Phytoremediation, Transgenic Plants and Microbes

Kambaska Kumar Behera

Abstract Phytoremediation is a promising technology using plants and microbes to clean up contaminated air, soil, and water. Pollutants pose a global threat for agricultural production, productivity, wildlife and human health. Environmental pollution increasing in many parts of the world. Many methods of preventing, removing and or correcting the negative effects of pollutants exist but their application has either been poorly implemented or not at all. For phytoremediation selected or engineered plants and microbes are used to treat efficiently low to moderate levels of contamination.

Phytoremediation uses the age-long abilities of selected plants and microbes to remove pollutants from the environment. Phytoremediation will probably become a commercially available technology in many parts of the world including India. Currently \$6–8 billion a year is spent on environmental cleanup in the US. In the United Kingdom £4 million are spent on air pollution control and £1.5 million on water-treatment plant, and this cost is expected to increase by 50 % over the next 5 years. The cost of phytoremediation has been estimated as \$25–\$100 per ton of soil, and \$0.60–\$6.00 per 1,000 gallons of polluted water, with remediation of organics being cheaper than remediation of metals. Phytoremediation also offers a permanent *in situ* remediation rather than simply translocating the problem. This review focuses on the major concerns such as phytoremediation technologies, plant and microbes in phytoremediation and, ecological considerations of phytoremediation.

Keywords Contaminant • Heavy metals • Phytoremediation • Phytoextraction • Phytostabilization • Phytovolatilization • Rhizofiltration • Rhizosphere • Soil pollution • Transgenic Plants

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K.K. Behera (✉)
Department of Bio-Science and Biotechnology, Banasthali University,
Banasthali, Rajasthan 304022, India
e-mail: kambaska@yahoo.co.in

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List of Abbreviations

AM	Arbuscular Mycorrhizae
EDTA	Ethylene diamine tetra acetic acid
PCBs	Polychlorinated biphenyls
PCE	Tetrachloroethylene
TCE	Trichloroethylene
TNT	2,4,6-Trinitrotoluene

1 Introduction

Phytoremediation is a novel strategy for the removal of toxic contaminants from the environment by using selected plants and microbes. This concept is increasingly being adopted as it is a cost effective and user-friendly alternative to traditional methods of treatment (Pilon-Smits and Freeman 2006). Toxic metal pollution and xenobiotics in water and soil is a major environmental problem and most conventional remediation approaches do not provide acceptable solutions (Wand et al. 2002). Rapid growth in population and massive industrialisation in recent years has resulted in pollution of the biosphere. Plants and microbes possess some characteristic features which enable them to absorb from soil and water, such heavy metals which are essential for their growth and development (Ghosh and Singh 2005).

Our planet is increasingly polluted with inorganic and organic compounds, primarily as a result of human activities. While inorganic pollutants occur as natural elements in the Earth's crust and atmosphere, anthropogenic activities such as industry, mining, motorized traffic, agriculture, logging, and military actions promote their release and concentration in the environment, leading to toxicity (Nriagu 1979; Wand et al. 2002). Organic pollutants in the environment are mostly man-made and xenobiotic, which are not normally produced or expected to be present in organisms (Pulford and Watson 2008). Many of them are toxic or carcinogenic. Sources of organic pollutants in the environment include accidental releases of fuels and solvents, industrial activities releases chemical and petrochemical, agriculture activities releases pesticides and herbicides and military activities releases explosives and chemical weapons, among others. Moreover, polluted sites often contain a mixture of both organic and inorganic pollutants (Ensley 2000; Reichenauer and Germida 2008). Currently \$6–8 billion a year is spent on environmental cleanup in the US, and \$25–50 billion per year worldwide with ejected 173 million tons of contaminants annually into the atmosphere (Glass 1999; Tsao 2003; http://www.edwardgoldsmith.org/1072/pollution-costs/2/). Most remediation activity still makes use of conventional methods such as excavation and reburial, capping, and soil washing and burning. However, newly emerging biological cleanup methods, such as phytoremediation, are often simpler in design and cheaper to implement (Chaudhry et al. 1998; Khan 2005). Phytoremediation incorporates a range of technologies that use plants to remove, reduce, degrade, or immobilize environmental pollutants from soil and water, thus restoring contaminated sites to a relatively clean, non-toxic environment. The cost of phytoremediation has been estimated as \$25–\$100 per ton of soil, and \$0.60–\$6.00 per 1,000 gallons of polluted water with remediation of organics being cheaper than remediation of metals. In many cases phytoremediation has been found to be less than half the price of alternative methods. Phytoremediation also offers a permanent in situ remediation rather than simply translocating the problem. However phytoremediation is not without its faults, it is a process which is dependent on the depth of the roots and the tolerance of the plant to the contaminant. Exposure of animals to plants which act as hyperaccumulators can also be a concern to environmentalists as herbivorous animals may accumulate contaminate particles in their tissues which could in turn affect a whole foodweb (http://arabidopsis.info/students/dom/mainpage.html). Phytoremediation depends on naturally occurring processes, in which plants detoxify inorganic and organic pollutants, via degradation, sequestration, or transformation. The different uses of plants and their associated microbes for environmental cleanup are discussed (Salt et al. 1998; Meagher 2000; Pilon-Smits 2005; Kramer 2010).

