

Field crops for phytoremediation of metal-contaminated land. A review

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Abstract The use of higher plants to remediate contaminated land is known as *phytoremediation*, a term coined 15 years ago. Among green technologies addressed to metal pollution, phytoextraction has received increasing attention starting from the discovery of hyperaccumulator plants, which are able to concentrate high levels of specific metals in the above-ground harvestable biomass. The small shoot and root growth of these plants and the absence of their commercially available seeds have stimulated study on biomass species, including herbaceous field crops. We review here the results of a bibliographical survey from 1995 to 2009 in CAB abstracts on *phytoremediation* and *heavy metals* for crop species, citations of which have greatly increased, especially after 2001. Apart from the most frequently cited *Brassica juncea* (L.) Czern., which is often referred to as an hyperaccumulator of various metals, studies mainly focus on *Helianthus annuus* L., *Zea mays* L. and *Brassica napus* L., the last also having the greatest annual increase in number of citations. Field crops may compensate their low metal concentration by a greater biomass yield, but available data from in situ experiments are currently very few. The use of amendments or chelators is often tested in the field to improve metal recovery, allowing above-normal concentrations to be reached. Values for Zn exceeding 1,000 mg kg⁻¹ are found in *Brassica*

spp., *Phaseolus vulgaris* L. and *Zea mays*, and Cu higher than 500 mg kg⁻¹ in *Zea mays*, *Phaseolus vulgaris* and *Sorghum bicolor* (L.) Moench. Lead greater than 1,000 mg kg⁻¹ is measured in *Festuca* spp. and various *Fabaceae*. Arsenic has values higher than 200 mg kg⁻¹ in sorghum and soybean, whereas Cd concentrations are generally lower than 50 mg kg⁻¹. Assisted phytoextraction is currently facilitated by the availability of low-toxic and highly degradable chelators, such as EDDS and nitrilotriacetate. Currently, several experimental attempts are being made to improve plant growth and metal uptake, and results are being achieved from the application of organic acids, auxins, humic acids and mycorrhization. The phytoremediation efficiency of field crops is rarely high, but their greater growth potential compared with hyperaccumulators should be considered positively, in that they can establish a dense green canopy in polluted soil, improving the landscape and reducing the mobility of pollutants through water, wind erosion and water percolation.

Introduction

Worldwide, soil is being seriously degraded as a result of increasing industrial, agricultural and civil activities. Soil contamination, both diffuse and localised, can lead to damage to several soil functions and contamination of surface- and groundwater. The main source of diffuse soil contamination is deposition from the atmosphere and flowing water or eroded soil itself. Further contamination may derive from direct application of pesticides, sewage sludge, fertilisers and manure, which often contain heavy metals. The soil functions most affected by contamination are buffering, filtering and transforming capacities (EEA 2003).

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Localised contaminated soils, currently called *brown-fields* (French et al. 2006) or *urban soils*, are frequently associated with abandoned industrial plant, accidental release of pollutants or inappropriate municipal and industrial waste disposal (EEA-UNEP 2000). In the mining industry, which is a major cause of soil degradation, the risk of contamination is associated with sulphur- and metal-bearing tailings stored in mining sites, and the use of chemicals such as cyanide in the refining process (EEA 2003).

Among various organic and inorganic pollutants, great worldwide concern about soil contamination regards heavy metals. In the European Union, contamination by metals accounts for more than 37% of cases, followed by mineral oil (33.7%), polycyclic aromatic hydrocarbons (PAH, 13.3%) and others (EEA 2007).

Many industrialised countries are now focusing on regulations to reduce the impact of pollution. For instance, the European Pollutant Release and Transfer Register (Regulation 166 EC 2006) lists possibly dangerous activities and pollutants and provides thresholds for releases into air, water, and soil for all main contaminants. As regards metals, the maximum amounts permitted are generally higher for air than for water or soil (Table 1).

The most highly developed remediation methods for metal-contaminated soils are physical or chemical, such as soil washing, excavation and reburial. *Phytoremediation*, which uses plants to take up metals, is a cheap alternative technology, which is solar-driven and performed directly in situ (Salt et al. 1998). Removing heavy metals through harvestable biomass is an efficient technique for inorganic pollutants. Plants used for this purpose should ideally combine high metal accumulation in shoots and high biomass production. Starting from the discovery of hyperaccumulator plants, which are able to concentrate high levels of specific metals in the above-ground biomass, there is

now great interest in crop species, which may solve the problem of the small biomass of hyperaccumulators. In this review, we present a summary of results from in situ experiments carried out with field crops in metal-contaminated soils.

Heavy metals in soils and plants

The term *heavy metal* generally refers to a specific group of elements with metallic properties (metals and semimetals), often associated with contamination and potential toxicity or ecotoxicity (Duffus 2002). Over the past two decades, this term has been used increasingly in the literature and in legislation related to chemical hazards and the safe use of chemicals. From the bibliographical survey made by Duffus (2002), the term *heavy metals* appears to be commonly applied to elements of density higher than $3.5\text{--}7\text{ g cm}^{-3}$ and high atomic number (higher than 20), and includes transition metals, some metalloids, lanthanoids and actinoids.

Although some metals are essential for plant and animal life, many are toxic at high concentrations, and awareness of the extent and severity of soil and water contamination they cause is growing. Besides their natural availability in soils, specific sources of heavy metals are mine tailings, leaded gasoline and lead-based paints (The Conservation Foundation 1987; Pirbazari et al. 1989), fertilisers, animal manure, biosolids, compost, pesticides, coal combustion residues and atmospheric deposition (Adriano 2001).

Metal(loid)s of environmental concern are As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb and others (Basta et al. 2005). Their anthropogenic application to soils is often related to the use of residuals, like biosolids, livestock manure and compost, adversely affecting human, crop and wildlife health (Adriano 2001). In plants, some metals play an important role as micronutrients, being essential for growth at low concentrations. Most of them are cofactors of enzymes and are involved in important processes such as photosynthesis (Mn, Cu), DNA transcription (Zn), hydrolysis of urea into carbon dioxide and ammonia (Ni), and legume nodulation and nitrogen fixation (Co, Zn, Co). Some are involved in flowering and seed production and in plant growth (Cu, Zn), especially when their availability is very low (Table 2). Interactions for uptake and transport may occur between metals or with macronutrients, depending on their relative concentrations. For instance, Cu reduces the uptake of Cd and Ni in soybean seedlings (Cataldo and Wildung 1978), whereas its uptake is inhibited by Cr, Cd, Co and Ni in barley. Nickel can compete with Cu, Zn and Co and, to a greater extent, with iron uptake (Cataldo et al. 1978). Lead is also an antagonist in the uptake of Fe, more than Mn and Co (Gaweda and

Table 1 Thresholds for release of inorganic pollutants to air, water and land in a single site

