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Biotechnological Approaches to Improve Phytoremediation Efficiency for Environment Contaminants

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1. Introduction

The realization, that plants serve the mankind by cleanup of the toxic contaminants, is quite old, but the problems of the contaminated land sites, water bodies and ground water and spoiled air worldwide have increased many folds due to anthropogenic activities during second half of the 20th century and hence deserve special attention. The environmental concerns of government and non-government agencies and the people at large have increased enormously, which have paved the way for the establishment of a large number of research institutes and commercial groups to develop new techniques and technologies for rapid cleanup of the contaminants from the sites identified for alarming contaminations. Phytoremediation, as a sustainable, cost effective and potential cleanup technology over the conventional methods, has emerged very fast as an alternative technology in the last decade (see Cunningham et al. 1995; Cunningham and Ow 1996; Salt et al. 1998; Saxena et al. 1999; Macek et al. 2000; Baker et al. 2000; Morikawa and Takahashi 2000; Singh et al. 2001; Morikawa et al. 2002; Kassal et al. 2002; Dhankhar et al. 2002; Maiti et al. 2004; Prasad 2004; Datta and Sarkar 2004; Schwitzguébel 2004; Pan et al. 2005).

Phytoremediation technology can be implemented *in situ* or *ex-situ* to cleanup a variety of the organic contaminants e.g.. petroleum hydrocarbons, gas condensates, crude oil, chlorinated compounds, pesticides, herbicides, explosive compounds as well as typical inorganic toxicants, such as heavy metals, metalloids, radionuclides, etc. (Morikawa and Takahashi 2000). Air pollutants like nitrogen and sulfur oxides, ozone and suspended particulate matters (SPMs) can also be ameliorated by growing efficient naturally occurring plants as well as more efficient genenetically modified plants (see Wellburn 1990; Morikawa and Takahashi 2000; Takahashi et al. 2001; Schwitzguébel 2004; Morikawa et al. 2005). Phytoremediation is considered as an aesthetically pleasing and solar energy driven cleanup technology, which causes minimal environmental disruption and *in situ* treatment preserves the topsoil (Morikawa and Takahashi 2000). It is inexpensive (60-80% or even less costly than conventional physio-chemical methods) and useful for treating a broad range of the environmental contaminants, especially at sites with shallow or low levels of contaminants. Possibly due to their static (non-mobile) nature, plants had to evolve their survival modes even in odd environments including sites contaminated with the xenobiotic substances, which are non-essential or even harmful for them. The natural adaptations and genetic mutations have evolved a wide range of preferential or general tolerance to the toxic substances in plants. Naturally occurring tolerance to plants is based on the mechanisms like phytostabilization, rhizodegradation, phytoaccumulation, phytodegradation, phytovolatization and evapotranspiration etc. which facilitate plants various means to avoid, escape, partition or remove the toxic contaminants as an adaptation measure. Such naturally evolved potential of plants, on the other hand, can be used for cleanup purposes. Bioprospecting of the suitable plant species and genotypes having higher tolerance, agroclimatic fitness, higher biomass and faster growth cycle is needed for various kinds of the contaminants.

In addition, to commercially exploite those naturally occurring plants selected for the remediation of the pollutants, some biotechnological approaches such as rhizosphere manipulations to increase bioavailability or biodegradation of the contaminants for higher uptake and rapid removal by the phytoremediator (Vassil et al. 1998; Chaudhary et al. 1998; de Souza et al. 1999; Singh et al. 2003; Saxena et al. 1999; Morikawa and Takashashi 2000; Geebelen et al. 2002; Piechalak et al. 2003; Thangavel and Subburaam 2004) and genetic engineering of plants to increase uptake, transport, partitioning, tolerance, *in situ* degradation, volatization or evaporation etc (Rugh et al. 1998; Zhu et al. 1999,a,b; Pilon Smits et al. 1999; Gleba et al. 1999; Zaal et al. 1999; Saxena et al. 1999; Morikawa and Takahashi 2000; Hirschi et al. 2000; Bizily et al. 2000; Hannink et al. 2001; Singh et al. 2001; Takahashi et al. 2001; Dhanker et al. 2002; Lee et al. 2003a,b; Pilon et al. 2003; Singh and Jaiwal 2003; Maiti et al. 2004; Datta and Sarkar 2004; Marikawa et al. 2002 2005; Pan et al. 2005) have been persued to increase the phtoremediation efficiency.

Such biotechnological efforts are also made to resolve the specific problems for the improvement of a phytoremediator to suit to the specific contaminant(s) and site(s) to make it commercially successful. This review is an attempt to analyse such approaches and efforts in the light of the present challenges towards the alarming contaminations of toxic heavy metals, major gaseous pollutants like nitrogen oxides, sulfur oxides and organic pollutants of agrochemicals and industrial origin (Fig. 1). We have confined our discussions largely on the higher plants and focused on the need to understand the key regulatory steps and mechanisms to produce superhyperaccumualtors of commercial grade by gene technologies. We have also discussed the needs of rhizosphere manipulations of plants for their better performance.

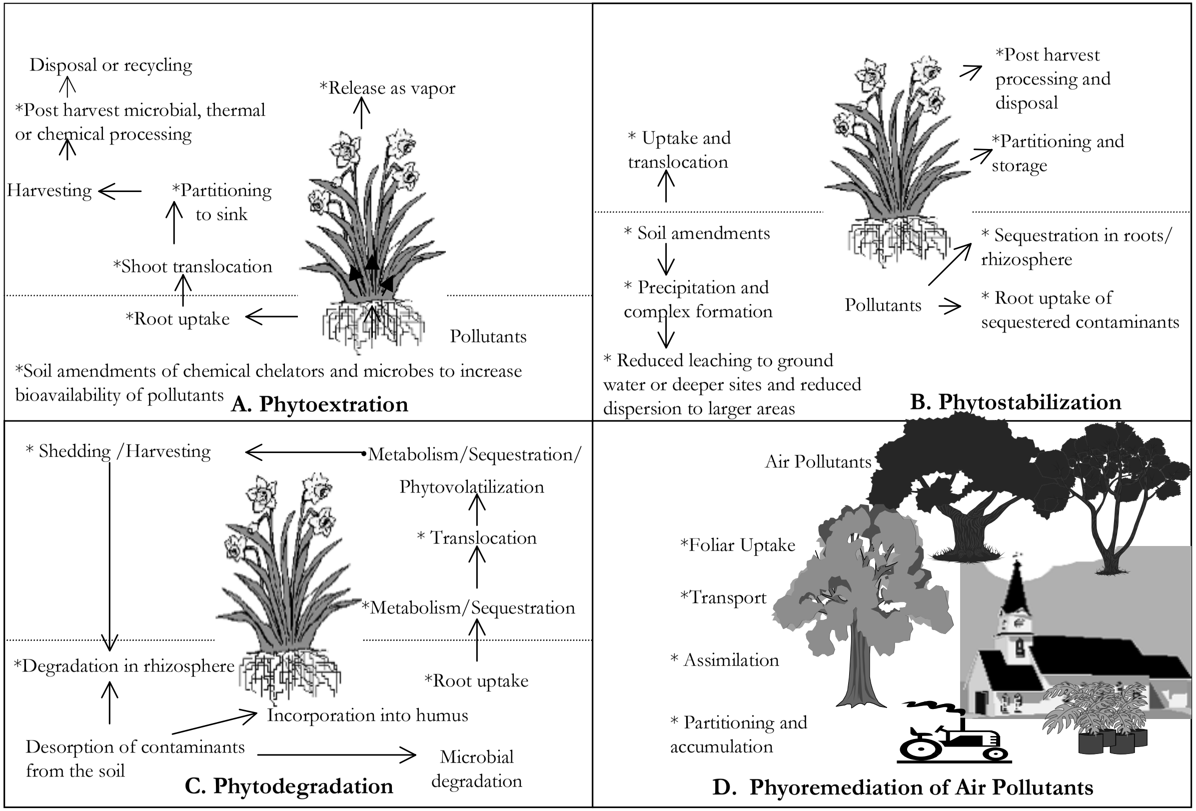

Fig. 1. Phytoremediation types and postulated sites for biotechnological interventions A. Phytoextraction / Phytovolatilization, B. Phytostabilization, C. Phytodegradation, D. Phytoremediation of air pollutants; *Sites for Biotechnological input

