Standard Article - doi: 10.3832/ifor0555-004 ©iForest - Biogeosciences and Forestry

Heavy metals and woody plants - biotechnologies for phytoremediation

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Soil contamination by heavy metals is among the most serious danger for the environment, and new methods for its containment and removal are claimed, in particular for agricultural soils. Phytoremediation is an emerging, potentially effective technology applicable to restoration of contaminated soils and waters. Besides hyperaccumulator herbaceous plants, several woody species are now considered of interest to this aim. Many woody plants are fast growing, have deep roots, produce abundant biomass, are easy to harvest, and several species revealed some capacity to tolerate and accumulate heavy metals. Biotechnologies are now available for investigating this potential and enlarge the possibilities of exploitation of trees for remediation. The use of *in vitro* cultures, the role of bacteria and mychorrhizas, the powerful tool of genetic engineering, are some of the aspects focused in this paper that open prospects of global relevance for a better understanding of the processes related to the uptake of heavy metals by woody plants. In recent years significant progress has been made in identifying native plants and developing genetically modified tree plants for the remediation of heavy-metal polluted environment. Despite the intensive research developed in the last years, few field trials demonstrated the feasibility of the approach described, therefore much efforts should be addressed to this goal.

Keywords: Engineering, *In vitro* culture, Michorrhyzas, Pollution, Trees

Introduction

Pollution of soil and agricultural land is a complex and serious phenomenon that in recent decades has increased its negative effects on the environment. Transfer of toxic elements to human food chain is a concrete danger that has to be faced, taking into account the possibility for plants to accumulate and translocate contaminants to edible and harvested parts (Kloke et al. 1984, Renzoni et al. 1998, Dudka & Miller 1999, McLaughlin et al. 1999, Puschenreiter et al. 2005). Traditional technologies for removal of pollutants can be successful in specific situations, but costs associated with these technologies are very high. There is an active effort to develop new, more cost-effective methods to remediate contamination of polluted soils, hence attention is now focusing on innovative biological technologies such as phytoremediation, based on the use of plants to extract, sequester and/or detoxify pollutants (Salt et al. 1998). The development of phytoremediation technologies is continuing, involving transgenic and non-transgenic approaches, as well as different biological, technical, social, and economical aspects. Biotechnologies applied to investigating the remediation capability of woody plants are increasingly showing their efficacy, hence some aspects of their exploitation are presented here.

The exploitation of *in vitro* culture, the role of endophytic bacteria and mychorrizas, and the efforts to enhance uptake capacity and tolerance to heavy metals by genetic engineering are therefore considered in the following chapters.

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Received: Sep 01, 2010 - Accepted: Nov 22, 2010

Citation: Capuana M, 2011. Heavy metals and woody plants - biotechnologies for phytoremediation. iForest 4: 7-15 [online: 2011-01-27] URL: http://www.sisef.it/iforest/show.php?id=555

Woody plants and phytoremediation

Twenty-five years ago, studies on phytoremediation techniques were rather scarce; now the scientific and social interest on this subject has increased substantially after the increasing pressure of public opinion.

The use of plants to decontaminate soils and waters has been developed only recently, the first reports appearing in the eighties, followed by more exhaustive articles during the nineties (Chaney 1983, Baker et al. 1994, Cunningham et al. 1995, Salt et al. 1995, Raskin et al. 1997). Much effort still has to be directed towards an understanding of the basic mechanisms and towards improving knowledge of the applications (Marmiroli et al. 2006), but its usefulness has been demonstrated in many sites and this technology is now used by several environmental companies (Glass 2000).

Phytoremediation is based on the removal of contaminants from the soil by mechanisms such as phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization (Salt et al. 1995), but the mechanisms involved in heavy metal remediation are limited to uptake, adsorption, transport and translocation, sequestration into vacuoles, hyperaccumulation and, in some cases, volatilization (Meagher 2000). Within this frame, studies on allocation plasticity and plant-metal partitioning can be of great significance (Audet & Charest 2008). When present at increased concentrations, both essential (copper, iron, manganese, molybdenum, zinc) and non-essential metals (*e.g.*, cadmium, chromium, lead, mercury) are toxic. Mercury and selenium can also be converted by plants into a volatile form to release and dilute into the atmosphere. Heavy metals cannot be metabolized, therefore the only possible strategy to apply is their extraction from contaminated soil and transfer to the smaller volume of harvestable plants for their disposal (Salt et al. 1995, Kumar et al. 1995, Raskin et al. 1997, Padmavathiamma & Li 2007); biomass can also be used in producing energy and, if economically profitable, metals can be eventually recovered (Zacchini et al. 2009).

