 76% of lead and 82% of nickel from an aqueous solution spiked with 10, and 5 mg L^{-1} of each metal, respectively [35]. Recent studies demonstrated that mesquite (*Prosopis* spp.), a desert plant species cultivated in hydroponics, was able to uptake Cr(VI) from the growing medium, transforming this metal to the less toxic Cr(III) species within the plant tissues [36].

3. Phytoremediation of heavy metal polluted soil

Current clean up technologies for the degradation, removal or immobilization of contaminants involve either bulk removal or in situ remediation. Soil movement requires expensive equipment, disturbs the ecosystem and is not well accepted by the community. On the other hand, the in situ technology depends on the cleaning cost per kg, the selectivity of the treatment agent, and the feasibility of recovering the chemicals used to treat the soil as well as the laden material from the surface and subsurface [37,38]. The use of acids or chelating agents might alter soil properties and its biological components [39,40] and might be either not selective or affected by ionic strength, flow rate, and type of contaminant [41]. Furthermore, soil cannot be used immediately after treatment.

Phytoremediation, on the other hand, can be accomplished in situ, is relatively inexpensive, environmentally friendly, and the soil can be utilized immediately after treatment application [10,42]. Although metals at high concentrations or in mixtures, as well as their low availability may represent a disadvantage for phytoremediation, certain strategies such as genetic improvement and the use of metal uptake enhancers are rapidly alleviating these limitations [43,44]. Furthermore, phytoremediation does not generate sludge and metals accumulated by plants can be recovered by metal extraction processes and incineration.

3.1. Heavy metal toxicity to plants

One of the disadvantages of phytoremediation is that heavy metals at high concentrations or within certain mixtures may deter plant growth and biomass production. Excess of heavy metals can affect plant growth in different manners. A summary of the main effects caused on plants by Cd, Cr, Cu, Hg, Ni, Pb, and Zn is presented in Table 2. As seen in this table, these heavy metals can disrupt the physiology and

Table 2
Main effect of heavy metals in plants

Metal	Effects
Cd	Decreases seed germination, lipid content, and plant growth; induces phytochelatins production [48,58–61]
Cr	Decreases enzyme activity and plant growth; produces membrane damage, chlorosis and root damage [43,62–65]
Cu	Inhibits photosynthesis, plant growth and reproductive process; decreases thylakoid surface area [48,51,52,66–69]
Hg	Decreases photosynthetic activity, water uptake and antioxidant enzymes; accumulates phenol and proline [50,69,70]
Ni	Reduces seed germination, dry mass accumulation, protein production, chlorophylls and enzymes; increases free amino acids [44,71–73]
Pb	Reduces chlorophyll production and plant growth; increases superoxide dismutase [45–47,49,74]
Zn	Reduces Ni toxicity and seed germination; increases plant growth and ATP/chlorophyll ratio [48,71,75,76]

morphology of plants. For example, Cr, Ni, and Pb have been found affecting chlorophyll production [44–46], while Cd, Cr, Cu, Ni and Pb decrease plant growth [47–49]. Other studies have proven that Cr, Cu, Hg, Ni, and Pb affect thylakoid surface area, electron transport and enzymatic activity, or reduce chlorophyll production [50–53]. However, plant species have developed certain strategies that allow them to grow and develop in metalliferous soils. Some of them have evolved in such a manner that they can be considered as metallophytes, even a few of them having metallo-dependent features [54]. Several species of *Thlaspi* are the most known metallophytes around the world. These species have been found growing in Cd, Pb, Zn, and Ni metalliferous soils in several European countries [55,56]. *Silene vulgaris* is another well documented metallicolous plant species [57]. Researchers have realized that such plants can accumulate far in excess toxic transition elements, characteristics that can be utilized to cleanup heavy metal polluted soils.

3.2. Heavy metal hyperaccumulator plant species

According to Reeves and Baker [2], the term hyperaccumulator was first applied by Jaffre et al. in 1976; but the present connotation concerning the concentration of more than 1000 mg kg^{-1} (0.1%) of metal in plant tissues was introduced by Brooks et al. in 1977, when they examined the Ni concentration in *Homalium* and *Hybanthus* from different sites throughout the world [2]. However, heavy metals such as Cd and Cr are considerable more toxic for plants than Ni; for this reason, scientists realized that the criterion could not be applied evenly. Lately, Baker et al. [77] established that the percentage of metal concentrated in dry leaves tissue should be the criterion to classify a plant as heavy metal hyperaccumulator. For example, plants containing more than 0.01% of Cd or more than 0.05% of Cr in dry leaf tissues (100 and 500 mg kg^{-1}, respectively) should be considered as hyperaccumulators of such metals. They established concentrations for Cd, Cr, Cu, Ni and Zn and summarized the number of families and species that accomplished with the criterion (Table 3). These researchers considered that metal concentration in dried leaves of hyperaccumulator plants should be at least an order of magnitude greater than the metal concentrations in leaves of nonaccumulator plants found on metalliferous soils.