2. Phytoremediation: The Processes, Potentials and Limitations

Phytoremediation is based on the fact that a living plant can be considered as a solar driven pump, which can extract, concentrate, degrade, volatize or vaporize soluble toxic substances from the soil, water or air through their natural water and mineral uptake, transport, partitioning, assimilation and transpiration systems. In addition, plants need to survive in several odd environments, and hence they posses more flexible metabolic systems evolved genetically or adopted physiologically to avoid, partition, degrade, store or exclude various undesired and toxic substances. They have developed various specific and general adaptation mechanisms to protect them from the abiotic and biotic stresses. The biotechnological approaches focus to exploit these evolved potentialities of the plants and other associated organisms and to modify their characteristics with some needed alterations in favour of the human needs. Cleanup of the toxic substances from the contaminated sites using the principles of phytoremediation can be achieved in many ways (see Table 1). The details of these processes have been discussed in many past and recent reviews (Brooks et al. 1979; Baker and Brooks 1989; Raskin et al. 1997; Salt et al. 1998; Saxena et al. 1999; Baker et al. 2000; Maiti et al. 2001; Raskin and Ensley 2000; Morikawa and Takahashi 2000; Prasad 2004; Thangavel and Subburaam 2004; Schwitzguébel 2004) and also in this chapter. The popularity of this technology is increasing with increase in the awareness for a need of sustainable environment around us. The remediation of soil pollution may involve a cost of 300 billion of dollars (Raskin et al. 1997; Maiti et al. 2004). Phytoremediation and other bioremediation techniques are not only significantly cost effective over the physical and chemical means of the soil, water or air remediations, they also reduce the risk from exposure to the hazardous constituents at waste and spill sites (Salt and Rauser 1995; Salt et al. 1995; Salt 2001; Raskin et al. 1994; Cunningham and Ow 1996).

The efforts to understand the physiological and molecular mechanisms involved in the processes of the phytoremediation by plants have come to the focus of attention more precisely with a view point to apply these *in situ* processes to enhance the phytoremediation potentials using biological and engineering strategies designed to optimise and improve the process (Schwitzguébel 2004). Several plant species have been explored and the treatment systems for decontamination of the toxicants from sites have been set up, but, most of them were used without exact understanding of the mechanisms involved. Certain woody plant species, shrubs, other perennials, and annual herbs including crop plants have been found suitable for the phytoremediation techniques (Table 2).

In addition to pulling out the toxic contaminants from the soil to metabolize, concentrate or evaporate, the phytoremediation techniques involve extensive pull out and evaporation of water from the plant covered sites. This high consumption

Table 1. Biotechnological approaches for the various modes of phytoremediation enhancement.

Mode	Meaning	Target	Possible Phytoremediation Enhancement strateies
Phytoextraction	The extraction of pollutants from soil, water or air and its higher accumulation and compartmentaion in harvestable plant parts	Toxic metals	Overexpression or insertion of uptake, transport, partitioning storage and binding related genes (including regulatory transcription factors and organ specific promoters)
Phytoaccumulation	The uptake and concentration of the contaminants within the roots or aboveground portions of the plants	-do-	-do-
Phytodegradation (Phytotrans-formation)	The partial or total degradation of complex organic molecules within the plants	Organic pollutants	Overexpression or insertion of uptake, transport, degradation and metabolism related genes and transcription factors.
Phytovolatilization	The uptake, transport and volatilization of volatile organics through stomata	Volatile pollutants or pollutants producing volatile products on catabolism	Insertion and overexpression of uptake, transport, degradation, metabolism and volatilization related genes and transcription.
Evapotranspiration	The uptake, transport and evaporation of pollutants through the transpiration pathways.	Contaminants reached to deeper sites or at wet, marshy sites	Gene manipulation to increase water uptake and transpiration rate
Phytostabilization	The reducing mobility of pollutants towards ground water or its dispersion in soil or water by enhancing precipitation or sequestering to the roots	To avoid leaching or dispersal and to concentrate pollutants in the rhizosphere of plants	Amendments of binders/sequesters and microbial population suitable for the purpose

Tree as pump	The use of trees to evaporate water and to extract pollutants from soil	Deep rooted pollutants from wasteland not expected to be used shortly	Genetic engineering for higher water uptake and enhanced transpiration rates
Phytostimulation (Rhizodegration)	The release of plant exudates/enzymes into the rhizosphere which stimulates the microbial and fungal degradations of organic pollutants	*Ex-situ* degradation of organic pollutants in rhizosphere of plants	Over expression /insertion of genes producing such microbial stimulants
Rhizofiltartion	The use of plant roots to absorb or adsorb pollutants from water and aqueous waste stream	Clean-up of shallow waterlogged areas or for municipal waste water treatment	Manipulation for desired and extensive root systems and higher uptake of the pollutants

and recycling of water can also prevents pollutant wash out and slows down the possible migration of toxic compounds through the soil and into the groundwater. In many cases, associated microflora play a important, if not the decisive, role in the treatment of the polluted sites (Siciliano and Germida 1998; Schwitzguébel 2004).

Though several plants have been identified from the natural plant populations as hyperaccumulators of toxic heavy metals (Prasad 2004 for a recent review), oxides of nitrogen (Morikawa et al. 2002, 2005) and organic pollutants (see Schwitzguébel 2004), bioprospecting for the natural phytoremediators has not been done adequately. For example, phytodiversity and the polluted sites are enormous in India, and many other developing countries, but-there have not been adequate work on biodiversity prospecting for the exploration of minerals and other natural resources and for the environmental cleanup (see Prasad 2004). Most of the knowledge generated on the different kinds of phytoremediation, improvements in phytoremediation potentials by engineering and biotechnological approaches and its commercialization, belongs to the countries which are more planned and environmentally careful, though many of them posses less plant diversity. Bioprospecting of the natural plant diversity for the environmental cleanup potentials will not only provide insights to use more appropriate phytoremediators, which are cheapest, sustainable and most acceptable in the public domain, but it will also provide very significant informations for gene pool available to produce superior quality genetically manipulated plants, more suitable for the commercial viability as phytoremediation systems. Generally fast growing plants with high biomass and different kinds of root system suitable to be used to cleanup the pollutants at different depth are considered as ideal phytoremediators. However, they should be tolerant enough for the target

Table 2. Some case studies and commercial phytoremediation field project based on websites (http:/www.mobot.org/jwcross/phytoremediation/phytorem-sponsors-corp.htm; Saxena et al. 1999; Morikawa and Takahashi 2000; Schwitzguébel 2004)