2 Plants and Phytoremediation

Plants are chemical factories that influence their environment not only by uptake of substances but also by exudation of many molecules that are produced in primary and secondary metabolism (Pilon-Smits 2005; Kramer 2010). This lively chemical and physical interaction of plants with their surrounding environment can be used for the remediation of contaminated sites. The contaminants may be taken up and metabolized by plants, immobilized on roots, or degraded by microorganisms living in the areas around the root of the plants. The methods that use plants for the remediation of contaminated sites are categorized under the term “phytoremediation”. The broader term “phytotechnology” is also used; however, this includes other methods such as constructed wetlands or ground cover plants for minimizing erosion (Zeng-Yei et al. 2010).

3 Phytoremediation Technologies

Phytoremediation explores plant’s innate biological mechanisms for human benefit. The subsets of this technology as applicable to remediation process are:

3.1 Phytoextraction

Phytoextraction is the removal of pollutants by the roots of plants, followed by translocation to above ground plant tissues, which are subsequently harvested

(Weyens et al. 2009). Continuous phytoextraction uses plants that accumulate high levels of pollutants over their entire lifetime. Induced phytoextraction enhances pollutant accumulation towards the end of the plant's lifetime, when they attain their maximal biomass, by adding chelators to the soil that reversibly bind the pollutant (usually a metal), releasing it from the soil and making it available for plant uptake. The technique is especially useful when dealing with toxic pollutants that cannot be biodegraded, such as metals, metalloids, and radionuclides (Dowling and Doty 2009). One category of plants that shows potential for phytoextraction, either as a gene source or for direct use, are the so-called hyper accumulators, plants that accumulate toxic elements to levels that are at least 100-fold higher than non-accumulator species (Baker and Brooks 1989; Peer et al. 2005). Hyper accumulator plants tend to grow slowly, which limits their usefulness for phytoremediation. Nevertheless, their growth rate may be improved through selective breeding (Chaney et al. 2007), and the transfer of metal hyper accumulation genes to high-biomass, fast growing species may also help to circumvent the problem (Le Duc et al. 2004, 2006). This technique saves tremendous remediation costs by accumulating low levels of contamination from a widespread area to an easily severable medium. Plants that are promising for phyto-extraction include the mustard plant and some varieties of broccoli and cabbage, which have the required tissue mass to absorb large quantities of metal, tend to pull the metal up into their shoots, and grow relatively quickly (Nakamura et al. 2008; Bi et al. 2011). Nickel and zinc appear to be most easily absorbed, although preliminary results for copper and cadmium are encouraging. The plants involved must have a relatively short lifecycle to facilitate the process which must be economically viable (Kramer et al. 1996).

3.2 Phytotransformation

It is the process by which plants chemically transform contaminants to more stable, less toxic, or less mobile forms. Metals like chromium can be reduced from the carcinogenic, highly mobile hexavalent form to the less toxic, non carcinogenic, less mobile trivalent form that easily binds to organic plant matter and renders the chromium fairly inert (Lee et al. 2006; Newman et al. 1997). The phytotransformation activities of plant mainly done by enzymes or enzyme co-factors (Dec and Bollag 1990). Dec and Bollag (1994) describe plants that can degrade aromatic rings in the absence of micro-organisms. Polychlorinated biphenyls (PCBs) have been metabolized by sterile plant tissues. Phenols have been degraded by plants such as potato (*Solanum tuberosum*),and white radish (*Raphanus sativus*) that contains peroxidase (Dec and Bollag 1994; Roper et al. 1996). Poplar trees (*Populus* spp.) are capable of transforming trichloroethylene in soil and ground water (Newman et al. 1997; Rosselli et al. 2003). Enzymes of particular interest for phytoremediation include: (1) dehalogenase (transformation of chlorinated compounds) (2) peroxidase (transformation of phenolic compounds) (3) nitroreductase (transformation of explosives and other nitrated compounds) (4) nitrilase