Reeves and Baker [2] analyzed the information available concerning the heavy metal concentration in plants and summarized the families and species considered as hyperaccumulators. In their analysis, these authors stated that some previous data, for example those concerning Cr hyperaccumulation by *Dicoma niccolifera*, *Sutera fodina* and *Leptospermum scoparium* appear to be incorrect. In the present review, we include the most recent information about plant species newly identified as Cd, Cu, Cr, Hg, Ni, Pb, and Zn hyperaccumulators (Table 4). With the exception of the references of Tang et al. [79] and Asensi et al. [80], the information presented in this table was generated from 2000 to 2004. As shown in this table, *E. crassipes* has been identified as a Hg hyperaccumulator [87], and new species of the Brassicaceae family have been added to the list of Cd, Ni, and Pb hyperaccumulators. In addition, desert plant species such as *Salsola kali* and *Prosopis* spp. from the families Chenopodiaceae and Fabaceae/Leguminosae, respectively have been added to the list of Cd and Cr hyperaccumulators [36,78]. Information concerning desert plants is considered very important because there are many heavy metal laden sites in desert areas and only a few plant species are able to thrive in desert ecosystems.

3.3. Possible use of plants for phytomining

The capability of plants to absorb metals of high commercial value has gained the attention of researchers around the world. The first attempt to use plants for phytoextraction had a connotation of cleaning up; however, scientists have realized that metals can be mined using plants. This technique is known as phytomining. Although the technology is still not well established, nickel mining via plants is already a patented technology [92]. There are also reports about plants that uptake gold and silver at high concentrations. Girling

Table 3
Number of metal hyperaccumulator plants

Metal	Criterion (% in leaf dry tissue)	No. of families	No. of species
Cadmium	>0.01	1	1
Chromium	>0.05	5	5
Copper	>0.1	16	35
Nickel	>0.1	36	151
Zinc	>1.0	5	13

Table 4
Family/species of cadmium, chromium, copper, mercury, nickel, lead, and zinc hyperaccumulator plants identified from 1997 to 2004

Cd	Cu	Cr	Hg	Ni	Pb	Zn
Asteraceae (Compositae)	Commelinaceae	Asteraceae (Compositae)	Pontederiaceae	Brassicaceae	Compositae	Crassulaceae
Chamomilla recutita (*Matricaria recutita*) [75], *Helianthus annuus* [81]	*Commelina communis* [79]	*Helianthus annuus* [85]	*Eichhornia crassipes* [87]	*Psychotria douarrei* [88]	*Dittrichia viscose* [89]	*Sedum alfredii* [91]
Brassicaceae *Arabidopsis halleri* [82]	Ericaceae *Erica andevalensis* [80]	Convolvulaceae *Convolvulus arvensis* [86]			Brassicaceae *Brassica pekinensis* [72]	
Chenopodiaceae *Salsola kali* [78]	Labiatae *Elsholtzia splendens* [83]	Geraniaceae *Pelargonium* spp. [84]			Fabaceae/Leguminosae *Sesbania drummondii* [90]	
Hypericaceae *Hypericum perforatum* [75]	Geraniaceae *Pelargonium* spp. [84]	Leguminosae/Fabaceae *Prosopis* spp. [36]			Geraniaceae *Pelargonium* spp. [84]	

and Peterson [93] informed that *Phacelia sericea* accumulated more than 3857 mg kg^{-1} of Au in root dry mass. Other reports indicated that alfalfa plants cultivated in agar-based media containing $KAuCl_4$ or $AgNO_3$ accumulated more than 370 mg kg^{-1} of Au and 120 mg kg^{-1} of Ag in the aerial part. These results open the possibility of using this plant in future gold and silver phytomining studies [94]. In addition, high resolution transmission electron microscopy (HRTEM) studies demonstrated that alfalfa plants are able to form gold and silver nanoparticles of different size and shape inside the plant tissues [95,96].

Starting in 2000, small-scale field trials using seeds of canola (*Brassica* sp.) and corn (*Zea mays*) have been run at several locations in New Zealand and Brazil [97]. The results of this pilot projects will bring more information about the real possibility of using plants for extracting precious metals.

3.4. Applications of XAS to phytoremediation

X-ray absorption spectroscopy (XAS) has been used to study different aspects of the coordination chemistry in many diverse phytoremediation systems [36,78,93–96,98–118,123–130]. The uptake and coordination of Cr, Ni, Cu, As, Au, and Se by mesquite, *Thalspi goesingense*, *Larrea tridentata*, *Pteris vittata*, *Medicago sativa*, and *Astragalus bisulcatus*, respectively, are only a few examples of phytoremediation systems studied using X-ray absorption spectroscopy [36,95,98,103,104]. The application of XAS to phytoremediation studies presents a series of advantages over other techniques. The main advantage of XAS would be the fact that there is no sample pretreatment; instead the samples are run on an as is basis either liquid, solid, or gas [119]. This part of the review will focus on the different applications of XAS including X-ray microprobe, micro-XAS, extended X-ray absorption fine structure (EXAFS), and X-ray absorption near edge structure (XANES) to study the coordination chemistry of metals in plants potentially usable for phytoremediation [100,108,111,114]. The theory behind each of the aforementioned techniques is excellently covered in depth in other articles and will not be discussed in detail within this review [119–122]. A brief introduction into the differences in XANES spectra will be supplied to exemplify the way that data can be extracted from the whole spectra and a brief overview of the data that EXAFS contains.

