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Phytoremediation of Metals in the Environment for Sustainable Development

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Phytoremediation is an environmental cleanup strategy in which selected green plants are employed to remove, contain, or render environmentally toxic contaminants harmless. This is an emerging biogeotechnological application based on the "green liver concept" and operates on the principles of biogeochemical cycling. Phytoremediation projects have been successfully implemented in the developed nations for the cleanup of metal polluted/contaminated soil/water including the restoration of degraded mines. Extensive diversity of native and non-native plants has been used in this strategy. This article would focus the relevance of phytoremediation technology for cleanup of metals in soils for sustainable development including some recent trends. Bioconcentration, phytoextraction, phytomining, hyper accumulation; natural accumulators, enhanced accumulation by using selected group of plants and soil amending chelators; phytostabilization, phytotransformation, and rhizofiltration; Rhizosphere biotechnology of metal hyper accumulating naturally occurring and transgenic plants and role of mycorrhizal fungi (both ecto- and arbascular mycorrihzae) are emphasized in this review. Phytoremediation is being used along with or, in some cases as substitute of expensive conventional chemical and mechanical cleanup methods. The *pros* and *cons* of phytoremediation technology are also discussed.

Key Words: **Biodiversity prospecting, Metal hyperaccumulators, Metal transporters, Metal tolerance, Phytoextraction, Phytoremediation, Phytostabilization, Transgenics, Phytovolatilization, Rhizofiltration**

Introduction

Even the supposedly most pristine environment in Arctic circle and Antarctica has not been spared by the globally transported anthropogenic pollutants/contaminants of three major groups viz, i) toxic trace metals (includes metalloids and radio nuclides) ii) acidifying gases (SOx), and iii) variety of persistent organic pollutants that play a major role in global climate change (AMAP 2002). This presentation would concentrate only on "trace metals, metalloids and radionuclides" which would intercept biogeochemical cycles (figure 1); hence, is a subject of utmost concern to "Man and Biosphere". Air (Atmosphere), water (Hydrosphere) and soil (Lithosphere) are not only the reservoirs of nutrients but also act as receptacles for a variety of inorganic and organic pollutants (figure 1). Noosphere is a man-dominated ecosystem, which releases variety of inorganic and organic pollutants. Phytosphere (green belts) is the sink for these pollutants.

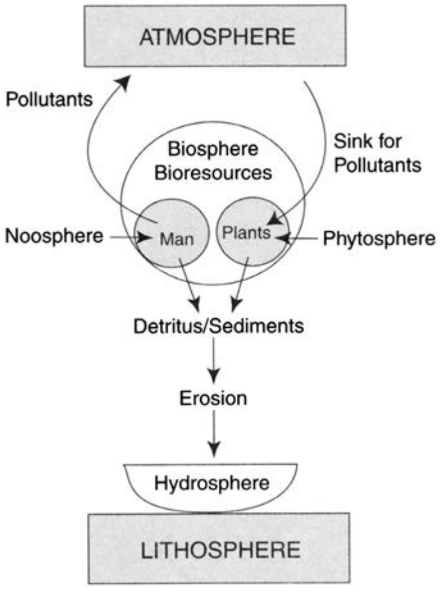

Figure 1 Concept of biogeochemical cycle on which bioremediation relies

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Phytoremediation technology is based on use of plants to cleanup metals; metalloids and radionuclide contaminated/polluted sites both in developed and transition nations (Bell et al. 1988, Iskandar 2002, Iskandar & Kirkham 2001, Kabata-Pendias 2001). This, strategy can be broadly termed as "Bioremediation" which involves soil micro flora and fauna. Bioremediation is being commercialized owing to its success in many real world ecosystems (Comis 1995, Cunningham & Berti 1993a,b). It is emerging as an innovative tool encompassing Biology, Geology, Biotechnology and allied disciplines with cutting edge applications for sustainable development (Cunningham & Ow 1996, Cunningham et al. 1995, Vangronsveld & Cunningham 1998, Groudev et al. 2001a,b, Mejare & Bulow 2000, Prasad & Freitas 1999, 2003). This review evaluates the global findings, primarily dealing with higher plants, focusing the salient developments, and linking the related branches of contemporary biotechnology highlighting the new levels of understanding. Suggestions for possible further research are also mentioned.

Though the phytodiversity and metal polluted sites are enormous in India, there have not been many research investigations from India in the area of metal tolerance in higher plants, biodiversity prospecting for mineral exploration and cleanup of metals in the environment (Aery & Tiagi 1986, Aravind & Prasad 2003a,b, Bhati & Singh 2003, Chandra Sekhar & Puvvada 1997, Chandra Sekhar et al. 1998, 2001, 2002, 2003a,b, Dhillon & Dhillon 2003, Prasad, 1995, 1996, 1997, 1998, 1999, 2001, 2002a,b, 2003, 2004a,b, Prasad et al. 2001a,b, Rama Kumar & Prasad 1999, Reddy & Prasad 1989, 1990, 1993, 1995). Contrastingly countries with less plant diversity have generated enormous data on metal detoxification mechanisms and their application for phytoremediation by appropriate filed and laboratory experiments and currently this knowledge is being commercialized (Baker et al. 1995, Brooks 1998, Chaney et al. 1997, Cunningham & Ow 1996, Cunningham et al. 1995, Dushenkov et al. 1995, 1997a,b, 1999, Ebbs & Kochian 1998, Entry et al. 1999, Ernst 2000, Glass 1999, 2000, Greger & Landberg 1999, Hossner et al. 1998, Huang & Cunningham 1996, Kramer & Chardonnes 2001, Kumar et al. 1995, Ma et al. 2001a,b, McGrath & Zhao 2003, Palmer et al. 2001, Pilon-Smits & Pilon 2000, 2002, Prasad & Freitas 1999,2000a,b,2003, Pulford & Watson 2003, Raskin & Ensley 2000, Rugh et al. 1996, Ruiz & Romero 2002, Salt et al. 1995a-c,1998, Schnoor 2000, Vangronsveld & Cunningham 1998, Wong 2003).

The following are some of the technical terms connected with phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) of metals in the environment (figure 2):

1. *Phytoremediation:* Use of plants to remediate contaminated soil, water and air.
2. *Phytoaccumulation:* The uptake and concentration of contaminants (metals or organics) within the roots or aboveground portion of plants.
3. *Phytoaccumulation coefficient:* Metal concentration in plant dry matter/extractable metal in soil.
4. *Phytoextraction*: The use of plants at waste sites to accumulate metals into the harvestable, above ground portion of the plant and, thus, to decontaminate soils.
5. *Phytoextraction coefficient*: The ratio of extractable metal concentration in the plant tissues (g metal/g dry weight tissue) to the soil concentration of the metal (g metal/g dry weight soil).
6. *Phytomining:* Use of plants to extract inorganic substances from mine ore.
7. *Phytostabilisation*: Plants tolerant to the element in question are used to reduce the mobility of elements, thus, they are stabilised in the substrate or roots.
8. *Phytovolatilization:* The uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant.

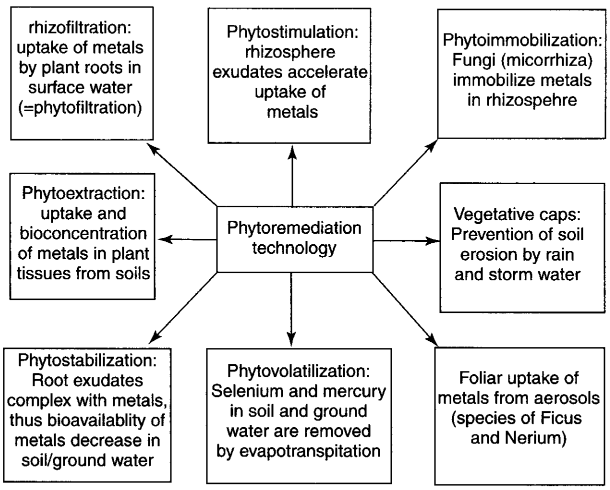

Figure 2 Various processes of phytoremediation technology

9. *Rhizofiltration/Phytofiltration*: Roots or whole plants of element accumulating plants absorb the element from polluted effluents and are later harvested to diminish the metals in the effluents
10. *Rhizosecretion:* A subset of molecular farming, designed to produce and secrete valuable natural products and recombinant proteins from roots

Phytoremediation involves diverse use of plants for *in situ* treatment of metal contaminated soils, sediments, water and air. It is best applied at sites with shallow contamination of metals (Bishop 1997, Bizily et al. 1999, Boyajian & Carreira 1997, Brennan & Shelley 1999, Brooks et al. 1998, Brown 1995, Brown et al. 1994, Chen et al. 2000, 2003, Conte & Piccolo 2003, Ebbs & Kochian 1997, Ebbs et al. 1997, Entry et al. 1996, Fitz & Wenzel 2002, Flathman & Lanza 1998, Francesconi et al. 2002, Garbisu & Alkorta 2001, Gisbert et al. 2003, Glass 1999, 2000, Gleba et al. 1999, Greger & Landberg 1999, Greger & Wang 2003, Hamlin 2002, Heaton et al. 1998, Hossner et al. 1998, Huang 1997, Huang et al. 1997, 1998, Kamnev & van der Lelie 2000, Karenlampi et al. 2000, Lanza & Flathman 2001, Lasat 2002, Lasat et al. 1997, 1998a,b, 2002, Linger et al. 2002, Madrid et al. 2003, McGrath & Zhao 2003, McGrath et al. 2001, McIntyre & Lewis 1997, Meagher 2000, Mejáre & Bülow 2000, Nedelkoska & Doran 2000a,b, Nie et al. 2002, Ouyang 2002, Palmer et al. 2001, Prasad 2003c, 2004a,b, Prasad & Freitas 1999, 2000a,b, 2003, Rangsayatorn et al. 2002, Raskin et al. 1994, 1997, Robinson et al. 1997a,b, Römkens et al. 2002). A model representing phytoremediation of metals by inducing metal tolerance or avoidance in plants (phytostimulation/phytotransformation) over a period of time is depicted in figure 3.

International Conference on the Biogeochemistry of Trace Elements (ICOBTE) is one of the important international events dedicated to this area, discusses the interdisciplinary science in this field, linking phenomena observed in the biogeosphere to physical and chemical reactions in the pedo- and lithosphere. So far, 7 international conferences have been held as follows: Orlando, USA (1990), Taipei, Taiwan (1993), Paris, France (1995), Berkeley, USA (1997), Vienna, Austria (1999) Guelph, Canada (2001) and Uppsala, Sweden (2003). Battelle, an USA based environmental consultancy agency organizes another important series of conference on the theme *in situ* and onsite remediation technology covering inorganics and organics.

Metal Accumulating Plants

Hyperaccumulator plants are geographically distributed and are found throughout the plant kingdom (Brooks 1998, Chaney et al. 1995). To date approximately 400 taxa, ranging in growth habit from annual herbs to perennials are known. Hyper accumulator plants have been identified on all continents, both in temperate and tropical environments (table 1). Natural occurrences of hyperaccumulators for Ni include New Caledonia, Cuba, Southeast Asia, Brazil, southern Europe and Asia Minor; for Zn and Pb include northwest Europe; and for Cu and Co include south-central Africa. Some families and genera are particularly well documented for Ni [Brassicaceae (*Alyssum* & *Thlaspi*), Euphorbiaceae (*Phyllanthus*, *Leucocroton*) and Asteraceae, Zn Brassicaceae (*Thlaspi*), and Cu and Co (Lamiaceae, Scrophulariaceae) (Brooks 1998). There are not many Cr hyper accumulators in nature, but there are numerous Ni hyper accumulators. A few Cr hyperaccumulators have been identified, partly because Cr exists predominantly in the +3 oxidation state and is very insoluble and much less available for plant uptake.

The ability of a plant to hyperaccumulate any one metal may infer some ability to accumulate other metals (Prasad & Freitas 2003). Some metals may interact competitively for accumulation (e.g., Zn and Ni in calamine and serpentine soils). The number of Ni hyper accumulator taxa are more than 300 in about 35 families (table 2). They commonly have 3-4% Ni in dry matter of leaves. *Alyssum betolonii*, which is endemic to serpentine soils, is known for its high concentration of

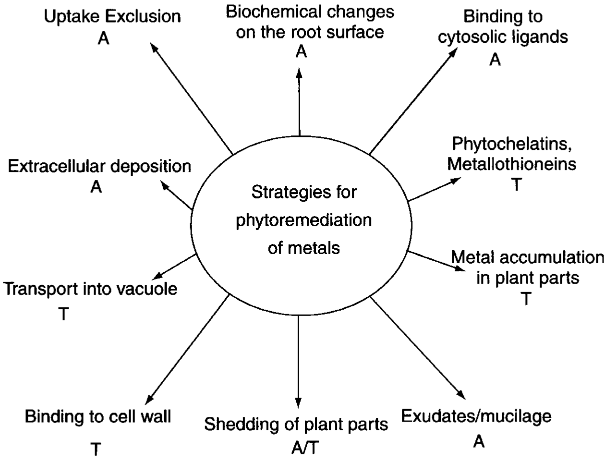

Figure 3 Avoidance and tolerance strategies (ecophysiological basis for the phytoremediation technology) exhibited by plants exposed to elevated concentrations of metals.

Table 1. *Plants that accumulate Lead (Pb), Nickel (Ni) and (Zn) >1 mg* g^{-1} *d.wt from Brassicaceae. Zinc Tabulation is based on ascending order of metal budget in plant metal wise (for details refer Palmer et al. 2001)*

Metal	Taxa	Distribution	Reference
	Lead (Pb)		
2740	*Thlaspicaerulescens* J.Presel & C.Presl	United Kingdom	Shimwell and Laurie 1972
8200	*Thlaspi rotundifolium* sub sp. *cepaeifolium* (Wulfen) Rouy & Fouc	Central Europe	Reeves and Brooks 1983
	Nickel (Ni)		
1050	*Cardamine resedifolia* L.	Italy	Vergnano Gambi and Gabbrielli 1979
1280	*Alyssum singarense* Boiss. & Hausskn.	Iraq	Brooks et al. 1979
2000	*Thlaspi bulbosum* Spruner ex Boiss.	Greece	Reeves and Brooks 1983
2440	*T.japonicum* H.Boissieu	Japan	Revees 1988
3000	*T.epirotum* Halacsy	Greece	Reeves and Brooks 1983
3140	*Pseudosempervivum sempervium* Boiss & Balansa) Pobed	Turkey	Reeves 1988
3420	*Alyssum tenium* Halacsy	Greece	Brooks and Radford 1978
3960	*A.fallacinum* Hausskn	Crete	Brooks and Radford 1978
4000	*Thlaspi ochroleucum* Boiss. & Heldr.	Greece	Reeves and Brooks 1983
4480	*Alyssum alpestre* L	Southern Europe	Brooks and Radford 1978
4550	*A.euboeum* Halacsy	Greece	Brooks and Radford 1978, Reeves et al. 1983a
4590	*A.obovatum* (C.A.Mey) Turez	Russia	Brooks et al. 1979
4900	*A.condensatum* Boiss. &Hausskn.	Iraq, Syria	Brooks et al. 1979, Reeves et al. 1983a
5530	*Thlaspi montanum* L. var. *montanum*	USA	Reeves et al.1983b
6230	*Alyssum virgatum* Nyar.	Turkey	Brooks et al. 1979
6600	*A.smolikanum* Nyar.	Greece	Brooks and Radford 1978
7080	*A.murale* Wealdst&Kit	Balkans	Brooks and Radford 1978 Brooks et al. 1979
7290	*A.oxycarpum* Boiss. & Balansa	Turkey	Brooks et al. 1979
7390	*A.giosnanum* Nyar.	Turkey	Brooks et al. 1979
7600	*A.peltarioides* subsp.*virgatiforme*Nyar.T.R.Dudley)	Turkey	Brooks et al. 1979, Reeves et al. 1983a
7700	*A.floribundum* Boiss.&Balansa	Turkey	Brooks et al. 1979
7860	*A.penjwinensis* T.R.Dudley	Iraq	Brooks et al. 1979
52120	*Thlaspi cypricum* Bornm.	Cyprus	Reeves 1988
8170	*A.anatolicum* Nyar.	Turkey	Brooks et al. 1979
9090	*A. akamasicum* B.L.Burtt	Cyprus	Brooks et al. 1979
10000	*A.serpylifolium* Desf	Spain	Brooks et al. 1981,Dudley 1986b
10200	*A.bertolonii* subsp.scutarinum Nyar	Balkans	Reeves et al. 1983b
10200	*A.syriacum* Nyar.	Syria	Brooks et al. 1979
10400	*A.crenulatum* Boiss	Turkey	Brooks et al. 1979
10900	*A.callichroum* Boiss. & Balansa	Turkey	Brooks et al. 1979
11400	Bornmulleria sp petri Greuter, Charpipn & Dittrich	Greece	Reeves et al. 1983a
11500	*Alyssum eriophyllum*, Boiss. & Hausskn.	Turkey	Brooks et al. 1979
11700	*A.discolor* T.R.Dudley & Huber-Morath	Turkey	Brooks et al. 1979
11800	*Thlaspi tymphaeum* Hausskn.	Greece	Reeves and Brooks 1983
11900	*Alyssum trapeziforme* Nyar.	Trukey	Brooks et al. 1979
12000	*Thlaspi goesingense* Halacsy	Greece	Reeves and Brooks 1983
12400	*T.graecum* Jord	Greece	Reeves and Brooks 1983
12500	*Alyssum heldreichii* Hausskn.	Greece	Brooks and Radford 1978
12500	*A.robertianum* Bernard ex Godron & Gren	Corsica	Brooks et al. 1979
13400	*A.bertolonii* Desv.	Italy	Minguzzi and Vergnano 1948, Palmer et al. 2001
13500	*A.cilicium* Boiss. & Balansa	Turkey	Brooks et al. 1979, Reeves et al. 1983a
13500	*A. huber-morathii* T R Dudley	Turkey	Brooks et al. 1979
13600	*Thlaspi kovatsii* Heuffel	Yugoslavia	Reeves and Brooks 1983, Baker and Brooks 1989

(contd.)

