

Transgenic Plants: Benefits, Applications, and Potential Risks in Phytoremediation

Viraj Gunarathne¹, Sonia Mayakaduwa², Ahamed Ashiq¹, Shyama Ranjani Weerakoon³, Jayanta Kumar Biswas⁴ and Meththika Vithanage¹

¹Ecosphere Resilience Research Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Colombo, Sri Lanka, ²School of Agriculture, Food and Wine, University of Adelaide, Adelaide, SA, Australia, ³Department of Botany, The Open University of Sri Lanka, Nawala, Sri Lanka, ⁴Department of Ecological Studies and International Centre for Ecological Engineering, University of Kalyani, Kalyani, West Bengal, India

5.1 INTRODUCTION

Contamination of soil, surface water, and groundwater with organic and inorganic pollutants has become a significant environmental issue. Although a number of methods including chemical/thermal treatments have been employed as remediation strategies, they have various limitations because of the high cost, low efficiency, generation of secondary pollutants, etc. (Arthur et al., 2005). Thus, phytoremediation, or use of plants as tools of bioremediation of soil and water by extracting, sequestering, or detoxifying contaminants, has been accepted as a favorable, cost-effective, and environmentally friendly approach (Cunningham et al., 1995; Singh et al., 2003). Plants can take up

a wide range of toxic metals, metalloids and organic contaminants. Then such contaminants are converted into nontoxic or less toxic products by metabolic processes in plant tissues (Meagher, 2000). Currently, several forms of phytoremediation such as phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization are being utilized (He and Yang, 2007). Phytoextraction is the process in which contaminants are accumulated from aboveground where the plant biomass induces the contaminants into the shoots after they are taken up by the plant roots from contaminated soil and water (Rafati et al., 2011). Rhizofiltration refers to the remediation of contaminated water by plant roots through adsorption, concentration, and

precipitation (Yadav et al., 2011). In phytostabilization, mobility of contaminants is reduced by plant adsorption or precipitation whereas phytovolatilization is carried out by the uptake of pollutants and releasing them into the atmosphere after conversion into a volatile form (Glick, 2003). Around 400 species of plant hyperaccumulators that can absorb high concentrations of contaminants through their roots are being used in phytoremediation. These plants can accumulate metals 50–100 times higher than normal plants (Chaney et al., 1997). The high detoxification capacity of hyperaccumulators has been attributed to the high bioconcentration factor and the efficient root-to-shoot transport system with high contaminant tolerance (McGrath and Zhao, 2003). Moreover, the ideal hyperaccumulators are resistant to pests/diseases, have extensive root systems, and can grow fast and produce high biomass (Watanabe, 1997).

Thus, improving plants' performance of contaminant remediation had been a challenge. In this respect, an approach of transgenic plants with enhanced phytoremediation capacity by genetic manipulation and plant transformation technologies was introduced (Ellis et al., 2004). Transgenic plants are defined as those that have been genetically modified using recombinant DNA technology to express a gene that is not native to the plant or to modify endogenous genes (Key et al., 2008). Accordingly, unique genes from hyperaccumulators are identified and subsequently transferred to plant species that have fast growth rate, deep rooting systems, tolerance to various climatic conditions/pests/diseases, etc. (Gullner et al., 2001; Cherian and Oliveira, 2005). Consequently, transformed plants can be regenerated by tissue culture techniques. So far, this has been identified as a highly effective approach to improve phytoremediation capacity. Therefore, this chapter aims to provide an overview of benefits and applications of transgenic plants

in phytoremediation. Current limitations and potential risks associated with the use of transgenic plants will also be highlighted.

5.1.1 A Brief History on the Use of Transgenic Plants for Phytoremediation

It has been reported that transgenic plants for phytoremediation were first developed to enhance heavy metal tolerance (Van Aken, 2008). For example, tobacco (*Nicotiana tabacum*) plants were modified by expressing a yeast metallothionein (MT) gene to increase cadmium tolerance whereas a mercury ion reductase gene were overexpressed in arabidopsis, a type of mustard (*Arabidopsis thaliana*) to enhance mercury tolerance (Misra and Gedamu, 1989; Rugh et al., 1996). Considerable attention was focused on poplar trees (*Populus* sp.), which have a fast growth rate and high biomass production. By that time, poplar plants had already been utilized in phytoremediation of selenium (Pilon-Smits et al., 1998). A plant pathogen (*Agrobacterium tumefaciens*) was used as a "natural genetic engineer" to carry out plant transformation. Consequently, a transgenic yellow poplar with overexpression of the bacterial gene encoding mercuric reductase was introduced for the remediation of mercury (Rugh et al., 1998). Glutathione (GSH) is a thiol containing compound that plays a crucial role in antioxidative and detoxification mechanisms in plant tissues (Noctor et al., 1998). Overexpression of an enzyme involved in glutathione synthesis (γ -glutamyl-cysteine synthetase) was found to increase cadmium accumulation in poplar plants (Arisi et al., 1997). Gullner et al. (2001) developed a transgenic poplar as well, to remediate herbicide chloroacetanilide by overexpression of the same enzyme. It had been confirmed that these transformed poplars contained high levels of glutathione and elevated their transport in phloem and xylem in comparison with wild

poplar (Herschbach et al., 1998). Fig. 5.1 is a pictorial representation of these transgenic plants at the cellular level.

5.2 ADVANTAGES OF TRANSGENIC PLANTS IN PHYTOREMEDIATION

As mentioned earlier, the concept of transgenic plants was introduced to overcome apparent limitations of natural hyperaccumulators including low efficiency in contaminant remediation, slow growth, low biomass production, etc. A brief scheme of the transgenic plants and possible contaminant mitigation pathways are summarized in Fig. 5.2.

Passive and active pathways are the routes taken for the absorption of the heavy metals by the plant roots. Diffusion of ions from the soil up to the epidermis is a passive

pathway, whereas the active pathway meets the demand of the concentration gradient through absorption by plants. Heavy metals in soils are nondegrading and can cause an ecosystem imbalance through bioaccumulation (Lugauskas et al., 2005). For instance, lead, which has no biological role, can be toxic to organisms and microbes (Sobolev and Begonia, 2008; Gall and Rajakaruna, 2013). High concentrations of nickel in soils coming from serpentines and through ultramafic bedrocks can cause permanent damage to the soil through its specific physiochemical properties and the mechanism caused by their binding with the soil causes microbial activities to decline, thereby reducing soil fertility and overall plant health (Mishra and Kar, 1974; Vithanage et al., 2014; Seneviratne et al., 2016).

The transgenic yellow poplar trees developed by Rugh et al. (1998) were reported to be highly efficient in mercury decontamination.