Table 1 Important enzymes of plant useful in transforming organic compounds

Sl.No.	Enzyme	Plants known to produce enzymatic activity	Application
1	Dehalogenase	Hybrid poplar (*Populus* spp.), algae (various spp.), parrot feather (*Myriophyllum aquaticum*)	Dehalogenates chlorinated solvents
2	Laccase	Stonewort (*Nitella* spp.), parrot-feather (*Myriophyllum aquaticum*)	Cleaves aromatic ring after TNT is reduced to triaminotoluene
3	Nitrilase	Willow (*Salix* spp.)	Cleaves cyanide groups from aromatic rings
4	Nitroreductase	Hybrid poplar (*Populus* spp.), Stonewort (*Nitella* spp.), parrot feather (*Myriophyllum aquaticum*)	Reduces nitro groups on explosives and other nitroaromatic compounds, and removes nitrogen from rings structures
5	Peroxidase	Horseradish (*Armoracia rusticana* P. Gaertner, Meyer & Scherb)	Degradation of phenols (mainly used in wastewater treatment)
6	Phosphatase	Giant duckweed (*Spirodela polyrhiza*)	Cleaves phosphate groups from large organophosphate pesticides

(transformation of cyanated aromatic compounds) and (5) phosphatise (transformation of organophosphate pesticides) (Frova 2003; Cobbett and Goldsbrough 2002; Fletcher et al. 2005; Subramanian et al. 2006). A list of important enzymes of plant involved in phytoremediation process listed in Table 1.

3.3 Phytostabilization

In this process plant minimize the mobility and migration of potential contaminants in soils. This process takes advantage of plant roots ability to alter soil environment conditions, such as pH and soil moisture content (EPA 1998, 1999; Kramer et al. 2000). Many root exudates cause metals to precipitate, thus reducing bioavailability. This is the most experimental form of phytoremediation, but has potential applicability for many metals, especially lead, chromium, and mercury are stabilized in the soil (Cunningham et al. 1995) and reduce the interaction of these contaminants with associated biota. The success of phyto-remediation is dependent on the potential of the plants to yield high biomass and withstand the metal stress. Besides, the metal bioavailability in rhizosphere soil is considered to be another critical factor that determines the efficiency of metal translocation and phytostabilization process (Ma et al. 2011a).

In recent years, several chemical amendments, such as ethylene diamine tetra acetic acid (EDTA), limestone have been used to enhance phyto-stabilization process (Barrutia et al. 2010; Wu et al. 2011). Even though these amendments increase the efficiency of phytostabilization process, some chemical amendments (e.g., EDTA) are not only phytotoxic (Evangelou et al. 2007) but also toxic to beneficial soil microbes that play important role in plant growth and development (Muhlbachova 2009; Ultra et al. 2005).

3.4 Phytovolatilization

Phytovolatilization is a mechanism by which plants convert a contaminant into a volatile form, thereby removing the contaminant from the soil or water (Singh et al. 1980; Toro et al. 2006; Terry et al. 1992) at the contaminated site. In this process plants, possibly in association with microorganisms, can convert selenium to dimethyl selenide which is the non toxic form (Kumar et al. 1995; Brooks et al. 1998). Dimethyl selenide is a less toxic, volatile form of selenium. Phytovolatilization may be a useful, inexpensive means of removing selenium from sites contaminated with high concentration selenium wastes (Zayed et al. 1998; Zhang and Moore 1997; Pilon-Smits and LeDuc 2009). Similarly, some transgenic plants (e.g., *Arabidopsis thaliana*) have converted organic and inorganic mercury salts to the volatile, elemental form (Watanabe 1997; van Hoewyk et al. 2008; Zeng-Yei et al. 2010).