Table 1 *(contd.)*

Metal	Taxa	Distribution	Reference
13700	*Alyssum markgrafii* O E Schulz	Albania	Brooks et al. 1979, Reeves et al. 1983a
14800	*Streptanthus polygaloides* A Gray	USA	Reeves et al. 1981
16200	*Thlaspi caerulescens* J Presl & C	Greece	Reeves and Brooks 198
16300	*Alyssum chondrogynum* B L Burtt	Cyprus	Brooks et al. 1979
16500	*A.dubertretii* gomb	Turkey	Brooks et al. 1979
16500	*A.carcium* T.R.Dudley & Huber-Morath	Turkey	Brooks et al. 1979
17100	*A.troodii* Boiss.	Turkey	Brooks et al. 1979
17600	*Pseudosempervium aucheri* (Boiss.) Pobed	Turkey	Reeves 1988
18100	*Alyssum constellatum* Boiss.	Turkey	Brooks et al. 1979
18300	*Thlaspi rotundifolium* (L.) Gaudin var.*corymbosum* (Gay)	Central Europe	Reeves and Brooks 1983a,b
18900	*Alyssum samariferum* Boiss. &Hausskn.	Samar	Brooks et al. 1979, Reeves et al. 1983a
18900	*Peltaria dumulosa* Post	Asia	Reeves et al. 1980
19200	*Bornmuellaria glabrescens* (Boiss.& Balansa) Cullen & T.R.Dudley	Turkey	Reeves et al. 1983a
19600	*Alyssum davisianum* T.R. Dudley	Turkey	Brooks et al. 1979
20000	*Alyssum cassium* Boiss	Turkey	Brooks et al. 1979
20800	*Thlaspi elegans* Boiss	Turkey	Reeves 1988
18300	*T. rotundifolium* (L.) Gaudin var.*corymbosum* (Gay)	Central Europe	Reeves and Brooks 1983
18900	*A.samariferum* Boiss. &Hausskn	Samar	Brooks et al. 1979, Reeves et al. 1983a
21100	*A.pinifolium* (Nyar.)T.R.Dudley	Turkey	Brooks et al. 1979, Reeves et al. 1983a
21300	*Bornmuellera baldaccii* (Degen) Heywood	Greece	Reeves et al. 1983a
22200	*Alyssum pterocarpum* T.R Dudley	Turkey	Brooks et al. 1979, Reeves et al. 1983a
22400	*A.lesbiacum* (P.candargy) Rech.f	Greece	Brooks et al. 1979
23600	*A.cypricum* Nyar	Cyprus	Brooks et al. 1979
24300	*A.masmenaeum* Boiss	Turkey	Brooks et al. 1979
26900	*Thlaspi jaubertii* Hedge	Turkey	Reeves 1988
27300	*T.caerulescens*	Germany, Belgium	Reeves and Brooks1983
29400	*Alyssum argenteum* All	Italy	Brooks et al. 1979, Reeves et al. 1983a
31000	*Thlaspi sylvium* (as *T.alpinum* subsp.*sylvium*)	Central Europe	Reeves and Brooks 1983
31200	*Bornmuellaria tymphea* (Hausskn.) Hausskn	Greece	Reeves et al. 1983a
34400	*Peltaria emarginata* (Boiss.)Hausskn.	Greece	Reeves et al. 1980
35600	*Thlaspi oxyceras* (Boiss.) Hedge	Turkey, Syria	Reeves 1988
	Zinc (Zn)		
1150	*Thlaspi idahoense* Payson	USA	Reeves et al. 1983b
1400	*Cochlearia pyrenaica* DC.	Belgium	Ramaut et al. 1972
1680	*Thlaspi violascens* Schott & Kotschy.	Turkey	Reeves 1988
3000	*Thlaspi montanum* L.	USA	Reeves et al. 1983b
3000	*T.ochroleucum* Boiss.&Helder.	Greece	Reeves and Brooks 1983
3090	*T.parvifolium* A.Nelson	USA	Reeves et al. 1983b, Reeves 1988
3520	*T. liaceum*	Turkey	Reeves 1988
3890	*T.magellanicum* Comm.Poir.	Argentina, Chile	Reeves 1988
10500	*Thlaspi bulbosum* Spruner ex Boiss.	Greece	Reeves and Brooks 1983
21000	*T.praecox* Wulfen	Bulgaria	Reeves and Brooks 1983
26700	*Arabidopsis thaliana* L	Pennsylvania,USA	Cannon 1960, Reeves 1988
16000	*Thlaspi stenocarpum* (boiss.) Hedge	Spain	Reeves and Brooks 1983
21000	*T.rotundifolium* subsp.*cepaeifolium* (Wulfen)	Italy	Reeves and Brooks 1983
11000	*T.rotundifolium* (L.) Gaudin	France	Reeves and Brooks 1983
39600	*T. caerulescens* J Presl & C Presl	Germany	Linstow 1924,Reeves & Brooks 1983

Table 2. *Plants that hyper accumulate metals and concentration limits (Brooks 1998, Baker et al. 1995, 1998, Hossner et al. 1998, Francesconi et al. 2002, Reeves & Baker 1998)*

Metal	Concentration in leaves mg/g DW	No. of taxa	No. of families	Examples
Cd	> 0.1	1	1	*Thlaspi caerulescens*
Pb	> 1	14	6	*Minuartia verna*
Co	> 1	28	11	*Aeollanthus biformifolius*
Cu	> 1	37	15	*Aeollanthus biformifolius*
Ni	> 1	317	37	*Alyssum bertolonii, Berkheya coddi*
Mn	> 10	9	5	*Macadamia neurophylla*
Zn	> 10	11	5	*Sedum alfredii Thlaspi caerulescens*
As	> 22	2	1	*Pteris vittata, Pityrogramma calomelanos*

Ni (> 10,000 mg kg^{-1} in leaves). The fact that serpentine (ultramafic) soils also contain other elements such as Cr has led to the assumption that the preferential accumulation of Ni in many species of *Alyssum* is due to a selective uptake mechanism (Sequeira & Pinto da Silva 1991) .

Brassica juncea (Indian mustard) - a high-biomass producing plant can accumulate Pb, Cr(VI), Cd, Cu, Ni, Zn, 90 Sr, B, and Se (Palmer et al. 2001, Prasad 2001, Kumar et al. 1995, Salt et al. 1998, Raskin & Ensley 2000). It produces biomass of over 20 times that of *Thlaspi caerulescens* (Salt et al. 1998). *Brassica juncea* had the best ability to transport lead to the shoots. *B. juncea* cultivars varied widely in their ability to accumulate Pb, with different cultivars ranging from 0.04% to 3.5% Pb accumulation in the shoots to 7 to 19% in the roots (Kumar et al. 1995). Except for sunflower (*Helianthus annuus*) and tobacco (*Nicotiana tabacum*), other non-Brassica plants had phytoextraction coefficients less than one.

Phytoremediation is being investigated and/or used commercially in a number of field scenarios (Glass 1999, 2000, Prasad 2001,2003,2004). Plant species are selected for phytoremediation based on their growth rates and yield, the depth of their root zone, and their ability to bioaccumulate contaminants and the type of rhizospheric changes they can initiate for decontamination (Prasad 2001,2003,2004). Variations in plant accumulation of metals are regulated by growth seasons, species and tissues of the plants (Benson et al. 1981, Ellis & Eslick 1997, Hagemeyer et al. 1994, Huang and Cunningham 1996). The ecophysiological, biochemical and molecular aspects underlying the success of phytoremediation are detailed in table 3 with key reference. Processes fostering phytoremediation are also enumerated in table 4 for specific cases of metals.

Phytoremediation of heavy metal contaminated soils in temperate regions has been reviewed recently by Pulford and Watson (2003). Tree crops suitable to tropical and sub-tropical ecosystems such as *Eucalyptus camaldulensis* were invstigated for their important attributes such as tolerance to a wide range of soil type, and *p*H, rapid growth and high wood yield for timber and fuel wood. Mixed effuents were found to be beneficial for improving soil health and for enhancing growth and biomass production of the tree crops (Latimer et al. 1996, Eklund 1995, El-Hasan 2003, Hagemeyer et al. 1994, Landeen & Mitchell 1986, Prasad et al. 2001). This kind of practice may come up as an alternative forestry/agroforestry related water technology for irrigation in water and biomass scarce areas (Bhati & Singh 2003, Lepp 1975, Marcantonio et al. 1998, Pulford & Watson 2003, Schnoor 2000, Zeleznick & Skousen 1996).

Phytoextraction

In this process, a crop species known to accumulate metals, metalloids or radionuclides is cultivated on metalliferous soil. The crop is harvested and the contaminant is recovered (Bañuelos et al. 1999, Brooks et al. 1998, Conte & Piccole 2003, Ebbs & Kochian 1998, Garbisu & Alkorta 2001, Huang et al. 1997, Nedelkoska & Doran 2000a,b, Kumar et al. 1995, Prasad 1998, McGrath & Zhao 2003). In order to have a practicable treatment alternative, one needs a vigorously growing plant (>3 tons dry matter/ha/yr) that is easily harvested and which accumulates large concentrations of metal in the harvestable portion (>1000 mg/kg metal). As a general rule, readily bioavailable metals for plant uptake include cadmium, nickel, zinc, arsenic, selenium, and copper. Moderately bioavailable metals are cobalt, manganese, and iron; while lead, chromium, and uranium are not easily bioavailable.

Table 3. *Ecophysiological, biochemical and molecular basis for phytoremediation*

Mechanism	Key reference (s)
Mycorrhizae	Bi et al. 2003, Entry et al.1999, Jan Colpaert 2001 in Prasad 2001, Khan 2001, Khan et al.2000, Riesen and Brunner 1996, Shetty et al. 1994, 1995, El Kherbawy 1998
Cell wall exudates	Briat and Lebrun 1999, Hall 2002
Plasma membrane (passive uptake and active efflux)	Hall 2002, Clemens et al. 2002
Biodiversity prospecting	Cannon 1960, Brooks, 1998, Prasad and Freitas 2003 Ferritins, metallothioneins, Prasad 1995, 1997, 1999, Brait and Lebrun 1999, Rama Kumar and Prasad 1999
glutathione derived peptides (phytochelatins)	Hartley-Whitaker et al. 2001, Cobbet and Goldsbrough 2002, Rama Kumar and Prasad 1999, Rauser 1995, 2003, Mejare and Bulow 2000, Reddy and Prasad 1990, Schmoger et al. 2000, Vatamaniuk et al.2000, Sanita di Toppi et al. 2002
Low molecular weight organic acids and amino acids	Hall 2002, Banks et al 1994, Brait and Lebrun 1999, Deiana et al. 2003, Delhaize et al. 1993, Hall 2002, Harmens et al. 1994, Huang et al. 1998, Kerkeb and Kramer 2003, Kramer et al.1996, Ma et al. 2001b, Larsen et al. 1998, Pigna et al. 2003, Wu et al.2003
Heat shock proteins	Reddy and Prasad 1993, Neumann et al. 1994, Reddy and Prasad 1995 Vacuolar compartmentation Clemens et al. 2002, Prasad 1995, 1997, 1999
Metal transporters (cation efflux family (formerly cation diffusion family)	Assuncão et al. 2001, Lasat et al.2000, Persans et al. 2001, Pence et al.2000, Guerinot 2000, Prasad 2002a, 2003a, Mc Grath and Zhao 2003
Genetic and transgenic strategies for metal hyper accumulation	Clemens 2001, Clemens et al.2002, Karenlampi et al.2000, Maywald and Weigel 1997, Misra and Gedamu 1989, Pollard et al.2002, Raskin 1996, Rugh et al. 1996, Pena and Seguin 2001, Gisbert et al.2003, Krämer and Chardonnens 2001
Tissue culture techniques	Brewer et al.1997, Nedelkoska and Doran 2000b, Shanks and Morgon 1999, Palmer et al. 2001
Rhizosphere biotechnology and Physiology andbiochemistry of metal tolerance and toxicity	Wenzel et al. 1999 in Prasad and Hagemeyer 1999, Wenzel et al. 2003 in Prasad 2003c, Prasad and Strzalaka 2002
Over expression of glutathione (precursor for metal sequestration)	Zhu etal. 1999a,b, Xiang et al. 2001
Limitations of chelate enhanced phytoextraction and concomitent limitations	Blaylock et al. 1997, Huang 1997, Huang et al.1997, Kock and Mitchel 1957, McGrath and Zhao 2003 Karmer et al. 1996, Madrid et al. 2003, Prasad 2003c, Römkens et al. 2002, Wu 1999

Use of soil amendments such as synthetics (ammonium thiocyanate) and natural zeolites mobilize metal ions and increased the metal bioavailability to plants for the uptake and accumulation (Gersevich & Petunkin 1993, Zorpas et al. 1999). EDTA and NTA (nitrilotriacetic acid) etc. have been used as chelators for rapid mobility and uptake of metals from contaminated soils by plants. Thus, usage of synthetic chelators significantly increased Pb and Cd uptake and translocation from roots to shoots facilitating phytoextraction of the metals from low grade ores. On the other hand synthetic cross-linked polyacrylates, hydrogels have protected plant roots from heavy metal toxicity and prevented the entry of toxic metals into roots (Huttermann et al. 2003 in Prasad 2003c, Laperche et al. 1997).

Different genotypes of plants behave differently in the uptake of metals (Benson et al. 1981). Greger (2003) demonstrated this phenomenon using *Salix* sp. For e.g. some clones have a high uptake of Zn and Cu, whereas others have a low uptake of Cd (Landberg & Greger 1994, Greger & Landberg 1999). This suggests, that the rhizosphere mechanisms regulate the release of metals from the soil colloids, and may be metal specific. Various clones of *Salix* spp. were cultivated in nutrient medium for three months. Subsequently these stem cuttings were transferred to a rhizobox system in which the root mass was connected to soil via a nylon net (25 µm) (figure 4). After 48-hours treatment, root exudates were collected from the rhizosphere by dipping the roots in redistilled water for 30 seconds. The solution was analysed for organic acids by ion chromatography, for Cu, Cd and Zn by AAS and for *p*H. The results showed that plant roots released organic acids viz. citric acid malic acid and succinic acid and changed the *p*H of the rhizosphere (Delhaize et al. 1993, Lee et al. 1977).

Arsenic is a toxic contaminant which is common in natural environments. Sorption/desorption of arsenate on/from different soil components may

Table 4. *Possible processes fostering phytoremediation*

Process fostering phytoremediation	Metal	Reference
Phytoextraction	Gold	Anderson et al. 1998
Metal accumulation	Gold, copper and zinc	Baker et al. 1988
Accumulation, phytovolatilization	Selenium	Bañuelos et al. 1991, 1992 a,b, 1993d, Bañuelos et al. 1997a,b, 1993c; Bañuelos and Meek 1990, Bañuelos et al. 1998, Brown and Shrift 1982, Lauchli 1993 ; Terry et al. 1992, Wu et al. 1988
Phytoremediation	Boron and selenium	Bañuelos et al. 1990, 1991, 1993a,b,
Accumulation and extraction	Boron	Bañuelos et al. 1995a,b, 2002
Root exudates and organic acids enhanced phytoremediatio	Aluminium	Barcelo and Poschenrieder 2002, Delhaize et al. 1993 Lee et al. 1977, Ma et al. 2001b, Mengoni et al. 2000
Accumulation	Air borne trace elements	Bargagli et al. 2003
Chelator enhanced uptake	Cadmium, Nickel and Chromium	Chen and Cutright 2001
Citric acid enhanced remediation	Toxic trace metals	Chen et al. 2003
Removal	Uranium	Dushenkov et al. 1997a
Phytoextraction	Zinc	Ebbs and Kochian 1998
Synthetic chelate enhanced phytoextraction	Lead	Huang 1997, Huang and Cunningham 1996 Huang et al. 1997
Phytoextraction	Toxic trace elements	Kumar et al. 1995
Phytoremediation	Uranium	Huang et al. 1998
Uptake	Radionuclide	Gouthu et al. 1997, Landeen and Mitchell 1986, Koranda and Robinson 1978
Bioavailability, root uptake, phytoremediation	^{137}Cs	Lasat et al. 1997, 1998b, Fesenko et al. 1997b
Phytoextraction	Toxic metals	Lasat 2002
Hyper accumulation	Arsenic	Ma et al. 2001a
Hyper accumulator	Copper	Malaisse et al. 1978
Phytoextraction	Toxic trace elements	McGrath and Zhao 2003
Uptake, accumulation and translocation	Arsenate	Hartley-Whitaker et al. 2001, Macnair et al. 1992, Meharg and Macnair 1991, 1992
Uptake	Chromium(III) (VI)	Mishra et al. 1997
Availability and Uptake	Technetium99	Echevarria et al. 1977, Murphy and Johnson 1993
Hyper accumulation, tolerance	Cadmium	Nedelkoska and Doran 2000a, b
Bioavailability and bioccumulation	Trace metals	Farago et al. 1977, Martin et al 1996, Prasad 1995
Accumulation	Nickel and zinc	Reeves 1988
Hyper accumulation	Lead and zinc	Reeves and Brooks 1983
Hyper accumulation	Nickel	Boyd and Martens 1994, Boyd et al. 1994, Brooks 1981, Dudley 1986a, b: Gabbrielli et al. 1990, 1991, Homer et al. 1991a,b, Jaffre et al. 1976, 1979, Kramer et al. 1997, Reeves et al. 1981, 1983, 1996, Robinson et al. 1997: Shetty et al. 1995.
Hyper accumulation	Zinc and cadmium	Brown et al. 1995a,b
Tolerance	Heavy metals	Arazi et al. 1999, Baker 1987Clemens 2001, El Aziz et al. 1991 Macnair 1993, Mengoni et al. 2000
Hyper accumulation	Copper and cobalt	Baker et al. 1983, Malaisse et al. 1978, 1979
Hyper accumulation	Zinc	Bashmakov et al. 2002