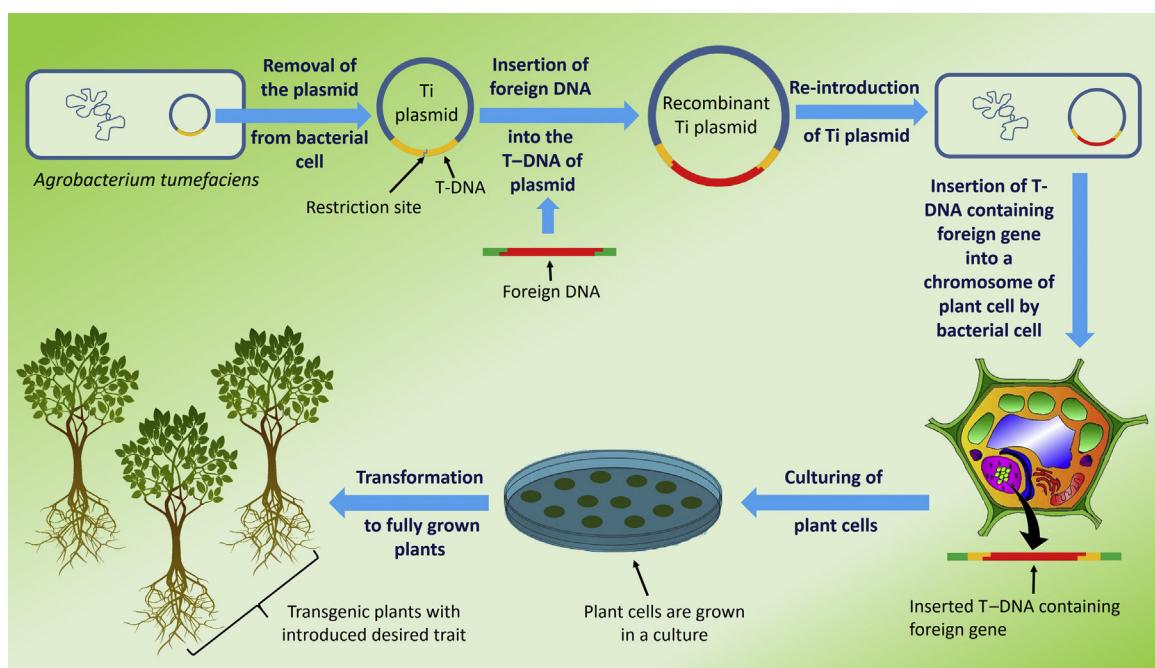


FIGURE 5.1 Scheme for plant transformation through *Agrobacterium* method.

Modified soybean plants by expression of *Escherichia coli* arsC and *E. coli* γ -glutamylcysteine synthetase showed threefold hyperaccumulation and almost 17-fold biomass production compared with wild plants (Dhankher et al., 2002). Moreover, *Arabidopsis* plants modified by bacterial genes including *E. coli* arsC gene accumulated two- to threefold arsenic in contaminated soil, compared with wild type (Dhankher et al., 2002). Pilon-Smits et al. (1999) modified Indian mustard (*Brassica napus*) by overexpressing APT-sulfurylase (APS) gene. In this study, it was reported that these transgenic plants accumulate selenium three times higher than the wild plant. Due to high biomass production, transgenic plants can accumulate and decontaminate pollutants for a long time period. Thus, they have been proven to be beneficial over time in reducing the treatment cost (Dimkpa et al., 2017). On the other hand, enhanced remediation capacity in transgenic plants leads to a decline in remediation costs and to a decrease in the use of harmful chemicals. Nonedible transgenic plant biomass can be further utilized for different purposes such as energy generation by using anaerobic digestion, prevention of soil erosion, land rehabilitation, stabilizing water canals, etc. (Trapp and Karlson, 2001). Green chemistry is another major advantage of transgenic plants. In this respect, transgenic hyperaccumulators can be used to extract different high value products including enzymes, polysaccharides, various chemicals, fuels, etc. to replace the use of toxic chemicals, and are able to obtain phytosiderophores for metal absorption. (Seneviratne and Vithanage; Escande et al., 2014). The phytosiderophores so produced mobilize the heavy metals in the rhizosphere like in the case of *Leersia hexandra*, which is a graminaceous plant (Liu et al., 2011). It can accumulate Cr (III) as well as Fe (III) and the differences in the valence state and ionic radius explain the mode of transportation by the plant forming different

complexes (Pillichshammer et al., 1995). The rhizosphere of the hyperaccumulator plants depends on the availability of the metals for phytoremediation through acidification and siderophore production, which in turn convert them to readily usable forms for the plants. Siderophores normally have greater affinity towards Fe than the other divalent cation (Seneviratne and Vithanage; Neubauer et al., 2000).

5.3 EMERGING APPLICATIONS OF TRANSGENIC PLANTS IN HEAVY METAL REMEDIATION

Genes involved in acquisition, translocation, sequestration, and detoxification of metals have been identified from a number of organisms particularly bacteria, yeast, or higher plant species (Vögeli-Lange and Wagner, 1990; Maestri and Marmiroli, 2011). Transfer of these genes into fast-growing, high biomass candidate plants has been identified to accelerate heavy metal remediation. In these transgenic plants, recombinant proteins that play viable roles in metal chelation [citrate, phytochelatin (PC), MTs], assimilation, and membrane transport can be overexpressed (Pilon-Smits et al., 1998). On the other hand, glutathione synthesis or sulfur metabolism can be enhanced in these plants (Maestri and Marmiroli, 2011). This may lead to increased or reduced metal uptake, translocation, or sequestration based on the type and location of the chelator. To describe, MT, a peptide capable of high affinity coordination of heavy metal ions, has been introduced to several plant species. Overexpression of MTs increases metal resistance, and thereby helps in metal accumulation in plants. It has been observed that tobacco plants modified with human MT have enhanced Cd, Zn, and Ni accumulation (Macek et al., 2002; Pavlikova et al., 2004a,b). Thus, MT transferred to tobacco increased Cd tolerance at the seedling

