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Review

Phytoextraction of heavy metals – the process and scope for remediation of contaminated soils

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Abstract

Contamination of lands with toxic heavy metals is a widespread environmental issue resulting from global industrialization. Conventional techniques for reclamation of such soils are expensive and environmental non-friendly. Phytoremediation is an emerging group of technologies utilizing green plants to clean up the environment from contaminants and has been offered as a cost-effective and non-invasive alternative to the conventional engineering-based remediation methods. There are different versions of phytoremediation viz. phytoimmobilization, phytostabilization, rhizofilteration, phytovolatilization and phytoextraction, the latter being most widely accepted for remediation of soils contaminated with toxic heavy metals. Different aspects of phytoextraction and scope of this technology for remediation of heavy metal-contaminated soils are reviewed discussing challenges and opportunities in realizing phytoextraction as an economically viable remediation method.

Keywords: Chelants, heavy metals, metal hyperaccumulators, phytoextraction, phytoremediation

Pollution of the biosphere with toxic heavy metal is a widespread ecological problem resulting from anthropogenic activities like fossil fuel burning, ore mining and smelting, industrial and municipal waste disposal, and agricultural activities (Nriago, 1979; Adriano, 2001; Kratz and Schnug, 2006). Accumulation of toxic heavy metals such as lead (Pb), arsenic (As), selenium (Se), cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), mercury (Hg) and uranium (U) in agricultural soils not only has detrimental effects on the ecosystem functioning but also poses potential health risks due to transfer of these contaminants into the food chain (Kabata-Pendias, 1992; Giller *et al*., 1998). In Western Europe, reportedly 300,000 sites have been contaminated with heavy metals (Gade, 2000; McGrath *et al*., 2001). In the U.S.A., 600,000 heavy metal-contaminated sites need reclamation (McKeehan, 2000), whereas 100,000 ha of croplands, 55,000 ha of pasture and 50,000 ha of forests have been lost due to heavy metal pollution (Ragnarsdottir and Hawkins, 2005). In China, one-sixth of the total arable land has been contaminated with heavy metals (Liu, 2006). In developing countries including India, Pakistan and Bangladesh, untreated effluents from industry and the raw municipal sewage are often disposed off in agricultural fields in the peri-urban areas which are commonly used for producing vegetables. However, in these countries, only limited data exist for the health risk assessment (Kahlown *et al*., 2006; Sharma *et al*., 2007; Kibria *et al*., 2007; Khan *et al*., 2007; Kashif *et al*., 2009).

Residence time of metals in soil is of the order of thousands of years. This is because unlike organic pollutants, metals are not degraded biologically but are transformed from one oxidation state or organic complex to another (Gisbert *et al*., 2003). Consequently, environmental concerns have resulted in the set up of strict guidelines to prevent the increasing concentrations of heavy metals in soils. Therefore, techniques are imperatively needed to reduce the level of toxic metals in contaminated soils, particularly those used for crop production. Conventional methods used for the remediation of metal contaminated soils are essentially based on civil or chemical engineering and include: vitrification; excavation followed by land filling; chemical treatments; and electrokinetics (Salt *et al*., 1995a; Glass, 1999; Kumpiene *et al*., 2008). However, remediation of heavy metal-contaminated soils with conventional methods is prohibitively expensive (Salt *et al*., 1995a). For example, the remediation cost for one ton of the polluted soil employing conventional methods *viz.* vitrification, land filling, chemical treatment and electrokinetics may be in the range of 75−425, 100−500, 100−500 and 20−200 US $, respectively, (Glass, 1999). Using conventional technologies, the projected 5-year cost for remediation of metal-contaminated sites in the U.S.A. was estimated to be 7.1 billion US $ (U.S. EPA, 1993). According to the European Environment Agency, the costs for the clean-up of contaminated sites in the Europe are estimated to be EUR 59−109 billion (Commission of the European Communities, 2002). Besides, the conventional

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remediation strategy is an *ex situ* approach that severely disrupts the soil structure, landscape and the ecosystem (Salt *et al*., 1995a). The high cost of remediation by conventional techniques has incited research on the development of innovative technologies, of which phytoremediation has been offered as an environmental friendly and cost effective alternative to the conventional remediation approaches. The cost of removing toxic metals by phytoextraction may be as low as 5−40 US $ per ton soil, *i.e.* a fraction of the costs associated with the conventional engineering based methods (Glass, 1999). Likewise, the remediation cost for one acre of metal-polluted land up to 50 cm depth was estimated to be 60,000−100,000 US $ by phytoextraction as compared to at least 400,000 US $ for the conventional method involving soil excavation, transport and storage as hazardous waste (Salt *et al*., 1995a).

Phytoremediation is the use of green plants for removing pollutants from the contaminated environment. For remediation of heavy metal pollution, the phytoremediation technique has different versions *viz.* (i) *phytoimmobilization* – uses plants to prevent the movement and transport of the dissolved contaminants within soil thus reducing the risks associated with contaminated run-off and leaching into the groundwater; (ii) *phytostabilization* – is used to mechanically stabilize heavy metal polluted soils to prevent contaminated run-off, bulk erosion and air-borne transport; (iii) *rhizofilteration* – in which plants absorb toxic metals from contaminated water bodies and concentrate them in roots; (iv) *phytovolatilization* – involves the plant uptake of metals from soil and their volatilization from the foliage; and (v) *phytoextraction* – employs metal accumulating plants which take up toxic metals from soil and concentrate them in the harvestable biomass (Baker and Brooks, 1989; Dushenkov *et al*., 1995, 1997, Salt *et al*., 1995a). Since phytoextraction is the most recognized and applied phytoremediation technique for the removal of toxic metals from soil, the present review is focused on various aspects of phytoextraction.

Phytoextraction employs two strategies *viz.* (i) continuous or natural phytoextraction utilizing natural metal hyperaccumulating plants and (ii) chemically induced phytoextraction in which metal hyperaccumulation in plants is triggered through chemical amendments (Lombi *et al*., 2001). In natural phytoextraction, hyperaccumulating plants have natural ability to extract high amounts of metals from soil, have efficient mechanism to translocate metals from roots to shoots, and can accumulate and tolerate high metal concentrations due to inherent mechanisms to detoxify metals in the tissues. On the other hand, in chemically induced phytoextraction, metal mobilization, root uptake, and translocation to shoots is facilitated by chemical amendments in soil; plants have mostly low tolerance to high metal concentrations thus surviving for a short period following metal hyperaccumulation (Nascimento and Xing, 2006). Another major difference between the natural and induced phytoextraction is that natural hyperaccumulators are mostly slow growers producing low biomass, whereas induced phytoextraction employs fast growing plants with high biomass yields (Nascimento and Xing, 2006).