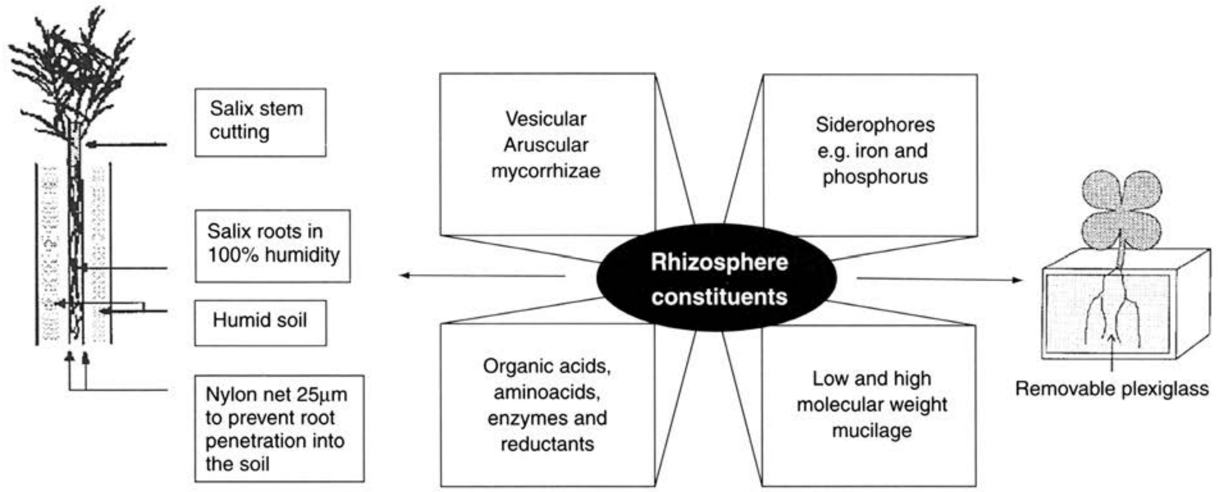

Figure 4 Rhizosphere constituents that would play a significant role in phytoremediation are amenable for collection and investigation using root box microcosm (redrawn based on Greger 2003)

have a dominant role in regulating arsenic mobility in soil-water-plant systems (Smith et al. 1998). To have information on the factors which may influence the mobility and potential toxicity of arsenic in soil environments, sorption/desorption of arsenate in the presence of inorganic phosphate, sulfate and low molecular mass organic ligands (LMMOLs) malate, oxalate, citrate and succinate has been investigated.

Organic ligands inhibited arsenate adsorption mainly at ligand/arsenate molar ratio > 1, when added before arsenate and more in acidic than in neutral or alkaline environments. The efficiency of LMMOLs in preventing arsenate sorption on the Andisol at *p*H 4.0 was as follows: citrate > malate > oxalate > succinate. The efficiency of organic ligands in preventing arsenate adsorption was also influenced by the nature and surface properties of variable charge minerals and soils (Pigna et al. 2003).

A novel approach by using synthetic cross linked polyacrylates and hydrogels has been examined on the metal-contaminated soils. A compound i.e. Stockosorb K400 when applied to hydrocultures of Scots Pine, *Pinus sylvestris*, which contained 1 μM of Pb, two useful functions have been observed: (i) the hydrogel increased the nutrient efficiency of the plants and, (ii) the detrimental effect of the heavy metal was completely remediated. Determination of the heavy metal content of the roots revealed that the uptake of the lead was greatly inhibited by the hydrogel. Analysis of the fine roots of 3- year-old spruce which had been grown for one vegetation period in lead-contaminated soil with and without hydrogel amendments showed that, the amendment of the soil with the crosslinked polyacrylate and hydrogels did indeed prevent the uptake of the lead into the stele of the fine roots.These amandements inhibited the heavy metal uptake by the plant root. The presence of hydrogels (Stockosorb K 400) in the culture solution enhanced the root length and decreased lead concentration in the roots of *Pinus sylvestris*. (Hüttermann et al. 2004, in Prasad 2004) (figure 5). Lead can be made greatly more bioavailable by the addition of EDTA to soils lead, chromium and uranium can be removed by binding to soils and root mass via rhizofiltration (Dushenkov et al. 1995).

Bioconcentration of heavy metals by plants is well established and today a large number of higher plants that hyperaccumulate heavy metals are known (Brooks 1998 Dahmani-Muller et al. 2001). Plants exhibit strategies when exposed to elevated concentrations of heavy metals and they are various classified as (i) indicators: plants in which uptake and translocation reflect soil metal concentration and show toxic symptoms (ii) excluders: plants that restrict the uptake of toxic metals into shoot over a wide range of background concentrations (iii) accumulators: plants in which uptake and translocation reflect background metal concentration without showing toxic symptoms and (iv) hyper-accumulators: plants in which metal concentration is up to 1% in dry matter (table 2). Thus, plants growing

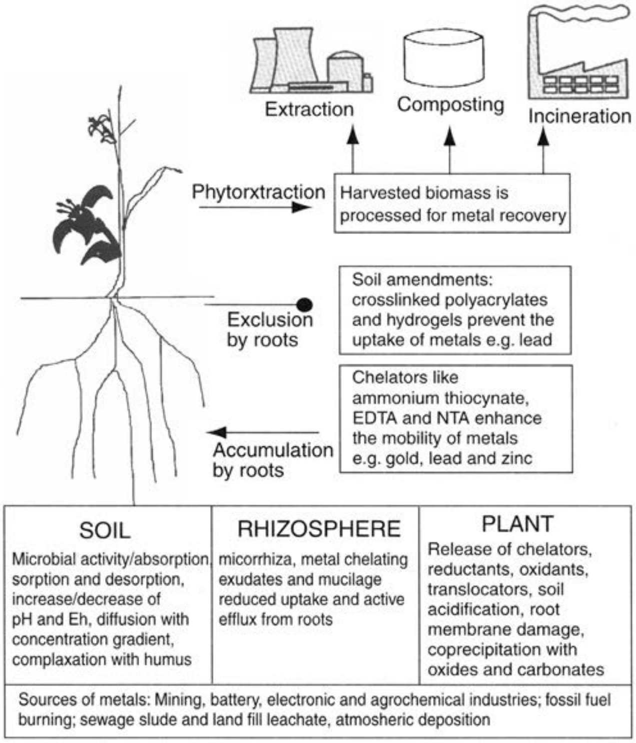

Figure 5. Phytoremediation for sustainable development. It consists of i) planting a speices that tends to accumulate and store, transpire or degrade the target contaminant, ii) letting the crop grow and iii) harvesting it. Cofiring of phytomass or incineration and composting are concentration methods. Liquid extraction is another seperation method. Variety of chelators have been tested to enhance phytoextraction. To achieve satisfactory results, the soil, rhizosphere and plant continuum processes need indepth investigation

in metal contaminated and polluted terrestrial ecosystems take up toxic heavy metals and complex them in their parts, thus, help in environmental decontamination. Phytoextraction has been successfully applied for selenium (Bañuelos et al. 1999).

Phytomining uses hyperaccumulating plants to remove precious metals where conventional mining would not be economically feasible. The sulfur oxidi-zing bacteria have long been used in "biomining" to a significant extent for mining of copper, lead and nickel etc.

Several commercial agencies are pursuing research on phytomining. The pioneering phytoextraction lab of late Prof. Robert Brooks at Massey University in New Zealand discovered that *Brassica juncea* and other hyperaccumulating plant species accumulate as much as 20 ppm of gold in greenhouse pot experiments when plants were supplemented with the metal solubilizing chelating agent such as ammonium thiocyanate. However, disposal of toxic cynide, the leaching reagent of choice for gold and silver extraction inturn poses serious environmental problem. However, vascular plants (*Salix viminalis* x *schwerinii* & *Sorghum bicolor*) possessing appropriate enzymes are used as a part of phytoremediation to detoxify cyanide by converting it to the amino acid asparagine (Korte et al. 2000).

To date there are several commercial ventures gaining economic benefits from phytoextraction, not only by recovering extracted metals from plant biomass, but also in using the biomass for energy generation (Glass 1999, 2000). Plants that hyperaccumulate heavy metals, metalloids and radionuclides could potentially recontaminate the environment if not treated or processed appropriately. Any treatment or processing should result in a minimal volume of contaminant material requiring any further treatment. Two treatment methods, cofiring with coal/incineration and composting are concentration methods. The third, liquid extraction is a separation method. A sub sample of harvested phytomass is burnt with coal under typical electrical power plant combustion conditions. Another fraction of phytomass was composted in containers and the third portion was subjected liquid extraction using chelation agents. Hyperaccumulators of metals contain leachable metals that would recontominate the environment thereby requiring some type of post phyto-remediation treatment. Cofiring with coal reduced the lead-contaminated mass by about 90% wt by concentrating the lead into small fly ash particles. Containers composting reduced the lead-contaminated material by about 26% wt, while extraction using chelating agents removed over 90% wt. of the lead in two sequential batch extractions. Hyperaccumulators have often been isolated from nature in areas of high contamination or high metal concentration. Among the earliest identified terrestrial hyperaccumulating species are members of the genus *Thlapsi* are known to accumulate Zn, Cd and Pb, and the *Alyssum* genus, which are Ni accumulators. For additional information reference be made to Prasad and Freitas 2003. Alemon-scented geranium (Pelargonium sp. 'Frensham') was reported to accumulate large amounts of Cd, Pb, Ni and Cu from soil in greenhouse experiments (Prasad 2004 in Prasad 2004a).

The commercial potential of hyperaccumulators derives from their rates of accumulation of metals, combined with their growth (i.e., biomass accumulation) rates: multiplying the metal accumulation rate (gm metal per kilogram of plant tissue) by the growth rate (kilograms of tissue per hectare per year), yields a value for metal removal from soil (gm or kg of metal per hectare per year). It has been estimated that this rate must be at least several hundreds and perhaps as much as 1,000 kg/ha/yr, to be commercially viable, and even then, remediation may take many years depending on intital metal concentrations and the depth of the contamination (Glass 1999, McGrath & Zhao 2003).

There are numerous reports in the literature of natural or selected species being able to accumulate larger concentrations of metal, in the range of 2-4% of dry weight. It has been estimated that accumulations of 1-2% might yield metal removal rates of 200-1,000 kg/ha/yr, depending on the growth rate of the plants, and thus be sufficient for commercial application.

MacGrath and Zhao (2003) discussed the potential of phytoextraction, since fast-growing hyper-accumulators that can produce a large biomass are known. However, higher growth and enhanced biomass production by most naturally occurring metal hyperaccumulators and the problem of limited solubility of metals in soils (i.e., the high affinity of metal ions for soil particles) are being addressed and there have been some significant developments in this area. The selection or creation of new plant varieties, including the use of genetic engineering tools have promising scope to enhance biomass production. Such efforts are also directed towards the use of artificially improved or selected varieties to enhance uptake of the metal contaminant (Cunningham & Ow 1996, Raskin 1996). The problem of limited solubility is being investigated through the use of chelators or other surfactants in the soil, or even combination of phytoremediation with other *in situ* techniques like electro-osmosis, to stimulate metal mobility in the soil.

Another strategy to enhance metal uptake by plants, being pursued by a number of research groups, is the use of symbiotic root-dwelling fungi. These fungi, called mycorrhizae, naturally live on the roots of many types of plants, and benefit their plant host by improving the plant's ability to take up water and certain types of nutrients, by effectively increasing the surface area of the root available for such uptake (Wenzel et al. 1999 in Prasad & Hagemeyer 1999, Wenzel et al. 2004 in Prasad 2004)

In order for plant roots to take up metals from soil, the soil-bound metal must first be solubilized (i.e., freed from the organic or inorganic soil components to which they are bound). This could be accomplished by secretion of metal-chelating molecules or metal reducates by the plant roots (Raskin & Ensley 2000). Once solubilized, the metal ions may enter roots via extracellular or intracellular pathways, with the intracellular pathways generally requiring the presence of an ion channel or a metal transport protein in the plasma membrane of the root cells. It has been speculated that some of these channels may be nonspecific, so that they can be used by different metals (Raskin & Ensley 2000). Once metal ions reach the root, they can either be stored in vacuoles in the root, often in chelated form, or they can be transported to the shoots. It is reported that some hyperaccumulating species accumulate metals both in roots or shoots or both in root and shoot (Prasad 1998).

Phytoremediation has been applied to treat soil and water contaminated with arsenic, an element used in pesticides and wood treatment. Very well characterized crop species such as Indian mustard and sunflowers are currently in use for the phytoremediation technology. Researchers from all over the world have now shown that plants can accumulate lead, mercury, arsenic, chromium, uranium, cesium, strontium, gold, zinc, selenium, manganese, calcium, iron, magnesium and other metals from soils into harvestable plant parts. When coupled with techniques involving soil amendments and hyperaccumulation inducing agents, then plants can take up heavy metals more than 1% (up to 3% in some cases) of their dry weight.

A variety of different metals can be taken up by natural hyperaccumulators. The most readily bioavailable metals include Cd, Ni, Zn, As, Se and Cu, while more moderately bioavailable metals are Co, Mn and Fe, Pb, Cr and U are generally not considered very bioavailable, but all are capable of phytoremediation to some extent: certain plants can take up Pb, but availability is enhanced by the use of chelators.

Hyperaccumulators are capable of uptake and translocation of metal contaminants in the soil by plant roots into the above ground portions of the plants. After the plants have been allowed to grow for several weeks or months, they are harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits. If plants are incinerated, the ash must be disposed of in a hazardous waste landfill.

Using *Brassica juncea* (Indian mustard), lead-contaminated shallow soils in Trenton, New Jersey have been remediated (Blaylock et al. 1997). Approxi-mately 50% of the lead was removed from the surface soil (~ 700 mg/kg) in one year for phytoextraction to be effective, one needs vigorously growing plants (> 3 tons dry matter/ acre/yr), an easily harvestable above-ground portion, and a plant that accumulates large amounts of metals (~1000 mg/kg) in above ground biomass. To achieve cleanup within three to five years, the plant must accumulate about ten times the level in soil (for example, if the level in soil is 500 mg/kg, then the concentration in the plant must be almost 5000 mg/kg to cleanup the soil in a few years). Some sites have metals that are bioavailable while others do not. Generally, cadmium, nickel, zinc, arsenic, and copper are relatively bioavailable while lead, chromium, and uranium are not take-up and translocated to the harvestable biomass. Plants which accumulate nickel, cobalt, copper, manganese, lead, zinc, and selenium have been reported in the literature (Brooks 1988). Zinc and boron are phytotoxic to some plants at levels above 200 mg/kg in soil. Addition of EDTA (0.5 to 10 kg EDTA/kg soil) has greatly enhanced the bioavailability of lead, but the enhancement must be weighed against the increased probability of lead migration to groundwater (Barona et al. 2001, Chen & Cutright 2001, Hong et al. 1999, Huang et al. 1997, Jiang et al. 2003, Vassil et al. 1998, Wu et al. 2003). Mathematical modeling of water movement and metals transport may be required to further understand the fate of lead under these conditions (Fiorenza et al. 2001).

Advantages

The plant biomass containing the extracted contaminant can be a resource. For example, biomass that contains selenium (Se), an essential nutrient, has been transported to areas that are deficient in Se and used for animal feed (Bañuelos et al. 1997a). In green house experiments, gold was harvested from plants (Anderson et al. 1998).

Limitations: (i) Metal hyperaccumulators are generally slow-growing with a small biomass and shallow root systems (ii) Plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass. Hyperaccumulators may accumulate significant metal concentrations e.g., *Thlaspi rotundifolium* grown in a lead-zinc mine area contained 8,200 g/ g Pb (0.82%) and 17,300 g/g zinc (Zn) (1.73%), and *Armeria maritima* var. *halleri* contained 1,300 g/ g Pb, dry weight basis (Baker et al. 1995 (iii) Metals may have a phytotoxic effect (Kumar et al. 1995) (iv) Phytoextraction studies conducted using hydroponically-grown plants, with the contaminant added in solution, may not reflect actual conditions of soil. Phytoextraction coefficients measured under field conditions are likely to be less than those determined in the laboratory (Kumar et al. 1995).