3.5. XANES spectroscopy

XANES spectroscopy contains very useful information on chemical oxidation state and coordination geometry of elements in complexes [119–122]. Fig. 1A shows the entire XAS spectrum of copper in an octahedral arrangement of oxygen atoms. The line in Fig. 1A shows the division of the XAS spectrum into XANES and EXAFS spectra, which occurs at approximately 50 eV beyond the absorption edge. XANES spectroscopy provides a three-dimensional picture of the electronic interactions between the element of interest and the nearest neighboring atom. This interaction is diagrammatically shown in Fig. 1B. In this figure, half of a copper octahedral molecule is shown with the arrows representing the interaction between the absorbing atom and the backscattering atoms. The various coordination geometries of chemical compounds are expressed by the electronic interactions of the absorber and the nearest neighboring atoms or the scattering atoms [119]. Examples of some different Cu geometries and their XANES spectra are displayed in Fig. 2 to show the differences observed in the XANES spectra. Fig. 2 shows two octahedral complexes of Cu, Cu(II) nitrate and Cu(II) tartrate. Slight differences can be observed in the XANES of the two compounds. However, the overall general shape and position of the absorption edges for both complexes are very similar. Also, in Fig. 2 a square planer Cu(II) complex is shown [Cu(II) phthalocyanine], which has a much different absorption edge feature and a different edge position. The Cu(II) phthalocyanine absorption edge is shifted to a lower energy than the other two Cu complexes because of donation of electrons from the π-system in the phthalocyanine. These

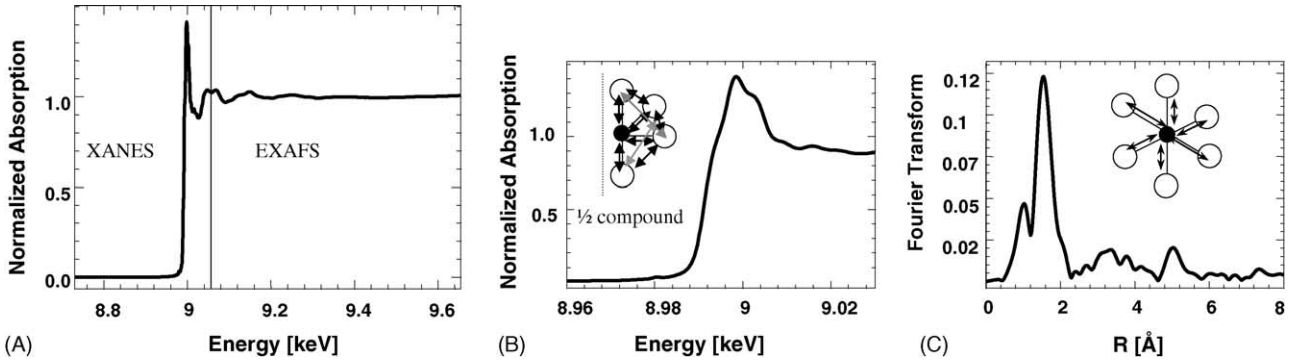

Fig. 1. (A) The entire XAS spectra. The line indicates the separation between the XANES and EXAFS spectra at approximately 50 eV after the inflection point of the absorption edge. (B) The XANES spectra obtained for a copper compound with an octahedral geometry (the solid black circle represents copper, and the white circles represent oxygen). The arrows represent the interactions of the different atoms in the compound. Only 1/2 of the compound is shown for simplicity. (C) The Fourier transformed EXAFS of a copper compound with an octahedral geometry (the solid black circle represents the copper atom and the white circles represent the oxygen atoms). The arrows represent the interactions that are visualized by the main peak in the Fourier transform.

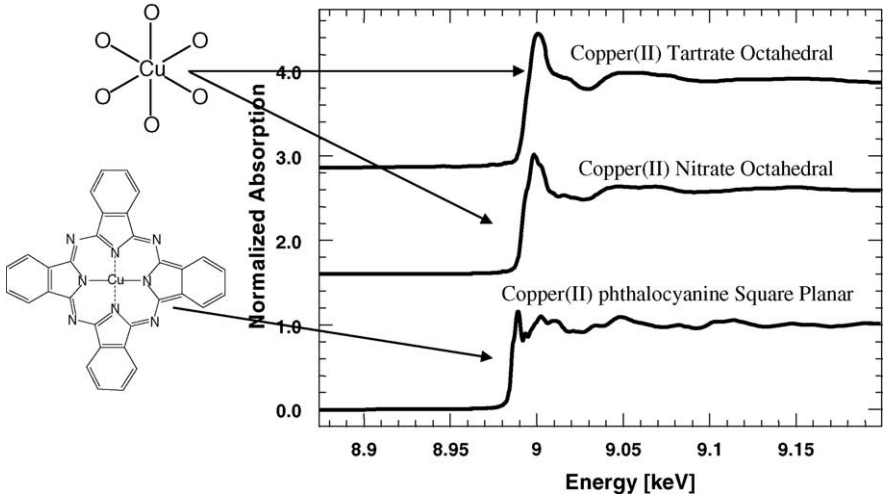

Fig. 2. Different XANES spectra obtained for copper compounds in octahedral and square planar geometries.

difference allow for the determination of both geometry and oxidation state of metals in different complexes. Through the use of linear combination X-ray absorption near edge structure (LC-XANES), the different geometries/oxidation states in an unknown compound can be quantified and determined [123–128]. LC-XANES fittings have been used in a number of different phytoremediation studies to quantify the metal species composition of plant-metal samples [36]. Using compounds of known geometries and oxidation states, the geometry and oxidation state of an unknown can be determined, as shown in Fig. 3. This figure shows a LC-XANES fitting of Cu absorbed by buffalo grass. The information determined from the XANES fitting is that the Cu is in an octahedral complex and is still present in the plus (II) oxidation state.

