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ROLE OF TRANSGENIC PLANTS IN PHYTOREMEDIATION : APPLICATIONS, CURRENT STATUS AND FUTURE PROSPECTIVES

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This review highlights the recent developments and achievements made in the field of phytoremedation particularly in the developing countries like India. Phytoremediation is nothing but cleaning the environment with the help of plants. Plant plays an important role in cleaning the environment. Phytoremediation is thought to be a cost effective and environmentally friendly technology to remove toxic metals from soils. In a recent study transgenic poplars have been developed with an enhanced uptake and metabolism of toxic volatile pollutants. Plants are autotrophic organisms capable of using sunlight and carbon dioxide as sources of energy and carbon. However, phytoremediation also suffers from several limitations, among which the most commonly evoked are the slow rate of removal, incomplete metabolism and potential increase in bioavailability of toxic contaminants.

Keywords: Cleaning; Environment; India; Phytoremediation; Transgenic plants.

Introduction

Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless[1]. Rapid industrialization coupled with increased urbanization and changed agricultural practices in developing countries like India have enhanced the levels of contaminants in the environment, with a consequent impact on human health. Cleaning up of the environment by removal of hazardous contaminants is a crucial problem, which needs multi-faceted approaches for reaching suitable solutions. Significant progress has been made in recent years in developing native or genetically modified plants for the remediation of environmental contaminants. Phytoremediation is thought to be a cost effective and environmentally friendly technology to remove toxic metals from soils. Phytoremediator should be fast growing, develop a large biomass, be tolerant to and accumulate high concentrations of toxic metals in the shoot, and be easily cultivated and harvested[2-3]. Heavy metals have different patterns of behaviour and mobility within a tree. Lead, chromium and copper tend to be immobilised and held primarily in the roots, whereas Cd, Ni and Zn are more easily translocated to the aerial tissues. Heavy metals cause problems at high concentrations and when they are sufficiently environmentally mobile, they can move between media (e.g. soil to water) or can be taken up by living organisms[1-3]. Plants often use pathways and enzymes similar to those of mammals, which led to the 'green liver' concept. However, being autotrophic organisms, plants do not actually use organic compounds for their energy and carbon metabolism[4]. As a consequence, they usually lack the catabolic enzymes necessary to achieve full mineralization of organic molecules, potentially resulting in the accumulation of toxic metabolites. Hence, the idea to enhance plant biodegradation by genetic transformation was developed, following a strategy similar to that used to develop transgenic crops[5-6]. The purpose of this review is to provide a summary of the recent advances in development of transgenic plants for phytoremediation. It also highlights about their mechanism, physiology and phytoremediation applications, limitations and future prospectives.

Phytoremediation: Importance and characteristics- Phytoremediation– the use of plants to remediate the pollutants has the advantage of cleaning up the environment, especially soils and solutions, coupled with its aesthetic, environmentally friendly and economic qualities[7-10]. Six main subgroups of phytoremediation have been identified[11-16]; (1) Phytoextraction: plants remove metals from the soil and concentrate them in the harvestable parts of plants.2) Phytodegradation: plants and associated microbes degrade organic pollutants.(3) Rhizofiltration: plant roots absorb metals from waste streams. (4) Phytostabilisation: plants reduce the mobility and bioavailability of pollutants in the environment either by immobilisation or by prevention of migration. (5) Phytovolatilisation: volatilisation of pollutants into the atmosphere *via* plants, for example, poplar, eucalyptus, Indian mustard, tobacco, and yellow poplar have been reported. 6) Evapotranspiration: this is the results of the combined effects of plants both to evaporate water on their leaf surfaces and to vaporize water at the stomata.

However, phytoremediation also suffers from several limitations, among which the most commonly evoked are the slow rate of removal, incomplete metabolism and potential increase in bioavailability of toxic contaminants. The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a 'green', sustainable process. Some species may be capable of mobilising metals from less-soluble soil fractions in comparison to nonhyperaccumulating species[16-19]. However, despite great promise, rather slow removal rates and potential accumulation of toxic compounds within plants might have limited the application of phytoremediation.