Pollutants and their compounds	Threshold for release (kg year^{-1})		
	To air	To water	To land
Arsenic	20	5	5
Cadmium	10	5	5
Chromium	100	50	50
Copper	100	50	50
Mercury	10	1	1
Nickel	50	20	20
Lead	200	20	20
Zinc	200	100	100

All metals are considered as total mass of element in all chemical forms (from Annex II, Regulation 166/2006/EC)

Table 2 Role and toxicity of metals in plants and humans

Metal	Essential for plants	Functions in plants	Toxicity threshold in plant tissues (mg kg ⁻¹ dw)	Some hyperaccumulators	Threshold for hyperaccumulation in above-ground biomass (mg kg ⁻¹ dw)	Essential for human	Toxicity symptoms in humans
As	No	–	>~20 (a)	<i>Pteris vittata</i> L. (f)	>1,000 (f)	No	Cancer (e.g. lung and skin); cardiovascular, gastrointestinal, hepatic and renal diseases
Cd	No	–	5–10 (b)	<i>Thlaspi caerulescens</i> J. & C. Presl. (e)	>100 (e)	No	Stomach irritations (vomiting and diarrhoea); lung damage; kidney diseases; cancer (probably)
Co	Yes	Cofactor of biosynthetic enzymatic activities; essential for <i>Rhizobium</i>	60–170 (c)	<i>Haumaniastrum robertii</i> (Robyns) P. A. Duvign. & Plancke (e)	>1,000 (e)	Yes	Contact dermatitis; mutagenic and carcinogenic effects
Cr	No	–	1–2 (b)	<i>Brassica juncea</i> (L.) Czern.; <i>B. napus</i> (L.); <i>Vallisneria americana</i> (d)	>20,000 (g)	Yes	Genotoxic carcinogens (Cr ⁶⁺); lung cancer
Cu	Yes	Constituent of enzymes; role in photosynthesis; involved in reproductive and in determining yield and quality in crops	15–20 (b)	<i>Brassica juncea</i> (L.) Czern.; <i>Vallisneria americana</i> Michx. (d)	>1,000 (e)	Yes	Inhibition of dihydrophil hydratase (in haemopoiesis); accumulation in liver and kidney
Mn	Yes	Constituent of enzymes; activation of enzymes; photosynthesis; reproductive phase; resistance against biotic and abiotic stress	170–2,000 (h)	<i>Agrostis castellana</i> Boiss. & Reuter (d)	>10,000 (e)	Yes	Neurological symptoms; affection of liver function
Ni	Yes	Constituent of enzymes; activation of urease	20–30 (b)	<i>Alyssum</i> spp.; <i>Thlaspi</i> spp. (e)	>1,000 (e)	Yes	Accumulation in lungs
Pb	No	–	10–20 (b)	<i>Thlaspi rotundifolium</i> (L.) Gaudin ssp. <i>Cepaeifolium</i> (Wulfen) Rouy & Fouc.; <i>T. caerulescens</i> J. & C. Presl.; <i>Alyssum wulfenianum</i> Bernh.; <i>Arrhenatherum elatius</i> (L.) Beauv.; <i>Festuca ovina</i> L. (e)	>1,000 (e)	No	Irreversible neurological damage; renal disease; cardiovascular effects; reproductive toxicity
Zn	Yes	Constituent of cell membranes; activation of enzymes; DNA transcription; involved in reproductive phase and in determining yield and quality of crops; resistance against biotic and abiotic stress; legume nodulation and nitrogen fixation	150–200 (b)	<i>Thlaspi</i> spp.; <i>Cardaminopsis</i> spp. (e)	>10,000 (e)	Yes	Inhibition of copper absorption; nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea, headaches

Hyperaccumulator plant species refer to temperate climates. Variations in phytotoxicity thresholds take into account interspecific and environmental variability

(a) Wallace et al. 1980; (b) Förstner 1995; (c) Li et al. 2009; (d) McCutcheon and Schnoor 2003; (e) Terry and Banuelos 2000; (f) Visoottiviseth et al. 2002; (g) Zayed and Terry 2003; (h) Adriano 1986

Capecka 1995) and can inhibit enzymes such as ureases. In rice, the competition in uptake between arsenate and phosphate, which may markedly reduce plant growth, is well known (Meharg and Hartley-Whitaker 2002). Interactions between metals for uptake across cellular membranes and vacuoles and transport depend on the expression and functionality of specific transporter families shared by various metals (Hall and Williams 2003).

Phytotoxicity is mainly associated with non-essential metals like As, Cd, Cr and Pb, which generally have very low toxicity thresholds (Clemens 2006) and lower values for hyperaccumulation (especially for Cd) than the other metals. The above-mentioned metals, except Cr, are also not essential for humans, and may enter the food chain through ingestion of contaminated edible products at various levels, depending on the metal in question. Arsenic, Cr and Pb are not easily transferred to above-ground plant biomass, mainly being stored in root cells (Marin et al. 1992; Tiwari et al. 2009; Melleme et al. 2009), whereas Zn is easily accumulated in green tissues like leaves (Probst et al. 2009).

Uptake of metals is mainly influenced by their bioavailable fraction rather than by the total amount in soil. Although the low availability of soluble forms of metals is desirable for food production, for phytoextraction, the opposite is required, for better efficiency in soil remediation. Metal availability depends on (1) the intensity of adsorption to soil particles; (2) the ability of plants to desorb and transfer metals to their tissues; and (3) interactions with soil microorganisms (Salt et al. 1998; Lasat 2002).

Bioavailable metals include a water-soluble fraction, which is in equilibrium with cation exchange sites of soil organic matter or clays, including forms chelated to inorganic or organic soil constituents (MacCarthy 2001). In general, both high cation exchange capacity (CEC) and soil pH reduce metal bioavailability, and thus mobility and possible leaching. Conversely, the anionic behaviour of As leads to its greater mobility in conditions of high pH and organic matter and reduced contents of iron and oxygen in soil (Hartley et al. 2009).

Organic matter forms metal complexes, so that it can either reduce metal mobility or increase availability when the complexes are soluble in the soil solution (Halim et al. 2003). The redox potential of soil is directly involved in metal availability, as anaerobic conditions generally induce precipitation of sulphides (Cd, Co, Cu, Ni, Pb, Sn, Zn), with the exception of Mn, which increases its availability (Suthersan 1997).

Within a certain interval of contamination, plants are able to defend themselves from heavy metals. Baker and Walker (1989) classified plants in three groups, excluders, indicators and accumulators, the concentrations of pollutants in shoot biomass being, respectively, lower, similar

and higher than that of the soil. When great amounts of metals enter plants, various mechanisms of detoxification are possible, such as reduced translocation (Angelova and Ivanov 2009), compartmentalisation (vacuoles, cell walls) (Lasat et al. 1998), chelation with phytochelatins (Cobbett 2000) and biotransformation (Tomsett et al. 1992).