3.5 Rhizodegradation

Rhizodegradation is a biological treatment of a contaminant by enhanced bacterial and fungal activity in the rhizosphere of certain vascular plants. The rhizosphere is a zone of increased microbial density and activity at the root/surface, and was described originally for legumes by Lorenz Hiltner in 1904 (Curl and Truelove 1986; Khan 2005). Plants and micro-organisms often have symbiotic relationships making the root zone or rhizosphere an area of very active microbial activity (Anderson et al. 1993; Anderson and Coats 1994; Schnoor et al. 1995; Siciliano and Germida 1998a, b; Khan 2005). Plants can moderate the geochemical environment in the rhizosphere, providing ideal conditions for bacteria and fungi to grow and degrade organic contaminants. Plant litter and root exudates provide nutrients such as nitrate and phosphate that reduce or eliminate the need for costly fertilizer additives. Plant roots penetrate the soil, providing zones of aeration and stimulate aerobic biodegradation (Moorehead et al. 1998; Singer et al. 2003; Newman and Reynolds 2004). Many plant molecules released by root die back and exudation resemble common contaminants chemically and can be used as co-substrates. The phenolic substances released by plants have been found to stimulate the growth of Polychlorinated biphenyl (PCB) degrading bacteria (Fletcher and Hedge 1995; Fletcher et al. 1995; Aken 2008; Aken et al. 2010). Recent studies have described enhanced degradation of penta-chlorophenolin the

Table 2 Commonly used plant species in phytoremediation of organic compounds

Name of the Plant	Common Name	Contaminant	Reference
(*Hordeum* v*ulgare* L. cv. Klages)	Barley	Hexachlorobenzene, PCBs, Pentachlorobenzene, Trichlorobenzene	McFarlane et al. (1987)
Panicum antidotale, Panicum maximum, Pennisetum Purpureum, Vetiveria zizynoides etc	Forage grasses	Chlorinated benzoic acids	Siciliano and Germida (1998a)
Myriophyllum aquaticum	Parrot feather	Tetrachloroethane (PCE), Trichloroethane (TCE), TNT	Best et al. (1997)
Populus hybrids	Hybrid poplar	Atrazine, nitrobenzene, TCE, TNT	Burken and Schnoor (1997)
Bromus catharticus	Prairie grass	2-chlorobenzoic acid	Topp et al. (1989)
Glycine max (L.) Merr. cv Fiskby v	Soyabean	Bromacil, nitrobenzene, phenol	Fletcher et al. (1990)
Myriophyllum spicatum	Eurasian watermilfoil	TNT	Hughes et al. (1997)
Helianthus annuus	Sun flower	Cd, Cr, and Ni	Turgut et al. (2004)
Jatropha curcas	Baigaba	Soil contaminated with lubricating oil	Agamuthua et al. (2010)

rhizosphere of wheat grass (*Agropyroncristatum*) (Ferro et al. 1994; Alkorta and Garbisu 2001), increased initial mineralization of surfactants in soil-plant cores (Knabel and Vestal 1992), and enhanced degradation of Trichloroethylene (TCE) in soils collected from the rhizospheres. Anderson et al. (1993) provides a review of microbial degradation in the rhizosphere. Thus, current research suggests the interaction between plants and soil microbes may be an important factor influencing biological remediation of contaminated soils.

Rhizofiltration: Rhizofiltration uses plant roots to filter contaminants directly out of waste streams, in either a hydroponic or a constructed wetland setting. Rhizofiltration is also suitable for inorganics, as the plant material can be replaced periodically. Erosion and leaching often mobilize soil contaminants, resulting in additional aerial or waterborne pollution. This process is used to reduce contamination in natural wetlands and estuary areas although the technology has been extended to engineered applications like gray water and wastewater treatment. It also includes the use of plants to absorb, concentrate, and remove toxic metals from polluted streams. Many submerged and floating aquatic plants are particularly adept for rhizofiltration. Also, flow-through rhizofiltration systems can be designed for removing contaminants from water by pumping the water through a trough planted with contaminant accumulating plants (Knox et al. 1984; EPA 2001). The water moves through the cycle until it is clean enough to be discharged. However, metals and other contaminants become concentrated in plant biomass, which eventually must be disposed (Table 2).

4 Characteristics of Plant Species for Phytoremediation

Populations of metal-tolerant, hyper accumulating plants can be found in naturally occurring metal-rich sites (Baker and Brooks 1989). However, these plants are not ideal for phyto-remediation since they are usually small and have a low biomass production. In contrast, plants with good growth usually show low metal accumulation capability as well as low tolerance to heavy metals.