In a recent study transgenic poplars have been developed with an enhanced uptake and metabolism of toxic volatile pollutants. Plants are autotrophic organisms capable of using sunlight and carbon dioxide as sources of energy and carbon. However, plants rely on the root system to take up water and other nutrients, such as nitrogen and minerals, from soil and groundwater. The ideal plant species to remediate a heavy metal-contaminated soil would be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest. This could be done by conventional plant breeding programmes or by genetic manipulation. Typically, transgenic plants exhibiting new or improved phenotypes are engineered by the over expression and/or introduction of genes from other organisms, such as bacteria or mammals. Historically, transgenic plants for phytoremediation were first developed is an effort to improve heavy metal tolerance; for example, tobacco plants (*Nicotiana tabacum*) expressing a yeast metallothionein gene for higher tolerance to cadmium, or *Arabidopsis thaliana* over expressing a mercuric ion reductase gene for higher tolerance to mercury. The first attempts to transform plants for phytoremediation of organic compounds targeted explosives and halogenated organic compounds in tobacco plants [11-27].

Applications to Air Pollution- Nitrogen dioxide (NO_2) is a major air pollutant, which forms photooxidants such as ozone by the photochemical reactions with hydroxyl radicals [28]. Plants are reported to assimilate the nitrogen in NO_2 to organic compounds, including amino acids. Genetic manipulation of plants is important to produce "wonder plants" that can clean up and serve as powerful sinks of air pollutants. Although, not all nitrogen derived from NO_2 is metabolized through a primary nitrate assimilation pathway, the major portion of NO_2 taken up by plants is assimilated through a primary nitrate assimilation pathway. Therefore, those enzymes involved in the primary metabolism of nitrate, such as nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS), which are respectively the first, second and third enzymes in primary nitrate metabolism, may play a key role in the metabolism of NO_2-nitrogen in plants. All genes for NR, NiR and GS are nuclear-encoded. Transgenic *Arabidopsis, Pittosporum tobira* and *Raphiolepis umbellata* plants or calli bearing an expression cassette of the complementary DNA (cDNA) of NiR gene from spinach or *Arabidopsis* have been produced and studied. Transgenic *Arabidopsis* plants bearing an expression cassette of the cDNA of tobacco NR gene or that of GS gene from *Arabidopsis* have also been studied[29-32]. N_2O is known to be a key atmospheric greenhouse gas that contributes to global climatic change through radiative warming and depletion of stratospheric ozone.

Trees for phytoremediation-Phytoremediation employs the use of plants, alone or together with their associated microorganisms, to degrade, contain or stabilize various environmental contaminants in soil, water, and air. The main characteristic of trees, that makes them suitable for phytoremediation, is their large biomass, both above and below ground. In addition to the direct stabilisation of the soil by the tree roots, the vegetation cover decreases the risk of soil loss by wind and water erosion. Leaf fall adds significant amounts of organic matter to the surface layers of the soil, promoting nutrient cycling, soil aggregation and water holding ability. Dead tree roots and root exudates also contribute to this. The large amount of water removed from soil by the transpiration stream decreases the downward flow through the soil, and so reduces leaching losses. Overall, therefore, the growing of trees can contribute positively to physically stabilising contaminated land. The evidence for chemical phytostabilisation is still sparse, and this is an important area requiring research in the future.

The long generation time of trees acts to prevent a rapid selection of heavy metal tolerant genotypes, the production of which is random or induced by the pollutant. Therefore, tree species are generally not able to adapt to high concentrations of heavy metals in the soil, resulting in the evolution of only a few metal-tolerant ecotypes. However, although tobacco and *A. thaliana* are good laboratory models, their small stature might not be suitable for field applications [30-35]. The wide genome of trees and facultative tolerance, such as the redistribution of roots to less contaminated zones of soil, allows survival of trees not selected for metal tolerance on polluted soils. Hence, there is particular interest in the genetic transformation of poplar trees (*Populus* sp.), which are fast growing plants with high biomass – ideal attributes for phytoremediation. Plant transformation is usually performed using the 'natural genetic engineer' *Agrobacterium tumefaciens*, a plant pathogen that has become the favourite vector for gene transfer to plants. However, *A. tumefaciens*-mediated transformation of forest trees is notoriously challenging, which explains why there have been only a few reports about the genetic modification of poplar plants. The first transgenic plants of poplars were developed for phytoremediation. Their transgenic line was designed to treat chloroacetanilide herbicides by the overexpression of a gamma-glutamylcysteine synthetase, an enzyme involved in glutathione synthesis. A characteristic feature of metalliferous soils in Europe is the absence of woody and tree species. A nutrient thin film hydroponic technique (NFT) was developed and that could be used for rapid screening of tree cuttings for tolerance to heavy metals based on biomass production in condition of metal exposure. Many species, such as *Salix caprea and S. cinerea*, and the hybrid *S. viminalis,* are known to colonise edaphically extreme soils. Acclimation of trees to metal stress has been studied using various indices such as cell suspension cultures and callus cultures, but seedling growth is the most commonly used index, despite their greater sensitivity to adverse conditions than mature trees. Ninety four clones of *Salix viminalis* and *Salix dasyclados* were tested for tolerance to heavy metals using a hydroponic system[36-38].