Phytostabilization

Establishment of rooted vegetation on mine tailings prevents windblown dust, an important pathway for human exposure at hazardous waste sites. Hydraulic control is possible, in some cases, due to the large volume of water that is transpired through plants which prevents migration of leachate towards groundwater or receiving waters. Metals do not ultimately degrade, so capturing them *in situ* is sometimes the best alternative at sites with low contamination levels (below risk thresholds) or vast contaminated areas where a large-scale removal action or other *in situ* remediation is not feasible. Vigorously growing plants are necessary to exert hydraulic control and immobilization at the site; plants cannot die or be removed during the phytostabilization design period. Low-level radionuclide contaminants can also be held in place by phytostabilization, and this alternative can result in significant risk reduction if their half-lives are not too long. Soil amendments such as phosphate, lime, and organic matter are sometimes needed to immobilize toxic metals such as lead, cadmium, zinc, and arsenic. Cadmium is readily translocated to leaves in many plants, which represents a risk to the food chain (Barman & Lal 1994, Brown et al. 1996, Chaney 1980, McLaughlin & Singh 1999) and this pathway may be the limiting consideration in applying phytostabilization at some metal contaminated sites. It involves the use of plants especially roots and/or plant exudates to stabilize, demobilize and bind the contaminants in the soil matrix, there by reducing their bioavailability. This approach is suitable for metal contaminated soils. Several plant species including common agricultural and horticultural crops have shown potential in phytostabilization. Field trails for *in situ* decontamination of heavy metal polluted soils using metal accumulating crops was proved to be advantageous, particularly with fibre yielding crops (Felix 1997, Prasad et al. 2003).

Phytostabilization relies on plants, or compounds they secrete, to stabilize low levels of contaminant that are present in soils (e.g., by absorption or precipitation), to prevent them from mobilizing or leaching in a manner that would endanger public health (Cunningham et al. 1995). Possible mechanisms might include sequestering the contaminant in or on cell wall lignin ("lignification"), absorption of contaminants to soil humus, via plant or microbial enzymes ("humification"), or other mechanisms whereby the contaminant is sequestered in the soil, e.g., by binding to organic matter. Phytostablilization is primarily applicable to metal contamination, and might best be used near the end of remediation by traditional means, or after site closure, for those sites where it is acceptable under regulatory guidelines to leave a non-bioavailabe portion of the contaminant remaining in the soil (e.g. a risk-based regulatory approach). The term also refers to the use of a vegetative cover, e.g., at a landfill, to prevent contaminants from leaching into groundwater or surface waters (Glass 1999).

Phytostabilization reduces the mobility of the contaminant and prevents migration to the ground water or air, and it reduces bioavailability for entry into the food chain. (Barman & Lal 1994, Bañuelos & Mayland 2000, Bunzl et al. 2001, Chandra Sekhar et al. 2001, Dudka & Miller 1999, Dudka et al. 1996). This technique can be used to reestablish a vegetative cover at sites where natural vegetation is lacking due to high metal concentrations in surface soils or physical disturbances to surficial materials. Metal-tolerant species can be used to restore vegetation to the sites, thereby decreasing the potential migration of contamination through wind erosion, transport of exposed surface soils, and leaching of soil contamination to ground water (Kock & Mitchell 1957).

Phytostabilization occurs through root zone microbiology and chemistry, and/or alteration of the soil environment or contaminant chemistry. Soil *p*H may be changed by plant root exudates or through the production of CO_2. Phytostabilization can change metal solubility and mobility or impact the dissociation of organic compounds. The plant affected soil environment can convert metals from a soluble to an insoluble oxidation state. Phytostabilization can occur through sorption, precipitation, complexation, or metal valence reduction. Plants can also be used to reduce the erosion of metal-contaminated soil. The term phytolignification has been used to refer to a form of phytostabilization in which organic compounds are incorporated into plant lignin (Cunningham et al. 1995). Compounds can also be incorporated into humic material in soils in a process likely related to phytostabilization in its use of plant material (Bollag & Myers 1992, Conte & Piccolo 2003).

Cattle provide remarkable Reclamation of mine tailings through the Development of Rhizosphere

In Globe Arizona, USA, copper mines rehabilitation and mine reclamation programme [Arizona Ranch, Resource Management and Mine Reclamation and Tucson (ASARCO Inc. Copper Operations)] is primarily based on cattle. Due to stringent environmental legislation the mine operators are under the obligation of restoration of metal polluted and contaminated sites. In order to curb the metal pollution several of the metal polluting industries and agencies are looking for low cost and effective measures rather than adopting conventional chemical methods which are cost prohibitive. To achieve mine reclamation, herds of cattle are impounded with electric fence on the sites of mine tailings, metal contaminated soils by providing fodder and water for varying durations. Cattle stabilize the soil by their hoofs and enrich nutrient status through dung and urination. This process is repeated at regular intervals in cycles often

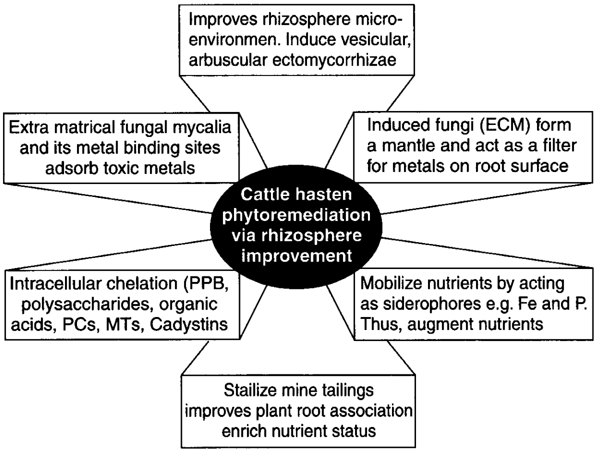

Figure 6 Cattle hasten bioremediation of mine tailings by improving the rhizosphere environment.

spreading for years. This exercise provided convincing evidence for cattle accelerated rhizosphere development and enriched soil microorganisms and nutrients (figure 6) (Dagget 1997).

Advantages: (i) It circumvents the removal of soil, (ii) It has a lower cost and is less disruptive than other more-vigorous soil remedial technologies, (iii) Revegetation enhances ecosystem restoration.

Limitations: (i) The contaminants remain in place. (ii) The vegetation and soil may require long-term maintenance to prevent re-release of the contaminants and future leaching, (iii) Vegetation may require extensive fertilization or soil modification using amendments (Zorpas et al. 1999),(iv) Plant uptake of metals and translocation to the aboveground portion must be avoided, (v) The root zone, root exudates, contaminants, and soil amendments must be monitored to prevent an increase in metal solubility and leaching, (vi) Phytostabilization might be considered to only be an interim measure, (vii) Contaminant stabilization might be due primarily to the effects of soil amendments, with plants only contributing to stabilization by decreasing the amount of water moving through the soil and by physically stabilizing the soil against erosion.

Plants that produce root exudates are shown to stimulate growth of beneficial microorganisms or stimulate cometabolism will be of more benefit than plants without such useful exudates (Barcelo et al. 2003, Barcelo and Poschenrieder 2002, Yoshitomi & Shann 2001). The type, amount, and effectiveness of exudates and enzymes produced by 'plantroots' will vary between species and even within species or varieties of one species.

Phytovolatilization

This method can be used only for those contaminants that are highly volatile. Mercury or selenium, once taken up by the plant roots, can be converted into non-toxic forms and volatilized into the atmosphere from the roots, shoots, or leaves. For examples, Se can be taken up by plants of the *Brassica* genus and other wetland plants, and converted (for example, by methylation to the volatile dimethyl selenium) into non-toxic forms which are volatilized by the plants: field testing has shown this to be a potentially effective method A similar mechanism can be exploited for Hg, although there are no naturally occurring plants that can accomplish this: the goal here is to engineer bacterial genes for mercury reduction into plants, and here too laboratory experiments are highly encouraging (Bizily et al. 1999, Heaton et al. 1998).

Phytovolatilization occurs as growing trees and other plants take up water and the contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Mercury has been shown to move through a plant and into the air in a plant that was genetically altered to allow it to do so. The thought behind this media switching is that elemental Hg in the air poses less risk than other Hg forms in the soil.

Advantages: (i) Contaminants could be transformed to less-toxic forms, such as elemental mercury and dimethyl selenite gas, (ii) Contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation.

Limitations: The contaminant or a hazardous metabolite might accumulate in vegetation and be passed on in later products such as fruit or lumber. Low levels of metabolites have been found in plant tissue.

Rhizofiltration

Rhizofiltration has been pioneered by Dr Ilya Raskin and the group at Rutgers University, NJ (USA) (Dushenkov et al. 1995, Kumar et al. 1995). It is effective in cases where wetlands can be created and all of the contaminated water allowed to flow through inclose contact with roots. Contaminants should be those that sorb strongly to roots such as, lead, chromium(III), uranium, and arsenic (V).

Rhizofiltration refers to the use of plant roots to sorb, concentrate, and precipitate metal contaminants from surface or groundwater. Roots of plants are capable of sorbing large quantities of lead and chromium from soil, water or from water that is passed through the root zone of densely growing vegetation.Shallow lagoons have been engineered as wetlands and maintained as facultative microbial systems with low dissolved oxygen in the sediment. Groundwater or wastewater is pumped through the system for the removal of contaminants by rhizofiltration. Wetlands have been used with great success in treating metals for many years (McGrath et al. 2001, Wenzel et al. 2003, Fitz & Wenzel 2002). Long-term utilization of wetland plants and sulfate-reducing conditions result in an increase in *p*H and a decrease in toxic metals concentrations for treatment of acid mine drainage. Root systems and sediments in wetlands are facultative (aerobic and anaerobic zones) which facilitates sorption and precipitation of toxic metals. (Dushenkov at al. 1995, Fitz & Wenzel 2002). Experimental evidence showing nonlinear kinetics of disappearance of metals from solution suggests that several different mechanisms operate simultaneously (Kumar et al. 1995). Surface absorption by the roots, the fastest and often the most prevalent mechanism, most likely depends on physico-chemical processes (e.g., ion exchange, chelation) and can even take place on dead roots.

Rhizofiltration is the use of plant roots to accumulate metals from water. Hydroponically cultivated plants rapidly remove heavy metals from water and concentrate them in the roots and shoots. Harvested plants containing heavy metals can be disposed of or treated to recycle the metal. Today scientists have identified plants demonstrating high biomass production and metal removal capacity for a wide variety of metals. Rhizofiltration has many of the benefits of other phytoextraction techniques, including low cost and minimal environmental disruption. A continuous flow system circulates the contaminated water through specially designed plant containment units. Periodically, older plants are harvested and replaced.

In its reliance on surface absorption as the primary mechanism for removing metals from waste streams, rhizofiltration is related to the process known as biosorption, in which microbial, fungal or other biomass, living or dead, is used to absorb large quantities of materials such as heavy metals. In addition to surface absorption, other, slower mechanisms underlying rhizofiltration may also occur: these might include biological processes (intracellular uptake, deposition in vacuoles, and translocation to the shoot), or precipitation of the metal from solution by plant exudates (the slowest mechanism).

There are several naturally occurring plant species with a natural propensity to accumulate high concentrations of metal. The desired species are those where root surface absorption is the primary, or fastest mechanism: in those plants where a substantial amount of shoot translocation occurs, more of the plant biomass becomes contaminated with the metal or radionuclide and must therefore be disposed as hazardous (or radioactive) waste, thus negatively affecting economics and efficiency. Rhizofiltration is believed to be effective (and perhaps most economically attractive) for dilute concentrations of contaminants in large volumes of water, and this feature may make it especially attractive for radionuclide contamination (Dushenkov et al. 1995, Kumar et al. 1995).

Rhizofiltration can be practiced *in situ* (e.g., on surface waters), but more likely would be operated in an aboveground artificial flow-through reactor, akin to hydroponics, and could thus be applicable to groundwater or industrial waste streams. At the conclusion of the process, plant roots and any other tissue containing metals can be harvested for disposal or metal recovery: any uncontaminated shoots can, in some cases, be used to regenerate new roots.

Advantages: (i) Either terrestrial or aquatic plants can be used. Although terrestrial plants require support, such as a floating plat-form, they generally remove more contaminants than aquatic plants, (ii) This system can be either *in situ* (floating rafts on ponds) or *ex situ* (an engineered tank system), (iii) An *ex situ* system can be placed anywhere because the treatment does not have to be at the original location of contamination.

Limitations: (i) The *p*H of the influent solution may have to be continually adjusted to obtain optimum metals uptake, (ii) The chemical speciation and interaction of all species in the influent have to be understood and accounted for, (iii) A well-engineered system is required to control influent concentration and flow rate, (iv) The plants (especially terrestrial plants) may have to be grown in a greenhouse or nursery and then placed in the rhizofiltration system, (v) Periodic harvesting and plant disposal are required, vi) Metal immobilization and uptake results from laboratory and greenhouse studies might not be achievable in the field.

Biodiversity prospecting for Phytoremediation and Metal Exploration

"Biodiversity prospecting" offers several opportunities of which the most important is to save as much as possible of the world's immense variety of ecosystems (Myers 1990). Biodiversity prospecting would lead to the discovery of wild plants that would not only clean metal polluted environments but also would help in mineral exploration (Aery & Tiagi 1986, Bargagli et al. 2003, Brooks et al. 1977a,b, Brooks 1972, Cannon 1959, 1960). Published literature indicate that wide variety of plants have been tested in field and laboratory (Prasad 2001). Remediation programmes relying on metal tolerant plants would be successful (Glass 1999, 2000).

The most successful monitoring methods for metals in the environment are based on bacterial heavy metal biosensors viz., a) gene based biosensors and b) protein based biosensors (Prasad 2001). Mosses, liverworts and ferns are also capable of growing on metal-enriched substrates. These plants possess anatomical and physiological characteristics enabling them to occupy unique ecological niches in natural metalliferous and man made environments. For example, groups of specialized bryophytes are found on Cu enriched substrates; so-called 'copper mosses' and come from widely separated taxonomic groups. Other bryophytes are associated with lead and zinc enriched substrates. However, the information about bryophytes growing on serpentine soils is rather scanty (Prasad 2001). Pteridophytes (ferns) are associated with serpentine substrates in various parts of the world. *Pteris vittata* (Brake fern), a fast growing plant is reported to tolerate soils contaminated with arsenic as much as 1500 ppm and its fronds concentrate the toxic metal to 22,630 ppm in 6 weeks (Ma et al. 2001a, Tu et al. 2003). Among angiosperms, about 400 metal hyperaccumulators have been identified which would serve as a reservoir for biotechnological application (Brooks 1998).

Brassicaceae had the highest number taxa i.e. 11 genera and 87 species that are established for hyper accumulation of metals (In Brassicaceae Ni hyper accumulation is reported in 7 genera and 72 species; Zn in 3 genera and 20 species) (Palmer et al. 2001, Prasad & Freitas 2003).

Considerable progress had been achieved recently in unraveling the genetic secrets of plants that hyper accumulate metals including genetic engineering for metal tolerance (Gisbert et al. 2003, Heaton 1998, Karenlampi et al. 2000, Macnair 1993, Macnair et al. 1992, Meharg & Macnair 1992, Mengoni et al. 2000, Peña & Séguin 2001, Pollard et al. 2002, Pollard & Baker 1996, Raskin 1996, Schat & Bookum 1992a). Genes responsible for metal hyperaccumulation in plant tissues have been identified and cloned (Moffat 1995, 1999). These findings are expected to identify new non-conventional crops, metallocrops that can decontaminate metals in the environment (Ebbs & Kochian 1998, Madejon et al. 2003, Raskin 1996). The fundamental aspects of microbe/plant stress responses to different doses of metals coupled with break through research innovations in biotechnology would successfully provide answers as to how to apply biodiversity for advancing phytoremediation technology.

Many of the hyperaccumulators inhabit in serpentine soils as well as in a wide range of environmental conditions. In addition to sepentinophytes, a number of wild brassicaceae are the best suitable species being hyperaccumulators for phytoremediation. Serpentine habitats and species are threatened worldwide. Mainly due to habitat loss, several serpentine endemic species have become extinct or they are highly threatened. These habitats, often regional hotspots of biodiversity should be preserved and actions to preserve these unique spots should be promptly implemented (Prasad & Freitas 1999, 2003).

Phytoremediation Research for Sustainable Development

Evaluation of promising agricultural crops e.g. kenaf and brassica based phytoremediaiton systems might be advantageous to Indian scenario for phytoextraction (Bañuelos et al. 1999, 2002, Ebbs & Kochian 1998, Linger et al. 2002), development of tree crop based (e.g. *Eucalyptus, Prosopis, Acacia* etc.) phytoremediation technology would be desirable (Bhati & Singh 2003, Greger & Landberg 1999, Latimer et al. 1996, Eklund 1995, El-Hasan 2003, Hagemeyer et al. 1994, Landeen & Mitchell 1986, Pulford & Watson 2003). A community of tree, legume and grass based phytoremediation systems would be advantageous (Barlow et al. 2000, Piechalak et al. 2002, Sahi et al. 2002) (figure 7).

Identification of low cost, effective and environ-mentally friendly chelators for enhanced accumulation of Pb and other metals in indian mustard and other promising crops (Blaylock et al. 1997), implications of use of chelators on ground water, (Barona et al. 2001) and development of

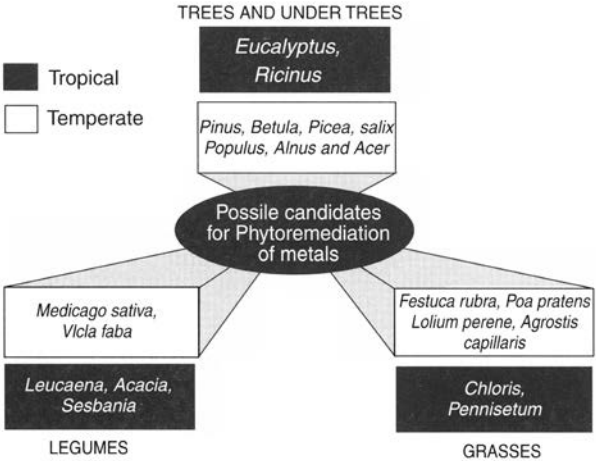

Figure 7 Possible candidates for phytoemediation of metals in temperate and tropical ecosystems

refined techniques for separating heavy metals from the phytomass of hyperaccumulators (Lakshmi & Clements 2003) needs critical investigation.