Metal hyperaccumulating plants – the remarkable biological resource for phytoextraction

Metal hyperaccumulators have the extraordinary capacity to accumulate high concentrations of heavy metals in the above-ground biomass. By virtue of this remarkable characteristic, phytoextraction could be economically viable alternative to the extreme expense of conventional remediation methods. Hyperaccumulators were originally defined as plant species capable of accumulating Ni in shoots in excess of 1000 mg kg^{-1} (Brooks *et al*., 1977). This concentration is almost 100 folds greater than that found in the non-accumulators even when growing in Ni-rich soils. According to this conventional criterion, a hyperaccumulator should tolerate and accumulate in the shoot tissue more than 10 mg kg^{-1} of Hg; 100 mg kg^{-1} of Cd; 1000 mg kg^{-1} of Co, Cr, Cu, Pb and Ni; and 10,000 mg kg^{-1} of Zn and Mn (Baker *et al*., 2000). An extreme example of hyperaccumulator is *Sebertia acuminata* (the New Caledonian Tree), which can accumulate as high as 250,000 mg kg^{-1} of Ni in the dried leaf sap (Jaffré *et al*., 1976). More than 400 plant species from 80 families have been reported as hyperaccumulators for a variety of metals (Baker and Brooks, 1989; Negri and Hinchman, 1996; Baker *et al*., 2000). The number of species known to accumulate one or more metals in excess of 1000 mg kg^{-1} is reported to be 1 for Cd; 2 for Tl; 4 for As; 14 for Pb; 20 for Se; 34 each for Co and Cu; and >320 for Ni (Anderson *et al*., 1999). There exists only one natural hyperaccumulator for Pb (Tamura *et al*., 2005), whereas for U, none has been reported to date, though hyperaccumulation of Pb and U can be induced in some plant species through soil application of chelating agents (Salt *et al*., 1995a; Huang *et al*., 1997, 1998; Blaylock *et al*., 1997). Some metal hyperaccumulators and their bioconcentration potential are listed in Table 1. The list includes natural hyperaccumulators as well as plants in which metal hyperaccumulation can be induced through chelator amendments in soil. Most of the data in Table 1 corresponds to studies carried out in soil, whereas some pertain to experiments conducted in hydroponics.

Table 1. Examples of metal hyperaccumulating plants and their bioconcentration potential

Metal	Plant species	Metal concentration (mg kg^{-1})^{a}	Reference
Arsenic	*Pteris vittate* L.	Up to 23,000	Ma *et al*., 2001
		Up to 13800	Tu *et al*., 2002
Cadmium	*Alyssum murale* Waldst. & Kit.	Up to 1300	Bernal *et al*., 1994
	Arabidopsis halleri L.	5722	Küpper *et al*., 2000
	Atriplex halimus L.	830	Lutts *et al*., 2004
	Colocasia antiquorum Schott	> 400	Kashem, *et al*., 2008
	Helianthus annuus L.	> 1500	Elkhatib, *et al*., 2001
	Pelargonium sp.	> 750	Dan *et al*., 2002
	Sedum alfredii Hance	570	Yang *et al*., 2004
	Thlaspi caerulescens J. *et* C. Presl	Up to 1,800	Baker *et al*., 1994
		3000	Baker and Walker, 1990
Chromium	*Leptospermum scoparium* J. R. & G. Forst.	> 30,000	Negri and Hinchman, 1996
	Pimelea suteri Kirk	Up to 30,000	Negri and Hinchman, 1996
Cobalt	*Aeollanthus biformifolius* De Wild.	> 1000	Brooks *et al*., 1992
	Haumaniastrum katangense (S.Moore) P.A.Duvign. & Plancke.	> 1000	Brooks *et al*., 1992
	H. robertii (Robyns) P.A.Duvign. & Plancke	10,200	Baker and Walker, 1990
Copper	*Aeollanthus biformifolius* De Wild.	> 1000	Brooks *et al*., 1992
	Haumaniastrum katangense (S.Moore)	> 1000	Brooks *et al*., 1992
	P.A.Duvign. & Plancke.	8356	Anderson *et al*., 1999
	Ipomoea alpina Rendle	12,300	Baker and Walker, 1990
Gold	*Brassica juncea* (L.) Czern.	100	Anderson *et al*., 1999
Lead	*Ambrosia artemisiifolia* L.	> 2000	Huang *et al*., 1997
	Armeria maritima (Mill.) Willd.	> 10,000	Baker and Brooks, 1989
	Arrhenatherum elatius (L.) P. Beauv. *ex* J. *et* C. Presl	24,000	Deram *et al*., 2000
	Brassica juncea (L.) Czern.	> 100,000	Raskin *et al*., 1994
	B. pekinensis (Lour.) Rupr.	> 7000	Xiong, 1998
	Fagopyrum esculentum Moench	4,200	Tamura *et al*., 2005
	Helianthus annuus L.	> 100,000	Raskin *et al*., 1994
	Pisum sativum L.	> 6500	Huang *et al*., 1997
	Sedum alfredii Hance	Up to 1182	Yang *et al*., 2002
	Solidago bicolor L.	> 2000	Huang *et al*., 1997
	Thlaspi caerulescens J. *et* C. Presl	Up to 1000	Baker *et al*., 1994
	T. rotundifolium (L.) Gaudin.	8,200	Baker and Walker, 1990
	Zea mays L.	> 2000	Huang *et al*., 1997
Manganese	*Macadamia neurophylla* (Guillaumin) Virot	51,800	Baker and Walker, 1990
Mercury	*Betula papyrifera* Marsh.	Up to 10,000	Negri and Hinchman, 1996
Nickel	*Alyssum bertolonii* Desv.	> 10,000	Robinson *et al*., 1997a
		> 100,000	Negri and Hinchman, 1996
	A. murale Waldst. & Kit.	> 10,000	Baker *et al*., 1988
		22,800	Chaney *et al*., 2008
	A. pintodasilvae Dudley	16,000	Chaney *et al*., 2008
	Berkheya coddii Roessler	17,000	Robinson *et al*., 1997b
	Pelargonium sp.	> 1190	Dan *et al*., 2002
	Psychotria douarrei (Beauvis.) Däniker	47,500	Baker and Walker, 1990
	Sebertia acuminata Pierre *ex* Baillon	Up to 250,000	Jaffré *et al*., 1976
	Thlaspi caerulescens J. *et* C. Presl	> 10,000	Baker and Brooks, 1989