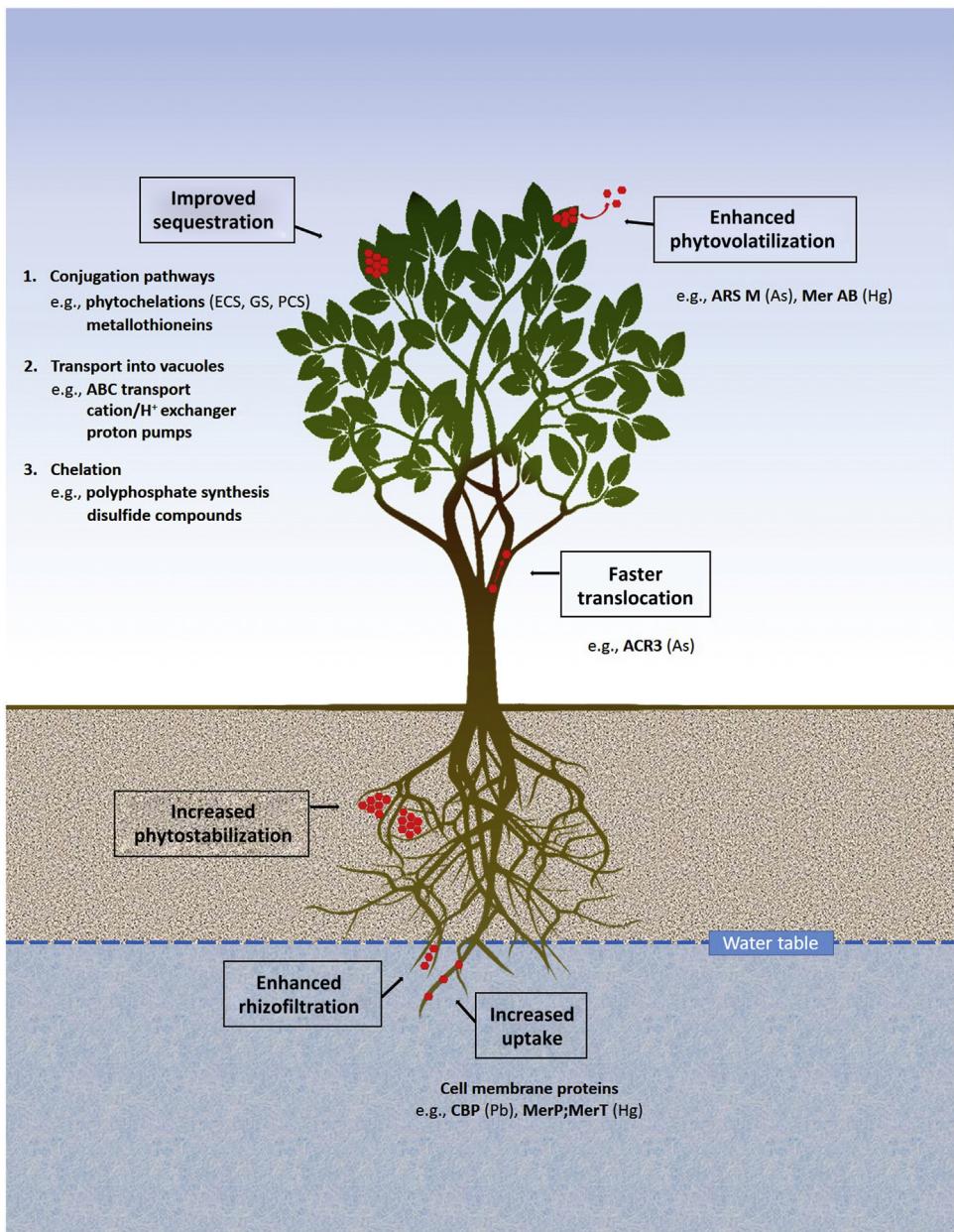


FIGURE 5.2 Scheme and advantages of transgenic plants for phytoremediation.

level (Pan et al., 1994). The highest effect of MT overexpression was reported by Hasegawa et al. (1997) who observed 16-fold Cd tolerance in cauliflower plants possessing transferred MT

from yeast. However, Elmayan and Tepfer (1994) reported a 60%–70% decrease in Cd accumulation in transgenic tobacco plants modified with human MT, compared with the control.

Moreover, metal binding peptides PCs have been overproduced in transgenic plants. They can sequestrate multiple metals in metal–thiolate complexes, playing a crucial role in metal detoxification (Clemens, 2006). These PCs are synthesized enzymatically during transpeptidation from glutathione. According to Gong et al. (2003), modified *A. thaliana* with a PC producing gene from *Triticum aestivum* showed an accelerated translocation of Cd into the shoots and reduced metal accumulation in roots, compared with wild type. Overexpression of the same gene in shrub tobacco enhanced its tolerance to Pb²⁺ and Cd²⁺ and accumulation of Cu, Zn, Cd, and Pb in shoots (Gisbert et al., 2003; Martínez et al., 2006). Further, long distance metal transport initiated by PCs was reported by Mendoza-Cózatl et al. (2008), who observed high PC and four times higher Cd levels in the phloem compared with the xylem in *Brassica napus*. Overexpression of *AtPCS1* gene in *A. thaliana* led to enhanced PC production twofold in comparison with the control (Lee et al., 2003). Also, overexpression of *AtPCS1* in *Arabidopsis* sp. and *Brassica juncea* was reported to enhance both GSH levels and arsenate and Cd tolerance respectively (Li et al., 2004; Gasic and Korban, 2007). Moreover, GSH, being a basic antioxidant molecule, can directly eliminate reactive oxygen species induced by metal ions in plant cells (Schutzendubel and Polle, 2002). The overexpression of GSH synthesizing enzymes such as γ -glutamyl-cysteine synthetase (γ ECS) or glutathione in transgenic mustard (*B. juncea*) enhanced both accumulation and tolerance of Cd (Zhu, 1999; Harada et al., 2001). On the other hand, reduced γ ECS resulted in decreased GSH levels in *A. thaliana* (Xiang et al., 2001). A transgenic tobacco modified by the overexpression of citrate synthase, which stimulates the production of metal chelator citric acid, was reported to enhance Al tolerance (De la Fuente et al., 1997). In these transgenic plants, Al excreted from root cells was

retained in an extracellular complex. Further, Han et al. (2009) transferred a gene (*OsCS1*) encoding for citrate synthase isolated from rice plants into tobacco and observed a high tolerance to Al. Another possible approach for transgenic remediation is transfer of genes encoding for phytosiderophores, which are crucial in acquisition of Fe and Zn. It was found that Fe uptake under Cd stress in maize could be achieved in maize plants, by modifying them with a key enzyme (nicotianamine synthase) in phytosiderophore biosynthetic pathway in barley and rice (Higuchi et al., 1999). Further, overproduction of phytosiderophores by overexpression of nicotianamine aminotransferase in rice plants was survived in Fe deficient soils (Takahashi et al., 2001). Transgenic plants modified to overexpress ferritin (an iron binding protein) exhibited nearly 1.3-fold iron levels in tobacco leaves and 3-fold in rice seeds (Goto et al., 1998). Introduction of a new metabolic pathway from another organism is another approach in transgenic heavy metal phytoremediation. Two bacterial genes, *MerA* and *MerB*, which encode genes for converting methylmercury to ionic mercury and for reducing ionic mercury to less toxic volatile elemental mercury, were transferred to *A. thaliana* plants (Rugh et al., 1996). These double transgenic plants showed a 50-fold more tolerance to mercury than wild plants and volatilized elemental mercury (Table 5.1).