In general, extensive root colonisation is essential for metal uptake, as it has been widely shown for nutrients in several plant species. Rhizosphere acidification and release of root exudates contribute to the absorption of several heavy metals (Lasat 2002), as reported in *Graminae* specifically for iron (Kanazawa et al. 1994) and zinc (Cakmak et al. 1996). Exudates may also be involved in the mechanisms of plant tolerance to heavy metals (Pellet et al. 1995; Larsen et al. 1998). Some root morphological traits, such as pattern of root density, maximum depth and specific root length, are considered crucial for adaptation to stress conditions (Fitter and Stickland 1991). Phenotypic root plasticity enables plants to cope with a wide range of soil factors and heterogeneity of soils. In this regard, dicotyledons exhibit greater plasticity than grasses (Eisenstat 1992; Taub and Goldberg 1996). Higher root proliferation is usually observed in favourable (e.g. fertilised) micro-sites, but root response also depends on the mobility of nutrients (Campbell and Grime 1989). As regards heavy metals, it has been noted that hyperaccumulator plants (e.g. *Thlaspi caerulescens* J. & C. Presl.) have preferential root growth in the zones where the metals are present (Schwartz et al. 1999; Whiting et al. 2000), thus increasing the efficiency of remediation in soils heterogeneously contaminated.

Phytoremediation

The term *phytoremediation*, from the Greek *phyto*, meaning “plant”, and the Latin suffix *remedium*, “able to cure” or “restore”, was coined by Ilya Raskin in 1994, and is used to refer to plants which can remediate a contaminated medium. Phytoremediation takes advantage of the plant’s ability to remove pollutants from the environment or to make them harmless or less dangerous (Raskin 1996). It can be applied to a wide range of organic (Anderson and Coats 1995; Schnoor et al. 1995) and inorganic contaminants. Phytoremediation is a general term including several processes (Table 3), among which phytoextraction and phytostabilisation are the most reliable for heavy metals.

Metal phytostabilisation

Phytostabilisation does not aim at removing contaminants from the soil, but at reducing their risks to human health and the environment. The establishment of a green canopy

Table 3 Differing areas of phytoremediation (from Salt et al. 1998; Dietz and Schnoor 2001)

Technology	Description
Phytoextraction	Uptake of pollutants from environment and their concentration in harvestable plant biomass
Phytostabilisation	Reduction of mobility and bioavailability of pollutants in environment
Phytovolatilisation	Removal of pollutants from soil or water and their release into air, sometimes as a result of phytotransformation to more volatile and/or less polluting substances
Phytotransformation	Chemical modification of pollutants as a result of plant metabolism, both <i>in planta</i> and <i>ex planta</i> , often resulting in their inactivation, degradation (phytodegradation) or immobilisation (phytostabilisation)
Rhizofiltration	Use of plant roots to absorb and adsorb pollutants or nutrients from water and wastewater (e.g. buffer strips)

in polluted soil has the effect of reducing the mobility of pollutants through water, wind erosion and water percolation. A significant fraction of metals can be stored at root level, especially in polyannual species (Vamerali et al. 2009) contributing to long-term stabilisation of pollutants. Much literature refers to phytostabilisation as a way of reducing mobility and excluding metals from plants. Application of soil amendments, such as phosphate fertilisers, organic matter, Fe- and Mn-oxyhydroxides and inorganic clay minerals, contributes to integrating the role of plants by reducing metal bioavailability, and thus preventing both plant uptake and leaching (Berti and Cunningham 2000). However, root sequestration of metals is not definitive, as removal of pollutants from the environment may last after tissue degradation (Vangronsveld et al. 1995; Arienzo et al. 2004). Phytostabilisation improves the chemical and biological characteristics of contaminated soil by increasing the amount of organic matter, nutrient levels, cation exchange capacity and biological activity (Arienzo et al. 2004). In several cases, a vegetation cover has been found to provide a cost-effective and environmentally sustainable method of stabilising and reclaiming toxic metal mine sites (Mench et al. 2003; Wong 2003).

Although this technique is effective in remediating metal-contaminated soils and sediments, King et al. (2006) showed that it fails when applied, for instance, to canal sediments because of the high mortality of various species such as poplars, willows and alders. According to Sutton and Dick (1987), soil acidity is the main constraint for the establishment of vegetation in these environments, although the application of organic residues (slight effect) and liming materials (Ye et al. 1999; Wong 2003) or lime-stabilised biosolids can attenuate the effects (Abbott et al. 2001; Basta et al. 2001; Adriano et al. 2004), allowing a vegetation cover to form (Brown et al. 2003, 2005).

The choice of plant species is an important task in a phytostabilisation-based technique (Rizzi et al. 2004). Plants must be able to develop extended and abundant root systems and keep the translocation of metals from roots to shoots as low as possible (Mendez and Maier 2008).

Metal phytoextraction

As heavy metals are the main inorganic contaminants, among existing phytotechnologies much interest is devoted to phytoextraction and its improvement (Adriano 1986, 1992; Alloway 1990; Meeuseen et al. 1994). Phytoextraction is a *green* technology, born 15 years ago from the studies of Raskin et al. (1994) and later of Brooks et al. (1998), which exploits the ability of plants to translocate a great fraction of metals taken up to harvestable biomass. Contaminated biomass may be used for energy production, whereas remaining ashes are dumped, included in construction materials, or subjected to metal extraction (phytomining; Brooks et al. 1998).

Although promising, phytoextraction has many limitations, deriving from scarce metal availability in soils, difficulties in root uptake, symplastic mobility and xylem loading, as well as the great energy cost required for detoxification and storage within shoots (Meagher 2000; Clemens et al. 2002).

Plants show differing morpho-physiological responses to soil metal contamination. Most are sensitive to very low concentrations; others have developed tolerance, and a reduced number show hyperaccumulation (Baker and Brooks 1989; Barceló et al. 1994; Brooks 1998). The latter capacity has practically opened up the way to phytoextraction (Garbisu and Alkorta 2003; Van der Lelie et al. 2001).

Metal accumulation is expressed by the metal biological absorption coefficient (BAC), i.e. the plant (harvestable)-to-soil metal concentration ratio (Blaylock et al. 1997). Besides convenient BAC, both the high bioconcentration factor (BCF, root-to-soil metal concentration ratio) and the translocation factor (TF, shoot-to-root metal concentration ratio) can positively affect phytoextraction. Tolerant plant species tend to restrict soil–root and root–shoot transfers, and therefore have much less accumulation in biomass, whereas hyperaccumulators actively take up and translocate metals into above-ground tissues. Plants with high BAC (greater than 1) are suitable for phytoextraction; those

with high BCF (higher than 1) and low TF (lower than 1) have potential for phytostabilisation (Yoon et al. 2006).