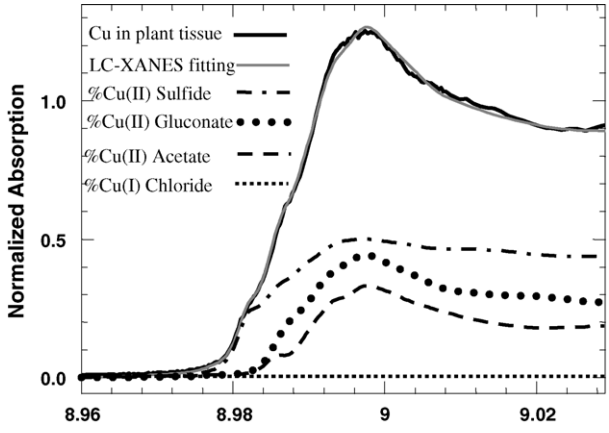

Fig. 3. LC-XANES fitting of copper within buffalo grass plant sample showing the varying composition of the sample with components of copper(II) acetate (25.4%), copper(II) gluconate (34.9%), and copper(II) sulfide (39.6%) and copper(I) chloride (0.0%).

3.6. EXAFS spectroscopy

EXAFS provides a two-dimensional representation of coordination between absorbing and backscattering atoms

within a chemical complex [119–122]. Fig. 1A shows the dissection of the entire XAS spectrum into the XANES and EXAFS regions. A diagrammatic representation of the interactions observed in the XANES spectrum is shown in Fig. 1B, where the solid black circle represents the absorbing atom and the white circles represent the backscattering atom. The arrows in the octahedral complex shown in Fig. 1B represent the simplest interactions between the absorbing and the backscattering atoms. This interaction creates a wave function that can be Fourier transformed to give a pseudo-radial distribution function as shown in Fig. 1C, where the major peak observed in the Fourier transform is the interatomic distance between Cu(II) and oxygen atoms at approximately 1.95 Å. The forward scattering and backscattering between the absorbing and backscattering atoms create the oscillations or the EXAFS spectrum. The oscillations can be represented by Eq. (1), also known as the EXAFS equation.

$$\chi = \sum \left(\frac{N}{kR^2}\right) S_0^2 F(K)\, \exp(-2k^2\sigma^2) \exp\frac{-2(R-\Delta)}{\lambda} \sin[kr + \delta(k)] \quad (1)$$

Eq. (1) calculates $\chi(k)$ or the fractional modulation of the X-ray absorption coefficient and accounts for all the interactions measured or calculated through summation of the specific interaction of a number of atoms [119–122]. The term N in the EXAFS equation corresponds to the total number of absorbing and backscattering atoms in the sample, S_0^2 accounts for the inelastic loss process, $F(K)$ is the energy dependence of the photoelectron scattering process, R^2 is the distance between the backscatter and absorber (Å^2), and k is the space term for calculating into k space or wavevector space (Å^{-1}). Furthermore, the amplitude of the EXAFS is directly related to the number of backscattering atoms in the sample. Although EXAFS has been reported to give excellent interatomic distances, the coordination numbers obtained from this technique usually have an error of ±20%. EXAFS contains a large amount of information that can be extracted from spectra that has been explained elsewhere [108–111].

3.7. X-ray microprobe and micro-XAS

X-ray microprobe has been applied to map numerous types of geological samples with a resolution better than 50 μm; however, more recently, the X-ray-microprobe has been applied to investigate the uptake of toxic elements by plants. Pickering et al. [100] have used the X-ray microprobe and micro-XAS techniques to investigate the uptake of Se by *A. bisulcatus*. In this study, maps of the Se distribution within the roots, shoots, and leaves, were produced based on concentration. With the combination of these two techniques, the authors were able to determine the oxidation state of Se in different parts of the plant. More importantly, this study showed the in plant reduction of selenate to organic forms of Se. In addition, it was found that the reduction of selenium varied with distance from stem. Thus, the highest selenate concentration was found at the center of the stems and the highest concentration of reduced organo-selenium compounds was observed in the leaves.

Howe et al. [108] used XANES and X-ray microprobe to investigate the localization and speciation of chromium in *Trifolium brachycalycinum* (clover). They showed that the Cr(VI) absorbed by clover was reduced within the plant to Cr(III). The complex Cr(III) within the tissues most closely resembled a hexacoordinated (octahedral) Cr–O complex. The results of the study indicated that the majority of the Cr(VI) reduction, approximately 75%, occurred at root level and Cr(III) was subsequently transported throughout the plant. Using the X-ray microprobe, they showed that the maximum Cr concentration was present in the veins of the leaves and the smaller concentration was found in the leaf margins.

Sarret et al. [111] used the X-ray microprobe, bulk, and micro-XAS techniques to investigate the forms of Zn accumulated in *Arabidopsis halleri*. This study showed the variation of Zn species within various plant sections. Some plants were collected from known Zn contaminated sites and others were germinated and greenhouse grown by the investigators. The bulk EXAFS showed that Zn primarily consisted of Zn malate and Zn phytate, with the Zn coordinated to six oxygen atoms in an octahedral arrangement. However, by adding citrate to the growth media to enhance Zn uptake, Zn citrate was identified in the sample spectra. It was also found that, under hydroponics conditions, the coordination of Zn within the plants was a combination of zinc-organic acids in tetrahedral arrangements. In addition, by using micro-EXAFS, the authors showed that the coordination was the same as in the bulk samples. Using X-ray microprobe, it was also determined that Zn was more concentrated on the trichomes, with a tetrahedral coordination to oxygen and carbon atoms present in the second shell of the complex.