Selenium	*Astragalus* sp.	Up to 10,000	Peterson, 1971
	A. pattersoni Gray *ex* Brand.	Up to 6000	Anderson *et al*., 1999
	A. pectinatus (Douglas *ex* Hook.) Douglas *ex* G. Don	4000	Shrift, 1969
	A. racemosus Pursh	14,900	Beath *et al*., 1937
Thallium	*Biscutella laevigata* L.	Up to 291	Anderson *et al*., 1999
	Iberis intermedia Guersent	Up to 1190	Anderson *et al*., 1999
Uranium	*Amaranth cruentus* L.	> 4000	Huang *et al*., 1998
	Atriplex confertifolia (Torr. & Frém.) S. Wats.	Up to 100	Anderson *et al*., 1999
	Brassica juncea (L.) Czern.	1788	Scholtz, 2006,
		> 5000	Huang *et al*., 1998
	B. chinensis L.	> 4000	Huang *et al*., 1998
	B. narinosa L.H. Bailey	> 3500	Huang *et al*., 1998
	Coprosma arborea Kirk	Up to 30,000	Negri and Hinchman, 1996
	Uncinia leptostachya Raoul	Up to 30,000	Negri and Hinchman, 1996
Zinc	*Arabidopsis halleri* L.	7429	Küpper *et al*., 2000
		32,000	Zhao *et al*., 2000
	Jatropa dioica Cerv.	> 6000	González and González-Chávez, 2006
	Polygonium aviculare L.	> 9000	González and González-Chávez, 2006
	Sedum alfredii Hance	Up to 5000	Yang *et al*., 2002
	Thlaspi caerulescens J. *et* C. Presl	Up to 21,000	Baker *et al*., 1994
		34,000	Brown *et al*., 1995
		51,00	Brown *et al*., 1994
	T. caerulescens subsp. calaminare (Lej.) Dvoráková	> 100,000	Negri and Hinchman, 1996

[a] All values are on shoot dry weight basis except *S. acuminata* for which values are based on the dry weight of leaf sap

The mechanism of metal hyperaccumulation

Metal accumulation in plants is a multi-step process that includes mobilization from soil into the soil solution; uptake by roots, xylem loading and transport to shoot; and distribution in aerial sinks followed by sequestration and storage in the leaf tissue (Clemens *et al*., 2002). For efficient metal hyperaccumulation, therefore, the plant must possess the ability to mobilize metals from soil into the solution phase; the ability to rapidly uptake metals by roots; high translocation rate from root to shoot; and the ability of extracellular storage or/and intracellular sequestration of metals in the leaf cells. These aspects have been reviewed by Salt *et al*. (1995a), Chaney *et al*. (1997), Clemens *et al*. (2002), Nascimento and Xing (2006), and are only briefly discussed in the following sections.

Metal Phytoavailability

The plant uptake of heavy metals from soil depends on the quantity factor, *i.e.* the total content of the potentially available metals in soil; the intensity factor, *i.e.* the activity and ionic ratios of metals in the soil solution; and the reaction kinetics, *i.e.* the rate of transfer from soil to the liquid phase to the plant roots (Brümmer *et al*., 1986). In soil, metals exist in five different pools: fraction 1, soluble *i.e.* metals in the soil solution (as free metal ions and metal complexes); fraction 2, exchangeable *i.e.* metals adsorbed on ion-exchange sites and on inorganic soil constituents; fraction 3, organic *i.e.* metals bound with the organic matter; fraction 4, insoluble *i.e.* metals precipitated mainly as oxides, carbonates and hydroxides; and fraction 5, residual *i.e.* metals incorporated in the silicate minerals. Anthropogenic contamination affects the metal content of fractions 1–4, whereas the fraction 5 reflects the background *i.e.* indigenous metal concentration (Salt *et al*., 1995a; Abollino *et al*., 2006). Only metals in the soluble fraction (fraction 1) and in some components of the exchangeable fraction (fraction 2) are readily available for plant uptake; those of fraction 3 and 4 can be released by different soil amendments; whereas metals in the fraction 5 are potentially non-available (Abollino *et al*., 2006). In general, the metal concentration in plants is correlated with the soil metal concentration in the soluble fraction, making this fraction as the most important indicator of the metal phytoavailability.

Phytoavailability of metals in soil is the first step for successful phytoextraction. A major proportion of metals in soil exists as the bound fraction and needs to be mobilized into the soil solution to make available for plant uptake. Although, this can be achieved artificially through soil amendments (chelant-induced phytoextraction), natural hyperaccumulators have the inherent capability to overcome this constraint by reducing the soil-bound metals by specific plasma membrane bound metal reductases; through root exudation of organic ligands like phytosiderophores and low-molecular weight organic acids (LMWOAs) which form metal complexes; and by acidifying the rhizosphere through the activity of proton pump and/or exudation of LMWOAs (Salt *et al*., 1995a; Nascimento and Xing, 2006; Quartacci *et al*., 2009). Roots of pea plants grown under Fe or Cu deficiency can reduce Fe(III) and Cu(II) thus increasing their uptake (Welch *et al*., 1993). Theoretically, root exudates should play a key role in mobilizing heavy metals thus facilitating their uptake by natural hyperaccumulators. However, the process of root exudation and composition of exudates in hyperaccumulating plants is relatively poorly understood for most of the environmentally relevant heavy metals. There are few reports pertaining to the role of specific root exudates in increasing the heavy metal mobility and uptake. Phytosiderophore like mugineic acid and avenic acid (which are exuded from roots of graminaceous plants in response to Fe and Zn deficiency) can mobilize Cu, Zn and Mn (Römheld, 1991). Nickel hyperaccumulation by *Thlaspi geosingense* has been attributed to the ligand-induced dissolution of Ni bearing minerals in the rhizosphere (Wenzel *et al*., 2002). The LMWOAs are of particular importance in mobilizing soil metals due to their dual function *viz*. soil acidification, and forming complexes with heavy metals. However, the metal complexing capacity of LMWOAs rather than soil acidification has been considered more important in metal mobilization and uptake by plants (Bernal *et al*., 1994; Quartacci *et al*., 2005). Mobilization of Cd and other heavy metals in soil by root exudates containing LMWOAs has been reported (Mench and Martin, 1991). The use of root-exuded LMWOAs sounds better for the public acceptance as these are degradable by microorganisms. However, the efficiency of organic acids in mobilizing metals in the rhizosphere soil is principally governed by their biodegradation rate, the aspect which is relatively poorly understood (Renella *et al*., 2004).

Root uptake

Metals enter into roots through either active (symplastic) or passive (apoplastic) pathway (Tandy *et al*. 2006; Lu *et. al*., 2009). In contrast to the apoplastic pathway in which metal ions or metal-chelate complex enter the root through inter-cellular spaces, the symplastic pathway is an energy dependent process mediated by specific or generic metal ion carriers or channels. In symplastic pathway, the non-essential metal ions compete for the transmembrane carrier used by essential heavy metals. For example, Ni and Cd compete for the transmembrane carrier used by Cu and Zn (Clarkson and Luttge, 1989). Even metal chelates like Fe-phytosiderophore can be transported by symplastic pathway via specialized carriers (Crowley *et al*., 1991). Not only hyperaccumulator plant species, but also the populations within a species may greatly differ in metal uptake. For example, Cd uptake by *Thlaspi caerulescens* ecotype 'Ganges', was not affected by presence of other divalent cations and Ca channel blockers, whereas in *T. caerulescens* ecotype 'Prayon' it was reduced in the presence of Zn (Lombi *et al*., 2002a).