Contaminant	Plant species and technique used	Institution/Industry/Company	Site name /Location
Removal of nitrogen	Poplar tree planting	CH2M HILL, Potland, OR, USA	Mill Greek, USA
Treatment of oily waste through land application	Rhizosphere amendants with rotation of grass, grains and clover crops on the sites two times each year .The crops are seasonally plowed into the soil with the applied waste to provide a stabilizing “green manure” nutrient source	-do-	Texaco, Anacortes, Washington, USA
Remediation of diesel contaminated soil	Cultivation of grass and clover and rhizosphere bioremediation	-do-	Daishowa paper Mill, Port Angeles,Washington,USA
Remediation of wood preservative wastes through plant cultivation (contaminants included pentachlorophenol (PCP) and PAH s)	Planting of native cottonwood, willow, alfalfa and several grasses in 1999 to 2001 added with rhizosphere bioremediation	-do-	Union Pacific Railroad, Laramine, WyominG, USA (140 Acre site)
Soil and ground water contamination with petroleum related organics, PAHs and chlorinated organics released by accidental spills in year 2000	Hybrid poplar trees, buried upto 10 feet below the surface and a sub-surface aeration system (to encourage deep rooting into ground water)	Ecolotree, Inc., Iowa city, IO, USA (Ecolotree (r) cap (Ecap) and Ecolotree (r) Buffer (EBuffer)	Milwaukee, Wisconsin, USA
Fertilizer and pesticide	440,12-18 feet tall bare root hybrid poplar were planted into 6’deep trenches	-do-	Illinois, USA (April, 1999)
Treated 80,000 gallons per day of municiple sewage contaning	South Burlington’s Living Machine	Living Technologies, Taos, NM, USA (Living Machines®)	Lake Champlain, USA (1995)

Trichloroethanol	Hybrid poplar	Occidental Petroleum Corp., Los Angles, CA USA& University of Washington; USA	Various sites in USA
Heavy metals	Indian mustard and sunflowers (the patented plants can take up heavy metals more than 3.5% of their dry weight)	Edenspace system carporation, Reston, VA, USA	Various sites in USA
Uranium soil contamination 47mg/kg	Sunflower (Accumulation in plants 764 mg/kg-1669mg/kg)	-do-	US Army site in Aberdeen, Maryland, USA
Arsenic	Fern *P. vittata* (brake fern). Phytoextraction in above ground part by more than upto 200 fold higher than other plants	.-do-	1.5 Acre site in New Jersey, North Carolina, USA (2001)
$^{89/90}$ Sr (radionuclide)	Specially selected Indian mustard ($^{89/90}$ Sr in plants was more than 10-15 fold higher that than in soil); Phytoextraction +soil amendments	-do-	Fort Greely, Alaska, USA
^{137}Cs (radionuclide)	-do-	-do-	Chernobyl Nuclear Power Plant accident in 1986 in Ukraine
Organic pollutants including dichlorobenzidine (a human carcinogen)	Mixed native species e.g. Willows and Poplars (13,000 trees)	Phytokinetics, Inc. North Logan, UT	Bofors-Nobel Superfund site, USA 20 Acre site)
Ground water treatment of chlorinated volatile organic	Poplar & willow trees (1000); 'Pump and treat' system (Evapotranspiration of contaminated water)+ Enhanced rhizosphere degradation	Solvent Recovery Services of New England (SRSNE)	Superfund site in Southington, Connecticut, USA (1998)
PATHs, heavy metals	Various	Stockholm University	Old gasworks site (Husarviken, Sweden)
Contaminations with wood presservatives including pentachlorophenol & polyaro-matic hydrocarbons	Perennial rye grass (*Lolium perenne*)		Mc Cormick and Baxter Superfund site, USA (1996-1998)

Cadmium, zinc, lead	Alpine pennycress (*Thlaspi caerulescens*) Take up Zn@125Kg/ha per year and Cd @ 2Kg/ha per year with optimum growth condition; Phytoextraction	Dr Chaney and coworkers	Pig's Eye landfill site in St Paul, Minnesota, USA
^{137}Cs and ^{90} Sr	Indian mustard and redroot pigweed (*Amaranthus retroflexus);* Phytoextration	-	Brookhaver National Lab New Jersy and in Ashtabula Ohio, USA
Lead and Cadmium	Indian mustard (*Brassica juncea*)	Phytotech, Florida State University, IETU	Czechowice oil refinery (Katowice, Poland)
Zinc and Cadmium	*Salix viminalis* (willow)	Swiss Federal Institute of Technology	Former landfill (Switzerland)
Nickel,copper,zinc ,cadmium	*Salix* species	University of Glasgow	Sewage disposal site (United Kingdom)
Zinc	H.annuus, Z.mays, C.halleri	International Graduate School Zittau	Zinc waste landfill (Hlemyzdi, Czech Republic)
Copper, zinc, cadmium	Improved tobacco	Several institutes	Zinc/Copper (Dornach, Switzerland)
Zinc, copper, lead, cadmium	Grasses for phytostabilization	Limburgs University	Zinc smelter site (Lommel, Belgium)
Zinc, copper, lead, cadmium	Grasses for phytostabilization	Limburgs University	Contaminated playing ground (Overpelt, Belgium)
Zinc, copper, lead, cadmium	*B. napus* for phytoextraction	Limburgs University	Zinc / Cadmium contaminated soil (Balen, Belgium)
Lead, cadmium, zinc, copper, Ti, Sb, As	Various plants	Several institutes	Guadiamar river area, Donana National Park (Aznalcollar mine, Spain)

Lead	Successive crops of sunflower and indian mustard planted in 24” deep *ex-situ* treatment cell on an impermeable concrete base .The single season phytoremediation treatment achieved the regulatory goal of 900 mg/kg. Total cost of phytoremediation treatment was less than $50 per cubic yard, which saved more that $1.1 million comparedto the estimated cost of excavation and disposal.	-do-	Daimler Chysler’s Detroit Forge Site, USA. (4300 cubic younds of soil with Pb^{+2} ranging from 75-3,450 mg/kg soil) in 1998
Lead	Sunflower and indian mustard were planted. A combined phytoextraction and Phytostabilization treatment for three years costed less than $40 per cubic yard of treated soil	-do-	Industrial facility in Connecticut, USA (1997-2000)
Lead	Indian mustard, Phytoextraction + rhizosphere amendments with EDTA	-do-	A Site at Trenton, NJ, USA(1996-1997)
BTEX	*Populus x Canadensis* (poplar)	Limburgs University	BTEX contaminated groundwater (Genk, Belgium)
Chlorinated organics	Various	Stockholm University	Eka Chemicals site, (Bohus, mercury Sweden)
Gasoline and diesel compounds	Poplars and willow	Technical University of Denmark	Old gas filling station (Axelved Denmark)
Cyanide, BTEX, PAHs and oil	Poplars and willow	Technical University of Denmark	Former municipal gasworks site
Pesticides	Poplars	Polish Academy of Sciences, Kornik ISTEA-CNR Bologna	Resort pollution by pesticides stored in bunkers (Niedwiady, Poland)

toxicant(s) to survive with prosperous vegetative growth on the contaminated site(s) and should be suitable for the agro-climatic conditions of the area under cleanup. It will be best to search out a naturally evolved phytoremediator with all such positive characters during the phytoprospecting, but it is likely that one may need to incorporate one or more character(s) artificially by genetic manipulations to achieve such goals.