A plant suitable for phytoremediation should possess the following characteristics: -

1. Ability to accumulate the metal (s) intended to be extracted, preferably in the above ground parts
2. Plants which do not translocate metals to the above-ground parts could be useful for phytostabilization and landscape recreation
3. Tolerance to the metal concentrations accumulated
4. Fastgrowth and effective for metal accumulating biomass and be ideally repulsive to herbivores to avoid the escape of accumulated metal (loid)s to the food chain
5. Have a widely distributed and highly-branched root system
6. Easy to cultivate and have a wide geographic distribution
7. Easily harvestable

5 Transgenic Plants and Phytoremediation

Transgenic plants are genetically modified organisms. In genetic engineering, plants are induced to take up a piece of DNA containing one or a few genes originating from either the same plant species or from any different species, including bacteria or animals (Kassel et al. 2002; Ruiz et al. 2003). The foreign piece of DNA is usually integrated into the nuclear genome, but can also be engineered into the genome of the chloroplast. Foreign DNA may cause an existing enzymatic activity to become up-regulated (over expression) or down-regulated (knockout/knockdown), or may introduce an entirely new enzymatic activity altogether. The expression of the introduced gene can be regulated by using different promoters. The gene product, a protein, may be present at all times, in all tissues (constitutive expression), or only in specific tissues (only in roots) or at specific times (only in the presence of light or a chemical inducer) (Cherian and Margaridaoliveira 2005). Moreover, using different targeting sequences, which function as “address labels”, the protein may be directed to different cellular compartments, such as the chloroplast, the vacuole, or the cell wall. In addition to the gene of interest, a marker gene is usually included in the gene construct so that transgenics can be selected for after the transformation event. Usually these marker genes confer herbicide or antibiotic resistance. The introduced genes integrate into the host DNA and are inherited by the offspring like any other gene. In the context of phytoremediation, it is desirable to engineer high-biomass producing, fast-growing plants with an enhanced capacity to tolerate pollutants. In addition, if a pollutant is remediated via accumulation, as is often the case for inorganics, transgenics may be engineered to possess improved pollutant uptake and root shoot translocation abilities. If the pollutant is remediated by degradation, as organics often are, enzymes that facilitate degradation in either the plant tissue or the rhizosphere (the region just outside of the root) may be over expressed. In cases where pollutants are volatilized, enzymes involved in the volatilization process may be over expressed. If a transgenic approach is to be used to breed plants with superior phytoremediation properties, it is necessary to understand the underlying mechanisms involved. Once potential rate-limiting steps have been identified by means of physiological and biochemical experiments, the specific membrane transporters or enzymes responsible can be singled out for over expression. If the genes encoding these proteins are available from any organism, they can be introduced into the plant and the transgenics can be compared with the wild type with respect to pollutant remediation. A great deal of research has been carried out to investigate mechanisms involved in plant uptake of inorganic and organic pollutants and their fate in the plant (Meagher 2000; Burken 2003). Generally, inorganics are taken up by transporters for essential elements, advertently if they are indeed essential, or in advertently if they are chemically similar to essential elements. Once inside the plant they may be detoxified by chelation and by compartmentation in a safe place such as the vacuole. Organics can move passively across plant membranes if they have the right degree of hydrophobicity, corresponding to a log Kow (octanol: water partition coefficient) of 0.5–3.0 (Wu et al. 2006). More hydrophilic organics cannot pass the hydrophobic interior of membranes passively, and there are usually no suitable transporters if they are foreign to the plant. Organic pollutants that do make it into the plant can be detoxified by enzymatic degradation. They may also be stored in the vacuole or cell wall, after enzymatic modification and conjugation to glutathione or glucose, the latter referred to as the “green liver model (Sandermann 1994; Coleman et al. 1997).

6 Microbes and Phytoremediation

A promising alternative to chemical amendments could be the application of microbe-mediated processes, in which the microbial metabolites/processes in the rhizosphere affect plant metal uptake by altering the mobility and bioavailability (Aafi et al. 2012; Glick 2010; Ma et al. 2011a; Miransari 2011; Rajkumar et al. 2010; Wenzel 2009; Yang et al. 2012). When considering approaches to alter heavy metal mobilization, there are several advantages to the use of beneficial microbes rather than chemical amendments because the microbial metabolites are biodegradable, less toxic, and it may be possible to produce them in situ at rhizosphere soils. In addition, plant growth promoting substances such as siderophores, plant growth hormones, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase produced by plant-associated microbes improve the growth of the plant in metal contaminated soils (Babu and Reddy 2011; Glick 2010; Glick et al. 2007; Kuffner et al. 2008; Lebeau et al. 2008; Luo et al. 2011, 2012; Ma et al. 2011a, b; Miransari 2011; Rajkumar et al. 2010; Wang et al. 2011; Wu et al. 2006). Overall the microbial activities in the root/rhizosphere soils enhance the effectiveness of phytoremediation processes in metal contaminated soil by two complementary ways: (i) Direct promotion of phytoremediation in which plant associated microbes enhance metal translocation (facilitate phytoextraction) or reduce the mobility/availability of metal contaminants in the rhizosphere (phytostabilization) and (ii) Indirect promotion of phytoremediation in which the microbes confer plant metaltolerance and/or enhance the plant biomass production in order to remove/arrest the pollutants.