Application of plant tissue culture and plant biotechnological tools such as somatic hybridization between compatible heavy metal hyper accumulators, and development of transgenic plants (Brewer et al. 1997), development of promising hairy root cultures for rhizofiltration of radionuclides (Eapen et al. 2003, Gleba et al 1999), transgenic crops for promising and low cost phytoremediation (Gisbert et al. 2003, Pilon-Smits & Pilon 2000, 2002) (figure 8).

Identification of indigenous plants that hyper accumulate heavy metals and eventually leading to phytomining (Botanical prospecting of ore deposits) (Brooks 1998, Brooks et al. 1998, Cannon 1960). Detailed studies on indigenous Brassicaceae for metal accumulation (Palmer et al. 2001), identification of indigenous sources of biomass for removal of specific metals from aqueous solutions (Prasad et al. 2001), identification of indigenous clays for sorption of metal contaminants (Churchmann et al. 1999), identification of plants capable of accumulating airborne elements would be worth attempting (Bargagli et al. 2003, El-Hasan et al. 2003, Keane et al. 2001). Analysis and examination of indigenous grass species inoculated with mycorrhizal fungi for their ability to accumulate metals, metalloids and radio nuclides (Entry et al. 1999, Khan 2001, Khan et al. 2000, Dushenkov et al. 1995, 1997a,b, 1999, Hossner et al. 1998), examination of arbuscular mycorrhizal associations for remedia-tion of metallifersous and coal fly ash (Bi et al. 2003, Veeken & Hamelers 2002 2002), plant community studies for tolerant to toxic trace metals for restoration of the degraded mines soils are warranted (Dahmani-Muller et al. 2000, Freitas et al. 2003, Wong 2003). In-depth analysis of indigenous ferns for arsenic hyper accumulation (Francesconi et al. 2002, Ma et al. 2001a, Zhang et al. 2002) would contribute to sustainable development of metalliferous soils.

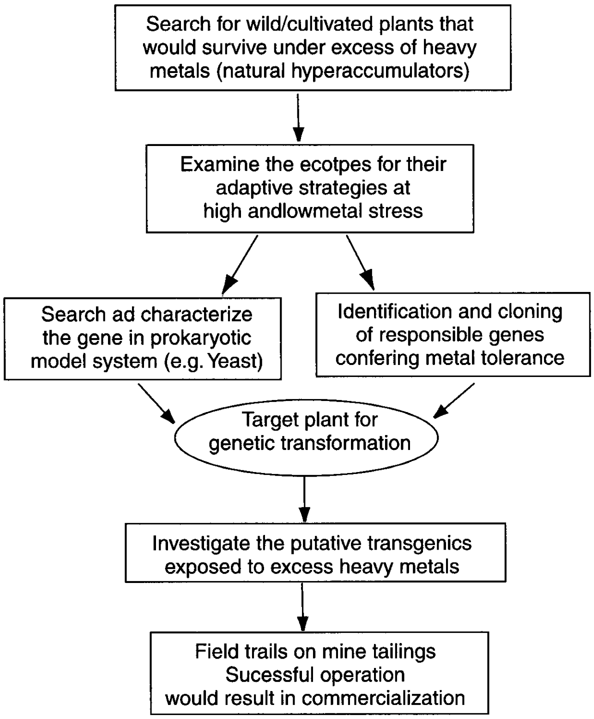

Figure 8 Feasible scheme for genetic improvement and transgenic production of plants for phytoextraction of metals

Studies on the molecular mechanisms (molecular farming) of plant metal tolerance (Clemens 2001, Clemens et al. 2002), expression of microbial genes from promising organisms e.g. *merB* in *Arabidopsis thaliana* that confer resistance to organo mercurials (Bizily et al. 1999), and production of genetically transformed tress over expressing 3-glutamyl cysteine synthetase will have potential role in phytoremdiation (Arisi et al. 2000), role of phytochelatins and metallothioneins (figure 9) (Briat & Lebrun 1999, Cobbett & Goldsbrough 2002, Prasad 2003a,b), plant ABC transporters (Guerinot 2000, Prasad 2002a, 2003a, Theodoulou 2000, Karenlampi 2000), role of citric (Chen et al. 2003), humic and fulvic acids would be the promising fields of investigation for phytoremediation of metal contaminated soils (Conte & Piccolo 2003).

Metal ions are complexed in the cytosol with glutathione (GSH) and the derived PCs are

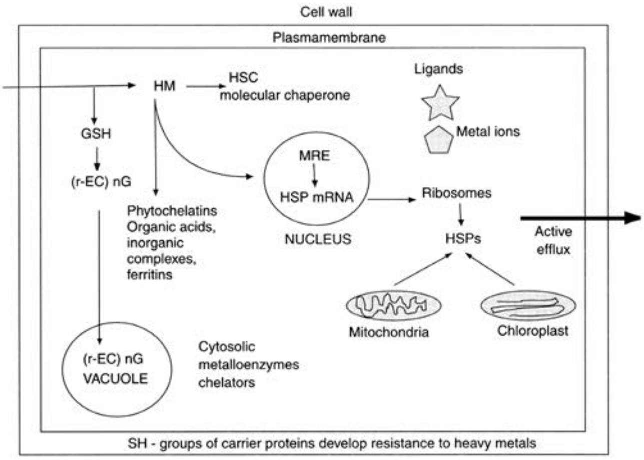

Figure 9 Cellular responses of heavy metal toxicity the physiological and molecular basis for the underlying mechanisms of phytoremediaton

transported into vacuole. These functions have been implicated in heavy metal complexation and transport (Prasad 1999, Rauser 1995, Reddy & Prasad 1990). Another cellular response observed was that heavy metals interact with metal regulating elements (MRE) at the promotor region located in MT genes. For example in soybean such sequences have been found in the upstream region of heat-shock gene coded for 17.5 kDa HSP. Some heat-shock genes and heat shock proteins (HSPs) have been induced by Cd ions in soybean. These HSPs have been implicated as molecular chaperones (Neumann et al. 1994, Reddy & Prasad 1993, Prasad 1997, Prasad 1999).

Studies on the ligands exuded by plants in rhizosphere (Deiana et al. 2003, Prasad 1998), rhizosphere biotechnology of indegenous plants to facilitate metal accumulation (Barceló et al. 2003, Deiana et al. 2003, Fitz & Wenzel 2002, Jauert P et al. 2002, McGrath et al. 1997, 2001, Mishra et al. 1997, Tepfer et al. 1989, Wackett & Allen 1995, Walker et al. 2003, Wenzel et al. 2003 (figure 4), plants with concurrent ability for uptake of heavy metals and persistent organic pollutants from soil (Mattina 2003) through soil-rhizosphere-plant continuum (figure 5), accumulation of potentially toxic elements in edible plants and their transfer to human food chain (Barman & Lal 1994, Dudka & Miller 1999, Yusuf et al. 2003) need focused research.

Development of modeling for environmental phytoremediation (Blaylock et al. 1995, Brennan & Shelley 1999, Fesenko et al. 1997a, Fiorenza et al. 2001, Ouyang 2002), identification of plant and animal indicators that might serve a realistic tool are warranted (Beeby & Richmond 2002, Bañuelos & Mayland 2000).

Species selective analysis and localization of metals in plant cell environment by sophisticated analytical tools would be advantageous to localize metals in plant cells, however expensive they might be such as: atomic absorption spectrometry AAS, atomic emission spectrometry AES, atomic fluorescence spectrometry AFS, anodic-stripping voltammetry ASV, energy dispersive X-ray fluorescence EDXRF, electro thermal atomic absorption spectrometry ETAAS, extended X-ray absorption fine structure spectroscopy EXAFS, flame AAS, flame AES flame AFS, particle-induced gamma-ray emission PIGE, particle-induced X-ray emission PIXE (Ager et al. 2002, Montes-Bayon et al. 2002, Schaumlöffel et al. in Prasad 2004a).

Conclusions

Inspite of certain limitations, there are several advantages of phytoremediation technology. There have been apprehensions and often metal accumu-lators are considered as if the contaminats are only transferred and are potentially toxic when the plants accumulate these contaminats. In this regard, industrial crops not used for food production e.g. fibre crops would be the best alternative land use option. Industrial crops such as *Brassica juncea, Armoracia rusticana, Arabidopsis halleri, Gossypium hirsutum, Helianthus annuus, Eucalyptus, Amaranthus, Cannabis sativa* and *Linum usitatissimum* based phytoremediation systems would contribute to sustainable development.

Agricultural production in metal/radionuclide contaminated soil is of human health concern because of high levels of metallic and radionuclide residues in agricultural food products. Therefore, interest arises in developing more integrated and ecologically-based approaches. In this context, industrial crops not used for food production such as fibre crops are thought of as an alternative option. Two fibre crops viz, hemp (*Cannabis sativa* L.) and flax (*Linum usitatissimum* L.) were prefereed for cultivation (Linger et al. 2002, Vandehove & Van Hees 2003). For both crops extensive knowledge is available on the use of the fibre for textiles and many other high value applications. Hemp has been investigated as a biofuel for energy production through thermal conversion and scores high on ecological and economic criteria. However, information on the radionuclide transfer to fibre crops is limited (Bañuelos et al. 1995a, 2002).

Higher soil potassium (K) levels decrease the Cs-transfer factor (TF), therefore, appropriate K-fertilisation will result in a decreased TF in most cases. Lower TF's were observed for flax than hemp, but the TFs to the usable plant parts both for hemp and flax, are low enough to allow for the production of a clean end product (fibre, seed oil, biofuel) even on heavily contaminated land (1480 kBq m^{-2}). Flax is a more demanding crop compared to hemp in terms of soil properties (more fertile, fine textured soils) and agronomy. Depending on soil type, climate conditions and requirements for final product use, one or the other crop will be an appropriate alternative land use (Linger et al. 2002, Vandehove & Van Hees 2003). Thus, fibre and energy crops and industrial crops that are amenable to genetic manipulation via *in vitro* culture techniques would certainly play significant role for the success of phytoremediation for sustainable development.

References

Aery N C and Tiagi Y D 1986 Bioindicator and accumu-lators in geobotanical and biogeochemical prospecting of metals; *Acta Biologia Hungarica* **371** 67-78

Ager F J, Ynsa M D, Domínguez-Solís J R, Gotor C, Respaldiza M A and Romero L C 2002 Cadmium localization and quantification in the plant *Arabidopsis thaliana* using micro-PIXE; *Nuclear Instruments and Methods in Physics Research Section B Beam Interactions with Materials and Atoms* **189** 494-498

AMAP 2002 *Arctic Monitoring and Assessment Programme, Arctic Pollution*, Oslo, Norway. p.112

Anderson C W N, Brooks R R, Stewart R B and Simcock R 1998 Harvesting a crop of gold in plants; *Nature* **395** 553-554

Aavind P and Prasad M N V 2004 Carbonic anhydrase impairment in cadmium-treated *Ceratophyllum demersum* L. (free floating freshwater macrophyte): toxicity reversal by Zinc; *J. Anal. At. Spectrom* **19** 52-57

Aavind P and Prasad M N V 2003b Zinc alleviates cadmium induced toxicity in *Ceratophyllum demersum* L.: a free floating fresh water macrophyte; *Plant Physiol. and Biochem.* **41** 391-397

Arazi T, Sunkar R, Kaplan B and Fromm H 1999 A tobacco plasma membrane calmodulin-binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic plants; *Plant J.* **20** 171-82

Arisi A C M, Mocquot B, Lagriffoul A, Mench M, Foyer C H and Jouanin L 2000 Responses to cadmium in leaves of transformed poplars over expressing γ-glutamylcysteine synthetase; *Physiol Plant* **109** 143-149

Assuncão A G L, Martins P D C, Folter S D, Vooijs R, Schat H and Aatrs M G M 2001 Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens; Plant Cell Environment* **24** 217-226

Baker A J M 1987 Metal tolerance; *New Phytol.* **106** 93-111

______ and Brooks R R 1989 Terrestrial higher plants which hyperaccumulate metal elements - A review of their distribution, ecology, and phytochemistry; *Biorecovery* **1** 81-126

______, Brooks R R, Pease A J and Malaisse F 1983 Studies of copper and cobalt tolerance in three closely related taxa within the genus *Silene* L. (Caryophyllaceae) in Zaire; *Plant Soil.* **73** 377-385

______, ______ and Reeves R D 1988 Growing for gold, copper and zinc; *New Sci.* **117** 44-48

______, McGrath S P, Sidoli C M D and Reeves R D 1995 The potential for heavy metal decontamination; *Mining Environ. Management* **3** 12-14

Banks M K, Waters C Y and Schwab A P 1994b Influence of organic acids on leaching of heavy metals from contaminated mine tailings; *J. Environ. Health* A **29** 1045-1056

Bañuelos G S and Mayland H F 2000 Absorption and distribution of selenium in animals consuming canola grown for selenium phytoremediation; *Ecotoxicol Environ Safety* **46** 322-328

Bañuelos G S and Meek D W 1990 Accumulation of selenium in plants grown on selenium-treated soil; *J. Environ. Qual.* **19** 772-777

______, Ajaw H A, Mackey B, Wu L L, Cook C, Akohoue S and Zambruzuski S 1997a Evaluation of different plant species used for phytoremediation of high soil selenium; *J. Environ. Qual.* **26** 639-646

______, ______, and Zambrzuski S 1997b Selenium-induced growth reduction in Brassica land races considered for phytoremediation; *J. Ecotoxicol. Environ. Saf.* **36** 282

______, ______, Wu L and Zambrzuski S 1998 Selenium accumulation by *Brassica napa* grown in Se-laden soil from different depths of Kesterson Reservoir; *J Soil Contamination.* **7** 4481-4496

______, Bryla D R and Cook C G 2002 Vegetative production of kenaf and canola under irrigation in central California; *Industrial Crops and Products;* **15** 237-245

______, Cardon G E, Mackey B, Ben-Asher J, Wu L L, Beuselinck P and Akohoue S 1993a Boron and selenium removal in boron-laden soils by fours prinkler-irrigated plant species; *J Environ. Qual.* **22** 786-792

______, ______, Phene C J, Wu L L, Akohoue S S and Zambrzuski S 1993b Soil boron and selenium removal by three plant species; *Plant Soil.* **148** 253-263

______, Dyer D, Ahmad R, Ismail S, Raut R N and Dagar J C 1993c In search of *Brassica* germplasm in saline semiarid regions of India and Pakistan for reclamation of selenium-laden soils in the U.S; *J. Soil Water Conserv.* **48** 530-534

______, Mackey B, Cook C, Akohoue S, Zambrzuski S and Samra P 1995a Response of cotton and kenaf with boron-laden water and soil; *Crop Sci.* **36** 158-164

______, ______, Wu L L, Zambrzuski S S and Akohoue S 1995b Bioextraction of soil boron by tall fescue; *J. Ecotoxicol. Environ. Safety* **31** 110-116

______, Mead R R and Akohoue S 1991 Adding selenium-enriched plant tissue to soil causes the accumulation of selenium in alfalfa; *J. Plant Nutr.* **14** 701-713

______, ______ and Hoffman G J 1993c Accumulation of selenium in wild mustard irrigated with agricultural effluent; *Agric. Ecosystems. Environ.* **43** 119-126

______, ______, Meek D W and Phene C J 1992a Relations between phosphorus in drip irrigation water and selenium uptake by wild mustard; *J. Environ. Sci. Health.* A **27** 283-297

______, ______, Wu L L, Beuselinck P and Akohoue S 1992b Differential selenium accumulation among forage plant species grown in soils amended with selenium-enriched plant tissue; *J. Soil Water Conserv.* **47** 338-342

______, Meek D W and Hoffman G J 1990 The influence of selenium, salinity, and boron on selenium uptake in wild mustard; *Plant Soil.* **127** 201-206

______, Shannon M C, Ajwa H, Draper J H, Jordahl J and Licht L 1999 Phytoextraction and accumulation of boron and selenium by poplar *Populus* hybrid coles; *Int. J. Phytochem.* **1** 81-96

Barceló J and Poschenrieder C and Tolrà R P 2003 Importance of phenolics in rhizosphere and roots for plant–metal relationships. *Proc. 7th Int. Conf. on the Biogeochem. of Trace Elements;* Uppsala '03. SYM02 - Biotic and Abiotic Processes in Soil Rhizosphere **2** 162-163

______ and ______ 2002 Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review; *Environ. Exp. Bot.* **48** 75-92

Bargagli R F, Monaci and Agnorelli C 2003 Oak leaves as accumulators of airborne elements in an area with geochemical and geothermal anomalies; *Environl. Pollut.* **124** 321-329

Barlow R, Bryan N, Andersland J and Sahi S 2000 Lead hyper accumulation by *Sesbania drummondii, Proc. Conf. on Hazardous Waste Res.* 112-114

Barman S C and Lal M M 1994 Accumulation of heavy metals (Zn, Cu, Cd and Pb) in soil and cultivated vegetables and weeds grown in industrially polluted fields; *J. Environ. Biol.* **15** 107

Barona A, Aranguiz I and Elías A 2001 Metal associations in soils before and after EDTA extractive decontamination implications for the effectiveness of further clean-up procedures; *Environ. Pollut.* **113** 79-85