3. Commercial Viability of Phytoremediation Projects

Phytoremediation has been carried out commercially or demonstrated at pilot scale at nearly 200 sites in USA involving all the contaminant categories (Glass 1999; Shekhar et al. 2004). A growing concern over the safe and sustainable environment has created a huge space globally for such eco-friendly techniques within a viable commercial set up. Several universities, research institutes, government bodies and private companies are collaborating to develop large scale economically viable projects for cleanup of the notorios toxicants contaminating various sites accidentally or slowly (Table 2). Such efforts and practices are, however, confined to developed countries which are getting better public perception and pressure for the sustainable eco-friendly developmental projects. Other parts of the world including most of the developing countries are yet to be adequately sensitized to the cause of the environmental cleanup and a central focus on the sustainable development which is a task ahead. It is evident, that phytoremediation, as a technology, will gain momentum throughout the world, as we don’t have better options to treat the contaminated water, air and land sites which are creating a high risk health hazards to human and live stocks and damaging green cover and plant productivity enormously.

Large scale phytoremediation of the contaminated sites has been achieved for heavy metals, organic xenobiotics and radionuclides (Table 2. Glass 1999, Dietz and Schnoor 2001; Schwitzguébel et al. 2002; Schwitzguébel 2004). Developing a commercial phytoremediation strategy needs attention to both pre-harvest (e.g. contaminant level monitoring, plant selection, decontamination rates, agro-climatic suitability of phytoremediator, groundwater capture zone, transpiration rate and required cleanup time etc.) and post harvest processing (e.g. harvestable biomass collection, leftovers and underground residues disposal and treatment removal of the contaminated plant materials etc.) steps. With minimal environmental disturbances, the phytoremediation techniques can be applied to a broad range of toxicants, which generate less secondary air or water waste as compared to other traditional methods. The organic pollutants may ideally be degraded to CO_2 and H_2O, reducing environmental toxicity. It is always beneficial for treating large volumes of water, air or land having low to moderate concentration of the contaminants. During land reclamation using phytoremediation, the topsoil is left in usable condition and may be developed for agricultural use as the soil remains intact at the site after contaminants are removed in contrast to conventional methods.

Rhizosphere amendments with chelators, bacteria and mycorrhizae have been used to enhance bioavailability of the contaminants to the remediating plants for large scale remediation strategies (Table 2. Chaudhary et al. 1998; Khan et al. 2000; Thangavel and Subburaam 2004; Schwitzguébel 2004). Rhizosphere manipulations to deal with various layers/depth of the contaminants and to provide sub-surface aeration etc. have been provided in some systems developed by companies dealing with this technology. Though hybrid poplar willows (*Salix* sp.), clover, alpine pennycress (*Thlaspi* sp.), grasses, Indian mustard, sunflower, geraniums, fern (*Pteris vittala*), perennial ryegrass, redroot pigweed etc. have been plants of choice for many commercial phytoremediation systems (Table 2), several new plants with higher efficiency and better suitability for phytoremediation can be searched out with the extensive phytoprospecting of new sites. In addition, genetically modified superior quality phytoremediators can be developed to handle specific situations. A large number of large scale demonstration/ treatment projects have established the commercial viability of phytoremediation as a sustainable and viable cleanup technology of present and for the future.

4. Rhizosphere Manipulations for Enhanced Bioavailability of the Toxic Substances

Amongst the major factors that can make a phytoremediation successful and commercial, rhizosphere manipulations for increased bioavailability of toxic substances have been a focus of attention in the recent past. In addition to genetic ability of the phytoremediating species /cultivars, optimal agronomic (soil and crop management) practices can increase the efficiency of the system (Li et al. 2000; Khan et al. 2000; Thangavel and Subburaam 2004; Datta and Sarkar 2004).

Heavy metals are one very significant category of the industrial contaminants, which are unique being selectively toxic, persistent and non-biodegradable (Baker and Brooks 1989; Bharti and Singh 1993, 1994; Kumar et al. 1993; Singh et al. 1994a,b, 1996, 1997a,b,c, 2001, 2003; Dabas et al. 1995; Bharti et al. 1996). The United States Environmental Protection Agency (USEPA) has indicated recently that the sites polluted with toxic heavy metals should receive priority for cleanup during the next few years (Eccles 1998). The contaminated land sites may consist of a heterogeneous mixture of different minerals, organic, organomineral substance and other solid components. The binding mechanisms of the heavy metals are, therefore, complex and vary with the composition of soil, soil acidity and redox conditions (Thangavel and Subburaam 2004). The bioavailability and mobility of heavy metals in soils is dependent upon the redistribution processes between solution and solid phases and among solid phase components. The rates of redistribution of metals and their binding intensity in soils were affected by the metal species, loading levels, ageing and soil properties (Eccles 1998; Han et al. 2003). The slow desorption of heavy metals in soil has been a major impediment to the successful phytoextraction of the metal contaminated sites (Thangavel and Subburaam 2004). Generally, only a fraction of soil metal is readily available (bioavaialable) for the plant uptake. The bulk of the metal in soil is commonly found as insoluble compounds unavailable for transport into roots from the aqueous phase. Cadmium and zinc are considered as easily mobile heavy metals as they occur primarily as soluble or exchangeable, readily bioavailable forms (Thangavel and Subburaam 2004). Copper, molybdenum and chromium are mainly bound in silicates and thus are slightly mobile. Lead occurs as insoluble precipiate (phosphates, carbonate and hydroxy-oxides), which are largely unavailable for plant uptake (Pitchel et al. 1999). It appears, therefore, that soluble, exchangeable and chelated species of trace elements are the most mobile in soils and these properties of the metals govern their migration and phytoavilability (Kabata-Pendias 1997). Binding and immobilization of the toxic metals within the soil matrix can significantly restrict their uptake and removal from the site. The bioavailability of the metals and other toxic substances, however, can be enhanced by manipulating the rhizosphere of the potential remediator plants by changing soil pH (lowering of pH is recommended to increase the bioavailability of heavy metals), adding chelating agents, using appropriate fertilizers (ammonium containing fertilizers), altering soil ion composition, adding adequate consortia of soil microbes and phytosiderophores and soil exudates managements (Table 3. Singh et al. 1996, 1999, 2001; Chaudhary et al. 1998; Khan et al. 2000; Thangavel and Subburaam 2004; Schwitzguébel 2004; Datta and Sarkar 2004).

Amendments of soil with ammonium containing fertilizers, organic and inorganic acids and elemental sulfur, HNO_3 and $CaCO_3$ lower the soil pH and enhance phytoaccumualtion of the toxic metals (Huang et al. 1997; Cristofaro et al. 1998; Chaney et al. 2000; Gao et al. 2003; Thangavel and Subburaam 2004), however, contrary reports are also available (Singh et al. 1996; Khan et al. 2000). Therefore, more precise and focused studies are needed to evaluate the independent effect of soil pH and soil amendments on hyperaccumulators yield and metal removal efficiency.

Artificial chelates e.g.. EDTA has been studied to enhance the heavy metal bioavailability and subsequent uptake and translocation to the shoots (Table 3. Fuentes 1997; Huang et al. 1997; Khan et al. 2000; Kayser et al. 2000). The chelates may be added at once a few days before harvest or gradually during the entire growth period. The uptake of Fe, Mn and Cu by maize plants was increased when EDTA or DTPA (1g/kg soil) was added in the soil prior to planting (Fuentes Bolomey 1997). Biosurfactants have also been shown to enhance the metal bioavailability in contaminated soil and sediments (Mulligan et al. 2001).