Hansel et al. [114] used a combination of X-ray microprobe and XAS to determine the spatial and temporal relationship between iron (Fe) and arsenic (As) species on roots of the aquatic plants *Phalaris arundinace* and *Typha latifolia*. By using the microprobe to map specific areas of the plant root, the investigation showed that As on the root surfaces consisted of approximately 82% arsenate and 18% of an iron-hydroxo-arsenic(III) complex, in small patches. However, the complex iron-oxide/hydroxide was also present as a continuous layer over the root surface.

3.8. Bulk XAS investigation in phytoremediation systems

Using bulk XAS, Gardea-Torresdey et al. [95,96] have investigated the uptake of gold (Au) and silver (Ag) by alfalfa plants. In the Au studies, it was found that tetrachloroaurate was reduced to Au(0) and nanoparticles were formed within the living plants. In another study using inactivated alfalfa biomass, these researchers were able to calculate the average

size of the Au nanoparticles using Eq. (2).

$$F_r = R^3(N_{\text{ratio}} - 1) + \frac{3}{4}dr^2 - \frac{1}{16}d^3 \quad (2)$$

This equation was developed by Borrowoski to determine the average size of Cu nanoparticles and has been found to be applicable to all face centered cubic (FCC) metals [129]. The results of the studies of Au uptake by alfalfa plants showed that the average coordination numbers below the coordination of bulk Au indicated the presence of Au(0) nanoparticles. In addition, by using high resolution transmission electron microscopy (HRTEM), it was found that the most common Au nanoparticles formed were icoshedral. However, all the low energy configurations of Au nanoparticles were found within the plants. In the studies of Ag uptake by alfalfa, Gardea-Torresdey et al. [95] confirmed the formation of Ag nanoparticles within the living plants. Using bulk XAS and Eq. (2), it was found that the average size of the Ag nanoparticles was of 9 Å. In addition, it was demonstrated that the Ag nanoparticles were trapped in specific areas within the plant. These studies have large implications to the phytomining of precious metals from low concentrations in soils or from previously mined areas containing precious metals at trace levels.

Pickering et al. [99] investigated the uptake and reduction of As by *B. juncea* (Indian mustard) under hydroponics growth conditions. These researchers showed that once As (III or V) is absorbed, it is converted and stored as an As(III)-tris-thiolate complex. They concluded that the coordinating ligand was either a glutathione or a phytochelatin. From this study, it was also observed that the addition of dimercaptosuccinate to the growth media resulted in a five-fold increase in the arsenic uptake. The fitting of the EXAFS spectra obtained from each of the samples showed that the As was coordinated to only three sulfur ligands within the plant. The presence of the three sulfur ligands indicates that the complexes are in a trigonal bypramidal geometry.

A different study carried out by Webb et al. [104] investigated the As uptake by *Pteris vittata* L. an As hyperaccumulating fern under soil growth conditions. In this study, it was shown that the As absorbed by the ferns, remained in the reduced oxidation state and was coordinated to oxygen ligands predominately, with minimal binding to sulfur moieties. In another study regarding As accumulation by *P. vittata* grown in hydroponics media contaminated with As(V), Lombi et al. [105] obtained similar results to those obtained by Web et al. [104]. The XANES analysis showed that the arsenic in the plants was present as 75% As(III) coordinated to oxygen ligands and 25% of the As was still present as As(V). In this study, no EXAFS were presented and thus, no comment on the coordinating ligands can be made.

Aldrich [118] studied the uptake and reduction of As(V) by mesquite. In this investigation, the author determined that As(V) was absorbed by mesquite and reduced to As(III). Moreover, the presence of arsenic–sulfur compounds was reported. The EXAFS results showed that the As(III) sulfur compounds contained three arsenic–sulfur bonds at an interatomic distance of approximately 2.3 Å, indicating that the compound had a trigonal bipyramidal geometry. In addition, the XANES analysis indicated that the As(V) was approximately 98% reduced in the roots, shoots, and leaves.

Chromium is another element of phytoremediation interest that has been studied using XAS. The major findings in Cr phytoremediation is the bioreduction from Cr(VI) to Cr(III) within the plants [36,106,107]. A study performed by Aldrich et al. [36] showed that mesquite plants were able to grow in hydroponics media and agar media containing 80 ppm of Cr(VI). The study also showed that the agar based growth media reduced the Cr(VI) by approximately 50%. However, only Cr(III) was found to be present in the roots, shoots, and leaves of the plants. In the hydroponics studies with 80 ppm of Cr(VI), it was determined that, in roots and shoots, about a 1–6% of the Cr still remained as Cr(VI). The rest of the chromium was found to be a combination of inorganic forms of Cr(III) and organic acid-ligated forms of Cr(III). In this study, the EXAFS data indicated that the average coordination number for the Cr in the plants was approximately 6, indicating that the Cr was present in a Cr(III)-oxygen octahedral complex inside the plants.