Metal tolerance for phytoremediation- Some plants can hyperaccumulate metal ions that are toxic to virtually all other organisms at low dosages [39-48]. This trait could be used to clean up metal-contaminated soils. Moreover, the accumulation of heavy metals by plants determines both the micronutrient content and the toxic metal content of our food. Complex interactions of transport and chelating activities control the rates of metal uptake and storage. In recent years, several key steps have been identified at the molecular level, enabling us to initiate transgenic approaches to engineer the transition metal content of plants [49-67]. Molecularly, the factors governing differential metal accumulation and storage are unknown. It is likely that vacuolar uptake is important [45-58]. Therefore, engineering vacuolar transporters, preferably in specific cell types, might result in significantly increased accumulation rates. The transport and storage forms of transition metals are largely unknown. Stem biomass production of three willow clones was enhanced by sludge application rate; it was also led to more uniform growth and a greater shoot number than in control plots [59-68]. Cadmium and Zn concentrations in the foliage generally varied with the concentrations in a particular sludge. Cadmium and Zn uptake was calculated to be about 1% of the amount applied, a degree of uptake not large enough to significantly decontaminate the soil [55-64]. The trees growing in substrate affected by the mining showed pronounced stunting, reduced leaf size and extensive necrotic and chlorotic spotting, and had concentrations more than 50 times higher for Cu, and 20 times higher for Pb and Zn. Senescence of trees usually produced an increase in metal levels due to concentration caused by loss of fluids. A characteristic of willow, which makes it a very suitable tree for use in phytoremediation, is that it can be frequently harvested by coppicing, yielding as much as 10–15 dry t ha per one year. Bushy *Salix* species with erect stems, rapid growth and good rooting ability are the most suitable for biomass coppice, with *S. viminalis* being one of the most widely used species. In addition to high biomass productivity, *Salix* trees also have an effective nutrient uptake, high evapotranspiration rate and a pronounced clone specific capacity for heavy metal uptake [55-63].

Absorption of heavy metals by trees -Trees differ in their ability to translocate heavy metals from the root to the shoot. It was acknowledged that the partitioning of metals between tissues may change as the soil metal levels increase. Wood and bark are important sinks for biologically available metals, with additional sink tissue being formed each growing season [63-69]. These tissues are slow to enter the decomposition cycle; accumulated metals can, therefore, be immobilised in a metabolically inactive compartment for a considerable period of time, if the contaminated trees are not reused for other purposes which accelerate the return of the heavy metals to the environment, such as in combustion. While metal concentrations in wood are frequently lower than in roots and bark, the fraction may represent a much more significant proportion of the total amount of metal in a tree. The success of *Salix* as a phytoextracting plant depends on its biomass production, metal accumulation capacity and the site of metal accumulation in the plant. Uptake of heavy metals by four varieties of *Salix,* used in woodchip production for energy, was measured. The trees were grown for 3 years on soil that had received sewage sludge for over 50 years. The main benefit from *Salix* growth on this metal contaminated site was reported to be site stabilisation, as metal uptake by the harvested biomass was not sufficient for phytoextraction to be realistic. Cadmium is highly zootoxic and is a common contaminant in the urban environment. Thus, the use of willow to remove Cd from moderately contaminated soil may be the most immediate practical application of phytoremediation. The bark and wood concentrations of heavy metals in 20 willow varieties were determined. Overall, the concentrations in the 3-year-old trees suggested certain clones have potential to take up significant quantities of metals.