Table 3. Changes in bioavailability of the environmental contaminants especially heavy metals in rhizosphere and their uptake and accumulation by plants leading to altered phytoremediation efficiency due to rhizosphere amendments

The toxic contaminant	Rhizosphere Amendments	Plant	Response	Reference
Cadmium	Iron	*Thlaspi caerulescens*	Decrease uptake by 3 folds	Lombi et al. (2002)
Cadmium and Zinc	Root exudates by the plant (organic legands)	*Thlaspi caerulescens*	Enhanced metal accumulation	Zhao et al. (2001)
Cadmium, Iron and Manganese	*Bacillus* sp., *Pseudomonas* sp. (Exude organic compounds)	*Brassica juncea*	Enhanced metal accumulation	Salt et al. (1995); Shekhar et al. (2004)
Iron, Manganese and Copper	EDTA	*Zea mays*	Enhance metal uptake	Fuentes (1997)
Iron, Manganese and Copper	Phytosiderophores	*Graminaceous species*	Enhance metal accumulation	Khan et al. (2000); Treeby et al. (1989); Ma and Nomoto (1996)
Lead	EDTA (0.5-1 mM)	*Pisum sativum*	2 fold increase in accumulation	Piechalak et al. (2003)
Lead	EDTA (0.25 mM)	*Brassica juncea*	75 fold higher Pb in plants than in hydroponics solution	Vassil et al. (1998)
Lead	EDTA (1 g/Kg soil)	*Garcinia cambogia*	Increased accumulation by 1.5 fold	Sekhar et al. (2004)
Lead	NaCl (6-12 EC)	*Vigna radiata*	Decreased accumulation by 3.5 to 5 fold	Singh et al. (2003
Lead	K_2HPO_4 (10 mM), $CaCl_2$ (10 mM), KNO_3 (10 mM)	*Vigna radiata*	Decreased metal accumulation in roots and leaves	Singh et al. (1994b)
Nickel	NPK fertilizers	*Alyssum bertolonii, Thlaspi caerulescens, Streptanthus polygaloids*	Enhanced biomass with same concentration of nickel in aerial parts	Bennett et al. (1998)

Selenium	Rhizosphere bacteria	*Brassica juncea*	4-5 fold higher Se accumulation and volatilization	de Souza et al. (1999)
Trace metals and organic pollutants	Mycorrhizae	Many plants	Enhance uptake, phytostabilization and Biodegradation of contaminants	Chaudhary et al. (1998); Schwitzguéb el, (2004)
Zinc	Lime stone, cattle manure and poultry litter	*Zea mays*	Reduced bioavailability	Pierzynski and Schwab (1993)
Zinc	Phytosiderophores	*Triticum aestivum*	Increased uptake	Zhang et al. (1991)

Another approach to enhance the rate of phytoremediation relates to the better agronomical management, which may yields an enhanced harvestable biomass of the remediating plants. Application of N-fertilizers (Bennett et al. 1998) to *Alyssum bertolonii, Streptanthus polygaloides* and *Thlaspi careulescens* have been shown to increase biomass very significantly without reducing the shoot nickel concentration. Addition of phosphate to soil may also help extract ion of Cr, Se and As by competing for the binding sites and thereby increasing bioavailability of the metals (Thangavel and Subburaam 2004).

Soil microbes have been found suitable to enhance the bioavailability and phytoremediation potential by complimenting the processes in many ways. Microbial activity in the rhizosphere of plants is several folds higher than in the bulk soil. Chemolithotrophic bacteria have been shown to enhance metal availability (Kelley and Tuovinen 1988). Several strains of *Bacillus* and *Pseudomonas* have been reported to increase cadmium accumulation by *Brassica juncea* (Salt et al. 1995). Naturally occurring rhizobacteria were found to promote Se and Hg accumulation in plants growing in wetland (de Souza et al. 1999). These microbes can grow more well, if organic manures are added to the soil. The mechanisms by which they increase the bioavailability and uptake of the heavy metals is not adequately elucidated yet, however, the possible mechanisms might include soil acidification and changes in the solubility of the metal complexes through their exudates (organic compounds exude from soil bacteria). The soil microbes may degrade organic pollutants and supply nutrients to plants for enhanced phytoremediation of the site.

It is generally considered that the majority of plants growing under natural conditions have symbiosis with mycorrhizae in roots, which result in increase in root surface area and nutrient acquisition (Khan et al. 2000). Mycorrhizal fungi have been reported in plants growing on heavy metal contaminated sites indicating its heavy metal tolerance and a potential role in the heavy metal phytoremediation (Table 3. Shetty et al. 1995; Weissenhorn and Leyval 1995;

Pawlawska et al. 1996; Chaudhary et al. 1998; Khan et al. 2000; Schwitzguébel 2004). Mycorrhizal fungal taxa, such as species like *Glomus*, *Gigaspora* and *Entrophospora*, have been reported to be associated with most of the plants growing in the heavy metal polluted habitats (Khan et al. 1990). The transport of the toxic metals absorbed by the mycorrhizal surface to the aerial part of the remediating plants is an obvious mechanism, which can enhance the total uptake and transport of the toxic metals in a defined period due to an increased surface area of the rhizosphere by the mycorrhizal associations.

Phytosiderophores (a class of organic compounds e.g. mugineic and avenic acids) exudated by roots of the many plants especially graminaceous species have been reported to enhance bioavailability of soil metals e.g.. Fe, Cu, Zn and Mn etc (Treeby et al. 1989; Thangavel and Subburaam 2004). Other kinds of root exudates can also reduce the rhizosphere soil pH and thus modulate the metal availability for uptake by the plants (Thangavel and Subburaam 2004), however, no direct evidence that indicates the involvement of root exudates in the phytoremediation has been documented.

5. Molecular Mechanisms of Uptake, Detoxification, Transport and Accumulation of Toxic Substances by Plants and Genetic Engineering for Enhanced Phytoremediation

Uptake of the toxic substances by the remediating plants is a pre-requisite for the phytoremediation. Following its bioavilability in the rhizosphere, their enhanced uptake and transport to the sink or metabolism sites can increase the efficiency of the phytoremediation of a selected plant. Transport proteins and intracellular high-affinity binding sites mediate the uptake of the metals and other substances across the plasma membrane. Many metal transporters genes have been cloned recently (Table 4. Datta and Sarkar 2004). Maser et al. (2001) have cloned genes of ZIP (Zn-regulated transporter/Fe-regulated transporter like proteins) family e.g.. *ZNT1* and *ZNT2*, from *Thlaspi careulescens*, which are highly expressed in roots of the accumulator plants, but their expression are not responsive to Zn status of the plants. Through functional complementation in yeast, it has been shown, however, that *ZNT1* protein mediates high affinity uptake of Zn and low-affinity uptake of Zn^{+2} and Cd^{+2}(Pence et al. 2000). The transcription (factors) activators, such as Zn-responsive element, have been suggested to play an important role in Zn hyperaccumulation in *T. careulescens* (Pence et al. 2000). An increased uptake of Cd by *T. careulescens* and *A. thaliana* by enhanced expression of *IRT1* gene, which is essential for Fe uptake has been demonstrated (Lombi et al. 2002; Vert et al. 2002; Connolly et al. 2002; Datta and Sarkar 2004).