An investigation about Cr uptake by the wetland plant *E. crassipes* showed also the bioreduction of Cr(VI) to Cr(III) [106]. In this investigation, it appeared that reduction of Cr(VI) occurred in the fine lateral roots of the plants. Only the presence of Cr(III) was detected within the plants roots, shoots, and leaves. In addition, the average coordination number of Cr(III) within the plants was of six presenting only the Cr–O binding type. It was also observed that Cr(III) translocated within the plants was bound to organic acid ligands, yet in the roots it appeared that the Cr was as hydrated Cr(III) ions.

In another study, Zayed et al. [107] demonstrated the accumulation and translocation of Cr within vegetable crops. They investigated different plant species and in all of them the bioreduction of Cr(VI) to Cr(III) was observed. More specifically, all the plants were able to bioreduce or bioconvert the toxic CrO_4^{2-} to non-toxic Cr(III) species. These researchers also found no reduction of the Cr(VI) in the hydroponics media, thus suggesting that all the Cr(VI) reduction occurred within the plants.

The phytoremediation of Cd has been studied using XAS to determine the uptake complexes, the production of phytochelatins, and the speciation of Cd within plants [31,58,78]. Pickering et al. [58] have investigated the phytochelatin systems and the associated model compounds using XAS. This study showed the absence of Cd–Cd bonds and the presence only of Cd–S bonds of 2.54 Å, which were found not to be indicative of a Cd-thiolate complex. The work also showed that the complexes had coordination numbers around 4 with a tetrahedral coordination. They also investigated this system using the sulfur K-edge which showed a low energy feature that was consistent with sulfur bound into a cluster.

Another study by de la Rosa et al. [78] investigated the uptake and translocation of Cd in *S. kali*. In this investigation, XAS spectra of whole plant parts (roots, stems, and leaves) were obtained. It was found that Cd within the plants remained in the (II) oxidation state. In addition, the EXAFS data showed that Cd was bound to O, S, and Cd. The researchers concluded that in *S. kali*, the Cd transport from roots to the aerial part might be occurring through the complexation with small organic acids. Moreover, the presence of coordination of Cd–S in stems and leaves of the plant suggested the formation of Cd-phytochelatin complexes.

Salt et al. [31] studied the uptake of cadmium by *B. juncea* using XAS. These researchers found that the percentage of Cd bound to phytochelatins was dependent on time. After 6 h, 34% of the Cd was found as Cd–S and a 60% after 72 h. In addition, it was found that the Cd inside the plant appeared to be in an octahedral geometry containing oxygen ligands. In another investigation using XAS, Salt et al. [115] studied the uptake of Cd, its mobility, and accumulation in Indian mustard, as well as the types of Cd-complexes formed within the plant. The EXAFS of the samples showed that, when analyzing the whole root tissue, Cd was found to be bound into a phytochelatin complex containing four S ligands. However, in the xylem sap the Cd was found to be coordinated to 6 O/N ligands.

The uptake and bio-transformation of Cu by *L. tridentata* (creosote bush) has also been studied using XAS [117]. In this study, the authors showed that Cu was absorbed and transported through the plant as Cu(II). However, in the leaves Cu was present in the (I) and (II) oxidation states. According to the results, the authors suggested that Cu was complexed to a phytochelatin. The EXAFS of the study showed that in roots, Cu was coordinated to 2 O (at 1.88 Å), 2 S (at 2.24 Å), and 3 Cu (at 3.72 Å) atoms. In the stems, Cu was found to be coordinated to 2 O (at 1.90 Å), 2 S (at 2.26 Å), and 1/2 of a Cu (at 2.71 Å) atoms. In the leaves of the plants, the coordination numbers were different being of 1 O, 4 S and 5 Cu (1 Cu atom at 2.78 Å and 4 Cu atoms at 3.72 Å) atoms. The authors suggested that Cu was bound into a multi-center-copper complex within the shoots of the plants.

XAS has also been used to study nickel phytoremediation and its localization and speciation within *Thlaspi* species [102,103]. In an investigation by Persans et al. [102], the role of histidine in Ni uptake by *T. geosingense*, a Ni hyperaccumulator, and *T. arvense*, a Ni non-hyperaccumulator, was studied. The XAS study on these plants showed that there were no major differences between the Ni coordination by histidine in the tissues. They concluded that the Ni hyperaccumulating phenotype did not show an over production of histidine in response to Ni exposure. However, the authors observed that the histidine concentration in the xylem and the shoot remained unchanged in the hyperaccumulator plant after Ni exposure. In addition, histidine concentrations in roots dropped to the levels observed in non-exposed non-hyperaccumulating species. Kramer et al. [103] have studied the subcellular localization and speciation of Ni in hyperaccumulating and non-hyperaccumulating *Thlaspi* species using XAS. Through this technique, the authors were able to identify the functional groups to which Ni was coordinated to within the *Thlaspi* species. The study indicated that the majority of the Ni within the leaves was coordinated to the cell walls and the remainder of the Ni was associated with citrate and histidine complexes.

Bracey et al. [110] studied the coordination of Zn binding by spinach carbonic anhydrase. The results of this study indicated that the Zn was bound into a Cys–His–Cys–H_2O complex. They developed five different models to fit the EXAFS data, which included coordination of the Zn directly to 4 S atoms, 3 S atoms and one O/N atom, 2 S atoms and 2 O/N atoms, 1 S atom and 3 O/N atoms, 4 O/N atoms, and 2 S atoms and 3 O/N atoms. The best fits of the EXAFS suggested that the Zn was coordinated to the 2 S atoms and the 2 O/N atoms within the complex, indicating a tetrahedral complex formed between the Zn and the carbonic anhydrase.