Root to shoot transport

Unlike non-hyperaccumulator plants, in which metals may be stored within root cells (thus becoming unavailable for xylem loading), hyperaccumulators efficiently transport metals from root to shoot. For example, variation in different ecotypes of *Sedum alfredii* for Zn hyperaccumulation was attributable to the difference in Zn transport across tonoplast in the root cells (Yang *et al*., 2006). Similarly, in Cd hyperaccumulating ecotypes of *Sedum alfredii* Cd uptake and xylem loading is an active process as compared to the non-hyperaccumulating ecotypes (Lu *et. al*., 2009). For translocation to shoot, metals must be loaded into xylem for which metals first have to cross the Casparian band on endodermis, which is a water impervious barrier that blocks the apoplastic flux of metals from the root cortex to the stele. Therefore, to cross this barrier and to reach the xylem, metals must move symplastically and this is a rate-limiting step in metal translocation from root to shoot. The endodermis, however, is not a perfect barrier against apoplastic transport of metals from the cortex into stele. Besides possessing 'passage cells' the endodermis may be leaky at least at two sites along the root axis *viz*, at the root apex, Casparian band is not fully developed (Huang and Van Steveninck, 1989); and the points in the basal roots where endodermis is ruptured transiently by lateral roots emerging from the pericycle of the stele through the cortex (Marshner, 1995). Disruption of endodermis can be induced through herbicides, which combined with chelants can enhance metal accumulation (Ensley *et al*., 1999). Besides, several studies on chelant-induced phytoextraction indicate that the root uptake of chelants and their metal complexes mainly follows the apoplastic route (Collins *et al*., 2002).

The xylem loading is a tightly regulated process mediated by membrane transport proteins which remain to be identified (Clemens *et al*., 2002). Once loaded into xylem, the long distance transport of metal ions may be retarded due to high cation-exchange capacity of the xylem cell walls. However, in metal accumulators, xylem loading as well as translocation to shoot is facilitated by complexing of metal with low-molecular weight chelators (LMWCs) e.g. organic acids (Senden *et al*., 1992), phytochelatins (Przemeck and Haase, 1991), and histidine (Krämer *et al*., 1996). A pH-dependent equilibrium exists between LMWCs, free hydrated metal cations and metal chelates in the mobile transpiration stream and the stationary metal binding sites in the cell wall of xylem vessels (Clemens *et al*., 2002). These organic ligands also play an important role in the metal tolerance by heperaccumulators. In induced phytoextraction also, the role of applied chelants on different components of hyperaccumulation is well established.

Metal unloading, trafficking and storage in leaves

Metal is transported (via flow of the xylem sap) to the apoplast of leaves from where it is distributed within the leaf tissue via apoplast or transporters-mediated uptake by symplast. Complexing with organic ligands, which may occur at any point along the transport pathway, converts the metal into less toxic form thus conferring high metal tolerance in hyperaccumulators (Peer *et al*., 2005). Nicotoianamine (Vacchina *et al*., 2003), citrate (Krämer *et al*., 2000), and histidine (Krämer *et al*., 1996) have been reported to form complexes with Ni; phytochelatins are known to complex with Cu (Curvetto and Rauser, 1979) and As (Raab *et al*., 2004); whereas Zn forms complexes with asparagine (Smirnoff and Stewart, 1987), phytate (Van Steveninck *et al*., 1987) and organic acids (Peer *et al*., 2005). Since most of the chelators (e.g. phytochelatins) use thiol group to complex the metals, sulfur biosynthesis pathways play a key role in the metal tolerance in several hyperaccumulators (Peer *et al*., 2005). Besides the above-mentioned mechanisms, upregulation of the anti-oxidant enzymes also confers tolerance to toxic heavy metals (Freeman *et al*., 2004).

In the leaf tissues, metals are sequestered in extra-cellular or sub-cellular compartments. About 35% of the Cd taken up by *T. caerulescens* was found in the cell walls and the apoplast in leaves (Cosio *et al*., 2005), whereas in Ni hyperaccumulator *Thlaspi geosingense*, Ni is sequestered in the cell wall as well as in vacuoles (Krämer *et al*., 2000). In the Zn-tolerant clone of *Deschampsia caespitosa*, more Zn was sequestered in vacuole as compared to the non-tolerant clone (Brookes *et al*., 1981). The cell types where metals are deposited vary with the metal as well as with the plant species. In *Thlaspi caerulescens*, Zn accumulation was several folds higher in vacuoles of the epidermal and sub-epidermal cells than of the mesophyll cells (Küpper *et al*., 1999); whereas in *Arabidopsis halleri*, Zn and Cd were sequestered preferentially in vacuoles of the mesophyll than the epidermal cells (Küpper *et al*., 2000). Arsenic in *Pteris vittata* is sequestered in vacuoles of the epidermal cells (Lombi *et al*., 2002b). Leaf trichomes may be the major sequestering sites for Cd in *Brassica juncea* (Salt *et al*., 1995b); for Ni in *Alyssum lesbiacum* (Krämer *et al*., 1997); and for Zn in *Arabidopsis halleri* (Küpper *et al*., 1999). However, proteins involved in the differential distribution of metals between leaf cells have not been identified (Clemens *et al*. 2000). Different approaches have been envisaged by Clemens *et al*. (2000) for engineering the plant metal homeostasis network to increase the metal accumulation in plants. For example, keeping in view the importance of vacuoles as the metal storage organelle, engineering tonoplast transporters in specific cell types might enhance the metal accumulation capability. Alternatively, creation of artificial metal sinks in shoots via expression of the cell wall proteins with high-affinity metal binding sites might be explored to increase the metal demand in shoots thus enhancing the accumulation in leaves (Clemens *et al*., 2000).

Natural phytoextraction

With exception to Ni phytoextraction, natural phytoextraction is mainly hampered by the low biomass of hyperaccumulators and it may require even decades to reduce the heavy metal concentration in soil to environmentally permissible levels. For example, the most efficient Zn hyperaccumulator *Thlaspi caerulescens* would require nine croppings to lower the Zn concentration of the polluted soil from 440 to 300 mg kg^{-1} (Mcgrath *et al*., 1993). To decontaminate a Ni and Zn contaminated soil, 13−14 years of continuous cropping of *T. caerulescens* were required (Baker *et al*., 1994). Brown *et al*. (1994) estimated a 28-year cultivation of *T. caerulescens* to completely remediate the soil containing 2100 mg Zn kg^{-1}. In a Cd and Zn-contaminated soil, a 14-month cropping of *T. caerulescens* removed almost 22% of Cd and 4% of Zn (McGrath *et al*., 2006). Phytotoxicity induced by the uptake of non-target metals may also limit the potential of phytoextraction. For instance, three crops of *T. caerulescens* though removed 43% of Cd and 7% of Zn from an industrially contaminated soil (19 mg Cd kg^{-1}, 2920 mg Zn kg^{-1} and 78 mg Cu kg^{-1}), its Cd and Zn extraction potential was severely affected by Cu toxicity in a sewage sludge contaminated agricultural soil that contained 42, 1756 and 1245 mg kg^{-1} of Cd, Zn and Cu, respectively (Lombi *et al*., 2001).