Metal hyperaccumulation and phytoremediation; mechanism - A relatively small group of hyperaccumulator plants is capable of sequestering heavy metals in their shoot tissues at high concentrations. Metal tolerance is one of the key prerequisites for phytoremediation. In recent years, major scientific progress has been made in understanding the physiological mechanisms of metal uptake and transport in these plants. However, relatively little is known about the molecular bases of hyperaccumulation. The major processes involved in hyperaccumulation of trace metals from the soil to the shoots by hyperaccumulators include: (a) bioactivation of metals in the rhizosphere through root–microbe interaction; (b) enhanced uptake by metal transporters in the plasma membranes; (c) detoxification of metals by distributing to the apoplasts like binding to cell walls and chelation of metals in the cytoplasm with various ligands, such as phytochelatins, metallothioneins, metal-binding proteins; (d) sequestration of metals into the vacuole by tonoplast-located transporters. Plants that take up heavy metals from the soil offer an alternative and less expensive method to strip heavy metals directly from the soil. Plants have constitutive and adaptive mechanisms for accumulating or tolerating high contaminant concentrations in their rhizospheres. The use of such plants to cleanup soils and water contaminated with pollutants, a technique known as phytoremediation, is emerging as a new tool for *in situ* remediation. Phytoremediation takes advantage of the fact that a living plant acts as a solar-driven pump, which can extract and concentrate certain heavy metals from the environment. This remediation method maintains the biological properties and physical structure of the soil. The technique is environmentally friendly, potentially cheap, visually unobtrusive, and offers the possibility of bio-recovery of the heavy metals. Phytoremediation strategies can offer suitable approaches for decontaminating polluted soil, water, and air by trace metals as well as organic substances [65-76]. Plants ideal for phytoremediation should be: (a) fast-growing, (b) have high biomass, (c) extensive root system, (d) be easy to harvest, and (e) tolerate and accumulate a range of heavy metals in their harvestable parts. To allow remediation within a reasonable period, metal uptake and plant yield have to be enhanced dramatically. This can be done by continuing the search for metal hyperaccumulators, as well as by engineering common plants with hyperaccumulating genes. However, this approach can be only used when the molecular mechanisms of metal uptake, tolerance, accumulation, and translocation are better understood. Hyperaccumulation of heavy metals by higher plants is a complex phenomenon. It involves several steps, such as: (a) transport of metals across the plasma membrane of root cells; (b) xylem loading and translocation; and (c) detoxification and sequestration of metals at the whole plant and cellular levels. The first hyperaccumulators characterized were members of the Brassicaceae and Fabaceae families. More than 400 plant species have been reported so far that hyperaccumulate metals and a considerable number of species show the capacity to accumulate two or more elements. While most of these plant species have been reported to accumulate Ni, some of them also accumulate Co, Cu, and Zn. A few species accumulate Mn and Cd. The mechanisms of metal hyperaccumulation in these plants are so far not fully understood. Generally speaking, the accumulation ability of a given metal is determined by the uptake capacity and intracellular transportation of increasing heavy metal mobilization in the rhizosphere need to be further studied.

Benefits and potentiality of trees for phytoremediation - The potential use of trees as a suitable vegetation cover for heavy metal-contaminated land has received increasing attention over the last 10 years. Trees have been suggested as a low-cost, sustainable and ecologically sound solution to the remediation of heavy metal-contaminated land, especially when it is uneconomic to use other treatments or there is no time pressure on the reuse of the land. Resistance was not species-specific, but rather clone- or hybrid-specific. Benefits can arise mainly from stabilisation of the soil or waste, although in some cases phytoextraction may be sufficient to provide clean up of the soil. Before these benefits can be realised, the trees must become established on a site. On highly contaminated soils, or on mining wastes, tree establishment may be inhibited by high concentrations of heavy metals. Under such conditions root immobilisation, which would normally protect a plant, may not be able to prevent toxic amounts of metal being translocated to the aerial parts of the plant. In less-contaminated soils, other factors may limit plant growth; such as macronutrient deficiencies and physical conditions, especially those properties leading to poor water holding, aeration and root penetration. Generally, willow achieved higher shoot growth increments than birch, and the greatest gains in survival occurred on the poorest growth medium (where topsoil was not used), whereas there was little advantage in their use on fertile sites with good drainage [77-79]. It was recognised that a low growth rate, with high survival, on these nutrient deficient sites is preferable to low survival coupled with a continued fertiliser application requirement to sustain growth. While the addition of organic amendments such as sewage sludge may aid revegetation, roots may not extend readily from a fertile layer into underlying contaminated material, and it may increase the weed problem in some young woodland areas. Once the trees have become established, the vegetation cover can promote physical stabilisation of a substrate, especially on sloping ground. Long-term stability of the land surface can be achieved as the standing trees decrease erosion of the substrate by wind and water [78-84].