Desirable characteristics for a plant species in phytoextraction are (1) fast growth and high biomass; (2) extended root system for exploring large soil volumes; (3) good tolerance to high concentrations of metals in plant tissues; (4) high translocation factor; (5) adaptability to specific environments/sites; and (6) easy agricultural management. All these traits are difficult to combine, and there are basically two available phytoextraction strategies, which make use of hyperaccumulators or biomass plant species, respectively. Hyperaccumulators, such as the well-studied *Thlaspi caerulescens* J. & C. Presl. and *Alyssum bertolonii* Desv. (McGrath 1998; Robinson et al. 1998) are able to take up specifically one or a few metals, generally producing a small shoot biomass with high metal concentrations (Baker and Brooks 1989; Reeves and Baker 2000). Instead, high-yielding biomass plant species can absorb a wide range of heavy metals at generally low concentration.

Hyperaccumulator plants

Hyperaccumulation is an unusual occurrence, ascertained in a narrow range of species which often grow in metal-rich sites, like serpentine and calamine (Brooks et al. 1977). By analysing the metal contents of several species from worldwide collections, Baker and Brooks (1989) suggested the following values of metal concentrations in shoots for hyperaccumulation without evident symptoms of toxicity: 100 mg kg⁻¹ for Cd, 1,000 mg kg⁻¹ for Ni, Cu, Co and Pb, and 10,000 mg kg⁻¹ for Zn and Mn (Table 2). Although these threshold values were fixed arbitrarily, they roughly lie at least at one order of magnitude greater than those found in common species (Salt and Kramer 2000). Hyperaccumulators are generally minor vegetation components in most European and North American habitats, but are sometimes relatively abundant in some locations in New Caledonia, Cuba and South Africa. Currently, more than 400 hyperaccumulator species are known, belonging to 45 different botanical families (Salt et al. 1998), among which the most frequent are *Brassicaceae*, with the genus *Thlaspi*, *Alyssum* and *Brassica* (e.g. *Thlaspi caerulescens* J. & C. Presl., *T. rotundifolium* (L.) Gaudin, *Brassica juncea* (L.) Czern., *Cardaminopsis* spp., *Alyssum* spp.), and *Fabaceae* (Baker et al. 2000). Among monocotyledons, *Poaceae* includes, for instance, *Agrostis castellanea* Boiss. & Reut., *Arrhenatherum elatius* (L.) Beauv., *Festuca ovina* L. as hyperaccumulators (Prasad and De Oliveira-Freitas 2003). The habitus of these plants varies from small annual herbaceous plants to perennial shrubs and trees, although they mainly occur as grasses in temperate climates. A few hyperaccumulator trees are found in New Caledonia, with *Psychotria douarrei* Beauv. accumulating up to 27,700 mg

Ni kg⁻¹ in its shrubs (Boyd et al. 1999) and *Sebertia acuminata* Pierre (*Rubiaceae*), which produces a latex with an extremely high Ni concentration (up to 26% in dry mass) (Jaffre et al. 1976). One of the most frequently investigated species is *Thlaspi caerulescens* J. & C. Presl., a hyperaccumulator of Zn and Cd (McGrath et al. 2006), which has often been studied for better understanding of the mechanisms of metal tolerance and for gene manipulation (Dushenkov et al. 2002; Guan et al. 2008).

Lack of information on the agricultural management of hyperaccumulators, together with slow-growing and poor shoot and root growth, increase the difficulties in the practical application of these species in remediation projects (Navari-Izzo and Quartacci 2001; Lasat 2002). There is also little knowledge on their rooting. In the case of *Thlaspi caerulescens* J. & C. Presl., preferential root development in soil regions where heavy metals are more concentrated was found to be a favourable trait for remediating heterogeneously contaminated soils (Schwartz et al. 1999; Whiting et al. 2000). Although hyperaccumulators have the advantage of tolerating high soil concentrations of specific contaminants, when further metals severely pollute the soil they die, like common species, as found for *Thlaspi caerulescens* J. & C. Presl. and *Haumaniastrum robertii* (Robyns) P.A. Duvign. & Plancke in a pluri-contaminated soil (Quartacci et al. 2003). The major limitations in application of phytoremediation involve the most phytotoxic metals, like Cd, Pb and Cr (Nanda-Kumar et al. 1995; Blaylock et al. 1997; Huang et al. 1997). Fast-growing herbaceous hyperaccumulators have been identified for Ni, with *Alyssum bertolonii* Desv. and *Berkheya coddii* Roessler able to reach 9 and 22 t ha⁻¹ of harvestable dry biomass, respectively (Robinson et al. 1997a, b). Relatively large biomass may also be obtained from the arsenic hyperaccumulator fern *Pteris vittata* L. when grown in favourable climates (Ma et al. 2001), reversing the generally negative correlation between plant biomass and metal concentration.

Crop species

The potential of some crops for phytoextraction purposes has been studied in the last few years (Baker et al. 1994; Ebbs and Kochian 1997; Ebbs et al. 1997), especially within *Brassicaceae*, in view of the large number of hyperaccumulators belonging to this family. Interesting Pb accumulations at shoot level have been found in *Brassica* species (*B. nigra* (L.) Koch, *B. carinata* A. Braun, *B. oleracea* L., *B. campestris* L., *B. juncea* (L.) Czern., *B. napus* L.) up to 3.5% of dry weight (Nanda-Kumar et al. 1995). Herbaceous or woody biomass species may be promising in view of their high-yielding ability, which can compensate for low concentrations of contaminants in their tissues

(Chaney et al. 1997), thus resulting in similar or even higher offtake of pollutants than hyperaccumulators.

In this paper, a bibliographical survey of the literature over the period 1995–2009 was carried out, in order to take a census of crop species involved in experimental research worldwide on *phytoremediation* and *heavy metals*, these terms being sought in CAB Abstracts database regardless of search field (Table 4). In the last 14 years, the most frequently cited species was *Brassica juncea* (L.) Czern. (148 citations), followed by *Helianthus annuus* L. (57), *Brassica napus* L. and *Zea mays* L. (both 39 citations). In the ranking, *Brassicaceae* (*Raphanus sativus* L., *Brassica carinata* A. Braun) and *Poaceae* (*Festuca* spp., *Lolium* spp., *Hordeum vulgare* L.) were again represented, whereas fewer citations were made of *Fabaceae* such as soybean, bean, alfalfa and pea. The greater interest in *Brassicaceae* derives from the fact that research on these species started earlier, together with the interesting concentrations they provide, especially for *Brassica juncea* (L.) Czern. The latter has been described as a hyperaccumulator for various metals such as Cd, Cr, Cu, Mn, Pb, Se and Zn. Apart from phytoextraction of radioisotopes (Cs^{137}) (McCutcheon and Schnoor 2003), the ability of sunflower to take up Cd, Pb and Zn has been reported by several authors (e.g. Fellet et al. 2007; Tassi et al. 2008). Maize was successfully experimented in phytoextraction of Cd and other metals such as Ni, Cu, Pb and Zn (Wu et al. 2007; Murakami and Ae 2009), often in association with the use of chelators, mycorrhizae, bacteria and other devices, such as

application of sulphur and co-planting with hyperaccumulators. Among the most represented families, interest focuses mainly on a few species for *Brassicaceae* and a greater number of *Poaceae*, which have been studied more recently, together with *Fabaceae*.