The most successful application of the natural phytoextraction has been reported for Ni, leading to phytomining of nickeliferous soils containing Ni content below the economic threshold for conventional mining. The pioneer studies on Ni phytomining (Nicks and Chambers, 1995, 1998) employing *Streptanthus polygaloides* have indicated that with optimized Ni phytomining a net return to the US grower ($ US 765 ha^{-1}, including metal and energy yields) could be well in excess of the average return obtained with cropping wheat ($ US 333 ha^{-1}, grain and straw). Robinson *et al.* (1997a) using NPK fertilizers showed a 3-fold increase in the dry matter yield (9 t ha^{-1}) of *Alyssum bertolonii*, which could extract 72 kg of Ni ha^{-1}. According to a model proposed by Robinson *et al.* (1997a), seven croppings of *A. bertolonii* would reduce the plant-available Ni pool by 30%. Yearly harvesting of the *Alyssum* crop and its incineration can produce, besides the energy dividend, S-free bio-ore with about 11% Ni, which is much higher than the Ni content of the conventional ores. An increase in the biomass yield due to NPK fertilization has been reported for another Ni hyperaccumulator *Berkheya coddii*, which can extract as much as 100 kg Ni ha^{-1} (Robinson *et al.*, 1997b). Although, natural hyperaccumulators for thallium *viz. Biscutella laevigata* (13,768 mg Tl kg^{-1}; biomass 4 t ha^{-1}) and *Iberis intermedia* (4,055 mg Tl kg^{-1}; biomass 10 t ha^{-1}) have been reported (Anderson *et al.*, 1999), these have not been exploited for phytoextraction of this rare but extremely toxic metal. The Chinese brake fern (*P. vittata*) was estimated to remediate an As-contaminated site in 10 years or less (Salido *et al.*, 2003).

Induced phytoextraction

The induced phytoextraction operates through high-biomass crops in which metal hyperaccumulation is triggered through soil amendments that increase the metal phytoavailability and translocation from root to shoot (Salt *et al.*, 1995a). The most commonly used high-biomass crops include sunflower, ryegrass, and various species of *Brassica*. Ryegrass and mustard can yield dry matter as high as 1.5 and 10 t ha^{-1}, respectively (Vandenhove and Hees, 2004). At neutral or alkaline pH, most of the metals in soil are not available to plants; particularly Pb and Cr are inherently immobile. However, the metal mobility and thus phytoextraction can be considerably enhanced by inorganic and organic amendments (Schmidt, 2003). Since inorganic and organic agents mobilize metals by different mechanisms, these are discussed separately.

Inorganic amendments

Inorganic agents mobilize metals mainly by desorption process (Brümmer *et al.*, 1986), which is affected either by lowering the soil pH (Gray *et al.*, 1999, Schremmer *et al.*, 1999), or by salt addition (Smolders *et al.*, 1998). The solubility of heavy metals is governed by soil pH, the amount and type of sorption sites and the total content of heavy metals in soil (Brümmer *et al.*, 1986; Hornburg and Brümmer, 1993; Gray *et al.*, 1999). The proportion of soluble Cd, Zn, Pb, and Cu increases at soil pH below 6.5, 5.3, 4.5 and 3.5, respectively (Hornburg and Brümmer, 1993). After desorption of metals into the soil solution and removal by plant uptake or leaching, further acidification will dissolve several soil minerals (Brümmer *et al.*, 1986), the extent of which depends on the buffering capacity of soils; in aerated soils the process follows the first-order kinetics for several metals (Aringhieri and Pardini, 1985). Since soil physicochemical characteristics strongly influence the soil's buffering capacity, the amount of protons needed to achieve the target pH may vary for different soils (Wang *et al.*, 2006). Moreover, the extreme acidification of soils (pH < 4) is detrimental for plant growth (Marschner, 1995). Therefore, to achieve efficient phytoextraction, pH optima should be worked out for different soil-plant systems. Decreasing the soil pH through application of mineral acids (Gray *et al.*, 1999) or elemental S (Wang *et al.*, 2006) is an effective strategy to desorb metals from soil into the solution phase thus increasing uptake by plants. Although, physiological acidification of rhizosphere by application of ammonium fertilizers has also been reported as a low-cost strategy to increase the heavy metal mobilization (Schremmer *et al.*, 1999), it is relatively less effective in enhancing phytoextraction as compared to the acidification of bulk soil. Moreover, to avoid nitrification of the applied ammonium, nitrification inhibitors may also be needed. The commercial nitrification inhibitors, however, are hardly effective at high soil temperatures (Ali *et al.*, 2008). Some metals in soil are also bound or adsorbed on oxides of Fe, Mn, and Al; dissolution of these oxides at low pH will simultaneously release these metals into the soil solution. Citric acid addition to a U-contaminated soil not only mobilized U but also released Fe and Al (Salt *et al.*, 1995b, Huang *et al.*, 1998). In some cases, natural hyperaccumulation may also be limited by the metal mobility in soil that needs to be enhanced through soil amendments. For example, after optimizing the soil pH through elemental S amendments, *T. caerulescens* extracted 36% of Cd from soil contaminated with 25 mg Cd kg^{-1} (Wang *et al.*, 2006).

Salt amendments like NaCl or KCl can increase phytoextraction by two mechanisms *viz.* exchange of metals from sorption sites in soil by the cationic component; and formation of stable metal complexes with the chloride anion (Schmidt, 2003). Addition of NaCl increased Cd concentration in the soil solution and accumulation in the leaf of Swiss chard (*Beta vulgaris* ssp. *Cicla*; Bingham *et al.*, 1983). In other studies enhanced accumulation of Cd in Swiss chard was attributed to the formation of chlorocomplexes of Cd instead of increased Cd concentration in the soil solution (Smolder *et al*., 1998). Application of KCl at 3 g kg^{-1} increased Cd accumulation in corn similar to that achieved by 0.6 g kg^{-1} of EDTA, suggesting KCl as a preferred amendment over EDTA because of the lower cost and relatively shorter persistence of the Cd-Cl complex in soil (Maxted *et al*., 2001). Application of 10 mmol kg^{-1} of NH_4Cl to a Zn-enriched soil though increased the metal solubility by 1.5 folds; it caused only a slight increase in the Zn accumulation by *Salix aurita* (Keller *et al*., 1999). Schmidt (2003) attributed the low efficiency of NH_4Cl in Zn phytoextraction to the small cation exchange effect (due to low NH_4Cl application rate) and the much lower extent of complexing Zn with Cl as compared to Cd. Since application of NaCl damages the soil structure more than other salts, Schmidt (2003) suggested the need of exploring other Cl^- salts for enhancing phytoextraction.