5.4 CURRENT LIMITATIONS

The main purpose of transgenic plants is of both environmental and economic significance, and is threefold: extraction of metals that may or may not be precious, for instance Ti, Ni, etc., to stabilize the environment without having any ecological risks; to improve the soil fertility by the process of phytoremediation; and provide for economic growth (Garbisu and Alkorta, 2003; Van Aken, 2009;

TABLE 5.1 A Summary of Use of Transgenic Plants in Heavy Metals/Metalloids Remediation

Contaminant	Gene	Source	Host Plant	Reference
Cd	γ -Glutamyl-cysteine synthetase glutathione synthetase GCS-GS operon	<i>Streptococcus thermophilus</i>	<i>Beta vulgaris</i>	Liu et al. (2015)
	γ -Glutamyl-cysteine synthetase ECS	<i>Escherichia coli</i>	<i>Populus tremula × P. alba</i>	He et al. (2015)
	Cysteine synthase RCS1	<i>Spinacia oleracea</i>	<i>Nicotiana tabacum</i>	Kawashima et al. (2004)
	Cysteine synthase RCS1	<i>Oryza sativa</i>	<i>Nicotiana tabacum</i>	Harada et al. (2001)
	Glutathione synthetase	<i>Escherichia coli</i>	<i>Nicotiana tabacum</i>	Bañuelos et al. (2005)
	<i>AtPCS1</i>	<i>Arabidopsis thaliana</i>	<i>Nicotiana tabacum</i>	Pomponi et al. (2006)
	<i>AtPCS1</i>	<i>Arabidopsis thaliana</i>	<i>Brassica juncea</i>	Gasic and Korban (2007)
	<i>AtPCS1</i> and MTL4	<i>Arabidopsis thaliana and Homo sapiens</i>	<i>Astragalus sinicus</i>	Ike et al. (2007)
	Glutathione synthetase and AsPCS1	<i>Saccharomyces cerevisiae</i> <i>Allium sativum</i>	<i>Arabidopsis thaliana</i>	Guo et al. (2008)
	<i>APS1</i>	<i>Arabidopsis thaliana</i>	<i>Brassica juncea</i>	Wangeline et al. (2004)
	HisCUP1	Recombinant fusion	<i>Nicotiana tabacum</i>	Pavlíková et al. (2004a,b)
	<i>TaPCS1</i>	<i>Triticum aestivum</i>	<i>Nicotiana glauca</i>	Martínez et al. (2006)
Ni	NA synthase NAS1	<i>Arabidopsis thaliana</i>	<i>Nicotiana tabacum</i>	Douchkov et al. (2005)
Mn	NA synthase NAS1	<i>Hordeum vulgare</i>	<i>Nicotiana tabacum</i>	Kim et al. (2005)
	CAX2	<i>Arabidopsis thaliana</i>	<i>Nicotiana tabacum</i>	Korenkov et al. (2007)
As	<i>APS1</i>	<i>Arabidopsis thaliana</i>	<i>Brassica juncea</i>	Wangeline et al. (2004)
	gshI and arsC	<i>Escherichia coli</i>	<i>Arabidopsis thaliana</i>	Dhankher et al. (2002)
	<i>AtPCS1</i>	—	<i>Brassica juncea</i>	Gasic and Korban (2007)
Hg	<i>APS1</i>	<i>Arabidopsis thaliana</i>	<i>Brassica juncea</i>	Wangeline et al. (2004)
	<i>merC</i>	<i>Acidithiobacillus ferrooxidans</i>	<i>Arabidopsis thaliana</i>	Sasaki et al. (2006)
	<i>merApe9</i> and <i>merB</i>	—	<i>Arabidopsis thaliana</i>	Bizily et al. (2003)
	<i>merApe9</i>	—	<i>Nicotiana tabacum</i>	He et al. (2001)
	<i>merP</i>	<i>Bacillus megaterium</i>	<i>Arabidopsis thaliana</i>	Hsieh et al. (2009)
	<i>merApe9</i> and <i>merBpe</i>	—	<i>Spartina alterniflora</i>	Czako et al. (2006)
	<i>merA</i> and <i>merB</i>	<i>E. coli Tn21</i>	<i>Nicotiana tabacum</i>	Ruiz et al. (2003)
Se	<i>SMT</i> and <i>APS1</i>	<i>Astragalus bisulcatus</i> and <i>Arabidopsis thaliana</i>	<i>Brassica juncea</i>	LeDuc et al. (2006)
	<i>SMT</i>	<i>Astragalus bisulcatus</i>	<i>Brassica juncea</i>	LeDuc et al. (2004)
	<i>CGS1</i>	<i>Arabidopsis thaliana</i>	<i>Brassica juncea</i>	Huysen et al. (2004)
Cu	<i>CUP1</i>	<i>Saccharomyces cerevisiae</i>	<i>Nicotiana tabacum</i>	Thomas et al. (2003)
	Glutathione synthetase 1	<i>Escherichia coli</i>	<i>Brassica juncea</i>	Bennett et al. (2003)
Pb	Glutathione synthetase 1	<i>Escherichia coli</i>	<i>Brassica juncea</i>	Bennett et al. (2003)
		<i>Saccharomyces cerevisiae</i>	<i>Arabidopsis thaliana</i>	Song et al. (2003)
Zn	Glutathione synthetase 1	<i>Escherichia coli</i>	<i>Brassica juncea</i>	Bennett et al. (2003)

Vangronsveld et al., 2009). However, while the objectives are all clear, there are several challenges, especially in the productivity of these transgenic plants for a specified metal or even adapting the plants to different environmental conditions.

The 400 species of hyperaccumulators, as mentioned above, that could take up higher concentrations of contaminants and stores in the shoot system showed numerous limitations as well. Conventional hyperaccumulators require a long time period, even decades, for remediation of polluted sites. Incomplete metabolism, toxic metabolites, less detoxification capacity, and possible increase of contaminant bioavailability are the other potential limitations of phytoremediation (Chaudhry et al., 2002; Cherian and Oliveira, 2005). The process of having to exactly make up the genetic material to produce these transgenic plants using the already sensitive hyperaccumulators is a tedious task. Although, at a practical level, it has been proven as a low-maintenance and low-cost process, it requires large areas and the transgenic plants are limited in number for the metabolism of a particular contaminant.