Plant associated-microbes can also immobilize the heavy metals in the rhizosphere through metal reduction reactions. Chatterjee et al. (2009) reported that the inoculation of Cr-resistant bacteria *Cellulosimicrobium cellulans* to seeds of green chilli grownin Cr (VI) contaminated soils decreased Cr uptake into the shoot by 37 % and root by 56 % compared with uninoculated controls. This study indicates that bacteria reduced the mobile and toxic Cr (VI) to nontoxic and immobile Cr (III) in the soil. According to Abou-Shanab et al. (2007) the lower Cr translocation from root to shoots of water hyacinth is indicative of a Cr reducing potential of rhizosphere microbes. In a similar study Di Gregorio et al. (2005) demonstrated the Se reducing potential of *Stenotrophomonas maltophilia* isolated from the rhizosphere of *Astragalus bisulcatus*. They reported that this bacterium significantly reduced soluble and harmful Se (IV) to insoluble and unavailable Se (0) and thereby reducing the plant Se uptake. These examples illustrate mechanisms, by which metal reducing microbes immobilize metals within the rhizosphere soil and reflect the suitability of these microbes for phytostabilization applications.

Besides, the synergistic interaction of metal oxidizing and reducing microbes on heavy metal mobilization in contaminated soils has also been studied. Beolchini et al. (2009) reported the inoculation of Fe-reducing bacteria and the Fe/S oxidizing bacteria together significantly increased the mobility of Cu, Cd, Hg and Zn by 90 % and they attributed this effect to the coupled and synergistic metabolism of oxidizing and reducing microbes. Though these results open new perspectives for the bioremediation technology for metal mobilization, further investigations are needed to utilize such bacteria in phytoextraction practices.

6.1 Endophytic Bacteria and Phytoremediation

Endophyte-assisted phytoremediation is a promising new field to improve remediation by utilizing microorganisms that live within plants to improve plant growth, increase stress tolerance, and degrade pollutants. These are the bacteria colonizing the internal tissues of plants without causing symptomatic infections or negative effects on their host (Schulz and Boyle 2006). Endophytic bacteria reside in apoplasm or symplasm. Although bacterial endophytes exist in plants variably and transiently (van Overbeek and van Elsas 2008), they are often capable of triggering physiological changes that promote the growth and development of the plant (Conrath et al. 2002).In general, the beneficial effects of endophytes are greater than those of many rhizobacteria (Pillay and Nowak 1997) and these might be aggravated when the plant is growing under either biotic or abiotic stress conditions (Barka et al. 2002; Hardoim et al. 2008). Endophytic bacteria have been isolated from many different plant species (Lodewyckx et al. 2002; Idris et al. 2004; Barzanti et al. 2007; Sheng et al. 2008; Mastretta et al. 2009); in some cases, they may confer to the plant higher tolerance to heavy metal stress and may stimulate host plant growth through several mechanisms including biological control, induction of systemic resistance in plants to pathogens, nitrogen fixation, production of growth regulators, and enhancement of mineral nutrients and water uptake (Ryan et al. 2009). Additionally observed beneficial effects due to bacterial endophytes inoculation are plant physiological changes including accumulation of osmolytes and osmotic adjustment, stomatal regulation, reduced membrane potentials, as well as changes in phospholipid content in the cell membranes (Compant et al. 2005). Further, the endophytic bacteria isolated from metal hyper accumulating plants exhibit tolerance to high metal concentrations (Idris et al. 2004). This may be due to the presence of high concentration of heavy metals in hyper accumulators, modulating endophytes to resist/adapt to such environmental conditions. It is also possible that the metal hyper accumulating plants may simultaneously be colonized by different metal-resistant endophytic bacteria ranging wide variety of gram-positive and gram-negative bacteria (Rajkumar et al. 2009).