Organic complexing agents

The organic complexing agents or chelants desorb metals from different soil components by forming water soluble complexes (Schmidt, 2003). Examples of chelating agents include: (i) synthetic aminopolycarboxylic acids (APCAs), *e.g.* ethylenediaminetetraacetic acid (EDTA), hydroxyethylethylenediaminetriacetic acid (HEDTA), 1,2-cyclohexylenedinitrilotetraacetic acid (CDTA) and diethylenetriaminepentaacetic acid (DTPA); (ii) natural aminopolycarboxylates, *e.g.* ethylenediamine disuccinate (EDDS) and nitriloacetic acid (NTA); and (iii) low molecular weight organic acids (LMWOAs), *e.g.* citric acid, oxalic acid, gallic acid and acetic acid. Efficiency of some chelants for enhancing phytoaccumulation of heavy metals is summarized in Table 2.

Chelants can be particularly useful in mobilizing heavy metals at high soil pH as the stability of metal-organic complex increases with increasing pH (Scheffer and Schachtschabel, 1998). The efficiency of metal solubilization by chelating agents depends on the stability constants of the metal-chelate complex and follows the order EDTA (and related compounds) > NTA > citric acid > oxalic acid > acetic acid (Wenger *et al*., 1998). Besides mobilizing metals in soil, chelants also facilitate metal translocation from root to shoot (Shen *et al*., 2002; Santos *et al*., 2006). After assimilation by root, entire metal-chelate complexes are translocated to shoots and this has been well established for Pb-EDTA (Sarret *et al*., 2001), Pb-EDDS (Tandy *et al*., 2006), Cu-NTA (Wenger *et al*., 2002), Cu-EDDS (Luo *et al*., 2005) and Zn-EDTA (Collins *et al*., 2002). Ethylenediaminetetraacetic acid has been the most investigated organic amendment in phytoextraction and has been successfully used to enhance the phytoextraction of Pb and other heavy metals (Blaylock *et al*., 1997; Shen *et al*., 2002; Wu *et al*., 2003; Santos *et al*., 2006). Relatively few studies demonstrate the heavy metal fractions targeted by chelating agents. For example, the EDTA targeted the Pb corresponding mainly to the exchangeable and the carbonate fractions, whereas Pb associated with oxide, organic and residual fractions was less effectively solubilized (Elless and Blaylock, 2000).

There are also risks associated with the use of synthetic APCAs under field conditions as the amount of metals mobilized by synthetic chelants often far exceeds the amount taken up by plants (Römkens *et al*., 2002). Not only the synthetic APCAs like EDTA are recalcitrant, their metal complexes are also highly stable and can persist in the soil pore water for several months (Lombi *et al*., 2001). The leaching of chelator or metal-chelator complexes into the groundwater poses a great environmental risk, which may be even higher during the periods of heavy irrigations or precipitation (Meers *et al*., 2005b). Few attempts have been made to reduce the leaching risk associated with the use of synthetic chelators. For example, Salt *et al*. (1998) suggested the use of chelator at the time of maximum crop biomass. Alternatively, formulation of slow-release EDTA granules by coating with silicates can reduce the risk of metal leaching (Li *et al*., 2005). Biodegradable APCAs e.g. EDDS, NTA, methylglycinediacetate (MGDA) and Hydroxyiminodisuccinic acid (HIDS) have been evaluated as environmentally safe alternatives to EDTA (Grčman *et al*., 2003, Tamura *et al*., 2005; Freitas and Nascimento, 2009; Rehman *et al*., 2009). For example, EDDS was more effective than EDTA in mobilizing Cu and Zn, whereas EDTA was more effective than EDDS for Pb and Cd (Luo *et al*., 2005). Organic acids like malic acid, oxalic acid and citric acid have been evaluated for mobilizing heavy metals like Cd, Zn, U, Cr and Ni in soils thus increasing not only the metal uptake but also translocation to shoot. Citric acid compared to EDTA was more effective in mobilizing Cr (Jean *et al*., 2008). In a study with serpentine-mine tailings, EDTA and DTPA though mobilized more Cr and Ni than LMWOAs (citric and oxalic acids) but reduced the biomass of *B. juncea* due to metal phytotoxicity (Hsiao *et al*., 2007). Of the various synthetic APCAs and LMWOAs tested, citric acid was most effective in mobilizing U from soil and increasing its uptake by *Brassica* spp. by more than 1000 folds (Huang *et al*., 1998). Comparing different biodegradable amendments for mobilizing U, Duquène *et al*. (2008) also reported citric acid as the most effective amendment causing up to 479 folds increase in the soluble U as compared to 10–100 folds increase induced by oxalic acid, EDDS and NTA. The efficiency of various organic complexing agents in phytoextraction also varies for different heavy metals. For mobilizing Cu and Pb, the effectiveness of various chelants followed an order EDTA

>> citric acid ≈ oxalic acid ≈ malic acid; for Zn, EDTA >> malic acid >> citric acid ≈ oxalic acid; and for Cd, EDTA >> malic acid >> citric acid >oxalic acid (Wu *et al*., 2003). Cadmium accumulation in sunflower was highest with citric acid followed by malic acid, CTAB and EDTA (Elkhatib *et al*., 2001), whereas accumulation of Pb by *B. juncea* increased in the order EDTA > CDTA > DTPA > EGTA > citric acid (Blaylock *et al*., 1997).

The degradation rate of metal-chelant complex depends on their stability constants, microbial activity and the concentration of free ionic metals (Schmidt, 2003). Moreover, heavy metal forms of chelates like Fe(II)-EDTA generally degrade much slower than chelates of other metals like Na, Ca, or Mg (Henneken *et al*., 1998). Even a biodegradable APCA, NTA decomposes at a much slower rate when complexed with Zn (Wenger *et al*., 1998) as

Table 2: Chelant-induced phytoextraction: comparing different organic complexing agents for enhancing phytoextraction of heavy metals by some high biomass crop plants