The other limitation is that the mechanisms and pathways have not been fully analyzed and thus the effectiveness of the extraction of the contaminants from different soils is mostly restricted by the measurable or available surface root systems up to a certain reachable root growth (Sheoran et al., 2009; Mahar et al., 2016). Weather and climatic conditions play a vital role for the phytoextraction rates due to their extreme sensitivity. Some of these trace elements are taken up by plants using mobilizing agents, chelates for instance, which have shown inductive properties in the phytoremediation but possess an adverse effect on the soil ecosystems depending on the dosage (Wei et al., 2008). Although there are numerous limitations involve for the formulation of transgenic plants, it posses a huge advantage to

retain the trace metals through the biomass obtained and process them further for recovery.

5.5 POTENTIAL RISKS

Even though transgenic plants have added benefits for effective remediation over native plant species, several kinds of public and scientific concerns have been emerging with respect to the potential environmental issues. The risks posed by transgenic plants on the environment can primarily be categorized as direct and indirect risks. In terms of direct risks, transgenic plants have high potential to become invasive plants or weeds, or have an influence on biodiversity (Auer, 2008).

Generally, transgenic plants possess high fitness (e.g., resistance for environmental stresses) that comes from transgenes and it makes them better competitors among native plant varieties (Ellstrand and Schierenbeck, 2006). The transgenes that are especially introduced to plants utilized for phytoremediation have been proven to give them an extensive ability to thrive in the extreme environments that mostly are present in polluted areas. So they have high ability to spread throughout certain areas that were introduced earlier, causing a potential risk for invasion into natural plant communities. Several scientific studies provide evidences for the concept that describes the adaptation and invasion of newly introduced genotypes for certain ecosystem, through gene flow and temporal variationsevidence for the of the adaptation and invasion of newly introduced genotypes for certain ecosystems, through gene flow and temporal variations (Ellstrand and Schierenbeck, 2006; Culley and Hardiman, 2007).

There are three main pathways that have been identified by previous studies (Johnson and Riordan, 1999; Auer, 2008) by which transgenic plants may become weeds. Some of the advanced traits offered through transgenic modification permit them to escape from

plantations by themselves. The gene flow from the transgenic population to the nontransgenic native populations that have sexual compatibility can possibly make a new weed population or increase the ability of spreading of the existing weed population. Influence of gene flow among transgenic crop cultivars and weeds to create increased weed problems has been documented (Simard et al., 2006; Zelaya et al., 2007). Moreover, the evolution of herbicide resistant weed varieties can occur due to frequent application of certain herbicides that are tolerable to the transgenic population.

Plant populations consisting of transgenic plants can affect the biodiversity in surrounding ecosystems in several ways. Deleterious transgenes, such as genes that are involved in male sterility or ripening ability of fruits, transferred to relative species through gene flow have the capacity for extinction of native populations (Hancock, 2003). The highly competitive transgenic varieties can replace their wild relatives making them extinct as well (Hegde et al., 2006).

Transgenic plants may have other direct potential risks, including effects on soil microorganisms and nutrient cycling, nearby insect populations, food supply for animal species and agricultural practices, depending on the plant variety and the transgenes they bear (Auer, 2008). The indirect risks can involve generating risks for other plant and animal species, natural community structures, and ecosystem functioning (Wilkinson et al., 2003).

5.5.1 Risk Assessment Theories and Practices

Risk can be simply explained as the probability of an undesirable event to happen. Risk assessment is a part of risk analysis, which includes two additional components, namely, risk management and risk communication (Johnson et al., 2007). Risk assessment systems usually rely on logical paces of identification

of problems, recognition of possible hazards, determination of potential exposure pathways, estimation of the magnitude of harms and expression of uncertainty (Auer, 2008). Risk assessment practices for transgenic plants are used by government agencies and stakeholders to detect probable risks from those plant varieties to the environment or humankind. Mostly, risk assessment practices used by government agencies consider a plant as a weed in certain mismanaged areas, its ability to invade natural areas, and its potential for gene flow through pollen (Auer, 2008).

Qualitative methods and quantitative methods such as probabilistic assessments are the main scientific approaches for risk assessment. Mostly, qualitative assessments center on expert judgments, empirical data, or combination of approaches. However, quantitative assessment approaches have limited use for risk analysis of transgenic plants owing to deficiencies of ecological data to construct mathematical models.

An ecological risk can be explained as the artifact of distinct undesirable influence on the environment and the path or mechanism that led to those undesirable consequences. Predictive ecological risk assessment is one such approach that utilizes present understanding for estimation of possible forthcoming threats from direct and/or indirect contact to genetically modified plants, genes of those plants, and gene products (Hayes, 2004; Auer, 2008).

However, the most accepted way to make a comparison of risk assessments for transgenic plant varieties is assessing the impacts of traditionally bred varieties (Conner et al., 2003). This is because in most cases the effects from transgenic plants are identical to the effects from traditionally bred varieties.

5.5.2 Challenges for Complex Risk Assessment

Challenges for risk assessment include difficulties regarding impact of transgenic plants

for natural plant communities, ecosystems, and nontarget organisms; characterization of special and temporal gene flow; and fitness of plants in fluctuating environmental conditions. The deficiencies of fundamental knowledge on ecological aspects of urban areas, gardens, and most natural habitats restrict the identification of exposure pathways, prediction of potential hazards, and planning of hypothesis-directed studies. However, the evaluation of direct and indirect risks from transgenic plants to non-transgenic varieties, community assemblage, and ecosystem functioning are considered as the most difficult tasks using experimental procedures (Auer, 2008).

The environmental impact assessment studies for transgenic plants provide ecologically important evidence but most of them are limited to relatively short periods of time and generally they are conducted at small scales (Snow and Palma, 1997). Monitoring and assessing of long-term ecological influences emerging from transgenic plants in a broad area of ecosystems can be considered as a challenging task. Each year dozens of new transgenic plant cultivars are being introduced throughout the world and a complete assessment of environmental impact from those plants in a broad way is a tedious project.

5.6 REGULATORY APPROACHES FOR TRANSGENIC PLANTS

In spite of forthcoming assurances and high public acceptance rate for transgenic plant varieties, a number of safety concerns have arisen on the topic regarding potential risks associated with the environment and mankind; these are the reasons for imposing a wide range of regulations and legislation. Worldwide, most regulatory approaches for transgenic plants have been established in the United States and Europe. The approaches adopted by those two regions have fundamental differences (Nap et al., 2003). Regulatory

approaches of the United States basically focus on the characteristics of genetically modified products whilst the European system relies on the generation process of genetically modified plants. Most other countries have made their own regulatory system based on the two main systems as stated above. In spite of the differences they possess, each risk assessment and regulatory approach recognizes the possibilities, probabilities, and consequences of damage in different scenarios.