Bashmakov D I, Lukatkin A S and Prasad M N V 2002 Zinc hyperaccumulating weeds from temperate Russia; *Zeszyty Naukowe PAN* **33** 309-313

Beeby A and Richmond L 2002 Evaluating *Helix aspersa* as a sentinel for mapping metal pollution; *Ecological Indicators* **1** 261-270

Bell J N B, Minski M J and Grogan H A 1988 Plant uptake of radionuclides; *Soil Use Manage.* **4** 76-84

Benson L M, Porter E K and Peterson P J 1981 Arsenic accumulation and genotypic variations in plants on arsenical mine wastes in S.W. England; *J. Plant Nutr.* **3** 655-666

Bhati M and Singh G 2003 Growth and mineral accumulation in *Eucalyptus camaldulensis* seedlings irrigated with mixed industrial effluents; *Bioresource Technology* **88** 221-228

Bi Y L, Li X L, Christie P, Hu Z Q and Wong M H 2003 Growth and nutrient uptake of arbuscular mycorrhizal maize in different depths of soil overlying coal fly ash; *Chemosphere* **50** 863-869

Bishop J 1997 Phytoremediation: A new technology gets ready to bloom; *Environ. Solutions.* **10** 29

Bizily S P, Rugh C L, Summers A O and Meagher R B 1999 Phytoremediation of methylmercury pollution: *merB* expression in *Arabidopsis thaliana* confers resistance to organimercurials; *Proc. Natl. Acad. Sci. USA* **96** 6808-6813

Blaylock M J, Salt D E, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley B D and Raskin I 1997 Enhanced accumulation of Pb in indian mustard by soil-applied chelating agents; *Environ. Sci. Technol.* **31** 860-865

______, Tawfic T A and Vance G F 1995 Modeling selenite sorption in reclaimed coal mine soil materials; *Soil Sci.* **159** 43-48

Bollag J M and Myers C 1992 Detoxification of aquatic and terrestrial sites through binding of pollutants to humic substances; *Sci. Total Environ.* **117/118** 357-366

Boyajian G E and Carreira L H 1997 Phytoremediation: A clean transition from laboratory to marketplace? *Nature Biotechnol.* **15** 127

Boyd R S and Martens S N 1994 Nickel hyperaccumulated by *Thlaspi montanum* var. *montanum* is acutely toxic to an insect herbivore; *Oikos.* **70** 21-25

Boyd R S, Shaw J J and Martens S N 1994 Nickel hyper accumulation in *S. polygaloids* (Brassicaceae) as a defense against pathogens; *Am. J. Bot.* **81** 294-300

Brennan M A and Shelley M L 1999 A model of the uptake, translocation, and accumulation of lead (Pb) by maize for the purpose of phytoextraction; *Ecological Engineering* **12** 271-297

Brewer E P, Saunders J A, Angle J S, Chaney R L and McIntosh M S 1997 Somatic hybridization between heavy metal hyperaccumulating *Thlaspi caerulescens* and canola; *Agron. Abstr* 154

Briat J F and Lebrun M 1999 Plant responses to metal toxicity; *CR Acad. Sci. Paris/Life Sci* **322** 43-54

Brooks R R 1972 *Geobotany and Biogeochemistry in Mineral Exploration;* (New York: Harper and Row)

______ (ed) 1998 *Plants that Hyperaccumulate Heavy Metals;* (Wallingford, UK: CAB International)

______ and Radford C C 1978 Nickel accumulation by European species of the genus *Alyssum; Proc. Roy. Soc. Lond.* **B200** 217-224

______, Chambers M F, Nicks L N and Robinson B H 1998 Phytomining; *Trends in Plant Science* **3** 359-362

______, Lee J, Reeves R D and Jaffre' R 1977a Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants; *J. Geochem. Explor.* **7** 49-57

______, Morrison R S, Reeves R D, Dudley T R and Akman Y 1979 Hyperaccumulation of nickel by *Alyssum linnaeua* (Cruciferae); *Proc. Roy. Soc. Lond.* **B203** 387-403

______, Shaw S and Marfil A A 1981 Some observations on the ecology, metal uptake, and nickel tolerance of *Alyssum serpyllifolium* subspecies from the Iberian Peninsula; *Vegetat.* **45** 183-188

______, Wither E D and Zepernick B 1977b Cobalt and nickel in *Rinorea* species; *Plant Soil.* **47** 707-712

Brown K S 1995 The green clean: The emerging field of phytoremediation takes root; *Bio. Sci.* **45** 579-582

Brown S L, Chaney R L, Angle J S and Baker A J M 1994 Phytoremediation potential of *Thlaspi caerulescens* and Bladder Campion for zinc and cadmium contaminated soil; *J. Environ. Qual.* **23** 1151-1157

______, Chaney R L, Angle J S and Baker A J M 1995a Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution; *Soil Sci. Soc. Am. J.* **59** 125-133

______, Chaney R L, Angle J S and Baker A J M 1995b Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-amended soils; *Environ. Sci. Technol.* **29** 1581-1585

Brown S L, Chaney R L, Lloyd C A, Angle J S and Ryan J A 1996 Relative uptake of cadmium by garden vegetables and fruits grown on long-term biosolid-amended soils; *Environ. Sci. Technol.* **30** 3508-3511

Brown T A and Shrift A1982 Selenium: Toxicity and tolerance in higher plants; *Biol. Rev. Cambridge Philos. Soc.* **57** 59-84

Bunzl K, Trautmannsheimer M, Schramel P and Reifenhäuser W 2001 Availability of arsenic, copper, lead, thallium, and zinc to various vegetables grown in slag-contaminated soils; *J. Environ. Qual.* **30** 934-939

Cannon H L 1959 Advances in botanical methods of prospecting for uranium in the western United States in *Proc. 1st Int. Geochem. Exploration Symp. at the Intl. Geol. Congr.*, Mexico City, pp. 235-241

______ 1960 Botanical prospecting for ore deposits; *Science* **132** 591-598

Chandra Sekher K and Puvvada G V K 1997 Studies on the metal binding properties of the seeds of *Strychnos potatorum* Linn; *NML Technical Journal* **39** 239-243

______, Chary N S and Kamala C T 2002 Application of *Garcinia cambogia*, a plant biomass for chromium removal and speciation studies; *Int. J. Env. Studies* **5**

______, ______, ______ and Anjaneyulu Y 2003a Utilisation of plant-metal interactions for environmental management: from a general disbelief to universal acceptance, PINSA-B, 7(1):

______, Kamala C T, Chary N S and Anjaneyulu Y 2003b Removal of heavy metals using a plant biomass with reference to environmental control; *Intl. J. Mineral Proc.* **68** 37-45

______, Natarajan K A, Subramaniam S and Modak J M 1998 Removal of metal ions using an industrial biomass with reference to environmental control; *Int. J. Mineral Proc.* **53** 107-120

______, Rajni Supriya K, Kamala C T, Chary N S and Nageswara Rao T 2001 Speciation, accumulation of heavy metals in vegetation grown on sludge amended soils and their transfer to human food chain; *Toxicol. Environ. Chem.* **82** 33-43

Chaney R L 1980 Health risks associated with toxic metals in municipal sludge; in *Sludge - Health Risks of Land Application* pp.59-83 eds. G Bitton, B L Damro, G T Davidson, J M Davidson (USA : Ann Arbor Sci. Publ., Ann Arbor, MI)

______, Brown S, Li Y M, Angle J S, Homer F and Green C 1995 Potential use of metal hyperaccumulators; *Mining Environ. Management.* **3** 9-11

______, Malik M, Li Y M, Brown SL, Brewer E P, Angle J S and Baker A J M 1997 Phytoremediation of soil metals; *Current Opinion in Biotechnol.* **8** 3279-3284

Chen H M, Zheng C R, Tu C and Shen Z G 2000 Chemical methods and phytoremediation of soil contaminated with heavy metals; *Chemosphere* **41** 229-234

Chen H and Cutright T 2001 EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus* annuus; *Chemosphere* **45** 21-28

Chen Y X, Lin Q, Luo Y M, He Y F, Zhen S J, Yu Y L, Tian G M and Wong M H 2003 The role of citric acid on the phytoremediation of heavy metal contaminated soil; *Chemosphere* **50** 807-811

Churchmann G J, Slade P G, Rengasamy P, Peter P, Wright M and Naidu R 1999 Use of fine grained minerals to minimize the bioavaliability of metal contaminants. Environ impacts of metals. Int Workshop, Tamil Nadu Agricultural University, Coimbatore, India, pp 49-52

Clemens S 2001 Molecular mechanisms of plant metal tolerance and homeostasis; *Planta* **212** 475-486

______, Palmgren M G and Krämer U 2002 A long way ahead understanding and engineering plant metal accumulation; *Trends in Plant Science* **7** 309-315

Cobbett C and Goldsbrough P 2002 Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis; *Annu. Rev. Plant Biol* **531** 159-182

Comis D 1995 Metal-scavenging plants to cleanse the soil; *Agric. Res.* **43** 4-9

Conte H M P and Piccolo A 2003 Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances; *Chemosphere* (In Press)

Cunningham S D and Berti W R 1993a Recommendations of contaminated soil with green plants : An Overview; *In Vitro Cellular and Development Biology Plant* **29** 1003

______ and ______ 1993b The remediation of contaminated soils with green plants: An overview; *In Vitro Cell. Devel. Biol - J. Tissue Cult. Assoc.* **29** 207-212

______ and Ow D W 1996 Promises and prospects of phytoremediation; *Plant Physiol.* **110** 715-719

______, ______ and Huang J W 1995 Phytoremediation of contaminated soils; *Trends Biotechnol.* **13** 393-397

Dagget D 1997 Convincing evidence (Cattle provide remarkable reclamation of mine tailings in Arizona); *Range* **5** 22-25

Dahmani-Muller H, Oort F and Balabane M 2001 Metal extraction by *Arabidopsis halleri* grown on an unpolluted soil amended with various metal-bearing solids, a pot experiment; *Envir. Pollut.* **114** 77-84

______, Oort F, Gélie B and Balabane M 2000 Strategies of heavy metal uptake by three plant species growing near a metal smelter; *Environ. Pollut.* **109** 231-238

Deiana S, Gessa C, Palma A, Premoli P and Senette C 2003 Influence of organic acids exuded by plants on the interaction of copper with the polysaccharidic components of the root mucilages; *Organic Geochemistry* **34** 651-660

Delhaize E, Ryan P R and Randall P J 1993 Aluminum tolerance in wheat (*Triticum aestivum* L.) II. Aluminum-stimulated excretion of malic acid from root apices; *Plant Physiol.* **103** 695-703

Dhillon K S and Dhillon S K 2003 Quality of underground water and its contribution towards selenium enrichment of the soil–plant system for a seleniferous region of northwest India; *J. Hydrology* **272** 120-130

Dudka S and Miller W P 1999 Accumulation of potentially toxic elements in plants and their transfer to human food chain; *J. Envron. Sci. Health B* **344** 681-708

______, Piotrowska M and Terelak H 1996 Transfer of cadmium, lead, and zinc from industrially contaminated soil to crop plants: A field study; *Environ. Pollut.* **94** 181-188

Dudley T R 1986a A new nickeliferous species *Alyssum* (Cruciferae) from Portugal: *Alyssum pintodasilvae* T R Dudley; *Feddes Repert* **97** 135-138

______ 1986b A nickel hyperaccumulating species of *Alyssum* (Cruciferae) from Spain: *Alyssum malacitanum* (Rivas Goday) T R Dudley, comb et stat nov.; *Feddes Report* **97** 139-142

Dushenkov V, Kumar P B A N, Motto H and Raskin I 1995 Rhizofiltration: the use of plants to remove heavy metals from aqueous streams; *Environ. Sci. Technol.* **29** 1239-1245

______, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I and Ensley B 1997 Phytoremediation: A novel approach to an old problem; *Studies Environ. Sci.* **66** 563

______, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting K C and Ensley B 1997a Removal of uranium from water using terrestrial plants; *Environ. Sci. Technol.* **31** 3468-3474

______, Mikheev A, Prokhnevsky A, Ruchko M and Sorochinsky B 1999 Phytoremediation of radiocesium-contaminated soil in the vicinity of Chernobyl, Ukraine; *Environ. Sci. Technol.* **33** 469-475

Eapen S, Suseelan K N, Tivarekar S, Kotwal S A and Mitra R 2003 Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor; Environ. Res.* **91** 127-133

Ebbs S D and Kochian L V 1997 Toxicity of zinc and copper to *Brassica* species: Implications for phytoremediation. *J. Environ. Qual.* **26** 776-781

______ and ______ 1998 Phytoextraction of zinc by oat *Avena sativa*, barley *Hordeum vulgare*, and Indian mustard *Brassica juncea; Environ. Sci. Technol.* **32** 802-806

______, Lasat M M, Brandy D J, Cornish J, Gordon R and Kochian L V 1997 Heavy metals in the environment - Phytoextraction of cadmium and zinc from a contaminated soil. *J. Environ. Qual.* **26** 1424-1430

Echevarria G, Vong P C, LeClerc-Cessac E and Morel J L 1997 Bioavailability of technetium-99 as affected by plant species and growth, application form, and soil incubation; *J. Environ. Qual.* **26** 947-956

Eklund M 1995 Cadmium and lead deposition around a Swedish battery plant as recorded in Oak tree rings; *J. Environ. Qual.* **24** 126-131

El Aziz R, Angle J S and Chaney R L 1991 Metal tolerance of *Rhizobium meliloti* isolated from heavy-metal contaminated soils;. *Soil Biol. Biochem.* **23** 795-798

El Kherbawy M, Angle J S, Heggo A and Chaney R L 1998 Soil *p*H, rhizobia, and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa (*Medicago sativa* L.); *Biol. Fertil. Soil.* **8** 61-65

El-Hasan T, Al-Omari H, Jiries A and Al-Nasir F 2003 Cypress tree *Cupressus semervirens* L. bark as an indicator for heavy metal pollution in the atmosphere of Amman City, Jordan; *Environt. Int.* **28** 513-519

Ellis R W and Eslick L 1997 Variation and range of mercury uptake into plants at a mercury-contaminated abandoned mine site; *Bull. Environ. Contam. Toxicol.* **59** 763-769

Entry J A, Vance N C, Hamilton M A, Zabowski D Z, Watrud L S and Adriano D C 1996 Phytoremediation of soil contaminated with low concentrations of radionuclides; *Water Air Soil Pollut.* **88** 167-177

______, Watrud L S and Reeves M 1999 Accumulation of ^{137}Cs and ^{90}Sr from contaminated soil by three grass species inoculated with mycorrhizal fungi;*Environ. Pollut.* **104** 449-457

Ernst W H O 2000 Revolution of metal hyperaccumulation and phytoremediation Hype; *New Phytol;* **146** 357-358

Farago M E, Clark A J and Pitt M J 1977 Plants which accumulate metals. I. The metal content of three Australian plants growing over mineralized sites; *Inorg. Chem. Acta.* **24** 53-56

Felix H 1997 Field trails for *in-situ* decontamination of heavy metal polluted soil using crops of metal-accumulating plants;. *Zeitschrift Pflanzenernahrung Bodenkunde.* **160** 525

Fesenko S V, Spiridonov S, Sanzharova N I and Aleksakhin R M 1997a Mathematical model of the biological availability of ^{137}Cs in the soils of grassland ecosystems;. *Eurasian Soil Sci.* **30** 34

______, Spiridonov S I and Alexakhin R M 1997b Dynamics of ^{137}Cs availability in a soil-plant system in areas of the composition of radioactive fallout; *J. Environ. Radioactivity.* **34** 287

Fiorenza S, Mahujchariyawong J and Ikeda S 2001 Modelling of environmental phytoremediation in eutrophic river - the case of water hyacinth harvest in Tha-chin River, Thailand; *Ecological Modelling* **142** 121-134

Fitz W J and Wenzel W W 2002 Arsenic transformations in the soil-rhizosphere-plant system fundamentals and potential application to phytoremediation; *J. Biotechnology* **99** 259-278

Flathman P E and Lanza G R 1998 Phytoremediation. Current views on an emerging green technology; *J. Soil Contamination* **7** 415-432

Francesconi K, Visoottiviseth P, Sridokchan W and Goessler W 2002 Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos* a potential phytoremediator of arsenic-contaminated soils; *The Sci The Total Environ.* **284** 27-35

Freitas H, Prasad M N V and Pratas J 2003 Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: environmental implications; *Environ. Intl.*

Gabbrielli R, Pandolfini T Vergnano O and Palandri M R 1990 Comparison of two serpentine species with different nickel tolerance strategies; *Plant Soil.* **122** 271-277

______, Mattioni C and Vergnano O 1991 Accumulation mechanisms and heavy metal tolerance of a nickel hyperaccumulator; *J. Plant Nutr.* **14** 1067-1080

Garbisu C and Alkorta I 2001 Phytoextraction a cost-effective plant-based technology for the removal of metals from the environment; *Bioresource Technology* **77** 229-236

Gersevich E G and Petunkin N I 1993 Use of natural zeolites for decrease of the radioactive contamination of soils and agricultural products. Republic Technol. Center Edition, Moscow, Pp. 1-172 (In Russian)

Gisbert C, Ros R, Haro A D, Walker D J, Bernal M P, Serrano R and Navarro-Aviñó J N 2003 A plant genetically modified that accumulates Pb is especially promising for phytoremediation; *Biochem. Biophysical Res. Comm.* **303** 440-445