Metal	**Total metal in soil**	**Chelant[a]**	**Plant species**	**Metal concentration in shoot**		**Reference**
	mg kg^{-1}			**mg kg^{-1}**		
				Control	**With chelate**	
Lead	6750	EDTA (4)	*Arrhenatherum elatius*	96	24000	Deram *et al*., 2000
	2450	HEDTA (2)	Pea	90	10600	Huang *et al*., 1997
	2450	HEDTA (2)	Corn	60	10200	Huang *et al*., 1997
	10600	EDTA (0.9)	Cabbage	126	5010	Shen *et al*., 2002
	10600	EDTA (0.9)	Mungbean	127	1170	Shen *et al*., 2002
	10600	EDTA (0.9)	Wheat	80	2650	Shen *et al*., 2002
	4000	CDTA (5.8)	*Helianthus annuus*	67	5200	Cooper *et al*., 1999
	4000	CDTA (5.8)	Redtop	25	3000	Cooper *et al*., 1999
	4000	CDTA (5.8)	Corn	36	2600	Cooper *et al*., 1999
	600	EDTA (2.92)	*Brassica juncea*	45	16,000	Blaylock *et al*., 1997
	600	DTPA (3.93)	*B. juncea*	45	10,000	Blaylock *et al*., 1997
	2500	EDTA (0.44)	Corn	90	500	Wu *et al*., 1999
Cadmium	150	NTA (1.91)	*B. juncea*	200	415	Quartacci *et al*., 2005
	150	NTA (3.82)	*B. juncea*	200	719	Quartacci *et al*., 2005
	200	Citric acid (1.92)	*B. juncea*	230	290	Quartacci *et al*., 2005
	200	Citric acid (3.84)	*B. juncea*	230	560	Quartacci *et al*., 2005
	100	EGTA (3.8)	*B. juncea*	220	2800	Blaylock *et al*., 1997
	100	EDTA (2.92)	*B. juncea*	220	1500	Blaylock *et al*., 1997
	100	CDTA (1.82)	*B. juncea*	220	1000	Blaylock *et al*., 1997
Uranium	750	Citric acid (3.84)	*B. juncea*	<5	5200	Huang *et al*., 1998
	750	Malic acid (2.68)	*B. juncea*	<5	2100	Huang *et al*., 1998
	750	Acetic acid (1.2)	*B. juncea*	<5	1700	Huang *et al*., 1998
	30	Citric acid (4.8)	*B. juncea*	21	1788	Scholtz, 2006
	30	Citric acid (4.8)	*H. annuus*	12	836	Scholtz, 2006
	205	Citric acid (4.8)	*B. juncea*	101	460	Scholtz, 2006
	205	Citric acid (4.8)	*H. annuus*	184	344	Scholtz, 2006
Molybdenum	4	Citric acid (4.8)	*B. juncea*	206	467	Scholtz, 2006
	4	Citric acid (4.8)	*H. annuus*	91	226	Scholtz, 2006
	173	Citric acid (4.8)	*B. juncea*	119	296	Scholtz, 2006
	173	Citric acid (4.8)	*H. annuus*	142	275	Scholtz, 2006
Arsenic	11	Citric acid (4.8)	*B. juncea*	2	24	Scholtz, 2006
	11	Citric acid (4.8)	*H. annuus*	5	14	Scholtz, 2006

[a] Figures in parentheses indicate chelant application rate (g kg^{-1})

compared to Na_3NTA (Tabatabai and Bremner, 1975). Biodegradability of organic complexing agents plays an important role in determining their efficiency as well as their safety in phytoextraction and this has been well documented for APCAs and organic acids (Meers *et al*., 2005a; Evangelou *et al*., 2008). While EDTA and citric acid both caused a rapid increase in the labile fractions of Cu, Zn, Cd and Pb, the half-life of metals solubilization decreased sharply with citric acid due to rapid degradation (Meers *et al*., 2005a). However, due to higher amount of metals mobilized with increasing EDTA application rate, *H. annuus* suffered from heavy metal stress, whereas metal phytoextraction by citric acid was also inefficient due to its rapid degradation (Lesage *et al*., 2005). The role of biodegradation in inefficiency of LMWOAs for phytoextraction has also been emphasized by Evangelou *et al*. (2008). To overcome the rapid biodegradation of LMWOAs, their successive application was ineffective as these are degraded by the same microorganisms that had already developed in response to the first application.

Besides biodegradation, the high buffering capacity of calcareous soils may require even higher concentrations of LMWOAs. For example, citric acid can be effective in mobilizing heavy metals only when its application rate exceeded the buffering capacity of soil (Lesage *et al*., 2005). Therefore, for phytoremediation of contaminated calcareous soils, prior acidification with mineral acids or elemental S may be envisaged as a useful strategy to lower the amount of citric-acid required. This has been demonstrated in studies with phytoremediation of U-contaminated soils for which citric acid has proved to be the most effective amendment (Huang *et al*., 1998; Shahandeh and Hossner, 2002). However, soil acidification alone (with mineral acids or elemental S) did not increase the U uptake by plants. Since U is taken up by plants as free uranyl cation ($UO2^{2+}$), which is the predominant U species at pH <5.5 (Ebbs *et al*., 1998a) and has strong tendency to bind to soil solids and organic matter thus rendered unavailable for plant uptake (Sheppard *et al*., 1989). Citric acid, which besides lowering the soil pH and releasing uranyl ion, forms binuclear complex with uranyl cation thus increasing the U solubility over 200 folds (Ebbs *et al*., 1998b) and U phytoaccumulation over 1000 folds (Huang *et al*., 1998). However, the extent of U solubilization is strongly dependent on the amount of the citric acid and the initial pH of soil. For instance, for soil with a pH of 7.7, doubling the citric acid application rate from 10 to 20 mmole kg^{-1} caused a 5-fold increase in the shoot U concentration by *B. juncea* (Huang *et al*., 1998). On the other hand, at pH 4–5, relatively low application rate (2 mmole kg^{-1}) citric acid was more effective in solubilizing U than at pH 6–8 (Ebbs *et al*., 1998b).

Phytotoxicity of chelants

The chelant phytotoxicity may often limit the phytoextraction potential that relies not only on the high metal concentration in shoots but also on the high biomass production (Nascimento and Xing, 2006). Although, there are conflicting reports regarding the phytotoxicity of chelating agent, biomass reduction by synthetic APCAs has been frequently reported. For example, EDTA and EDTA-heavy metal complexes are toxic to the soil microflora (Grčman *et al*., 2001) as well as to plants causing drastic growth reduction in several plant species (Chen and Cutright, 2001; Nascimento *et al*., 2006; Jean *et al*., 2008). Biomass reduction in different plant species has also been reported with other synthetic APCAs like EGTA (5–10 mmol kg^{-1}), DTPA (5–10 mmol kg^{-1}), EDDHA (1.39 mmol kg^{-1}, HEDTA (1.45 mmol kg^{-1}), EDTA (1.32 mmol kg^{-1}) and CDTA (5–10 mmol kg^{-1}) (Blaylock *et al*., 1997; Huang *et al*., 1997; Sun *et al*., 2009). Degradable APCAs like EDDS and NTA are also sometimes toxic. For instance, EDDS, applied at 5–10 mmol kg^{-1} caused severe growth reduction in *Zea mays* (Luo *et al*., 2005), whereas its application in 4 splits each of 10 mmol kg^{-1} caused biomass reduction in *B. rapa* (Grčman *et al*., 2003). Plant growth stage has significant effects on the tolerance against synthetic APCAs and this varies with plant species. For example, moderate rates of EDTA increased Cd phytoextraction by *Solanum nigrum* when applied near flowering stage (Sun *et al*., 2009). The other degradable APCA, NTA sowed no toxicity symptoms when applied in the range of 1–20 mmol kg^{-1} (Quartacci *et al*., 2005), whereas in an other study it caused moderate to severe growth reduction with application rate of 2.7–26.6 mmol kg^{-1} (Kulli *et al*., 1999). The LMWOA, citric acid applied in the range of 3–20 mmol kg^{-1} was not phytotoxic (Huang *et al*., 1998; Shahandeh and Hossner, 2002; Wu *et al*., 2003; Quartacci *et al*., 2005). At higher application rate (25 mmol kg^{-1}) though citric reduced the dry matter yield of mustard and ryegrass, it enhanced U phytoextraction by both species (Vandenhove and Hees, 2004).