Until now, research has been carried out continuously for almost all species, with an increasing rate of citations per year, which varies from 155% for oilseed rape to 3% for maize (Fig. 1), suggesting that this citation rate may be an index of species efficiency. Until now, most results come from hydroponic- or pot-greenhouse or laboratory experiments, making it difficult to transfer data to

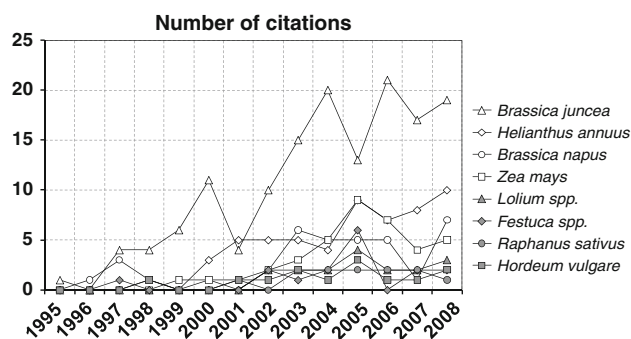


Fig. 1 Dynamics of number of citations (CAB Abstracts database) over last 14 years for most frequently studied field crops in phytoremediation. Keywords (regardless of search field): *phytoremediation* and *heavy metals*

Table 4 Number of citations per crop species found in a bibliographical survey in CAB Abstracts database over period 1995–2009 (until April)

Family	Species	No. of citations	Average rate of variation in citations (% year ⁻¹)	Period	
				From	To
<i>Asteraceae</i>	<i>Helianthus annuus</i> L.	57	+88	2000	2009
<i>Brassicaceae</i>	<i>Brassica juncea</i> (L.) Czern.	148	+10	1995	2009
	<i>Brassica napus</i> L.	39	+155	1996	2009
	<i>Raphanus sativus</i> L.	13	+39	1998	2008
	<i>Brassica carinata</i> A. Braun	11	+17	1997	2008
	<i>Sinapis alba</i> L.	4	–	2003	2007
	<i>Glycine max</i> (L.) Merr.	9	+10	2002	2007
<i>Fabaceae</i>	<i>Phaseolus vulgaris</i> L.	8	+5	2001	2008
	<i>Medicago sativa</i> L.	7	+18	2000	2007
	<i>Pisum sativum</i> L.	2	–	2003	2007
	<i>Zea mays</i> L.	39	+3	1999	2009
<i>Poaceae</i>	<i>Lolium</i> spp.	19	+22	2002	2009
	<i>Festuca</i> spp.	16	+15	1997	2008
	<i>Hordeum vulgare</i> L.	13	+16	2001	2008
	<i>Sorghum</i> spp.	9	+100	2002	2009
	<i>Triticum</i> spp.	9	+12	2000	2005
	<i>Avena sativa</i> L.	4	–	1998	2008
	<i>Oryza sativa</i> L.	2	–	2008	2009

Regardless of database search field, keywords were *phytoremediation* and *heavy metal(s)*

open-field conditions, although site-specific results are progressively increasing.

In an attempt to provide realistic results, Table 5 lists data from field and some pot experiments only. For field crops, data on metal concentrations are available mainly for Cu, Zn, Pb and Cd, which are frequently found in polluted sites; less information is available for As, and only a few experiments deal with Co, Cr and Ni. The use of amendments or chelators is often tested in the field to improve metal uptake, allowing above-normal concentrations to be reached. Among the most frequently studied metals, interesting Zn concentrations (higher than $1,000 \text{ mg kg}^{-1}$) are found in *Brassica* spp., *Phaseolus vulgaris* L. and *Zea mays* L., and Cu (above 500 mg kg^{-1}) in *Zea mays* L., *Phaseolus vulgaris* L. and *Sorghum bicolor* (L.) Moench. Concentrations of Pb greater than $1,000 \text{ mg kg}^{-1}$ are found in *Festuca* spp. and *Fabaceae* such as *Medicago sativa* L.. On the basis of the generally low concentrations, Cd phytoextraction for crops does not seem to be reliable, as contents of 20 mg kg^{-1} in maize and about 50 in alfalfa and bean were reached. Arsenic shows concentrations of more than 200 mg kg^{-1} in sorghum and soybean. Much of this literature reports

impaired shoot growth (Clemente et al. 2005) and the need to use chelators to improve metal recovery (Luo et al. 2005, 2008). In many cases, organic amendments and fertilisation also aimed at increasing soil fertility (Marchiol et al. 2004) and soil pH (Clemente et al. 2005) in markedly degraded sites.

The use of field crops for phytoremediation purposes should not consider the use of products for animal feed or human consumption, although in many cases risks are small, since translocation to grains of various metals is limited. For instance, negligible Cd and Cu contents in maize kernels and oilseed rape seeds were found in a pluri-contaminated soil by Wang et al. (2002). We also found small concentrations of metals in sunflower achenes and maize kernels in field plots in a soil severely contaminated with Cd, Pb, Cu, Mn and Zn (Mosca et al. 2004). From these results, obtained without application of amendments, interesting Zn and Mn concentrations (Table 6) and total offtake (about 3 kg ha^{-1} for both metals) were found in sunflower and maize, but translocation to maize kernels was relatively poor (Mn: 12%; Zn: 18% of the above-ground contents) (Fig. 2).