5.7 FUTURE RESEARCH DIRECTIONS

It is well documented that transgenic plants have exceptional capacity to contribute to the revitalization process of contaminated lands through the phytoremediation process. However, the risk inherent with the transgenic plant varieties should be minimized and their application and capabilities towards more effective and more efficient remediation processes must be achieved through future research. Studies on the mechanisms of phytoremediation of these transgenic plants must be further looked into to have a comprehensive understanding as to whether a particular trace element or other contaminant can be induced into a plant's roots and thus whether it can be used for remediation.

Environmental risk assessment of those transgenic plants introduced for effectual phytoremediation must be associated with complete and long-term field testing as a critical step in research and development. The research and development process must look closely at the prevention technologies for further spreading of modified genes from transgenic plants to natural plant communities. Therefore, the development of further mechanisms such as genetic use restriction technologies, which can be utilized to control the dispersion of transgenic plants in natural habitats, should be facilitated.

References

- Arisi, A.-C.M., et al., 1997. Modification of thiol contents in poplars (*Populus tremula* × *P. alba*) overexpressing enzymes involved in glutathione synthesis. *Planta* 203 (3), 362–372.
- Arthur, E.L., et al., 2005. Phytoremediation—an overview. *CRC. Crit. Rev. Plant. Sci.* 24 (2), 109–122.
- Auer, C., 2008. Ecological risk assessment and regulation for genetically-modified ornamental plants. *CRC. Crit. Rev. Plant. Sci.* 27 (4), 255–271.
- Bañuelos, G., et al., 2005. Field trial of transgenic Indian mustard plants shows enhanced phytoremediation of selenium-contaminated sediment. *Environ. Sci. Technol.* 39 (6), 1771–1777.
- Bennett, L.E., et al., 2003. Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. *J. Environ. Qual.* 32 (2), 432–440.
- Bizily, S.P., et al., 2003. Subcellular targeting of methylmercury lyase enhances its specific activity for organic mercury detoxification in plants. *Plant Physiol.* 131 (2), 463–471.
- Burken, J.G., Schnoor, J.L., 1997. Uptake and metabolism of atrazine by poplar trees. *Environ. Sci. Technol.* 31 (5), 1399–1406.
- Chaney, R.L., et al., 1997. Phytoremediation of soil metals. *Curr. Opin. Biotechnol.* 8 (3), 279–284.
- Chaudhry, Q., et al., 2002. Prospects and limitations of phytoremediation for the removal of persistent pesticides in the environment. *Environ. Sci. Pollut. Res.* 9 (1), 4.
- Cherian, S., Oliveira, M.M., 2005. Transgenic plants in phytoremediation: recent advances and new possibilities. *Environ. Sci. Technol.* 39 (24), 9377–9390.
- Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* 88 (11), 1707–1719.
- Conner, A.J., et al., 2003. The release of genetically modified crops into the environment. *Plant J.* 33 (1), 19–46.
- Culley, T.M., Hardiman, N.A., 2007. The beginning of a new invasive plant: a history of the ornamental Callery pear in the United States. *AIBS Bull.* 57 (11), 956–964.
- Cunningham, S.D., et al., 1995. Phytoremediation of contaminated soils. *Trends Biotechnol.* 13 (9), 393–397.
- Czako, M., et al., 2006. Transgenic *Spartina alterniflora* for phytoremediation. *Environ. Geochem. Health* 28 (1–2), 103–110.
- De la Fuente, J.M., et al., 1997. Aluminum tolerance in transgenic plants by alteration of citrate synthesis. *Science* 276 (5318), 1566–1568.
- Dhankher, O.P., et al., 2002. Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthetase expression. *Nat. Biotechnol.* 20 (11), 1140.
- Dimkpa, C., Bindraban, P., McLean, J.E., Gatere, L., Singh, U., Hellums, D., 2017. Methods for rapid testing of Plant and Soil Nutrients. In: Lichtfouse, E. (Ed.), *Sustainable Agriculture Reviews*, vol 25. Springer, Cham.
- Douchkov, D., et al., 2005. Ectopic expression of nicotianamine synthase genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco. *Plant Cell Environ.* 28 (3), 365–374.
- Ellis, D.R., et al., 2004. Production of Se-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase. *BMC Plant Biol.* 4 (1), 1.
- Ellstrand, N.C., Schierenbeck, K.A., 2006. Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica* 148 (1–2), 35–46.
- Elmayan, T., Tepfer, M., 1994. Synthesis of a bifunctional metallothionein/β-glucuronidase fusion protein in transgenic tobacco plants as a means of reducing leaf cadmium levels. *Plant J.* 6 (3), 433–440.
- Escande, V., et al., 2014. Ecological catalysis and phytoextraction: symbiosis for future. *Appl. Catal. B: Environ.* 146, 279–288.
- Gall, J.E., Rajakaruna, N., 2013. The physiology, functional genomics, and applied ecology of heavy metal-tolerant Brassicaceae, Brassicaceae: Characterization, Functional Genomics and Health Benefits, 2013. Nova Science Publishers, Inc., New York, pp. 121–148.
- Garbisu, C., Alkorta, I., 2003. Basic concepts on heavy metal soil bioremediation. *EJMP & EP (Eur. J. Miner. Process. Environ. Protect.)* 3 (1), 58–66.
- Gasic, K., Korban, S.S., 2007. Transgenic Indian mustard (*Brassica juncea*) plants expressing an *Arabidopsis* phytochelatin synthase (*AtPCS1*) exhibit enhanced As and Cd tolerance. *Plant Mol. Biol.* 64 (4), 361–369.
- Gisbert, C., et al., 2003. A plant genetically modified that accumulates Pb is especially promising for phytoremediation. *Biochem. Biophys. Res. Commun.* 303 (2), 440–445.
- Glick, B.R., 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* 21 (5), 383–393.
- Gong, J.-M., et al., 2003. Long-distance root-to-shoot transport of phytochelatins and cadmium in *Arabidopsis*. *Proc. Natl Acad. Sci.* 100 (17), 10118–10123.
- Goto, F., et al., 1998. Iron accumulation in tobacco plants expressing soyabean ferritin gene. *Transgenic. Res.* 7 (3), 173–180.
- Gullner, G., et al., 2001. Enhanced tolerance of transgenic poplar plants overexpressing γ-glutamylcysteine synthetase towards chloroacetanilide herbicides. *J. Exp. Bot.* 52 (358), 971–979.
- Guo, J., et al., 2008. Overexpressing GSH1 and AsPCS1 simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana*. *Chemosphere* 72 (7), 1020–1026.