Glass D J 1999 *US and international markets for phytoremediation,1999-2000* DJ Glass Associates Inc, Needham, MA, USA, pp. 266

______ The 2000 *Phytoremediation Industry* pp 100 (Needham, MA, USA: D J Glass Assocaites Inc.)

Gleba D, Borisjuk M V, Borisjuk L G, Kneer R, Poulev A, Skarzhinskaya M, Dushenkov V, Logendra S, Gleba Y Y and Raskin I 1999 Use of plant roots for phytoremediation and molecular farming; *Proc. Natl. Acad. Sci. USA* **196** 5973-5977

Gouthu S, Arie T, Ambe S and Yamaguchi I 1997 Screening of plant species for comparative uptake abilities of radioactive Co, Rb, Sr and Cs from soil; *J. Radioanal. Nucl. Chem.* **222** 247

Greger M 2003 Properties of *Salix* roots that influence geochemical release of heavy metals in soil; in *Proc. 7th Internat. Conf. Biogeochem. of Trace Elements;* Uppsala p.126-127

______ and Landberg T 1999 Use of willow in phytoremediation; *Int. J. Phytorem.* **1** 115-123

______ and Wang Y 2003 Phytoremediation of toxic metals. COST action 837 WG2 +WG 4. Stockholm University. Stockholm. Pp. 60

Groudev S N, Georgiev P S, Spasova I I and Komnitsas K 2001a Bioremediation of a soil contaminated with radioactive elements; *Hydrometallurgy* **59** 311-318

Groudev S N, Spasova I and Georgiev P S 2001b *In situ* bioremediation of soils contaminated with radioactive elements and toxic heavy metals; *Int. J. Mineral. Processing* **62** 301-308

Guerinot M L 2000 The ZIP family of metal transporters; *Biochimica et Biophysica Acta* **1465** 190-198

Hagemeyer J, Schafer H and Breckle S W 1994 Seasonal variations of nickel concentrations in annual xylem rings of beech trees (*Fagus sylvatica* L.); *Sci. Total Envir.* **145** 111-119

Hall J L 2002 Cellular mechanisms for heavy metal detoxification and tolerance; *J. Exp. Botany* **53** 1-11

Hamlin R L 2002 Phytoremediation literature review. environmental awareness in the United States. http://www.Umass.Edu/Umext/Soils and Plant

Harmens H, Koevoets P L M, Verkleij J A C and Ernst W H O 1994 The role of low molecular weight organic acids in the mechanism of increased zinc tolerance in *Silene vulgaris* (Moench) Garcke; *New Phytol.* **126** 615-621

Hartley-Whitaker J G, Vooijs A R, Bookum W T, Schat H C and Meharg A A 2001 Phytochelatins are involved in differential arsenate tolerance in *Holcus lanatus; Plant Physiology* **126** 299-306

Heaton, A C P, Rugh C L, Wang N and Meagher R B 1998 Phytoremediation of mercury - and methylmercury - polluted soils using genetically engineered plants; *J. Soil Contamination.* **7** 497-510

Homer F A, Morrison R S, Brooks R R, Clemens J and Reeves R D 1991a Comparative studies of nickel, cobalt, and copper uptake by some nickel hyperaccumulators of the genus *Alyssum; Plant Soil.* **138** 196-205

______, Reeves R D Brooks R R and Baker A J M 1991b Characterization of the nickel-rich extract from the nickel hyperaccumulator *Dichapetalum gelonioides Phytochem.* **30** 2141-2145

Hong P K A, Chelsea K, Andrew Li, Banerji S K and Regmi T K 1999 Extraction, recovery, and biostability of EDTA for remediation of heavy metal-contaminated soil; *J. Soil Contamination* **8** 81-103

Hossner L R, Loeppert R H and Newton E J 1998 *Literature review: phytoaccumulation of chromium, uranium, and plutonium in plant systems.* Amarillo National Resource Center For Plutonium. Amarillo, TX 79101

Huang J W 1997 Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction; *Environ. Sci. Technol.* **31** 800-805

______ and Cunningham S D 1996 Lead phytoextraction: Species variation in lead uptake and translocation; *New Phytol.* **134** 75

______, Blaylock M J, Kapulnik Y and Ensley B D 1998 Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyper accumulation in plants; *Environ. Sci. Technol.* **32** 2004-2008

Huang J W, Chen J, Berti W R and Cunningham S D 1997 Phytoremediation of lead contaminated soil: role of synthetic chelates in lead phytoextraction; *Environ. Sci. Technol.* **31** 800-805

Iskandar I K 2000 Environmental restoration of metal-contaminted soils. pp 320. (Boca Raton: CRC Press)

Iskandar I K and Kirkham M B 2001 *Trace elements in soil; Bioavailability, Flux and Transfer*; pp.304 (Boca Raton: CRC Press)

Jaffre' T, Brooks R R, Lee J and Reeves R D 1976 *Sebertia acuminata*: A hyperaccumulator of nickel from New Caledonia; *Science* **193** 579-580

______, ______ and Trow J M 1979 Hyperaccumulation of nickel by Geissois species from New Caledonia, indicator plants; *Plant Soil.* **51** 157-162

Jauert P, Schumacher T E, Boe A, and Reese R N 2002 Rhizosphere acidification and cadmium uptake by strawberry clover; *J. Environ. Qual.* **31** 627-633

Jiang X J, Luo Y M, Zhao Q G, Baker A J M, Christie P and Wong M H 2003 Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil; *Chemosphere* **50** 813-818

Kabata-Pendias A 2001 Trace Elements in Soils and Plants; pp 432 (Boca Raton: CRC Press)

Kamnev A A and van der Lelie D 2000 Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation; *Review. Biosci. Rep.* **20** 239-258

Karenlampi S, Schat H, Vangronsveld J, Verkleij J A C, Van Der Lelie C, Mergeay M and Tervahauta, A I 2000 Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils; *Environ. Pollut.* **107** 225-231

Keane B M H, Shann C J R and Rogstad S H 2001 Metal content of dandelion *Taraxacum officinale* leaves in relation to soil contamination and airborne particulate matter; *The Sci. The Total Environ.* **281** 63-78

Kelly R J and Guerin T F 1994 Metal accumulating plants and implications for the mining industry; *Australian J. Mining* 38-40

Kerkeb L and Kramer U 2003 The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea; Plant Physiology* **1312** 716-724

Khan A G, Kuek C, Chaudhry T M, Khoo C S and Hayes W J 2000 Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation; *Chemosphere* **41** 197-207

______ 2001 Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil; *Environ. Int.* **26** 417-423

Korte F, Spiteller M and Coulston F 2000 The cyanide leaching gold recovery process is a non-sustainable technology with unacceptable impacts on ecosystems and humans: The disaster in Romania; *Ecotox. Environ. Safety.* **46** 241-245

Kock D C and Mitchell R L 1957 Absorption of metal chelates by plants; *Soil Sci.* **84** 55-62

Koranda J J and Robinson W L 1978 Accumulation of radionuclides by plants as a monitor system; *Environ. Health Perspec.* **27** 165-175

Kramer U, Smith R, Wenzel W W, Raskin I and Salt D E 1997 The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense* halacsy; *Plant Physiol.* **115** 16-41

______ and Chardonnens A N 2001 The use of transgenic plants in the bioremediation of soils contaminated with trace elements; *Microbiol. Biotechnol.* **55** 661-672

______, Cotter-Howells J D, Charnock J M, Baker A J M and Smith J A C 1996 Free histidine as a metal chelator in plants that accumulate nickel.; *Nature* **373** 635-638

Kumar P B A N, Dushenkov V, Motto H and Raskin I 1995 Phytoextraction: The use of plants to remove heavy metals from soils; *Environ. Sci. Technol.* **29** 1232-1238

Lakshmi K and Clements L D 2003 Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part I Preparation of synthetic hyperaccumulator biomass; *Biomass and Bioenergy* **24** 69-79

Landberg, T and Greger M 1994 Can heavy metal tolerant clones of *Salix* be used as vegatation filters on heavy metal contaminated land? in *Willow vegetation filters for municipal wastewaters and sludges. A biological purification system. Report 50*, pp. 133-144. eds Paronsson K Perttu (Swedish University of Agricultural Sciences, Department of Ecology and Environmental Res. Uppsala)

Landeen D S and Mitchell R M 1986 Radionuclide uptake by trees at Radwaste Pond in Washington State; *Health Physics* **50** 769-774

Lanza G R and Flathman P E 2001 Phytoremediation technologies. in Hazardous and radioactive waste treatment technologies handbook. ed. 13 pp. CRC Press, Boca Raton, Florida, USA

Laperche V, Logan T J, Gaddam P and Traina S J 1997 Effect of apatite amendments on plant uptake of lead from contaminated soil; *Envir. Sci. and Technol.* **31** 2746-2753

Larsen P B, Degenhardt J, Tai C Y, Stenzler L, Howell S H and Kochian L V 1998 Aluminum-resistant Arabidopsis mutants that exhibit altered patterns of Al accumulation and organic acid release from roots; *Plant Physiol.* **117** 9-18

Lasat M M 2002 Phytoextraction of toxic metals: A review of biological mechanisms; *J. Envir. Qual.* **31** 109-120

______, Baker A J M and Kochian L V 1998a Altered zinc compartmentation in the root symplasm and stimulated Zn^{2+} absorption in to the leaf as mechanisms involved in zinc hyperaccumulation in *Thlaspi caerulescens; Plant Physiol.* **118** 875-883

Lasat M M, Norvell W A and Kochian L V 1997 Potential for phytoextraction of ^{137}Cs from a contaminated soil; *Plant Soil.* **195** 99-106

______, Pence N S, Garvin D F, Ebbs S D and Kochian L V 2000 Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens; J. Exp. Bot.* **51** 71-79

______, Ebbs S D, Kochian L V and Cornish J and Fuhrmann M 2002 Uptake of Cesium-137 and Strontium-90 from contaminated soil by three plant species, Application to Phytoremediation; *J. Environ. Qual.* **31** 904-909

______, Fuhrmann M, Ebbs S D, Cornish J E and Kochian L V 1998b Phytoremediation of a radiocesium-contaminated soil: evaluation of cesium^{137} bioaccumulation in the shoots of three plant species; *J. Environ. Qual.* **27** 165-169

Latimer S D, Devall M S, Thomas C, Ellgaard E G, Kumar S D and Thein L B 1996 Dendrochronology and heavy metal deposition in tree rings of baldcypress; *J. Environ. Qual.* **25** 1411-1420

Lauchli A 1993 Selenium in plants: Uptake, functions, and environmental toxicity; *Botanica Acta.* **106** 455-468

Lee J, Reeves R D, Brooks R R and Jaffre' T 1977 Isolation and identification of a citrate-complex of nickel from nickel-accumulating plants; *Phytochem.* **16** 1503-1505

Lepp N W 1975 The potential of tree-ring analysis for monitoring heavy metal pollution patterns; *Environ. Pollut.* **9** 49-62

Linger P, Müssig J, Fischer H and Kobert J 2002 Industrial hemp *Cannabis sativa* L. growing on heavy metal contaminated soil, fibre quality and phytoremediation potential; *Industrial Crops and Products* **16** 33-42

Linstow O 1924 Die naturliche Anreicherung von metallsazen und anderen anorganiscgen verbindungen in den pflanzen; *Feddes Rep* **31** 1-51

Ma L Q, Komar K M, Tu C, Zhang W, Cai Y and Kennelley E D 2001a A fern that hyperaccumulates arsenic; *Nature* **409** 579

Ma J F, Ryan P and Delhaize E 2001b Aluminium tolerance in plants and the complexing role of organic acids; *Trends Plant Sci.* **6** 273-278

McLaughlin M J and Singh B R 1999 Cadmium in soils and plants. A global perspective. pp. 1-9. in: M J McLaughlin and B R Singh Eds. Cadmium in soils and plants; (The Netherlands: Kluwer Academic Publishers, Dordrecht)

Macnair M R 1993 Transley review No. 49. The genetic metal tolerance in vascular plants; *New Physiologist* **124**, 541–559

______, Cumbes Q J and Meharg A A 1992 The genetics of arsenate tolerance in Yorkshire fog, *Holcus lanatus* L; *Heredity.* **69** 325-335

Madejón P, Murillo J M, Marañón T, Cabrera F and Soriano M A 2003 Trace element and nutrient accumulation in sunflower plants two years after the Aznalcóllar mine spill; *The Science of The Total Environment* (In Press)

Madrid F, Liphadzi M S and Kirkham M B 2003 Heavy metal displacement in chelate-irrigated soil during phytoremediation; *J. Hydrology* **272** 107-119

Malaisse F, Gregoire J, Morrison R R and Reeves R D 1978 *Aeolanthus biformifolius* De Wild : A hyperaccumulator of copper from Zaire;. *Science* **199** 887-888

______, Reeves R D, Morrison R S and Brooks R R 1979 Copper and cobalt uptake by metallophytes from Zaire; *Plant Soil* **53** 535

Marcantonio F, Flowers G, Thien L and Ellgaard E 1998 Lead isotopes in tree rings: chronology of pollution in Bayou Trepagnier, Louisiana; *Environ. Sci. Technol.* **32** 2371-2376

Martin H W, Young T R and Adriano D C 1996 Evaluation of three herbaceous index plant species for bioavailability of soil cadmium, nickel and vanadium; *Plant Soil.* **182** 199-207

Mattina M J I, Lannucci-Berger W, Musante C and White J C 2003 Concurrent plant uptake of heavy metals and persistent organic pollutants from soil; *Environl Pollut.* (In Press)

Maywald F and Weigel H J 1997 Biochemistry and molecular biology of heavy metal accumulation in higher plants; *Landbauforschung Volkenrode.* **47** 103

McGrath S P and Zhao F J 2003 Phytoextraction of metals and metalloids from contaminated soils; *Current Opinion in Biotechnology* **14** 1-6

______, ______ and Lambi E 2001 Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils; *Plant and Soil* **232** 207-214

______, Shen Z G and Zhao F J 1997 Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils; *Plant Soil.* **188** 153-159

McIntyre T and Lewis G M 1997 The advancement of phytoremediation as an innovative environmental technology for stabilization, remediation, or restoration of contaminated sites in Canada: a discussion paper; *J. Soil Contam.* **6** 227-241

Meagher R B 2000 Phytoremediation of toxic elemental and organic pollutants; *Current Opinion in Plant Biology* **31** 53-162

Meharg A A and Macnair M R 1991 Uptake, accumulation, and translocation of arsenate in arsenate-tolerant and non-tolerant *Holcus lanatus* L. *New Phytologist.* **117** 225-231

______ and ______ 1992 Genetic correlation between arsenate tolerance and the rate of influx of arsenate and phosphate in *Holcus lanatus* L; *Heredity.* **69** 336-341

Mejáre M and Bülow L 2000 Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals; *Trends in Biotechnology* **19** 67-73

Mengoni A, Gonnelli C, Galardi F, Gabbrielli R and Bazzicalupo M 2000 Genetic diversity and heavy metal tolerance in populations of *Silene paradoxa* L. Caryophyllaceae: a random amplified polymorphic DNA analysis; *Mol. Ecol.* **9** 1319-1324

Minguzzi C and Vergnano O 1948 Il cpntenuto di nichel nelle ceneri di *Alyssum bertlonii* Desv Atti; *Soc. Toscana Sci. Natl. Pisa Mem. Ser. A* **55** 49-74

Mishra S, Shanker K and Prakash S 1997 A study on the uptake of trivalent and hexavalent chromium by paddy rice (*Oryza sativa*): Possible chemical modifications in rhizosphere; *Agric. Ecosystems Environ.* **62** 53

______ and Gedamu L1989 Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants; *Theor. Appl. Genet.* **78** 161-168

Moffat A S 1995 Plants proving their worth in toxic metal cleanup; *Science* **269** 302-303

______1999 Engineering plants to cope with metals; *Science* **285** 369-370

Montes-Bayon M, Grant T D Meija J and Caruso J A 2002 Selenium in plants by mass spectrometric techniques: development in bioanalytical methods; *J. Anal. At. Spectrom.* **17** 1015-1023

Murphy C E and Johnson T L 1993 Vegetative uptake of technetium-99 from buried, solidified, low-level radioactive waste; *J. Environ. Qual.* **19** 793-799

Myers N 1990 The Biodiversity challenge: expanded hot-spots analysis; *The Environmentalist* **10** 243-256

Nedelkoska T V and Doran P M 2000a Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining; *Minerals Engineering* **13** 549-561

Nedelkoska T V and Doran P M 2000b Hyper accumulation of cadmium by hairy roots of *Thlaspi caerulescens*; *Biotechnol Bioeng.* **67** 607-615

Neumann D, Lichtenberg O, Günther D, Tschiersch K and Nover L 1994 Heat-shock protein induced heavy-metaltolerance in higher plants; *Planta* **194** 360-367

Nie L, Shah S, Rashid A, Burd G I, Dixon G and Glick B R 2002 Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2; *Plant Physiology and Biochemistry* **40** 355-361

Ouyang Y 2002 Phytoremediation modeling plant uptake and contaminant transport in the soil-plant-atmosphere continuum; *J. Hydrology* **266** 66-82

Palmer C E, Warwick S and Keller W 2001 Brassicaceae (Cruciferae) family - plant biotechnology and phytoremediation; *Int. J. Phytoremed* **3** 245-287

Peña L and Séguin A 2001 Recent advances in the genetic transformation of trees; *Trends in Biotechnology* **19** 500-506

Pence N S, Larsen P B, Ebbs S D, Lasat M M, Letham D L D, Garvin D F, Eide D and Kochian L V 2000 The molecular basis for heavy metal hyperaccumulation in *Thlaspi caerulescens; Proc. Natl. Acad. Sci. USA* **97** 4956-4960

Persans M W, Salt D E, Kim S A and Guerinot M L 2001 Phylogenetic relationships within cation transporter families of *Arabidopsis; Plant Physiol.* **126** 1646-1667

Piechalak A, Tomaszewska B, Baralkiewicz D and Malecka A 2002 Accumulation and detoxification of lead ions in legumes; *Phytochemistry* **60** 153-162

Pigna M, Del Gaudio S D and Violante A 2003 Role of inorganic and organic ligands on the mobility of arsenate in soil environments. SYM01 Arsenic in Soil and Groundwater Environment:Biogeochemical Interactions; Proc. *7th Intern. Conf. Biogeochem. of Trace Elements;* Uppsala **2** 24-25

Pilon-Smits E and Pilon M 2000 Breeding mercury-breathing plants for environmental cleanup. *Trends Plant Sci.* **5** 235-236

______ and ______ 2002 Phytoremediation of metals using transgenic plants; *Critical Reviews in Plant Sciences* **21** 439-456

Pollard A J, Powell K D, Harper F A and Smith J A C 2002 The genetic basis of metal hyperaccumulation in plants; *Critical Reviews in Plant Sciences* **21** 539-566

______ and Baker A J M 1996 Quantitative genetics of zinc hyperaccumulation in *Thlaspi caerulescens; New Phytologist.* **132** 113-118

Prasad M N V 1995 Cadmium toxicity and tolerance to vascular plants; *Envir. Exp. Bot.* **35** 525-545

______ 1996 Variable allocation of heavy metals in phytomass of crop plants - human health implications; *Plzensky Lékarsky Sbornik, Suppl.* **71** 19-22. Czech Republic Medical Association (Prague: Charles University Press)

______ 1997 Trace metals in *Plant Ecophysiology* (ed) M.N.V. Prasad pp. 207-249 (New York, USA: John Wiley and Sons)

______ 1998 Metal-biomolecule complexes in plants: occurrence, functions and applications; *Analusis* **26** 25-28

______1999 Metallothioneins and metal binding complexes in plants; in *Heavy Metal Stress in Plants: from Molecules to Ecosystems,* pp. 51-72 eds M N V Prasad and J. Hagemeyer (Berlin, Heidelberg, New York: Springer-Verlag)

Prasad M N V 2000 Phytoremediation as a potential tool for environmental geotechnology and sustainable development; *5th Int. Symp. "Environmental Geotechnology and Global Sustainable Development".* Belo Horizonte/Minas Gerais, Brazil. on CD-ROM.