Salt et al. [101] have used XAS to investigate the ligands involved in coordinating Zn in the hyperaccumulator *T. caerulescens*. In this study, Zn was found to be coordinated predominately to histidine within the roots of the plant. However, in the plants xylem sap Zn was found predominately as free hydrated ions with small amounts of the Zn coordinated to organic acids. Finally, in the stems of the plants, Zn was found to be mainly bound to organic acids with small portions of free hydrated Zn and some bound to histidine. The EXAFS of the whole root material showed that the first shell coordination consisted of 6 O/N ligands. The sap of the plants showed a coordination of approximately 6 O ligands at around 2.08 Å. Also, Zn in leaf tissues showed a coordination of approximately 6 O atoms at 2.06 Å. These EXAFS results indicate that the Zn was present in octahedral arrangements within the plants after uptake.

With the aid of XAS, Kelly et al. [109] have studied the nature of Zn complex accumulated by *Datura innoxia*. The results showed that Zn in *D. innoxia* was found to be coordinated to free histidine residuals. The unfiltered EXAFS showed within the plants a 4 or 5 coordinate system for Zn with N/O ligands at 2.0 and 1.9 Å on average. The multiple scattering in the EXAFS showed very similar peaks to that of a Zn-histidine model compound. The results suggested that either a new Zn-binding protein was being produced within the plant or a Zn metalloenzyme was over expressed within the cells of the plants.

In another study by Sarett et al. [112], the forms of Zn and Pb accumulated by *Phaseolus vulgaris* in the presence and absence of EDTA were investigated using XAS. In this research, it was found that Zn was predominately present as zinc-phosphate dehydrate regardless of the form of Zn supplied in the growing media. In addition, the results of the EXAFS showed that the plants grown in a Zn-EDTA solution had coordination of 4 O ligands at approximately 2.0 Å in both the root and leaves with a second coordination shell of P at 3.1 and 3.5 Å. Very similar results were obtained for the plants grown using a Zn-sulfate solution. The results from the

Pb EXAFS showed that, in the leaves of the plants grown in a Pb nitrate solution, Pb was mainly found as Pb carbonate. However, the leaves of the plants grown in the Pb-EDTA solution contained a mixture of a Pb-EDTA complex and an unknown and undetermined Pb complex.

Sharma et al. [130] have investigated the Pb uptake by *Sesbania drummondii* using XAS. In this plant species, it was determined that only Pb–O complexes were formed within the roots and leaves of this plant. It was also reported that in roots, Pb showed a coordination of approximately 10 atoms, 8 of which were O atoms and 2 of which were C atoms. Moreover, it was shown that Pb could be bound in a form very similar to that of Pb acetate. It was also reported that in the leaves, the Pb coordination number was of 6, where 2 of the coordinating atoms were S with interatomic distances of 2.85 Å. In addition, a LC-XANES fitting was performed on the samples showing that the composition of the Pb within the plants was extremely varied. Features from Pb(II) acetate, Pb(II) sulfide, Pb metal and Pb(II) nitrate were included. It was observed that the plants transformed the Pb(II) nitrate into a Pb(II)-organic acid complex.

XAS was also used to study the mineralogy of the presence of Pb-phosphate grains in the roots of *Agrostis capillaris* L. used for Pb phytoremediation [131]. The XANES data showed that the Pb on the roots of the plants exhibited a spectrum very similar to that of Pb-hydroxyapatite and a polymorphite structure. The EXAFS showed the polymorphite oxygen shells at 2.30, 2.51, 2.71, and 3.04 Å, and the inclusion of a chlorine shell at 3.05 Å were required to obtain a reasonable fit. These researchers [131] concluded that the precipitation of the mineralized Pb on the roots might be a defensive mechanism of the plants to tolerate high levels of Pb or it might be a passive interaction of P with heavy metals within the plants.

Mercury is another element of phytoremediation interest studied using XAS. Although there have been many studies on Hg phytoremediation, the studies involving XAS are limited. In one study, XAS was used to investigate the accumulation of Hg by water hyacinths (*E. crassipes*) [87]. This study showed that the Hg within the plant was initially ionically coordinated to O ligands within the roots and it was concluded that the coordinating ligands were more likely organic acid ligands. In this case, the authors only used XANES analysis to determine the coordination environment of the Hg within the plants. They reported that Hg was only coordinated to S ligands and no presence of Hg–O bonds was detected.