- Han, Y., et al., 2009. One novel mitochondrial citrate synthase from *Oryza sativa* L. can enhance aluminum tolerance in transgenic tobacco. *Mol. Biotechnol.* 42 (3), 299–305.
- Hancock, J.F., 2003. A framework for assessing the risk of transgenic crops. *Bioscience* 53 (5), 512–519.
- Harada, E., et al., 2001. Transgenic tobacco plants expressing a rice cysteine synthase gene are tolerant to toxic levels of cadmium. *J. Plant. Physiol.* 158 (5), 655–661.
- Hasegawa, I., et al., 1997. Genetic improvement of heavy metal tolerance in plants by transfer of the yeast metallothionein gene (*CUP1*). *Plant Nutr. Sustain. Food Prod. Environ.* Springer, Dordrecht, pp. 391–395.
- Hayes, K.R., 2004. Best Practice and Current Practice in Ecological Risk Assessment for Genetically Modified Organisms. CSIRO Division of Marine Research, Hobart.
- He, J., et al., 2015. Overexpression of bacterial γ -glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. *New Phytol.* 205 (1), 240–254.
- He, Y.K., et al., 2001. Differential mercury volatilization by tobacco organs expressing a modified bacterial merA gene. *Cell Res.* 11 (3), 231.
- He, Z.-L., Yang, X.-E., 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *J. Zhejiang. Univ. Sci. B* 8 (3), 192–207.
- Hegde, S.G., et al., 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. *Evolution* 60 (6), 1187–1197.
- Herschbach, C., et al., 1998. Overexpression of γ -glutamylcysteine synthetase, but not of glutathione synthetase, elevates glutathione allocation in the phloem of transgenic poplar trees. *Plant Cell Physiol.* 39 (4), 447–451.
- Higuchi, K., et al., 1999. Cloning of nicotianamine synthase genes, novel genes involved in the biosynthesis of phytosiderophores. *Plant Physiol.* 119 (2), 471–480.
- Hsieh, J.-L., et al., 2009. Expressing a bacterial mercuric ion binding protein in plant for phytoremediation of heavy metals. *J. Hazard. Mater.* 161 (2–3), 920–925.
- Huysen, T., et al., 2004. Exploring the selenium phytoremediation potential of transgenic Indian mustard overexpressing ATP sulfurylase or cystathionine- γ -synthase. *Int. J. Phytoremediation.* 6 (2), 111–118.
- Ike, A., et al., 2007. Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant rhizobia with the MTL4 and the PCS genes. *Chemosphere* 66 (9), 1670–1676.
- Johnson, K.L., et al., 2007. How does scientific risk assessment of GM crops fit within the wider risk analysis? *Trends. Plant. Sci.* 12 (1), 1–5.
- Johnson, P.G., Riordan, T.P., 1999. A review of issues pertaining to transgenic turfgrasses. *HortScience* 34 (4), 594–598.
- Kawashima, C.G., et al., 2004. Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. *Biotechnol. Lett.* 26 (2), 153–157.
- Key, S., et al., 2008. Genetically modified plants and human health. *J. R. Soc. Med.* 101 (6), 290–298.
- Kim, S., et al., 2005. Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. *Plant Cell Physiol.* 46 (11), 1809–1818.
- Korenkov, V., et al., 2007. Enhancing tonoplast Cd/H antiport activity increases Cd, Zn, and Mn tolerance, and impacts root/shoot Cd partitioning in *Nicotiana tabacum* L. *Planta* 226 (6), 1379–1387.
- LeDuc, D.L., et al., 2004. Overexpression of selenocysteine methyltransferase in *Arabidopsis* and Indian mustard increases selenium tolerance and accumulation. *Plant Physiol.* 135 (1), 377–383.
- LeDuc, D.L., et al., 2006. Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. *Environ. Pollut.* 144 (1), 70–76.
- Lee, S., et al., 2003. Overexpression of *Arabidopsis* phytochelatin synthase paradoxically leads to hypersensitivity to cadmium stress. *Plant Physiol.* 131 (2), 656–663.
- Li, Y., et al., 2004. Overexpression of phytochelatin synthase in *Arabidopsis* leads to enhanced arsenic tolerance and cadmium hypersensitivity. *Plant Cell Physiol.* 45 (12), 1787–1797.
- Liu, D., et al., 2015. Enhanced heavy metal tolerance and accumulation by transgenic sugar beets expressing *Streptococcus thermophilus StGCS-GS* in the presence of Cd, Zn and Cu alone or in combination. *PLoS. One.* 10 (6), e0128824.
- Liu, J., et al., 2011. Characteristics of chromium (III) uptake in hyperaccumulator *Leersia hexandra* Swartz. *Environ. Exp. Bot.* 74, 122–126.
- Lugauskas, A., et al., 2005. Effect of copper, zinc and lead acetates on microorganisms in soil. *Ekologija* 1, 61–69.
- Macek, T., Macková, M., Pavlíková, D., Száková, J., Truksa, M., Singh Cundy, A., et al., 2002. Accumulation of cadmium by transgenic tobacco. *Acta Biotech.* 22 (1–2), 101–106.
- Maestri, E., Marmiroli, N., 2011. Transgenic plants for phytoremediation. *Int. J. Phytoremediation.* 13 (sup 1), 264–279.
- Mahar, A., et al., 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. *Ecotoxicol. Environ. Saf.* 126, 111–121.
- Martínez, M., et al., 2006. An engineered plant that accumulates higher levels of heavy metals than *Thlaspi caerulescens*, with yields of 100 times more biomass in mine soils. *Chemosphere* 64 (3), 478–485.
- McGrath, S.P., Zhao, F.-J., 2003. Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol.* 14 (3), 277–282.