Table 5 Metal concentrations in herbaceous crops from field/site experiments in recent bibliography (later than 2000)

Species	Metal concentrations (mg kg^{-1})								Treatments	Phytotoxicity	References
	As	Cd	Co	Cr	Cu	Ni	Pb	Zn			
<i>Brassica carinata</i> A. Braun	12	12		9.8	37	7.6	50	1,650		Yes	Marchiol et al. 2004; Soriano and Fereres 2003
<i>Brassica juncea</i> (L.) Czern.	30	10		5.2	71		55	2,029	Cow manure (As, Cu, Zn) or mature compost (Pb)	Yes	Clemente et al. 2005; Marchiol et al. 2004
<i>Brassica napus</i> L.	5.8	11		9	40	7	39	1,400		No	Marchiol et al. 2004;
<i>Festuca</i> spp.					106			90			Álvarez et al. 2003
<i>Glycine max</i> (L.) Merr.	230	2.4			440		72	430		No	Fellet et al. 2007
<i>Helianthus annuus</i> L.	20	0.64	0.71		70		5	150	Mineral fertilisation (Co)	No	Fellet et al. 2007; Marchiol et al. 2007
<i>Hordeum vulgare</i> L.	20	0.44			16		27	334		–	Soriano and Fereres 2003
<i>Lolium perenne</i> L.					29		140	59	Organic amendment	No	Alvarenga et al. 2009
<i>Medicago sativa</i> L.	85	53			77		2,177		EDTA (5 mM) for Pb only	–	Pajuelo et al. 2007
<i>Oryza sativa</i> L.					34		6	90		–	Murakami and Ae 2009
<i>Phaseolus vulgaris</i> L.		53			2,230		1,000	1,440	Hot EDTA (Cd, Pb), EDDS (Cu, Zn)	–	Luo et al. 2005; Luo et al. 2008
<i>Pisum sativum</i> L.							1,390		EDTA	–	Chen et al. 2004
<i>Raphanus sativus</i> L.		9.4		5	34	6.5	28	1,450		Yes	Marchiol et al. 2004
<i>Sorghum bicolor</i> (L.) Moench	240	3.7	1.8		540		100	580	Pyrite cinders (Cd)	No	Fellet et al. 2007; Marchiol et al. 2007
<i>Triticum secalotriticum</i> Wittm.	21	1.9			27.5		37	588		–	Soriano and Fereres 2003
<i>Zea mays</i> L.	30	20			1,220		257	1,200	EDDS (Cu, Zn) or EDTA (Cd, Pb)	No	Fellet et al. 2007; Luo et al. 2005

Possible treatments and phytotoxicity are listed

Table 6 Contents of metals in crops in a pluri-contaminated site in Milan (Italy) (site experiment)

Metal	<i>Brassica napus</i> L. var. <i>oleifera</i> D.C.	<i>Helianthus annuus</i> L.	<i>Linum usitatissimum</i> L.	<i>Raphanus sativus</i> L. var. <i>oleiformis</i> Pers.	<i>Zea mays</i> L.
Cd	1.19 c	2.27 b	5.04 a	1.19 c	0.40 d
Co	0.05 ab	0.08 a	–	0.01 b	–
Cr	0.53 ab	0.58 a	0.48 ab	0.61 a	0.35 b
Cu	7.76 b	15.7 a	7.62 bc	5.26 c	5.15 c
Mn	103 ab	170 a	36.6 ab	27.1 b	28.4 ab
Mo	0.98 a	0.06 b	–	1.15 a	–
Ni	1.04 a	0.92 a	0.69 ab	0.61 b	0.45 b
Pb	0.71 ab	0.93 a	0.16 c	0.85 a	0.23 c
Zn	201 ab	243 a	128 b	148 b	120 b

Data refer to shoots (oilseed rape, linseed) and to all above-ground biomass 'residues + grains' (sunflower, maize, fodder radish) at harvest. LSD test at $P \leq 0.05$. Letters: Comparison among species for same metal. Source: Mosca et al. 2004

Improving phytoextraction

Many efforts have been made to improve phytoextraction of heavy metals, so that the effects of a great variety of treatments to biomass species are still being studied. Besides genetic improvements, plant responses to chelators, hormones and mycorrhizae have been investigated in order to assess plant tolerance and metal uptake, and the most appropriate doses and ways of application of these means.

Genetic engineering

Genetic engineering applied to crops aims at manipulating the plant's capacity to tolerate, accumulate and metabolise pollutants. Many genes involved in the acquisition, allocation and detoxification of metals have been identified and characterised from a variety of organisms, especially bacteria and yeasts (Ehrlich 1997). Transgenic plants have been engineered to overproduce recombinant proteins playing possible roles in chelation, assimilation and membrane transport of metals. Enhanced tolerance and accumulation have been achieved through overproduction

of metal chelating molecules such as citrate, phytochelatins, metallothioneins, phytosiderophores and ferritin, or overexpression of metal transporter proteins.

Enhanced aluminium tolerance has been achieved by increasing organic acid synthase gene activity. Han et al. (2009) isolated a full-length OsCS1 gene encoding for citrate synthase, which is highly induced by Al toxicity in rice (*Oryza sativa* L.). Insertion of OsCS1 in several independent transgenic tobacco lines and its expression increased citrate efflux and conferred great tolerance to aluminium.

Overexpression of either gamma-glutamylcysteine synthetase or glutathione synthetase in transgenic *Brassica juncea* (L.) Czern. resulted in higher accumulation and tolerance of various metals such as Cd, Cr and As, considered alone or mixed together (Reisinger et al. 2008).

An attempt to improve tolerance to Cd, Zn and Ni was made by introducing a metallothionein gene in tobacco (Dorlhac de Borne et al. 1998; Pavlikova et al. 2004). Macek et al. (2002) also showed that Cd accumulation significantly increased in tobacco plants bearing the transgene coding for the polyhistidine cluster combined with yeast metallothionein.

Another promising approach is the introduction of genes encoding for phytosiderophores. A first step in this direction was achieved by Higuchi et al. (1999), who isolated genes encoding for nicotianamine synthase, a key enzyme in the phytosiderophore biosynthetic pathway in barley and rice. The increase of iron acquisition mediated by phytosiderophores was found to provide an advantage under Cd stress in maize (Meda et al. 2007). Overproduction of ferritin through genetic modification also led to increased Fe uptake as well as Cd, Mn and Zn, but only at alkaline pH (Sappin-Didier et al. 2005). This was due to high pH Fe deficiency, which stimulates metal uptake and translocation in shoots through an increase in root ferric reductase and H^+ -ATPase activities.

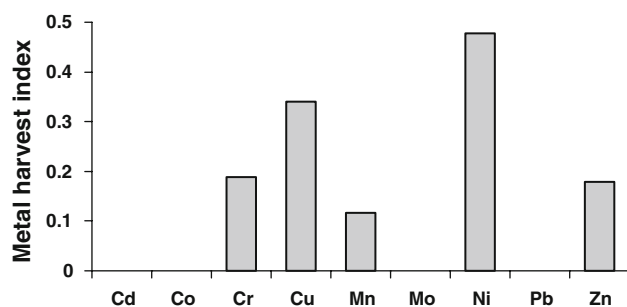


Fig. 2 Fraction of above-ground metals accumulating in maize grain at harvest in a pluri-contaminated soil in Milan (Italy). Source: Mosca et al. 2004

Chelating agents

The addition of natural and/or synthetic chelators has been extensively experimented in phytoextraction, in order to increase metal bioavailability, uptake and translocation of metals. This goal may be achieved by adding both inorganic and organic agents to the soil, although the latter appears to be more effective in increasing the solubility of metals (Schmidt 2003; Quartacci et al. 2005, 2006).