It must be stressed that some processes can limit the efficacy of plants in phytoremediation, such as the availability of the toxic metal ions in the soil for root uptaking, their rate of translocation from roots to shoots and the level of tolerance, the rate of chemical transformation into less toxic compounds (Prasad 2003). At the basis, we find the mechanisms implicated in plant metal tolerance and homeostasis (reviewed by Clemens 2001). Remediation using plants may take longer than other technologies, but the most relevant limitation is that it is most suited to cases where contaminants are present at shallow levels within the root layer.

Phytoremediation technology has been recently extensively reviewed (Salt et al. 1998, Meagher 2000, Dietz & Schnoor 2001, Barceló & Poschenrieder 2003, Suresh & Ravishankar 2004, Newman & Reynolds 2005, Pilon-Smith & Freeman 2006, Audet & Charest 2007a) and several species have been classified as hyperaccumulators and extensively investigated (Reeves 1992, Peer et al. 2005). However, on a large scale, metal uptake by trees can be more effective, mainly because of a deeper root system and a greater yield of biomass (Greger & Landberg 1999, Fischerová et al. 2006). High productivity and elevated uptake and translocation of pollutants to the harvestable biomass are the basis for efficient *in situ* restoration by means of vascular plants (Lasat 2002, Pulford & Dickinson 2005, Chaney et al. 2007).

Some woody species can be advantageously used also for phytoremediation of soils and groundwater from organic pollutants (Corseuil & Moreno 2001) and hydrocarbons (Thompson et al. 1998, Yoon et al. 2006). The potential in phytoremediation of metal contaminated soils expressed by forest trees has been assessed for several species in recent years (Arduini et al. 1996, Pisano & Rockwood 1997, Kozlov et al. 2000, Maurice & Lagerkvist 2000, Prasad & Freitas 2000, Kopponen et al. 2001, Rosselli et al. 2003, Pulford & Watson 2003, French et al. 2006, Meers et al. 2007, Unterbrunner et al. 2007, Brunner et al. 2008, Domínguez et al. 2008). Resistance to metals often appears to be clone- or hybrid-specific rather than species-specific (Punshon & Dickinson 1999).

Poplars are particularly suitable for remediation purposes (Dix et al. 1997, Burken & Schnoor 1998, Schnoor 2000), having already been considered for trials on metal tolerance in *in vivo* (Lingua et al. 2005) and *in vitro* observations (Franchin et al. 2007). Salicaceae are also reported to grow even in severe soil conditions and to accumulate heavy metals (Pulford & Watson 2003, Berndes et al. 2004). Many studies have thus been focused on the use of willows and poplars in phytoextraction (Riddell-Black 1994, Labreque et al. 1995, Bañuelos et al. 1999, Robinson et al. 2000, Aronsson & Perttu 2001, Granel et al. 2002, Klang-Westin & Perrtu 2002, Hammer et al. 2003, Vyslouzilová et al. 2003, Vervaeke et al. 2003, Madejou et al. 2004, Sebastiani et al. 2004, Kuzovkina et al. 2004, Robinson et al. 2005, Giachetti & Sebastiani 2006, Dos Santos Utmazian et al. 2007, Wieshammer et al. 2007, Jensen et al. 2009). These species can be advantageously exploited in short rotation coppice cultures (SRC), a strategy whose application in phytoremediation presents interesting and economically promising perspectives (Scarascia-Mugnozza et al. 1997, Paulson et al. 2003, Laureysens et al. 2004a, Laureysens et al. 2004b, Rockwood et al. 2004, Dickinson & Pulford 2005, Witters et al. 2009).