- Meagher, R.B., 2000. Phytoremediation of toxic elemental and organic pollutants. *Curr. Opin. Plant. Biol.* 3 (2), 153–162.
- Mendoza-Cózatl, D.G., et al., 2008. Identification of high levels of phytochelatins, glutathione and cadmium in the phloem sap of *Brassica napus*. A role for thiol-peptides in the long-distance transport of cadmium and the effect of cadmium on iron translocation. *Plant J.* 54 (2), 249–259.
- Mishra, D., Kar, M., 1974. Nickel in plant growth and metabolism. *Bot. Rev.* 40 (4), 395–452.
- Misra, S., Gedamu, L., 1989. Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants. *Theoret. Appl. Genet.* 78 (2), 161–168.
- Nap, J.P., et al., 2003. The release of genetically modified crops into the environment. *Plant J.* 33 (1), 1–18.
- Neubauer, U., et al., 2000. Siderophores, NTA, and citrate: potential soil amendments to enhance heavy metal mobility in phytoremediation. *Int. J. Phytoremediation.* 2 (4), 353–368.
- Newman, L.A., et al., 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environ. Sci. Technol.* 31 (4), 1062–1067.
- Noctor, G., et al., 1998. Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. *J. Exp. Bot.* 49 (321), 623–647.
- Pan, A., et al., 1994. Expression of mouse metallothionein-I gene confers cadmium resistance in transgenic tobacco plants. *Plant Mol. Biol.* 24 (2), 341–351.
- Pavlikova, D., et al., 2004a. The evaluation of cadmium, zinc and nickel accumulation ability of transgenic tobacco bearing different transgenes. *Plant Soil Environ.* 50 (12), 513–517.
- Pavliková, D., et al., 2004b. Cadmium tolerance and accumulation in transgenic tobacco plants with a yeast metallothionein combined with a polyhistidine tail. *Int. Biodeterior. Biodegrad.* 54 (2–3), 233–237.
- Pillichshammer, M., et al., 1995. Biosorption of chromium to fungi. *Biometals* 8 (2), 117–121.
- Pilon-Smits, E., et al., 1998. Selenium volatilization and assimilation by hybrid poplar (*Populus tremula* × *alba*). *J. Exp. Bot.* 49 (328), 1889–1892.
- Pilon-Smits, E.A., et al., 1999. Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. *Plant Physiol.* 119 (1), 123–132.
- Pomponi, M., et al., 2006. Overexpression of *Arabidopsis* phytochelatin synthase in tobacco plants enhances Cd²⁺ tolerance and accumulation but not translocation to the shoot. *Planta* 223 (2), 180–190.
- Rafati, M., et al., 2011. Phytoremediation potential of *Populus alba* and *Morus alba* for cadmium, chromium and nickel absorption from polluted soil. *Int. J. Environ. Res. Public Health* 5 (4), 961–970.
- Rugh, C.L., et al., 1996. Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. *Proc. Natl Acad. Sci.* 93 (8), 3182–3187.
- Rugh, C.L., et al., 1998. Development of transgenic yellow poplar for mercury phytoremediation. *Nat. Biotechnol.* 16 (10), 925.
- Ruiz, O.N., et al., 2003. Phytoremediation of organomercurial compounds via chloroplast genetic engineering. *Plant Physiol.* 132 (3), 1344–1352.
- Sasaki, Y., et al., 2006. Generation of mercury-hyperaccumulating plants through transgenic expression of the bacterial mercury membrane transport protein MerC. *Transgenic Res.* 15 (5), 615.
- Schutzendubel, A., Polle, A., 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *J. Exp. Bot.* 53 (372), 1351–1365.
- Seneviratne, M., et al., 2016. Plant growth promotion by *Bradyrhizobium japonicum* under heavy metal stress. *S. Afr. J. Bot.* 105, 19–24.
- Seneviratne, M., Vithanage, M., 2015. The role of siderophores on plants under heavy metal stress: a view from the Rhizosphere. *Res. Rev.: J. Botan. Sci.* 4 (3), 23–29.
- Sheoran, V., et al., 2009. Phytomining: a review. *Miner. Eng.* 22 (12), 1007–1019.
- Simard, M.-J., et al., 2006. Transgenic *Brassica napus* fields and *Brassica rapa* weeds in Quebec: sympathy and weed-crop in situ hybridization. *Botany* 84 (12), 1842–1851.
- Singh, O., et al., 2003. Phytoremediation: an overview of metallic ion decontamination from soil. *Appl. Microbiol. Biotechnol.* 61 (5–6), 405–412.
- Snow, A.A., Palma, P.M., 1997. Commercialization of transgenic plants: potential ecological risks. *Bioscience* 47 (2), 86–96.
- Sobolev, D., Begonia, M., 2008. Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. *Int. J. Environ. Res. Public Health* 5 (5), 450–456.
- Song, W.-Y., et al., 2003. Engineering tolerance and accumulation of lead and cadmium in transgenic plants. *Nat. Biotechnol.* 21 (8), 914.
- Takahashi, M., et al., 2001. Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nat. Biotechnol.* 19 (5), 466.
- Thomas, J.C., et al., 2003. Yeast metallothionein in transgenic tobacco promotes copper uptake from contaminated soils. *Biotechnol. Prog.* 19 (2), 273–280.
- Trapp, S., Karlson, U., 2001. Aspects of phytoremediation of organic pollutants. *J. Soils Sediment.* 1 (1), 37.

- Van Aken, B., 2008. Transgenic plants for phytoremediation: helping nature to clean up environmental pollution. *Trends Biotechnol.* 26 (5), 225–227.
- Van Aken, B., 2009. Transgenic plants for enhanced phytoremediation of toxic explosives. *Curr. Opin. Biotechnol.* 20 (2), 231–236.
- Vangronsveld, J., et al., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. *Environ. Sci. Pollut. Res.* 16 (7), 765–794.
- Vithanage, M., et al., 2014. Metal release from serpentine soils in Sri Lanka. *Environ. Monit. Assess.* 186 (6), 3415–3429.
- Vögeli-Lange, R., Wagner, G.J., 1990. Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves: implication of a transport function for cadmium-binding peptides. *Plant Physiol.* 92 (4), 1086–1093.
- Wangeline, A.L., et al., 2004. Overexpression of ATP sulfurylase in Indian mustard. *J. Environ. Qual.* 33 (1), 54–60.
- Watanabe, M.E., 1997. Phytoremediation on the brink of commercialization. *Environ. Sci. Technol.* 31 (4), 182A–186A.
- Wei, S., et al., 2008. Agro-improving method of phytoextracting heavy metal contaminated soil. *J. Hazard. Mater.* 150 (3), 662–668.
- Wilkinson, M.J., et al., 2003. Risk assessment of GM plants: avoiding gridlock? *Trends. Plant. Sci.* 8 (5), 208–212.
- Xiang, C., et al., 2001. The biological functions of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. *Plant Physiol.* 126 (2), 564–574.
- Yadav, B.K., et al., 2011. Rhizofiltration of a heavy metal (lead) containing wastewater using the wetland plant *Carex pendula*. *CLEAN—Soil, Air, Water* 39 (5), 467–474.
- Zelaya, I.A., et al., 2007. Transfer of glyphosate resistance: evidence of hybridization in *Comyzza* (Asteraceae). *Am. J. Bot.* 94 (4), 660–673.
- Zhu, Y.L., 1999. Overexpression of glutathione synthetase in *Brassica juncea* enhances cadmium tolerance and accumulation. *Plant Physiol.* 119, 73–79.