Trees have massive root systems, which help to bind the soil, and the addition of litter to the surface quickly leads to an organic cover over the contaminated soil. In addition, transpiration of water by the trees reduces the overall flow of water down through the soil, thus, helping to reduce the amounts of heavy metals that are transferred to ground- and surface waters. Phytostabilisation of a heavy metal-contaminated substrate may also be achieved by causing chemical changes to specific metals, which result in their becoming less bioavailable. Trees have been shown to meet all of these requirements, the first three in particular. While a high metal content in agricultural crops is not desirable, and indeed is potentially dangerous, a higher metal content in trees is acceptable, as long as normal physiological activity is not affected [80-98].

Very recently, the phytoremediation of volatile environmental pollutants with transgenic poplar trees over expressing a mammalian cytochrome P450 has been reported [80-91]. Cytochrome P450s constitute a large enzyme superfamily commonly involved in the metabolism of toxic compounds. The development of transgenic tobacco plants expressing a human cytochrome P450 and capable of metabolizing trichloroethylene (TCE) 640-fold faster than wild type plants. The same group later reported the introduction of a rabbit cytochrome P450 in transgenic hairy root cultures of *Atropa belladonna*, which also exhibited a faster metabolism of TCE. In the current study, the genetic transformation of hybrid poplar plants (*Populus tremula* x *Populus alba*) overexpressing mammalian cytochrome P450 2E1 (CYP2E1) has been well studied[78-88]. The engineered trees were capable of the enhanced metabolism of five volatile toxic compounds: TCE, vinyl chloride, carbon tetrachloride, chloroform and benzene. Among the different transgenic clones tested, the most efficient one, line 78, expressed CYP2E1 at a 3.7- to 4.6-fold higher level and exhibited the highest level of TCE metabolism (>100-fold higher than in non-transgenic controls). When cultivated in hydroponic solution spiked with toxic compounds, line 78 was capable of extracting 90% of TCE (compared with <3% extracted by non-transgenic controls), 99% of chloroform (compared with 20% by controls) and 92–94% of carbon tetrachloride (compared with 20% by controls). Enhanced metabolism of organic pollutants in transgenic plants is associated with a faster uptake, which can be explained by a steeper concentration gradient inside plant tissues. Transgenic plants were also shown to remove volatile compounds from contaminated air at a higher rate than non-transgenic controls: 79% of TCE (none removed by controls), 49% of vinyl chloride (compared with 29% by controls) and 40% of benzene (compared with 13% by controls). Transgenic poplars (CYP2E1) enhance both the uptake and the metabolism of several toxic solvents and could, therefore, help to overcome a major limitation inherent to phytoremediation – namely, the threat that accumulated toxic compounds would volatilize or otherwise contaminate the food chain[80-86]. The first report, about genetic engineering of plants for phytoremediation applications, constitutes a milestone in the field for several reasons: first, is one of the very few studies describing the successful development of transgenic poplars, which is technically challenging; second, the technology is efficient for the treatment of several important organic pollutants likely to be found in mixture in the environment; and third, it constitutes the achievement of a pioneer work initiated by the same group a decade ago. With federal regulations limiting the use of transgenic forest trees, further developments of phytoremediation are likely to involve genetic use restriction technologies (GURTs) for controlling the dispersion of transgenes in the environment. As for transgenic crops, risks inherent to genetically modified organisms have to be minimized and balanced with the increasing needs of an ever-expanding human population [82-87].

Local authorities, private companies and other bodies involved with the remediation of contaminated land should be encouraged to use phytoremediation, especially if budgets are limited and the alternative is that no treatment is carried out. There is an opportunity to use these sites as demonstration and research areas. There is still much fundamental and applied research needed to underpin phytoremediation technology, but this could be undertaken in conjunction with actual remediation schemes, which would achieve the dual purpose of treating contaminated sites and providing demonstration sites to show the application of phytoremediation [83-89].