6.2 Arbuscular Mycorrhizae and Phytoremediation

AM fungi are ubiquitous soil microbes occurring in almost all habitats and climates, including metal contaminated soils (Chaudhry and Khan 2002; Mastretta et al. 2006) and are considered essential for the survival and growth of plants growing in nutrient especially phosphorus deficient derelict soils. However, polluted wastelands contain reduced population diversity and numbers of autochthonous AM strains which are heavy metal tolerant (Chaudhry and Khan 2003). Studies with AM fungi have focused on their ability to enhance nutrient uptake in a nutrient deficient soil and have ignored the role they may play in phytoremediation. The prospect of fungal symbionts existing in metal contaminated soils has important implications for phytoremediation (mycorrhizo-remediation) of metal contaminated soils as AM fungi help plant growth through enhanced nutrient uptake. Plant species belonging to plant families *Chenopodiaceae, Cruciferaceae, Plumbaginaceae, Juncaceae, Juncaginaceae, Amaranthaceae* and few members of *Fabaceae*, are believed not to form a symbiosis with AM (Smith and Read 1997). In some cases, arbuscular mycorrhizal fungi have been shown to increase uptake of metals (Liao et al. 2003; Whitfield et al. 2004; Citterio et al. 2005) and arsenic (Liu et al. 2005; Leung et al. 2006) in plants but other studies showed no effect (Trotta et al. 2006;

Wu et al. 2007) or decreased concentrations in plant tissues. The contrasting results are difficult to evaluate and may be partly due to different experimental settings (Liu et al. 2005; Leung et al. 2006) versus field studies (Trotta et al. 2006; Wu et al. 2007) as in the case of arsenic uptake in *Pteris vittata* inoculated with arbuscular mycorhizal fungi.

6.3 Importance of Endophytic Bacteria

(i) Genetic engineering of endophytic bacteria is easier than the genetic engineering of plants. In addition, if strains are selected that can successfully colonize multiple plants, only one bacterial line would need to be created.
(ii) Gene expression within endophytes might be useful as a site-monitoring tool. Using plants as soil and groundwater samplers would yield both active and passive sampling characteristics at a low cost. Specific gene expression within endophytes, such as that possible with quantitative polymerase chain reaction, might then be an effective measurement tool. This approach would lessen the need for expensive sampling and analysis on heterogeneous sites.
(iii) Bacterial endophytes might function more effectively than bacteria added to soil would because of a process known as bioaugmentation. The plant provides aready-made environment for endophytic bacteria so competition pressure against colonization of the desired organism, as often occurs in soils, would be reduced.
(iv) If bacterial lines are carefully selected so that the strains are at a competitive disadvantage when not living as a plant endophyte, the movement of engineered genes in the environment would be greatly reduced.

7 Advantage and Disadvantage of Phytoremediation

Advantages	Disadvantages
It works on a variety of organic and inorganic compounds	It may take several years to remediate
It can be either in *situ/ex situ*	It may depends on climatic conditions
The technique is easy to implement and maintain	The technique restricted to sites with shallow contamination within rooting zone
Less costly compared to other treatment methods	Harvested biomass from phytoextraction may contain hazardous waste
Ecofriendly and aesthetically pleasing to the public	Consumption of contaminated plant tissue is also a concern
Reduces the amount wastes to be landfilled	Possible effect on the food chain

8 Ecological Considerations

Many ecological issues need to be evaluated when developing a remediation strategy for a polluted site. In particular, one has to consider how the phytoremediation efforts might affect local ecological relationships. As described above and shown in Fig. 1, phytoremediation-related processes can change the location or chemical makeup of contaminants in the polluted area. The question is, how do those processes affect the ecological interactions among the biota in the ecosystem? The choice of plant species for remediation will, of course, greatly influence which ecological partners and interactions will be present at the site, and consequently the fate of the pollutant. The direct ecological partners of phytoremediator plants include bacteria, fungi, animals, and other plants, all occurring inside, on, or in the vicinity of the roots and shoots of the phytoremediator plants (Fig. 1). These partners may be affected positively or negatively by the ongoing phytoremediation process. If the plants stabilize or degrade the pollutant, there by limiting its bioavailability and concentration, the phytoremediation process will probably benefit other organisms in the area. If, on the other hand, the plants accumulate the pollutant or its degradation products in their tissues, this may adversely affect microorganisms that live on or inside the plant (Angle and Heckman 1986), as well as root and shoot herbivores, and pollinators. Volatilization of a pollutant will simultaneously dilute and disperse the pollutant, which may affect ecosystems both on and off the site (Li et al. 2003; Lai et al. 2008). In addition to the direct ecological partners of the phytoremediator plants, the phytoremediation processes may also affect other trophic levels. If a