Table 4. Strategies for genetic engineering of plants to produce superior transgenic plants for phytoremediation of the environmental contaminants

Plant genotype	Foreign gene introduced	Promoter	Vector	Response obtained	Phytoremediation efficiency of transformed plants	References
Brassica juncea L. cv 173874	*Arabidopsis APSl* encoding ATP-sulfurylase	CaMV, 35S	*Agrobacterium tumefaciens*	Overexpression of plastidic ATP sulfurylase	2-3 fold higher Se accumulation in shoots and 1.5 fold higher Se in roots as compared to the wild type plants	Pilon-Smits et al. (1999)
,,	*E. coli gshII* encoding glutathione synthetase (GS)	,,	,,	Overexpression of cytosolic glutathione synthetase	3 fold high Cd accumulation in transformed plants	Zhu et al. (1999a)
,,	*E. coli gshI* encoding γ-glutamyl cysteinethione synthetase (GS)	,,	,,	Overexpression of γ -glutamyl cysteine synthetase targeted to the plastids	Increased tolerance to Cd, higher accumulation of phytochelatins, glutathione and total non-protein thiols, and accumulated more Cd (40-90% higher) in shoot than wild plants	Zhu et al. (1999b)
,,	*E. coli gor* gene encoding gluthione reductase (GR)	,,	,,	Overexpression of gluthione reductase targeted to the plastids (cpGR) as well as cytosol (cystGR)	Reduced Cd uptake and /or translocation: Cd levels in shoots of (cpGR) plants were half as high as those in wild type shoots. Two times higher root glutathione levels in transformed (cpGR) plants than in wild type	Pilon-Smits et al. (2000)

Nicotiana tabaccum and *B. juncea*	Mouse metallothionein1 (*MT1*) and human *MT2* genes	CaMV, 35S	,,	Increased cadmium tolerance and accumulation	Increased cadmium tolerance 10µM to 200 µM	Pan et al. (1994), Misra and Gedamu (1989)
Arabidopsis thaliana ecotype c-24	Chimeric plasmid pSNIRH containing spinach *NiR* cDNA and hygromycin phosphotransferase *hph* gene	CaMV, 35S and *NOS* polyadenylation signal	*A. tumefaciens*	Overexpression of *NiR* cDNA in transgenic plants	40% increase was observed in NO_2 assimilation	Takahashi et al. (2001) Morikawa et al. (2002)
B. oleracea var. botrytis	Yeast *cup1* gene	-	,,	,,	16 fold higher Cd tolerance and accumulation	Hasegawa et al. (1997)
B. juncea	γ- glutamyl cysteine synthetase (γ-ECS) and glutathione synthetase (GS)	-	-	Increased cadmium and zinc uptake	Accumulated 1.5 fold more Cd and 2 fold more Zn in green house experiments based on the field contaminated soils compared to the wild type Indian mustard	Bennett et al. (2003)
Arabidopsis thaliana	*Arabidopsis* PC synthase (*At PCS1*) gene	-do-	,,	Increased phytochelatin synthesis and higher tolerance to Cd	Increased cd phytoremediation	Lee et al. (2003b)
A. thaliana	Zinc transporter (*ZNT*), a putative vacuolar transporter, which encode a Pb-II/CdII/Zn II pump	-do-	,,	Increase Zn, Pb, and Cd tolerance	Two fold higher Zn accumulation in roots	van der Zaal et al. (1999)
A. thaliana	-do-	-do-	*A. tumefaciens*	Lower accumulation	Accumulated more Cd in	Lee et al.

				of Cd in shoot protoplast	vaculoes	(2003a)
N. tabaccum	Calcium vacuolar transporter *Arabidopsis antiporter CAX2*	-	-	Higher tolerance to Mn^{+2} levels	Accumulated more Ca^{+2},Cd^{+2} and Mn^{+2}	Hirachi et al. (2000)
N. tabaccum and *A. thaliana*	Bacterial genes *ars c* from *E.coli*	-	,,	Higher cadmium tolerance than wild type	Higher cadmium tolerance than wild type	Dhankher et al. (2003)
A. thaliana	Bacterial genes (*E.coli*) arsenate reductase (*ars c*) and γ- glutamyl cysteine synthetase (*γ-ECS*) together	Two different promoters; SRS1p and CaMV, 35S	,,	Higher arsenic tolerance	4-17 fold higher arsenic hyperaccumulation in shoots	Dhankher et al. (2002)
A. thaliana	Mouse Se-cysteine lyase (*pSLY*) and *pSCH*)	CaMV, 35S	,,	Expression in the cystol or chloroplast of *Arabidopsis* resulted 2 fold (cytosolic lines) or 6 fold (chloroplastic lines) higher SL activities in transgenic plants than wild type and enhanced tolerance to Se	Higher Se- volatilization than wild type	Pilon et al. (2003)
B. juncea	Cystathionine-gamma-synthase (*CGS*) gene from *A. thaliana*	-	*A. tumefaciens*	Higher Se tolerance than wild type	2-3 fold higher Se volatilization than wild type	van Huysen et al. (2003)
Lycopersicon	1-aminocyclopropane-1-		,,	Produces lower	Higher metal accumulation	GrichKo et al.

esculeutum and *B. juncea*	carboxylate (ACC) deaminase gene			levels of ethylene and protects from deleterious effects of six metals e.g. Cd^{+2}, Co^{+2}, Cu^{+2}, Mg^{+2}, Ni^{+2}, Pb^{+2} or Zn^{+2}	than the wild types	(2000), Nie et al. (2002)
N. tabaccum	Murine monoclonal antibody *IgG1* gene	-	-	Higher metal uptake	Higher level of phytoremediation	Drake et al. (2002)
A. thaliana	Yeast vacuole transporter *YCF1* gene	-	-	Enhance tolerance to Pb^{+2} and Cd^{+2}	Higher accumulation in transgenic plants	Song et al. (2003)
A. thaliana	Mercuric ion reductase *merA* genes from *E.coli*		,,	Transgenic plants expressing *merA* gene was more tolerant to $HgCl_2$ and Au^{+3} and volatilized elemental mercury	Higher mercury phytoremediation	Rugh et al. (1996)
A. thaliana	Mercuric ion reductase *merB* genes from *E.coli*		,,	Transgenic plants were more tolerant to methyl mercury and other organomercurials	Phytoremedation of organomercurials; can grow on 10 fold higher methyl mercury than wild type	Bizily et al. (1999)
A. thaliana	Both of the above genes		*A. tumefaciens*	-do-	Transgenic plants can grow on 50 fold higher methyl mercury than the wild type plants	Bizily et al. (2000)

A. thaliana	Both, *merA* and *merB* genes of bacteria origin		,,	Transfer to the chloroplast genome resulted in high levels of tolerance to the organomercurials compound, phenylmercuric acetate (PMA) and increased biomass	The use of chloroplast transformation to enhance Hg phytoremediation is particularly beneficial because it prevents the escape of transgenes via pollen to the related weeds or crops and there is no need for codon optimization to improve transgene expression	Ruiz et al. (2003)
N. tabaccum	Bacterial nitroreductase gene (*pNITRED3*)	CaMV, 35S promoter modified *nfsI* and *nos* termination sequences	,,	Increased tolerance to 2,4,6-trinitrotoluene (TNT)	Remediation/Detoxification of TNT (recalcitrant military explosive)	Hannink et al. (2001)

The metal transporters e.g. metal (or CPx-type) ATPases, that are involved in the overall metal ion homeostasis and tolerance in plants and natural resistance associated macrophase (Nramp) family of proteins and cation diffusion facilitator (CDF) family of proteins have been characterized in a wide range of organisms including plants (Belouchi et al. 1997; Tabata et al. 1997; Alonso et al. 1999; Guerinot and Eide 1999; Thomine et al. 1999; van der Zaal et al. 1999; Williams et al. 2000; Datta and Sarkar 2004). CPx-type metal ATPases have been implicated in the transport of essential as well potentially toxic metals like Cu, Zn, Cd and Pb etc across the cell membranes (Williams et al. 2000). They share a common feature of a conserved intra-membranous cystein-proline-cystein, cystein-proline-histidine or cystein-proline-serine(CPx) motif, which is thought to function in the metal transduction. These transporters use ATP to pump a variety of charged substrates across the cell membranes and are distinguished by the formation of a charged intermediate during the reaction cycle. *Arabidopsis* P-type ATPases (PAA1) was the first CPx-ATPases reported in the higher plants (Tabata et al. 1997; Datta and Sarkar 2004).