Use of transgenic plants for phytoremediation- With the rapid growth in the global population making it increasingly difficult to provide sufficient amounts of food, one potential solution is the use of genetically modified (GM) organisms, which might support starving populations through increased crop yield. However, the launch of GM foodstuffs has been impeded, in particular, by the reluctance of different regional jurisdictions to permit the application of GM plants. Another solution, therefore, might be to use remediation techniques to convert contaminated areas into suitable agricultural land and thereby increase the sites available for food production[85-91]. Phytoremediation using conventional plants (grasses, sunflower, corn, hemp, flax, alfalfa, tobacco, willow, Indian mustard, poplar, etc.) shows good potential, especially for the removal of pollutants from large areas with relatively low concentrations of unwanted compounds: areas for which it is not cost-effective to use traditional physical or chemical methods. However, gene transfer has already led to the production of GM crop varieties on hundreds of millions of hectares. This irreversible fact, together with recently improved attitudes towards GM plants (even within the EU), where GM food has traditionally been viewed with distrust), has resulted in calls for the large scale implementation of transgenic plants that can prevent or remove contamination more effectively. The generation of transgenic plants for environmental protection involves the two quite separate fields of pollution prevention and pollution removal, with specifically tailored plants already existing for both purposes. Pollution- preventing GM plants can significantly reduce the amount of agrochemicals needed for crops, thus reducing environmental pollution. Examples include Roundup Ready soya, which enables the use of more environmentally- friendly herbicides, as well as *Bacillus thuringiensis* (Bt)-corn and Bt-cotton, which minimise pesticide use. Pollution removing GM plants, which deal with contaminations caused by explosives, chlorinated solvents, mercury, selenium, phenolics, etc. have been reported. These plants have been developed to contain either transgenes responsible for the metabolisation of organic compounds (thereby leading to the accumulation of less toxic or less recalcitrant compounds) or transgenes that result in the increased accumulation of inorganic compounds. Once optimised this in harvestable parts and thus either enable their removal or prevent their migration to sites where they may pose a danger to human health. The first generation of commercially available transgenic plants (*e.g.* plants expressing the Bt toxin) were able to reduce the loss of crop yield caused by insect damage at the same time as reducing the amount of pesticide required. As both these and herbicide-resistant plants have been the subject of numerous reviews, and their advantages or disadvantages discussed extensively [93-98]. The transformed plants were examined for fatty acid content showing substantial presence of the precursor, which was further converted to alcohol by the enzyme normally present in tobacco plants. The effectiveness of this semi-synthetically prepared mixture has since been successfully trialled in field tests in northern Bohemia. Phytoremediation is not solely a function of plants but must always be considered in combination with the effect of rhizospheric microorganisms[90-100]. Although, they have an inherent ability to detoxify some xenobiotics (*i.e.* to make them non-phytotoxic), plants, compared with microorganisms, generally lack the mechanisms necessary for the complete degradation/ mineralisation of toxic compounds. The potential of genetic engineering to enhance the biodegradation of xenobiotics has been recognised since the early 1980s, with initial attempts being focused on microorganisms. However, there are two main problems with the introduction of GM microorganisms: the legislative barriers blocking their release into the environment and the poor survival rate of those engineered strains that have been introduced into real contaminated soil. The latter problem reflects the inadequate level of knowledge that currently exists about the consortia of microorganisms present in real soil and the ways in which they interact. The survival rate of introduced bacterial species might, however, be improved by the use of strains that have a selective advantage over others, such as strains supported by plants: for example, root colonisers. The use of plants, rather than microorganisms, as genetically engineered environmental cleanup biosystems might also help to overcome the legislative barriers. However, some species, for as yet unknown reasons, are simply more sensitive to contamination than others, so not all plants are equally well suited to metabolise or accumulate pollutants. For remediation purposes, besides their ability to take up, accumulate or metabolise the xenobiotics, one of the most important criteria is the ability of the plant to selectively support the metabolism and survival of degrading bacteria in the rhizosphere. Only recently developed methods of detection, such as stable isotope probing, have enabled us to obtain a deeper insight into the effect of pollutants and plants on microorganisms. Meta genomics, for example, has brought new insights into the presence and activity of degrading microorganisms within rhizosphere consortia, enabling the tracking of responses to compounds released by plants [97-108]. The genetic modification of microorganisms to improve their performance in the rhizosphere represents a challenging possibility that should not be abandoned simply because their release into the environment is currently restricted. Plants exploit their natural metabolic mechanisms to take up essential trace metals. Cations or oxyanions must either be accumulated in harvestable parts or transformed into less-toxic forms. Although, hyperaccumulators, such as *Thlaspi caerulescens,* can uptake sufficient levels of metals to make harvesting and metal recovery economic, they are often limited by their small biomass; the amount of pollutant they can remove from soil, is a function of their tissue concentration multiplied by the quantity of biomass formed. Despite this, and despite the fact that no universal phytoremediation plant exists, plants that are selective and only capable of accumulating certain elements, are already being used in the cleanup of a broad spectrum of hazardous elements. The transgenic tobacco accumulated twice the amount of cadmium in above-ground biomass than did the controls. A possible enhancement to this approach, currently being tested, involves the cloning of short (cysteine-rich) metal-binding sequences into plants to improve their metal-binding properties[99-107]. More recently, plants have been constructed, that express bacterial enzymes capable of TNT transformation and RDX (hexahydro-1,3,5-trinitro- 1,3,5 triazine, an explosive nitroamine widely used in military and industrial applications) degradation. In the great environmental cleanup required, the future lies in tailored phytoremediation-specific plants able to support microbial activities in the rhizosphere. However, to exploit these possibilities on a large scale, it will first be necessary to achieve changes in the existing legislation, overcome regulatory barriers and educate the public into improving their opinion of GM plants [100-107].