______ (ed) 2001 *Metals in the Environment - Analysis by biodiversity;* pp. 504 (New York: Marcel Dekker Inc.)

______2002a Zinc transporting genes in plants; *Zeszyty Naukowe PAN* **33** 363-366

Prasad M N V 2002b Bioresources for remediation and monitoring of metals in the environment; *Proc. Int. Conf. Bioresources and Environmental Stress.* Research Institute for Bioresources, Okayama University, Kurashiki, Japan. Pp. 7-10

______ 2003 Phytoremediation of metal-polluted ecosystem - Hype for commercialization; *Russ. J. Plant Physiol.* **50** 686-701

Prasad M N V (ed) 2004a *Heavy Metal Stress in Plants: from biomolecules to Ecosystems.* 2nd edn. (New Delhi: Springer-Verlag Heidelberg Narosa) 1 462

______2004b Phytoremediation of metals and radionuclides in the environment: the case for natural hyperaccumulators, metal transporters, soil-amending chelators atransgenic plants; in *Heavy Metal Stress in Plants: from Biomolecules to Ecosystems* (2nd ed) ed. M N V Prasad (New Delhi: Springer-Verlag. Heidelberg. Narosa)

______ and Freitas H 1999 Feasible biotechnological and bioremediation strategies for serpentine soils and mine spoils; *Electronic J. Biotechnology* **2** 36-50 (Online journal). Website http://www. ejbiotechnology.info

______and ______ 2000a *Quercus ilex* L. (Holly oak) phytomass utility for cleanup of metal contaminated aquatic and terrestrial sites. 5th Int. Symp. "Environmental Geotechnology and Global Sustainable Development". Belo Horizonte/Minas Gerais, Brazil on CD-ROM

______ and ______ 2000b Removal of toxic metals from the aqueous solution by the leaf, stem and root phytomass of *Quercus ilex* L. (Holly Oak); *Environ. Pollution* **110** 277-283

______ and ______ 2003 Metal hyperaccumulation in plants – Biodiversity prospecting for phytoremediation technology; *Electronic J. Biotechnology* http:// www. ejbiotechnology.info (Online) **6** (3)

______ and Hagemeyer J (eds) 1999 Heavy metal stress in plants: from molecules to ecosystems; Springer-Verlag. Heidelberg. pp. xiii +401

______, Greger M and Landberg T 2001a *Eucalyptus* seedlings accumulate toxic metals in hydroponic system- Sixth International conference on the Biogeochemistry of Trace elements, University of Guelph, Ontario, Canada.

______, ______ and ______ 2001b *Acacia nilotica* L. bark removes toxicmetals from solution: Corroboration from toxicity bioassay using *Salix viminalis* L. in hydroponic system; *Int J Phytoremediation* **3** 289-300

Prasad M N V, Greger M and Landberg T 2003 Assessment of metal toxicity in cotton seedlings for possible application in phytoremediation; Proc. 7th Intern. Conf. on the Biogeochem. *Trace Elements* pp. 190-191 Sweden: Uppsala)

______and Strzalka K (eds) 2002 *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*; pp 460 (Dordrecht: Kluwer Academic Publishers)

Pulford I D and Watson C 2003 Phytoremediation of heavy metal-contaminated land by trees-a review; *Environment International* **29** 529-540

Rama Kumar T and Prasad M N V 1999 Metal-binding properties of ferritin in *Vigna mungo* (L.) Hepper (Black gram): Possible role in heavy metal detoxification. *Bull. Envirn. Contam. Toxicol.* **62** 502-507

Ramaut J L, Petit J and Maquinay A 1972 *Cochlearia pyrenacia* – plante calaminaire? *Naturalistes Belg* **53** 475-478

Rangsayatorn N, Upatham S, Kruatrachue, M, Pokethitiyook P and Lanza G R 2002 Phytore-mediation Potential of *Spirulina* (Arthrospira) *platensis*: Biosorption and toxicity studies of cadmium; *Environ. Pollut.* **119** 45-53

Raskin I 1996 Plant genetic engineering may help with environmnetal cleanup; *Proc. Natl. Acad. Sci. USA* **93** 3164-3166

______ and Ensley B P 2000 *Phytoremediation of toxic metals - using plants to clean up the environment*; (New York: Wiley)

______, Kumar P B A N, Dushenkov V and Salt D E 1994 Bioconcentration of heavy metals by plants; *Curr. Opinion Biotechnol.* **5** 285-290

______, Smith R D and Salt D E. 1997 Phytoremediation of metals: Using plants to remove pollutants from the environment; *Curr. Opin. Biotechnol.* **8** 221-226

Rauser W E 1995 Phytochelatins and related peptides; *Plant Physiol.* **109** 1141-1149

______ 2003 Phytochelatin-based complexes bind various amounts of cadmium in maize seedlings depending on the time of exposure, the concentration of cadmium and the tissue; *New Phytologist* **158** 269

Reddy G N and Prasad M N V 1989 Cadmium inducible proteins in *Scenedesmus quadricauda*; *Curr. Sci.* **58** 1380-1382

______ and ______1990 Heavy metal binding proteins/petides: Occurrence structure, synthesis and functions. A review; *Environ. Exp. Bot.* **30** 251-264

______ and ______ 1993 Tyrosine is not phosphorylated in cadmium-induced HSP cognate in maize (*Zea mays* L.) seedlings: Role in chaperone function ? *Biochem. Arch.* **9** 25-32

______ and ______ 1995 Cadmium-induced proteins and protein phosphorylation changes in rice (*Oryza sativa* L.); *J. Plant Physiol.* **145** 67-70

Reeves R D 1988 Nickel and zinc accumulation by species of *Thlaspi* L., *Cochlearia* L., and other genera of the Brassicaceae; *Taxon.* **37** 309-318

______ and Brooks R R 1983 Hyperaccumulation of lead and zinc by two metallophytes from mining areas of central Europe; *Environ. Pollut. Ser. A.* **31** 277-285

Reeves R D, Brooks R R and Dudley T R 1983a Uptake of nickel by species of *Alyssum, Bornmuellera* and other genera of old world *Tribus alyssaeae*; *Taxon.* **32** 184-192

______, ______, and Press J R 1980 Nickel accumulation by species of *Peltaria* Jacq. (Cruciferae); *Taxon* **29** 629-633

______, MacFarlane R M and Brooks R R 1983b Accumulation of nickel and zinc by western North American genera containing serpentine-tolerant species; *Am. J. Bot.* **70** 1297-1303

Riesen T K and Brunner I 1996 Effect of ectomy-corrhizae and ammonium on ^{134}Cs and ^{85}Sr uptake into *Picea abies* seedlings; *Environ. Pollut.* **93** 1-8

Robinson B H, Brooks R R, Howes A W, Kirkman J H and Gregg P E H 1997a The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining; *J. Geochem. Explor.* **60** 115-126

______, Chiarucci A, Brooks R R, Petit D, Kirkman J H, Greg P E H and De Dominicis V 1997b The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel; *J. Geochem. Explor.* **59** 78-86

Römkens P, Bouwman L, Japenga J and Draaisma C 2002 Potentials and drawbacks of chelate-enhanced phytoremediation of soils; *Environ. Pollut.* **1161** 109-121

Rugh, C L, Wilde H D, Stacks N M, Thompson D M, Summers A O and Meagher R B 1996 Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene; *Proc. Natl. Acad. Sci.* **93** 3182-3187

Ruiz J M and Romero L 2002 First evidence of arsenic phytoremediation; *Trends in Plant Science* **7** 384

Sahi S V, Bryant N L, Sharma N C and Singh S R 2002 Characterization of lead hyperaccumulator shrub, *Sesbania drummondii*; *Environ Sci Technol* **36** 4376-4680

Salt D E, Prince R C, Pickering I J and Raskin I 1995a Mechanisms of cadmium mobility and accumulation in Indian Mustard; *Plant Physiol.* **109** 1427-1433

______, Blaylock M, Kumar P B A N, Dushenkov V, Ensley B D, Chet I and Raskin I 1995b Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants; *Biotechnol.* **13** 468-474

______, Prince R C, Pickering I J and Raskin I 1995c Mechanism of cadmium mobility and accumulation in Indian mustard; *Plant Physiology* **109** 1427-1433

______, Smith R D and Raskin I 1998 Phytoremediation. *Annu Rev Plant Physiol Plant Mol Biol* **49** 643-668

Sanità Di Toppi L, Prasad M N V and Ottonello S 2002 Metal chelating peptides and proteins in plants; in *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants* Pp. 59-93 eds. M N V Prasad and K.Strzalka (Dordrecht: Kluwer Academic Publishers)

Sanità Di Toppi L, Gremigni P, Pawlik Skowronska B, Prasad M N V and Cobbett C S 2003 Responses to heavy metals in plants – Molecular approach; in *Abiotic Stresses in Plants* pp. 133-156 eds. L. Sanità Di Toppi, and B. Pawlik Skowronska (Dordrecht: Kluwer Acad Publishers)

Schat H and Bookum T W M 1992 Genetic control of copper tolerance in *Silene vulgaris; Heredity.* **68** 219-229

Schmöger M E V, Oven M and Grill E 2000 Detoxification of arsenic by phytochelatins in plants; *Plant Physiol* **122** 793-801

Schnoor J L 2000 Phytostabilization of metals using hybrid poplar trees; in *Phytoremediation of Toxic Metals - using Plants to Clean-up the Environment* pp 133-150 ed. I Raskin and BD Ensley (New York: John Wiley & Sons Inc.)

Sequeira E M D and Pinto da Silva A.R. 1991 The ecology of serpentinized areas of north-east Portugal; in The Ecology of Areas with Serpentinized Rocks. A world review pp. 169-197 eds B A Roberts and J Proctor (Dordrecht: Kluwer Academic Publishers)

Shanks J V and Morgan J 1999 Plant 'hairy root' culture; *Current. Opinion Biotechnol.* **10** 151-155

Shetty K G, Hetrick B A D and Schwab A P 1995 Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants; *Environ. Pollut.* **88** 307-314

_______, _______, Hoobler D and Schwab A P 1994 Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil; *Environ. Pollut.* **86** 181-188

Shimwell D W and Laurie A E 1972 Lead and zinc accumulation of vegetation in the southern Pennines; *Environ. Pollut.* **3** 291-301

Smith E, Naidu R and Alston A M 1998 Arsenic in the soil environment: a review; *Adv. Agron..* **64** 149-195

Tepfer D, Metzger L and Prost R 1989 Use of roots transformed by *Agrobacterium rhizogens* in rhizosphere research: applications in studies of cadmium assimilation from sewage sludges; *Plant Mol. Biol. Int. J. Mol. Biol. Biochem. Genet. Eng.* **13** 295-302

Terry N, Carlson C, Raab T K and Zayed A M 1992 Rates of Se volatilization among crop species; *J. Environ. Quality* **21** 341-344

Theodoulou F L 2000 Plant ABC transporters; *Biochim. Biophys Acta* **1464** 79-103

Tiagi Y D and Aery N C 1995 Plant indicators of heavy metals; *Symp. Biomonitoring State Environ.* 207-222

Tu C, Ma L Q, Zhang W, Cai Y and Harris W G 2003 Arsenic species and leachability in the fronds of the hyperaccumulator Chinese brake *Pteris vittata* L., *Environ. Pollut.* (In Press)

Vandehove H and Van Hees M 2003 fibre crops as alternative landuse for radioactively contaminated aarable land; *Proc. 7th Internat. Conf. on the Biogeochem. of Trace Elements; Uppsala'* **2** 356-357

Vangronsveld J and Cunningham S D 1999 *Metal-Contaminated Soils: In-situ in Activation and Phytorestoration;* pp 265 (Heidelberg, Berlin: Springer-Verlag)

Vassil A D, Kapulnik Y, Raskin I and Salt D E 1998 The role of EDTA in lead transport and accumulation by Indian Mustard; *Plant Physiology* **117** 447-453

Vatamaniuk J O K, Mari S, Lu Y P and Rea P A 2000 Mechanism of heavy metal ion activation of phytochelatin PC synthase.blocked thiols are sufficient for PC synthase-catalyzed transpeptidation of glutathione and related thiol peptides; *J. Biol. Chem.* **27540** 31451-31459

Veeken A and Hamelers B 2002 Sources of Cd, Cu, Pb and Zn in biowaste; in *The Science of The Total Environment* **300** 87-98

Vergnano Gambi O and Gabrielli R 1979 Ecophysiological and geochemical aspects of nickel, chromium and cobalt accumulation in the vegetation of some Italian ophiolitic outcrops; *Ofioliti* **4** 199-208

Wackett L P and Allen D L 1995 Comment on "Bioremediation in the Rhizosphere"; *Environ. Sci. Technol.* **29**551-552

Wenzel W W, Bunkowski M, Puschenreiter M and Horak O 2003 Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil; *Envirn. Pollut.* **123** 131-138

Wong M H 2003 Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils; *Chemosphere* **50** 775-780

Wu J, Hsu F C and Cunningham S D 1999 Chelate assisted Pb phytoextraction: Pb availability, uptake and translocation constraints; *Environ. Sci. Technol* **33** 1898-1904

Wu L H, Luo Y M, Christie P and Wong M H 2003 Effects of EDTA and low molecular weight organic acids on soil solution properties of a heavy metal polluted soil; *Chemosphere* **50** 819-822

Wu L L, Huang Z Z and Burau R G 1988. Selenium accumulation and selenium-salt co-tolerance in five grass species; *Crop Sci.* **28** 517-522

Xiang C B L, Werner E L, Christensen M and Oliver D J 2001 The biological functions of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels; *Plant Physiology* **1262** 564-574

Yoshitomi K J and Shann J R 2001 Corn *Zea mays* L. root exudates and their impact on ^{14}C-pyrene mineralization; *Soil Biology and Biochemistry* **33** 1769-1776

Yusuf A A, Arowolo T A and Bamgbose O 2003 Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria; *Food and Chemical Toxicology* **413** 375-378

Zeleznick J D and Skousen J G 1996 Survival of three tree species on old reclaimed surface mines in Ohio; *J. Environ. Qual.* **25** 1429-1435

Zhang W, Cai Y, Tu C and Ma L Q 2002 Arsenic speciation and distribution in an arsenic hyperaccumulating plant; *The Science of The Total Environment* **300** 167-177

Zhu Y L, Pilon-Smits E A H, Jouanin L and Terry N 1999a Overexpression of glutathione synthetase in indian mustard enhances cadmium accumulation and tolerance; *Plant Physiol.* **119** 73-80

Zhu Y L, Pilon-Smits E A H, Tarun A S, Weber S U, Jouanin L and Terry N 1999b Cadmium tolerance and accumulation in indian mustard is enhanced by over expressing gamma – glutamyl cysteine synthetase; *Plant Physiology* **1214** 1169-1177

Zorpas A A, Constantinides T, Vlyssides AG, Aralambous I and Loizidou M 1999 Heavy metal uptake by natural zeolite and metals partitioning in sewage sludge compost; *Bioresource Technology* **71** 113-119