The uptake, coordination, reduction, and volatilization mechanisms of Se in plants have also been studied using XAS [100,113,116]. Although Se is not a heavy metal, it is a toxic non-metal of environmental interest. As mentioned earlier, Pickering et al. [100] have studied the Se uptake and transformation using XAS and microprobe techniques. Pilon-Smits et al. [113] studied the uptake and volatilization of Se using the poplar hybrid *Populus tremula*. In this study, Se was supplied as selenate, selenite, and selenomethionine. They found that Se volatilization rate was dependent on the form of Se supplied. In addition, it was found that plants supplied with selenomethionine absorbed higher concentrations of Se than plants supplied with the inorganic Se forms. XAS studies revealed that, when Se was supplied in the form of selenate and selenomethionine, this Se species remained unchanged. However, when the poplar was supplied with selenite, a species similar to selenomethionine was detected in plant tissues. On the other hand, Ellis et al. [116] used XAS to investigate the production of selenium-methylselenocysteine in transgenic plants. The authors over expressed the *A. bisulcatus* selenocysteine methyltransferase and transferred it to the non-Se-accumulating *A. thaliana*. It was found that after this treatment, the non-accumulating plants were able to uptake and tolerate different concentrations of Se. The results also showed that, in plant shoots of the non-accumulating plants, Se was present as Se-methylselenocysteine and gamma-glutamylmethylselenocysteine. The XAS results also showed that about 70% of the Se absorbed remained as the selenate form, in both the controls and the genetically modified plants. The results of the Se composition confirmed that the reduction of selenate to selenite is the rate-limiting step in the incorporation of Se into selenium-cysteine complexes.

3.9. Commercial application of phytoremediation

Although information generated from laboratory and greenhouse investigations illustrate the benefits of phytoremediation, commercial applications of this technique are relatively few. According to Van der Lelie [43], "phytoremediation will only be accepted if its success is demonstrated". So far, phytoremediation has demonstrated its assets in many pilot projects developed throughout Europe and the United States. Table 5 shows some locations where plants have successfully been utilized to restore heavy metal contaminated sites. As seen in this table Cd, Cu, Ni, Pb, and Zn, are the most common metals extracted using plants. Species from the genus *Brassica* seem to be the most effective in cleaning Pb polluted sites, whereas most of the species tested show capability for Cd and Zn removal. Though the aim of most of the pilot projects is phytoextraction, in some cases such as in Lommel, Belgium and the United Kingdom, the purpose was phytostabilization [43]. In addition, plants have also demonstrated their potential use as vegetative cover for water pollution prevention [20].

Based on these pilot project results, commercial applications of phytoremediation are growing. According to Glass [18], the United States market for metal phytoremediation in soil in 1997 was probably $1–2 million, ranged $15–25 million by 2000, and will be $70–100 million by 2005 [18]. However, the market for phytoremediation of industrial wastewaters is relatively low. It has been estimated that in 1997, this market was practically zero, and Glass [18] estimated that by 2005, the demand would not be greater than $2 million. Evidently, phytoremediation is advancing, but according to Glass [18], phytoremediation will be definitely accepted through social processes and sufficient information about specific set-

Table 5
Examples of American and European heavy metal sites with phytoremediation field projects

Location	Plants	Contaminants	Application
Trenton, NJ [132]	*Brassica juncea*	Pb	Phytoextraction
Anderson, SC [132]	*Populus deltoids* × *P. balsamifera* (hybrid poplar) grasses	Several heavy metals	Phytostabilization
Beaverton, OR Landfield reclamation [20]	*Populus* spp. (cottonwood)	Unspecified	Vegetative cover/water pollution prevention
Katowice, Poland [133]	*Brassica juncea*	Cd, Pb	Phytoextraction
Switzerland (Landfield) [134]	*Salix viminalis*	Cd, Cu, Zn	Phytoextraction
United Kingdom [43]	*Salix* spp.	Ni, Cu, Zn, Cd	Phytoextraction, phytostabilization
Hlemyzdi, Czech Republic [43]	*H. annus*, *C. sativa*, *Z. mays*, *C. hallery*	Zn	
Dornach, Switzerland [43]	Improved *Nicotiana* spp. plants (tobacco)	Cu, Cd, Zn	
Lommel, Belgium [43]	Grasses	Zn, Cd, Pb, Cu	Phytostabilization
Balen, Belgium [43]	*Brassica napus*	Zn, Cd, Pb	Phytoextraction

tings for specific contaminants. However, Gardea-Torresdey [135] states, "phytoremediation will allow science to contribute towards a giant step in the treatment of this country's hazardous waste sites".

4. Conclusions

The use of plants in metal extraction (phytoremediation) has appeared as a promising alternative for heavy metal removal from soil and water via extraction, containment and immobilization, and volatilization. Plants such as *E. crassipes* and *A. filiculoides* Lam. have demonstrated high capability to absorb Cd, Cu, Ni, and Zn from water ponds, whereas several species of *Thlaspi*, *S. vulgaris*, *Prosopis* spp., *S. kali*, *B. Juncea*, *B. napus*, *Salix* spp., and *Populus* spp. have proven to be efficient absorbing these metals from polluted soils. More recently, plants have shown that they also can be used as an environmentally friendly alternative for extracting precious metals. Although the information generated from laboratory and greenhouse investigations, as well as from many pilot projects developed throughout Europe and the United States is vast, commercial applications of phytoremediation are relatively low. It has been estimated that by 2005, the United States market for soil metal phytoremediation will probably be of $70–100 million, while for wastewater metal removal it will be of up to $2 million. The use of phytoremediation is increasing, but it will be definitely accepted through social processes and sufficient information about specific settings for specific contaminants. However, phytoremediation will allow science to contribute towards a giant step in the treatment of this country's hazardous waste sites.

Perhaps more important, the results of phytoremediation XAS investigations have produced a better understanding of the types of complexes that are created when toxic elements are absorbed by plants. In addition, a better understanding of the phytochelation system and its coordination complexes had been developed through XAS. Also, XAS allows the study of the coordination chemistry of metals within phytoremediation systems for understanding the complex process but also gives investigators a means to fine tune these systems.

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