Reducing the effects of environmental stressors on plant growth is advantageous in agriculture and horticulture, as well as in more newly developed areas of environmental management such as phytoremediation of contaminated soil. The functioning of transgenic canola and *P. putida* UW4 under field conditions, was consistent with laboratory studies that examined the effects of *P. putida* UW4 and transgenic tomato plants on flooding and the effects of transgenic canola exposed to Ni-spiked soil [106].

However, yet known plants species accumulating high levels of heavy metals in their biomass such as *Thlaspi caerulescens*, *T. goesingense* or *Cardaminopsis halleri* are slow growing, low biomass-producing species of no value for agronomic use. Therefore, development of transgenic crop plants with hyperaccumulating capability due to either introducing/overproducing genes encoding heavy metal binding peptides and proteins or overexpressing metal transporter proteins has been proposed as a promising tool for use in phytoremediation efforts.

Cytochrome P450 monooxygenases (P450s) metabolize herbicides to produce mainly non-phytotoxic metabolites[106]. Although rice plants endogenously express multiple P450 enzymes, transgenic plants expressing other P450 isoforms might show improved herbicide resistance or reduce herbicide residues. Mammalian P450s metabolizing xenobiotics are reported to show broad and overlapping substrate specificity towards lipophilic foreign chemicals, including herbicides. Phytoremediation is the use of plants and plant growth as a technique for detoxifying environmental polluted soils, sediments, and aquatic sites contaminated with organic and inorganic pollutants[107]. Phytoremediation costs much less than physical and chemical remediation treatments and has proven to be a sustainable technology for bioremediation. Phytoremediation is best suited for sites with shallow contamination (b5 m depth), moderately hydrophobic pollutants (logKow=0.5–3), short-chain aliphatic chemicals, and excess nutrients. Most pesticides are moderately hydrophobic, so phytoremediation is one possible method of removing pesticides from contaminated water and soil [108]. However, field trials have suggested that the rate of contaminant removal using conventional plants is insufficient. In plants, pollutants can be remediated through several biochemical processes–

adsorption, transport, and translation; hyperaccumulation; or transformation and mineralization–that protect the plants themselves from toxic organic foreign chemicals. Over-expression of endogenous plant genes or transgenic expression of bacterial or animal genes is required to significantly increase the remediation ability of plants[109].

The use of plants to clean-up contaminated soil and water could provide a cheap and effective technology for bioremediation. There are several ways, how to increase the efficiency of plant xenobiotic removal–e.g. genetically modified plants carrying a suitable gene from various organisms. The ability of plants to degrade polychlorinated biphenyls (PCBs) has been reported. PCBs are persistent toxic organic pollutants present in the environment. The over-expression of several plant and bacterial genes in transgenic plants has greatly enhanced these natural plant remediation systems. The small number of laboratories working on these problems at present cannot, however, hope to impact global pollution. Greatly expanded research programs focused on the basic and applied problems affecting each class of pollutants are needed for significant progress to be made. In particular, more quantitative data from massbalance studies are needed to determine the rate-limiting steps in the mineralization of organic pollutants. Once the rate-limiting steps in uptake, transport, or transformation have been identified, more informed construction of transgenic plants expressing plant, animal, or bacterial genes will result in dramatic improvements in phytoremediation capabilities [110].

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