Though the physiological role of the metal ATPases in higher plants is not precisely demonstrated, most CPx –type ATPases identified have been involved in the Cu or Cd transport. Since *Arabidopsis* CPx-ATPases show fairly low similarities to each other, they are specific for transporting different substrates (Datta and Sarkar 2004). The ATPases located in plasma membrane may function as efflux pumps removing potentially toxic metals from the cytoplasm, or may also be present at the various intracellular membranes and be responsible for the compartmentalization of the metals, e.g. sequestration in the vacuoles, golgi or endoplasmic recticulum (Datta and Sarkar 2004). To control the intracellular levels of the metals, regulation of transporters, which could occur in higher plants, similarly as has been observed in the bacteria and yeast, at the transcriptional level (control on initiation rates, mRNA stability, differential mRNA splicing) or at the post translational level (control on targeting and/or stability) have been postulated, though the precise mechanisms for the regulation of the metal transport by CPx-ATPases in higher plants is not known (Williams et al. 2000; Datta and Sarkar 2004).

Another divalent metal ion transporters of Nramp family, encoded by *Nramp* genes, have been identified in rice and *Arabidopsis* (Belouchi et al. 1997; Alonso et al. 1999). Cation diffusion facilitator (CDF) proteins have also been involved in the transport of Zn, Co, Cu and Cd in bacteria and plants e.g.. poplar (Blaudez et al. 2003). Related Zn transporters *ZAT1*, which may have a role in Zn sequestration in plants, have been reported in *Arabidopsis* (van der Zaal et al. 1999). Enhanced Zn resistance has been demonstrated in transgenic plants overexpressing *ZAT1*. constitutively throughout. Zinc transporter (ZIP) proteins have also been found to be involved in Zn and Fe uptake (Guerinot and Eide 1999). The metal uptake which may lead to an enhanced phytoremediation efficiency can be increased by increasing number of uptake sites, specific transporters and regulators of the transport system, intracellular high affinity binding sites by incorporating/over-expressing the target genes in the plants by genetic engineering (Table 4). However, a comprehensive understanding of the metal transport processes in plants is essential for formulating the effective strategies to develop genetically engineered plants that can be used commercially for rapid cleanup of the contaminated sites.

The toxic heavy metal detoxification mechanisms involve chelation of metals by a ligand, followed by the sequestration of the metal-ligand complexes into the vacuoles. Intracellular metal complex formations have been reported with peptide and protein legands, such as metallothioneins (MTs) and phytochelatins (PCs). Metallothineins are first identified in mammalian tissues as Cd –binding peptides and subsequently in the plants (Murphy and Taiz 1995; Foley et al. 1997; de Borne et al. 1998; Garcià-Hernández et al. 1998; Salt et al. 1998; Datta and Sarkar 2004). Phytochelatins are a family of sulfur rich peptides, first identified in yeast and subsequently in a wide variety of plant species including angiosperms (both monocots and dicots), gymnosperms, algae, fungi and marine diatoms but not in animals (Rauser 1995; Cobett 2000; Vatamaniuk et al. 2002; Datta and Sarkar 2004 and references therein). Molecular-genetic studies on yeast and *Arabidopsis* PCs have revealed significant insights during the last decade (Rauser 1995; Cobbett 2000). PCs are induced rapidly in cells and tissues on exposure to a range of metal ions (cations), such as Cd, Ni, Cu, Zn, Ag, Hg and Pb and anions, such as arsenate and selenite (Rauser 1995, 1999; Friederich et al. 1998; Ha et al. 1999; Leopold et al. 1999; Cobbett 2000; Hartley-Whitaker et al. 2001; Cosio et al. 2004; Hussain et al. 2004; Küpper et al. 2004; Raab et al. 2004; Song et al. 2004; Datta and Sarkar 2004).

The PCs are synthesized from glutathione by adding a terminal glycine (gly) into the dipeptides $(\gamma\text{-Glu-Cys})_n$ by the action of enzyme phytochelatin synthase. PCs form a family of structures with increasing repetitions of the γ-Glu-Cys dipeptide, followed by a terminal Gly;$(\gamma\text{-Glu-Cys})_n\text{-Gly}$, where $_n$ has been reported as being as high as 11, but is generally in a range of 2-5 (Cobbett 2000).

It has been demonstrated that GSH deficient mutants of *Arabidopsis* are deficient in PCs and are found Cd-sensitive (Cobbett et al. 1998). Metal ion induced and GSH dependent PC synthase activity that related to the metal tolerance has been shown in *Silene cucubalis* (Grill et al. 1989), tomato (Chen et al. 1997), pea (Klapheck et al. 1995) and *Arabidopsis* (Howden et al. 1995a,b). In Azuki beans (*Vigna angularis*), an essentiality of PC synthase for Cd tolerance has been demonstrated (Inouhe et al. 2000). PC synthase genes *AtPCS1* in *Arabidopsis*, whose expression mediated an increased Cd accumulation (Vatamaniuk et al. 1999) and *TaPCS1* in wheat that increased Cd-resistance and accumulation (Clemens et al. 1999) were reported simultaneously. Both *AtPCS1* and *TaPCS1* mediated Cd tolerance has been found GSH dependent and function in vacuole-deficient mutants, suggesting a cytosolic localization. These genes mediate *in vivo* PC biosynthesis in yeast

(Datta and Sarkar 2004). The role of GSH and PCs in hyperaccumulation of the heavy metals in plants has been demonstrated using transgenic approach in few plants (Table 4).

The availability of amino acids, especially that of sulfur amino acids and regulation of PC synthase activity, is considered as the most important regulatory mechanism of the PC biosynthetic pathways. Another important molecular event that regulates hyperaccumulation of toxic heavy metals in plants relates to sequestration of the metals in the vacuoles. The PC-metal complexes are driven by various membrane transporters (Cobbett 2000; Blaudez et al. 2003; Küpper et al. 2004; Cosio et al. 2004; Raab et al. 2004: Datta and Sarkar 2004). These membrane transporters include CPx-type ATPases, Nramp family of proteins and CDF family proteins as discussed earlier. More detailed insights on the characterization, isolation, cloning and regulation of transport of the PC-metal complexes from source to sink are needed to achieve better phytoremediation efficiency of the heavy metals using biotechnological approaches. Free histidine (His) has been reported to be Ni-chelator in *Alyssum lesbiacum* and *Brassica juncea* and it has been found to enhance release of Ni into the xylem during its transport to aerial parts (Kerkeb and Krämmer 2003). However, Persans et al. (1999) have reported that the Ni-hyperaccumulation phenotype in *Thlaspi goesingense* could not be related to the overproduction of His in response to nickel.

The phytoremediation mechanisms for most of the heavy metals thus seem to be governed by the ion transport and hyperaccumulation in the vacuolar sinks of the tolerant plants. Phytodegradation and phytovolatilization are the preferred mechanisms for the cleanup of organic xenobiotics (Morikawa and Takahashi 2000; Schwitzguébel 2004). These processes, however, also rely on the movement of the pollutants into plant roots and subsequent translocation into other tissues and parts of the plants, where the detoxification and metabolization take place (Schroeder et al. 2002). Higher plants have evolved many genes and enzymes, which have potentials to metabolize or degrade different kinds of xenobiotic compounds. Xenobiotic metabolism in plant cells proceeds through different partially linked stages (Schwitzguébel 2004 for a recent review). The reductive, oxidative and hydrolytic enzymes introduce functional groups (-OH, $-NH_2$, -SH) into lipophilic substrates in phase I reactions. Hydrolytic reactions, catalysed by esterases or amidases, are quite common and the multiple isoforms of substrate inducible enzymes have been reported. The oxidation reactions (epoxidation, O- or N-dealkylation, aryl- or alkylhydroxylation, N-or S-oxidation) appear to be catalysed by the cytochrome P450 mono-oxygenases. This process seems to be the most important in xenobiotics, phytoremediation. These enzymes are microsomal in localization and have been characterized well in mammalian systems. In plants, they are induced by wounding, pathogenesis and chemical stresses e.g. organic xenobiotic compounds. The wide range of transferases catalyze removal of glucosyl moieties, amino acids, malonic acid or glutathione residues in Phase II reaction. The herbicide and other xenobiotics metabolites containing these residues can be deposited as “bound residues” in the extracellular matrix/cell wall, or stored as water soluble metabolites in the vacuoles (Phase III) (Schwitzguébel 2004).