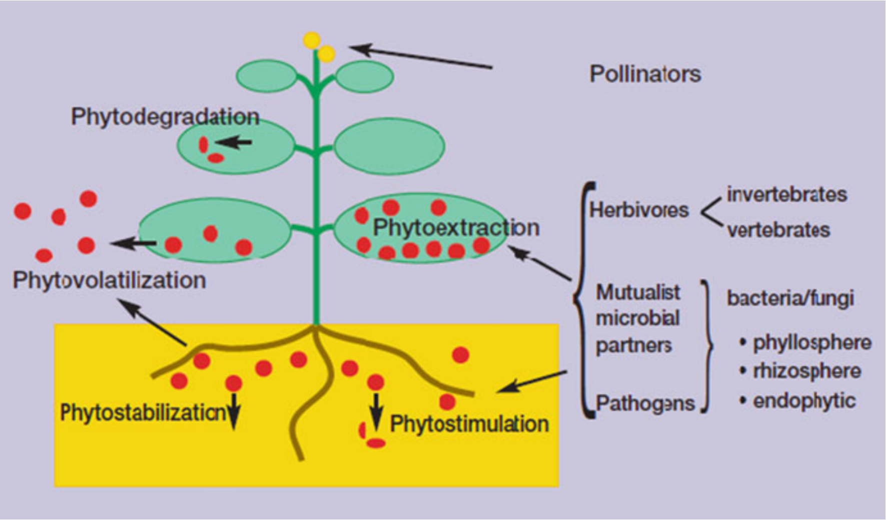

Fig. 1 Schematic overview of phytoremediation methods. Shown on the *right* are some ecological partners of the plants that may influence phytoremediation

pollutant is accumulated by the plant, this may facilitate its entry into the food chain, as depicted in Fig. 1. Conversely, these ecological partners may affect the remediation process positively or negatively, by interacting with the pollutant directly or with the plants. Herbivores or pathogens may hamper plant growth and thus the phytoremediation efficiency. On the other hand, rhizosphere or endophytic microorganisms may make pollutants more bio available for plant uptake, or may assist in the biodegradation process. While it is known that plant–microbe consortia often work together in remediation of organic pollutants (Olson et al. 2003; Barac et al. 2004; Van Aken et al. 2004; Taghavi et al. 2005), much still remains to be discovered about the nature of the interactions and the molecular mechanisms involved (e.g., signal molecules, genes induced).

Chaney et al. (1997) calculated that metal tolerance and hyper accumulation would be more important to phytoremediation than high biomass production. For an effective development of phytoremediation, each element must be considered separately because of its unique soil and plant chemistry. On the other hand, metals rarely occur alone and adaptive tolerance may be needed for several metals simultaneously, even though phytoextraction of only one metal would be the goal. In some cases it might be desirable also to extract more than one metal at the same time. To merge the high metalloid accumulation capacity with such preferable plant anatomy and growth characteristics, efforts are being made for the genetic manipulation of candidate plants in order to improve their uptake, translocation and tolerance.

9 Conclusion

A polluted site and pollutant poses a risk to the environment as well as to the biota. This risk is correlated with the toxicity and concentration of the pollutant, the likeliness of its mobilization and spread by water and wind, and the proximity of sensitive and interaction to the ecosystems. The remediation strategies available for site specific cleanup will vary in their effectiveness in alleviating the existing risks and in the characteristics of their associated risks, and will also have different timelines and price tags. For each individual site, these initial risks will need to be addressed and evaluated in order to design an optimal remediation approach. Once the remediation strategy is decided, steps must be taken to lessen the associated risks. In the case of phytoremediation, careful choice of plant species and management practices are key to promoting ecological restoration and preventing pollutant dispersal. Where possible, native plant species with effective remediation properties and that provide natural hydraulic control (e.g., trees) and soil stabilization (e.g., grasses) should be selected. Drip irrigation can be used to prevent leaching, and fencing will minimize pollutant entry into the food chain. Phytoremediation is an interdisciplinary technology that will benefit from research in many different areas. Much still remains to be discovered about the biological processes that underlie a plant's ability to detoxify and accumulate pollutants. Better knowledge of the biochemical mechanisms involved may lead to: (1) the identification of novel genes and the subsequent development of transgenic plants with superior remediation capacities; (2) a better understanding of the ecological interactions involved (e.g., plant microbe interactions); (3) the effect of the remediation process on the existing ecological interactions; and (4) the entry and movement of the pollutant in the ecosystem. In addition to being desirable from a fundamental biological perspective, this knowledge will help improve risk assessment during the design of remediation plans (including the additional risks of transgenic plants) as well as alleviation of the associated risks during remediation.

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