Challenges and opportunities

An ideal plant for metal phytoextraction should be fast growing, have high biomass and deep root system, be easy to harvest, and be able to tolerate and accumulate a range of heavy metals in the harvestable component (Clemens *et al*., 2002). However, no plant is known to date that meets all these criteria. Although, successful application of natural hyperaccumulators has been limited to few metals, there is a hope of commercial hype as the phytoextraction technology is at its infancy stage. Keeping in view that known metal hyperaccumulators comprise only < 0.2% of angiosperms (Baker and Whiting, 2002), there is great potential for biodiversity prospecting to discover more efficient natural hyperaccumulators. Besides, there is also need to explore various agronomic strategies to enhance the biomass potential of natural hyperaccumulators. Increasing the biomass yield and phytoextraction potential of natural hyperaccumulators by optimizing NPK fertilization seems to be a viable option (Barrutia, *et al*., 2009). Besides, the role of plant growth regulators *e.g.* cytokinins, indolebutyric acid, naphthylacetic acid, gibberellic acid and indole-3-acetic acid also needs to be explored for increasing the biomass and thus phytoextraction potential of natural hyperaccumulators. Enhanced phytoextraction of heavy metals by non-hyperaccumulators due to foliar sprays of growth regulators is well documented (Fuentes *et al*., 2000; Tassi *et al*., 2008; Hadi *et al*., 2010).

In the context of high biomass species, a strategy could be to improve their phytoextraction potential through introduction of genes conferring metal hyperaccumulation and tolerance. Several key steps have recently been identified at the molecular level indicating the possibility of engineering metal hyperaccumulation in plants (Clemens *et al*., 2002). In the absence of known genes for phytoextraction, while this could be achieved through somatic and sexual hybridization, it would require long-term efforts to develop a 'molecular tool-box' composed of the desired genes (Salt *et al*., 1995a). Although chelant-induced phytoextraction by high biomass crop species has always been considered as a promising alternative to the natural phytoextraction, it has not yet been widely evaluated under field conditions. There are indeed several constraints that challenge the commercialization of chelant-assisted phytoextraction. For example, to overcome the phytotoxicity and leaching problems associated with the use of chelants, split-application of chelants reduces the desired enhancement effects, whereas the sub-irrigation drainage systems to cope with the leaching risk is not cost effective (Evangelou *et al*., 2007). Besides, the costs of chelating agents required to operate phytoextraction at the field level have not been duly addressed. The calculated amounts of different chelating agents required under field conditions are provided in Table 3. For example, in most studies carried out with citric acid, the latter was effective at 20 m-mol kg^{-1}, which is equivalent to > 8 tons ha^{-1}. Therefore, the cost effectiveness of chelant-assisted phytoextraction needs reappraisal.

Although, external application of LMWOAs like citric acid has been well documented for mobilizing heavy metals in soils and increasing their uptake by plants (Huang *et al*., 1998, Ebbs *et al*., 1998b), the potential of citric acid-exuding plants has not been exploited for metal phytoextraction. Members of the family Proteacae e.g. *Lupinus* species are well known for exuding high amounts of LMWOAs that play a key role in mobilizing P in P-deficient soils (Neumann *et al*., 1999). Under P-deficient conditions, the proteoid roots of *L. albus* predominantly exude citric acid, which may be as high as 23% of the acquired C (Dinkelaker *et al*., 1989). Therefore, it should be worth exploring citric acid-exuding plant species with characteristics desirable for phytoextraction. Alternatively, co-culturing citric acid-exuding plants with known metal hyperaccumulators may also be exploited for increasing the metal phytoavailability and uptake by the hyperaccumulator thus minimizing the external input of citric acid.

Beneficial role of bacteria also needs to be exploited to enhance the phytoextraction of heavy metal polluted soils. Soil inoculation with Cd-resistant strains *Pseudomonas* sp. RJ10 and *Bacillus* sp. increased Cd and Pb phytoextraction by hyperaccumulating tomato plant (He, et al., 2009). *Pseudomonas* sp. is known to produce rhamnolipid, which is a metal sequestering agent and has a strong affinity for Cd. Rhamnolipid compared to citric acid may remain in the soil long enough to enhance metal phytoextraction, but is not recalcitrant like EDTA to raise concerns regarding metal leaching (Wen *et al*., 2009).

Phytoextraction could also be combined with profit making operations like forestry and bioenergy production (Ginneken *et al*., 2007). For example, hydroponically grown castor bean (*Ricinus communis*), which is known to accumulate Cd (Melo *et al*., 2009) and Pb (Romeiro, *et al*. 2006) needs to be explored in metal-contaminated soils. Phytoextraction of Cd by a high-biomass tree 'carambola' ((*Averrhoa carambola*) presents a feasible option to clean up Cd-contaminated agricultural soils. In a soil slightly contaminated with Cd, the tree would extract 50% of the total soil Cd in 13 years (Li *et al*., 2009). To produce biofuels, valorization of the bioenergy crops like poplar, willow trees, castor, *Jatropha* and *Brassica* spp. can be carried out by different energy recovery techniques like incineration, gasification, anaerobic digestion and pure plant oil production (Evangelou *et al*., 2007; Ginneken *et al*., 2007). Such a combined approach *i.e.* phytoextraction with bioenergy production though sounds attractive, investigations to realize this approach are still at initial stages (Ginneken *et al*., 2007).

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Table 3: Amount of different chelating agents required for induced phytoextraction

Chelant type	Compound	Amount applied		
		mmol kg^{-1}	g kg^{-1}	kg ha^{-1a}
Synthetic APCAs	Na_2-EDTA (372.24)[b]	1	0.372	838
		5	1.860	4188
		10	3.720	8375
	HEDTA (278.26)	1	0.278	626
		5	1.390	3131
		10	2.780	6261
	DTPA (393.34)	1	0.393	885
		5	1.965	4425
		10	3.930	8850
	CDTA (364.36)	1	0.364	820
		5	1.820	4099
		10	3.640	8198
	EGTA (380.35)	1	0.380	856
		5	1.900	4279
		10	3.800	8558
	HEIDA (177.16)	1	0.177	399
		5	0.885	1993
		10	1.770	3986
Natural APCAs	EDDS (358.19)	1	0.358	806
		5	1.790	4030
		10	3.580	8058
	NTA (191.14)	1	0.191	430
		5	0.955	2151
		10	1.910	4301
LMWOAs	Citric acid (192.13)	1	0.192	432
		5	0.960	2162
		10	1.920	4323
	Malic acid (134.09)	1	0.134	302
		5	0.670	603
		10	1.340	1207
	Oxalic acid (90.04)	1	0.090	203
		5	0.450	1013
		10	0.900	2026
	Acetic acid (60.05)	1	0.060	135
		5	0.300	675
		10	0.600	1350

[a] Considering the arable (0–15 cm) soil layer with a bulk density of 1.5 g cm^{-3}

[b] Figures in parentheses represent the molecular weight

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