One of the major limitations in the phytoremediation of the organic pollutants, especially for the soil contaminants, has been realized as the poor understanding of the soil chemistry of these pollutants, their mobilization in the rhizosphere, their uptake and the transport within the plants (Cunningham et al. 1996; Sicilano and Germida 1998; Trapp and Karlson 2001; Mehmannavaz et al. 2002; Campanella et al. 2002; Harvey et al. 2002; Muratova et al. 2003; Schwitzguébel 2004). Rhizosphere microbes can play an important role in enhancing the bioavailability of the organic pollutants for the plant uptake. Uptake of hydrophobic xenobiotics of larger size can be facilitated by the primary microbial biodegradations in the rhizosphere. The hydrophobic persistent organic pollutants like polychlororinated dibenzo-p-dioxins and furans (PCDD/Fs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) with present log K_{ow} value above 4 are taken up by roots and transported to shoots by the transpiration stream of plants like Zucchini (*Cucurbita pepo*) (Campanella and Paul 2000). A proteinaceous molecule able to increase apparent aqueous solubility and binding during transport of such organic compounds have been found in the xylem sap and leaf extracts (Campanella and Paul 2000; Campanella et al. 2002).Hybrid poplar (*Populus* species) have also been demonstrated to remediate organic pollutants including trichloroethylene (TCE), a potential carcinogen commonly found in ground water and the contaminated sites (Kassel et al. 2002). Although many organic pollutants are metabolized or degraded to less toxic substances and accumulated in the phytoremediating plants, certain volatile organic chlorinated compounds e.g. BTEX (benzene, toluene, ethylbenzene, xylene), and MTBE (methyl tert-butyl ether) etc. can be released to the atmosphere. However, volatilization undermines the merits for phytoremediation for these applications (Schwitzguébel 2004). For such problems, rhizodegradation is usually attempted as a solution. However, large root absorption area, big root tip mass, high biomass with high enzymatic capabilities can make plants as ideal cleaning system of soil-based organic pollutants too, if bioavailability, uptake, transport and its metabolism can be regulated upto the desired extent. Though some success have been achieved to develop large scale commercial phytoremediation projects for cleanup of the sites or groundwater contaminated with organic xenobiotics (Table 2. Glass 1999; Trapp and Karlson 2001; Schwitzguébel 2004), but still this area needs more attention in the future.

Isolation, characterization and cloning of most appropriate genes from the organisms across the taxonomic boundaries, adequate promoters and regulatory genes (e.g. transcription factors), efficient genetic transformation and *in vitro* regeneration protocols can be seen as biotechnological approaches to resolve such problems of persistent organic pollutant, contamination. Plant genetic engineering has emerged as a technology which can create new potential character in a plant from a distantly related organism (beyond taxonomic boundaries) or even using synthetic genes and promoters. Many appropriate genes of foreign origin have been transferred in the plants like *Arabidopsis thaliana, Nicotiana tabaccum, Brassica juncea, Brassica oleracea* var *botrytis, Lycopersicon esculeutum* etc. to enhance the phytoremediation efficiency of these plants (Table 4. Raskin 1996; Rugh et al. 1996; Arazi et al. 1999; Arisi et al. 2000; Meagher 2000; Nedelkoska and Doran 2000; Assuncao et al. 2001). The genes of choice are related to the regulatory genes of sulfur metabolism, glutathione biosynthesis for the synthesis of binding peptide and proteins, uptake and transport proteins for the partitioning, targeting and metabolizing proteins/enzymes etc. which have enhanced significantly the potential of the phytoremediation using transgenic plants. Transgenic plants so far have been developed for the hyperaccumulation of toxic heavy metals e.g. Hg, As, Pb, Cd, Co, Ni, Zn, Cu etc. air pollutants e.g. NO_2 and SO_2 and organic pollutants e.g. 2,4,6-trinitrotoluene and organomercurials etc. The literature available on the genetic engineering of plants for phytoremediation indicate clearly that this technology can be used successfully to enhance rhizosphere degradation, bioavailbility, uptake, transport, targeting, partitioning, storage and hyperaccumulation of toxic pollutants of various kinds and also to resolve the problems associated with post harvest, management and recycling of the contaminated phytomass. It can combine the various characters of ideal phytoremediation in one plant which has fast growth, higher biomass, suitability for easy post harvest, agroclimatic adaptations and desired root size and root depth alongwith high efficiency to remediate specific contaminants as well as mixture of many contaminants. Rhizosphere management can also be enhanced by introducing genes for required plant exudates and microbial strains for better potential for supplementing phytoremediation by enhancing bioavailbility and solubility of the pollutants.

A lot of challenges are to be addressed, by the biotechnologists to meet out the commercial needs and to utilize an optimal potential of this technology. The major limitations of plant genetic engineering as a technology have been the availability of most appropriate genes based on wider prospecting of huge biodiversity, novel promoters and transcription regulators (transcription factors regulating larger metabolic pathways), genes for factors regulating post translation modification, targeting and transport proteins and peptides and the factors for the storage management of the metabolites etc. In addition, removal of non-required or deleterious associated genes (e.g. selectable and visible markers) and avoidance of pollen mediated flow of foreign gene e.g. chloroplast transformation etc. will be a focal attention in the recent future. Addressing these challenges environmental safer, free from any health hazards, high potentials economic phytoremediation can be developed using extensive bioprospecting and genetic engineering in recent future.

6. Conclusion

Phytoremediation is an eco-friendly cost-effective technology, as compared to classical physical, chemical and even to the microorganisms-based bioremediation techniques. It is useful for the remediation of sites contaminated with non-biodegradable toxic heavy metals, hazardous air pollutants like oxides of nitrogen and sulfur, and photoxidants like ozone, recalcitrant organic pollutants, like chlorinated pesticides, organophosphate, insecticides, petroleum hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), sulphonated biphenyl (PCBs) and chlororinated solvents (TCE, PCE) etc.

Amongst the major limitations of the technique, tolerance level of plants to high contamination zones, treatment of only bioavailable fraction of the contaminants and remediation of the contaminants largely from within a meter of the surface of the soil and within a few meters of the surface of the groundwater can be counted. The agro-climatic and hydrological conditions may also limit the plant growth on the treatment site and chances of entering of the contaminants in food chain through animals /insects that eat plant material containing the contaminants need to be attended while advocating for this technology. Plant biomass and agricultural vegetable wastes can also be used as adsorbant systems for the remediation of waterbodies from organic and inorganic pollutant’s contaminations. Due to the low cost of the technique, the low disturbance in the *in situ* treatments, a higher probability for the public acceptance and an easy handling, this technology indicates a strong potential as a natural, or improved, solar energy driven remediation approach for the treatments of the various kinds of the pollutants.

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