Among various synthetic chelators, ethylenediamine tetraacetate (EDTA) has been tested more intensively (Blaylock et al. 1997; Huang et al. 1997; Grčman et al. 2001). This chelator has shown its ability to mobilise heavy metals from the soil–solid phase through stable metal complexes available for uptake in pore water.

Metal–chelator complexes are taken up along an apoplastic pathway (Tanton and Crowdy 1971), and they pass through the Casparian strip. The Casparian strip is not fully formed near the tip of roots, and it is also disrupted close to lateral branches (Tanton and Crowdy 1971; Haynes 1980). Through this pathway, components from the solution can enter the stele which houses the xylem without passing through a cell membrane (Haussling et al. 1988). In some species, water and solutes can also enter the xylem through passage cells, a small number of unsuberised endodermic cells (Clarkson 1996).

In several cases, chelators have been found to increase metal translocation from roots to shoots, as revealed by Ensley et al. (1999) with EDTA and organic acids. In other experiments, improvements of metal mobility in soil and accumulation in roots only were observed (Lombi et al. 2001).

Application of EDTA has been found to improve the uptake of several metals, particularly Pb—as shown, for instance, in *Brassica juncea* (L.) Czern., with concentrations 1,000–10,000 times greater than those of controls (Blaylock et al. 1997). Due to the persistent nature of EDTA in the environment, the risk of metal leaching to surface- and groundwater may rise markedly (Chen et al. 2004; Sun et al. 2001), as well as toxicity to soil biota (Grčman et al. 2001).

As an alternative to EDTA, its structural isomer ethylenediamine disuccinate (EDDS) has recently been proposed to enhance phytoextraction (Grčman et al. 2003; Luo et al. 2005). The [S,S]-isomer of EDDS is readily biodegradable in soil (Schowanek et al. 1997; Bucheli-Witschel and Egli 2001) and its mineralisation, including metal chelates (Vandevivere et al. 2001) in sludge-amended soil has been estimated to be complete in 28 days, with a half-life of 2.5 days (Jaworska et al. 1999). The affinity of EDDS is mainly oriented towards Cu and secondly to Zn, and this chelator shows a solubilisation effect greater than that of EDTA at equimolar concentrations and pH 7 (Tandy et al. 2004). No toxic effects of EDDS or the Cu–EDDS complex have been found on soil biota (Kos and Leštan

2004; Vandevivere et al. 2001). Tandy et al. (2006) found decreased concentrations in shoots of essential metals like Cu and Zn, and greater values for Pb in EDDS-assisted phytoextraction. These authors suggested that, in the presence of EDDS, the three pollutants are taken up by the non-selective apoplastic pathway as metal–EDDS complexes. In the absence of chelator, essential metal uptake is primarily selective along the symplastic pathway. This shows that synthetic chelating agents do not necessarily increase uptake of heavy metals, when soluble concentrations are equal in the presence and absence of chelates.

Some of the restrictions concerning chelate-assisted phytoextraction may be overcome by using easily biodegradable and poorly phytotoxic compounds, such as nitrilotriacetate (NTA) and low molecular weight organic acids, such as acetic, citric, oxalic, fumaric and succinic acids (Krishnamurti et al. 1997; Kulli et al. 1999; Kayser et al. 2000; Chen et al. 2003; Wenger et al. 2003). The chelating effect of organic acids roughly follows the order citric > malic > acetic, indicating that the corresponding dosage should be increased from at least 2 mmol kg⁻¹ of soil (citric acid) to 15 mmol kg⁻¹ (tartaric acid) (Gao et al. 2003). Nitrilotriacetic acid combines high biodegradability with good chelating strength. In soils, it was found to degrade as fast as citric acid, and rapidly even in anaerobic conditions and at low temperatures (Ward 1986). The chelating effect of nitrilotriacetic acid is weaker than that of EDTA, but greater compared with low molecular weight organic acids (Wenger et al. 2003; Quartacci et al. 2005; Ruley et al. 2006). Several studies have focused on the use of nitrilotriacetic acid as a ligand to assist metal phytoextraction (Wenger et al. 2003; Meers et al. 2004; Quartacci et al. 2005, 2006; Ruley et al. 2006), showing worse metal accumulation than with EDTA on *Brassica carinata* A. Braun and contamination by Cu, Pb and Zn (Quartacci et al. 2007).

Low molecular weight organic acids are natural compounds in plant root exudates and can improve ion solubility and uptake thanks to their metal chelating properties, their indirect effects on microbial activity (stimulation), and the physical properties of the rhizosphere (Wu et al. 2003). The positive influence of organic acids has been shown in durum wheat, the Cd concentration of which is proportional to the abundance of these acids in the rhizosphere (Cieśliński et al. 1998). Citric acid also reduces the toxicity of Cd in radish, and stimulates its translocation from roots to shoots by converting the metal into more easily transported forms (Chen et al. 2003).

Environmental- and plant-safe use of chelators should consider modest dosages—from 3 mmol kg⁻¹ of soil for EDTA (Luo et al. 2006) to 5 mmol kg⁻¹ for EDDS (Quartacci et al. 2007; Bandiera et al. 2009a)—applied close to harvest (about 1 week before) and preferably near the root system.

Humic acids

Humic substances are widespread natural molecules that have a potential role in phytoremediation as alternatives to synthetic chelators. Humic acids represent the fraction of humic substances insoluble in water in acidic conditions, which become soluble and extractable at higher soil pH. Molecules of humic acids are characterised by acidic groups such as carboxyl and phenol OH functional groups (Hofrichter and Steinbüchel 2001), which play an important role in the transport, bioavailability and solubility of heavy metals (Lagier et al. 2000). At the same time, humic acids can contribute to environmental protection by reducing the physical mobility (diffusion, mass flow) of various metal species (e.g. Cu, Pb, Zn, Ni) in soil, and thus limiting the consequent risk of percolation into groundwater (Halim et al. 2003). Contradictory results have been obtained from the application of humic acids. Bianchi et al. (2008) found increased mobilisation of Cu and Zn, associated with both negligible phytotoxicity in *Paspalum vaginatum* Sw. and improved metal extraction. Better uptake of Mo in forage in a polluted valley in Austria (meadow rendzina) was observed after application of large quantities (10 g kg^{-1} w/w, i.e. 1%) of humic acids, as a consequence of the increased extractable fraction of Mo (+28%) (Neunhäuserer et al. 2001). Increased concentrations of humic acids in soil, from 0.125 to 1.25 mg kg^{-1} , improved the solubility of Hg in modified Hg-contaminated mine tailings, especially when thiosulphate salts (ammonium and sodium) were added to the medium (Moreno et al. 2005). The same authors showed that translocation of Hg to the shoot varied significantly among plant species. The high translocation rate observed in *Brassica juncea* (L.) Czern. in the presence of ammonium thiosulphate was suppressed when humic acids were also added to the nutrient solution, probably because of the retention of Hg-humic acid complexes at root level.

Improved phytoextraction efficiency for Cd was found by Evangelou et al. (2004) after the application of high dosages of humic acids, through increased Cd bioavailability in soil. The addition of humic acids at a very high dose (20 g kg^{-1} of soil) increased Cd concentration in the shoot from 30.9 to 39.9 mg kg^{-1} in *Nicotiana tabacum* L., but not at a dose of 10 g kg^{-1} —probably due to soil acidification or the formation of Cd-humic acid complexes easily absorbed by plants.

The hormone-like effect of humic substances has also recently been investigated in crops (Delfine et al. 2005) and forest species (Pizzeghello et al. 2000)—with contrasting results, depending on plant species, amount and method of application, soil pH, and interactions with soil microflora. In *Raphanus sativus* var. *oleiformis*, we found that low doses of humic acids to the soil (0.1 g kg^{-1}) had a growth-

promoting effect, especially on roots, whereas high doses were phytotoxic (Bandiera et al. 2009b). At this low rate, humic acids increased the amounts of various heavy metals taken up by the plant and their translocation to the shoot, with major benefits for Cu and Pb.

Auxins

The use of plant growth-promoting substances may potentially improve the phytoremediation of trace elements, since they positively increase both shoot biomass and root extension, which may allow greater acquisition of metals. Among hormones affecting plant growth, auxins play the major role, being involved in cell division, growth, maturation, organ differentiation, and several physiological processes (Trewavas 2000). Auxins are also directly involved in cation uptake, having plasma membrane H^+ -ATPase as final target (Hager 2003). H^+ -ATPase acidifies the apoplast, whereas the cytoplasm becomes alkalised (Tode and Hartwig 2001). Acidification of the apoplast weakens the cell wall, and the electrochemical gradient created across the plasma membrane leads to the opening of cation channels or activates membrane ion transport proteins, which results in influx of cations (Liphadzi et al. 2006).

Very few studies regarding the use of auxins in phytoremediation are available in the literature. Among these, López et al. (2005) found increased Pb concentrations in roots (+40%) and leaves ($28\times$) of *Medicago sativa* L. when the plants received $100 \mu\text{M}$ of indoleacetic acid in addition to EDTA in the hydroponic solution. Liphadzi et al. (2006) also found improvements in metal uptake in *Helianthus annuus* L. when indoleacetic acid was given to leaves and soil, regardless of concentrations of 3 or 6 mg L^{-1} (i.e. $17\text{--}34 \mu\text{M}$). In non-EDTA-amended soil only, these authors observed increases in root growth which were associated with higher leaf Mn and Ni contents.

Plant growth can also be enhanced through bacterially produced phytohormones. For instance, Sheng and Xia (2006) found increased growth of both root and shoot and Cd accumulation in *Brassica napus* L. as a result of soil inoculation with Cd-resistant bacteria. In the experiment of Dimkpa et al. (2008), production of siderophores by various *Streptomyces* strains was found to promote auxin synthesis via chelation of metals, such as Cd, Cu, Ni, having the potential to inhibit the synthesis of this hormone.

Mycorrhizae

Mycorrhizae have various positive effects on plants. Increases in nutrient uptake and production of hormones such as cytokinins and gibberellins may be exploited in phytoremediation. Mycorrhization occurs naturally in a

very large number of species—more than 90%—with the exception of the *Brassicaceae* family (Arshad 2007).

Mycorrhizal fungi are ubiquitous in soils, including disturbed or contaminated land, although pollution causes changes in the diversity and prosperity of their populations. High concentrations of heavy metals can delay, reduce or prevent mycorrhizal colonisation and spore germination. The presence of heavy metals in soils allows the selection of tolerant plant and fungi species, and the simultaneous presence of various metals can cause synergic or antagonist interactions, thus increasing or lowering the toxicity of one metal. Ectomycorrhizal fungi are sensitive to heavy metals, and interactions between metals may modify metal toxicity. For instance, reduced Cd toxicity was detected in the presence of Zn (Colpaert and Van Assche 1992; Brunnert and Zadrazil 1985; Hartley et al. 1997). The toxicity of Cd, Pb, Zn and Sb in combination was equal to that of Cd alone (Hartley et al. 1997).

Mycorrhizal fungi can improve phytoextraction by making heavy metals more available for plant absorption. Giasson et al. (2005) reported that, in the presence of a form of Zn unavailable for plants (ZnCO_3), the endomycorrhizal hyphae of *Glomus intraradices* can move Zn to water-soluble species. This phenomenon was even more evident with Cd. Zinc saturation was reached in *G. intraradices* colonised roots at around 400 mg kg^{-1} , regardless of initial ZnCO_3 concentrations; Cd saturation was not reached.

Improved phytoextraction by mycorrhization may be achieved by several mechanisms: (1) better plant growth and biomass production; (2) increased plant tolerance to metals; and (3) greater metal concentrations in plant tissues. Baum et al. (2006) showed improvements in Zn phytoextraction in willows after inoculation with *Paxillus involutus* (ectomycorrhizae). The association between *Elsholtzia splendens* Nakai and various arbuscular mycorrhizal fungi (*Gigaspora* spp., *Scutellospora* spp., *Acaulospora* spp., *Glomus* spp.) increases both root and shoot growth and metal concentrations and is more effective than symbiosis with *Glomus caledonium* alone in enhancing the removal of Cu, Zn, Pb and Cd (Wang et al. 2005). In the same way, sunflower inoculated with *Glomus intraradices* achieves greater Cr removal, due to enhancement of both biomass production and Cr concentration (Davies et al. 2001).

Conclusions

Re-use of abandoned metal-polluted sites is often limited by the dangerous presence of several contaminants. These lands can gain in environmental value when cultivated for phytoremediation purposes, also allowing opportunities for managing the risks of pollutant dispersion in the long term.

In this regard, field crops represent a reliable alternative to hyperaccumulators, although the process still lasts a long time.

In order to solve the main restrictive factors of phytoextraction with field crops, such as activation of unavailable heavy metals in soils and the low uptake and translocation of target metals, some strengthening measures should be taken. For this reason, phytoextraction is linked with genetic engineering and advanced agricultural practices. The use of chelators to enhance metal bioavailability, the application of phytohormones to increase plant growth, and mycorrhization may all facilitate the application of phytoextraction at a commercially large scale.

Indeed, the choice of species, one or several, and adjustment of cultivation technique need thorough study of the plant's potential and adaptability to a specific environment. Results are not easily predictable, and preliminary experiments in micro- and mesocosms can only give some indications. The time-scales for remediation using phytoextraction are generally long, but there is current evidence that this process may be integrated with metal phytostabilisation, especially for tap-rooted species.

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