Use of *in vitro* cultures for research on phytoremediation

The inherent difficulties of experimenting on very large long-lived organisms such as forest trees, motivates the development of model systems. Besides the exploitation of hydroponic cultures, the *in vitro* model systems using shoot and cell cultures of plants demonstrated to be a useful tool for investigating efficiency of metal uptake and translocation (Castiglione et al. 2007). Cell and organ culture, in fact, as well as hydroponics, allow very fast accumulation of data in comparison with whole plant experiments under field conditions (Golan-Goldhirsh et al. 2004), and offer the advantage of testing the effects of contaminants under controlled conditions (Harms 1992). Hydroponic screening is often used to evaluate tolerance, accumulation and translocation in plants. Watson et al. (2003) demonstrated in *Salix* that results obtained in hydroponics and in field experiments are comparable. It is always advisable, however, to confirm data obtained by hydroponic tests by field performance trials. Using this technique, several studies have concerned, for instance, the response of willows to a metal cocktail (Watson et al. 1999) and of willows and poplars to the presence of cadmium (Šottníková et al. 2003, Lunácková et al. 2003, Dos Santos Utmazian et al. 2007, Zacchini et al. 2009), the response of a clone of *Populus* x *euramericana* to high concentrations of copper (Borghi et al. 2007), the mechanism of resistance to aluminium of *Picea abies* (Heim et al. 1999), the determination of the role of glutathione reductase metabolism in the defence of poplar (*Populus deltoides* x *P. nigra*) against high zinc concentration (Di Baccio et al. 2005).

As stated by Golan-Goldhirsh et al. (2004), the use of *in vitro* systems enables dissection of the complex system of plant, soil, and microbial interaction in order to evaluate the effect of stress factors on metabolism, specific enzymes and metabolites involved in plant response to the pollutant. For many woody species, moreover, the application of *in vitro* propagation techniques, allows for fast plant production and the application of promising genetic engineering programs (Confalonieri et al. 2003, Lyyra et al. 2006).

High concentration of zinc has been found to negatively affect the photosynthetic machinery in poplar: inhibition of adventitious root formation and leaf chlorosis indicated that the clone used was tolerant to external concentrations less than or equal to 1 mM (Castiglione et al. 2007), while in *Eucalyptus globulus* moderate concentrations of this metal were shown to either enhance or have no effect on rooting (Schwambach et al. 2005). Phytoremediation potentials of poplar lines (*Populus nigra* and transgenic *P. canescens)* were investigated using *in vitro* leaf discs cultures and found that Zinc^{2+} was phytotoxic only at high concentrations (10^{-2} to 10^{-1} M) in all *P. canescens* lines, but *P. nigra* was more sensitive (Bittsanszky et al. 2005). Cadmium added to the culture medium was shown to reduce the fresh and dry weights and the shoot length of white birch, while root length was not affected (Fernández et al. 2008). Copper at a concentration of 0.05 mM, manganese at 0.80 mM, and zinc at 0.12 mM showed a negative effect on shoot growth (number of shoots per explant and shoot length) in *Ailanthus altissima*, considered a fast-growing and contamination-resistant species (Gatti 2008). Zinc was found toxic in aspen (*Populus tremula* x *tremuloides*) cultures at 0.5 mM concentration, while lead at the same concentration did not show toxic effects and was accumulated at 3500 µg per g of biomass (Kalisová-Spirochová et al. 2003). *In vitro* studies were also developed to investigate the effects of high concentrations of zinc and copper on the biosynthesis and accumulation of polyamine in *Populus alba* (Franchin et al. 2007). On the basis of leaf symptoms, rate of adventitious root formation and ethylene production, it was found that Zn at 0.5-1 mM concentration was transiently toxic, while at 2-4 mM was increasingly toxic. Free and conjugated putrescine and spermidine accumulated proportionally to toxicity; also Cu strongly reduced rooting already at 5 µM and caused severe, dose-dependent toxicity symptoms (shoot chlorosis and necrosis) using concentrations up to 500 µM. In *in vitro* growing microshoots of *Populus alba*, the effect of high concentrations of cadmium, copper, zinc, and arsenic was investigated, showing differences in the response of different clones (Di Lonardo et al. 2011). Axenic poplar tumor cell cultures were tested for demonstrating the capability of taking up trichloroethylene (TCE) and degrading it to several known metabolic products (Newman et al. 1997), while poplar (*Populus deltoides×P. nigra*) *in vitro* culture has been used for developing mathematical models to define degradation pathways of nitramine compounds within plant cells (Mezzari et al. 2004). Metal tolerance was detected in a callus culture established from *Acer rubrum* seedlings growing in soil contaminated by zinc, cadmium, nickel and arsenic. A positive linear correlation was found between zinc resistance of callus and total Zn in soil beneath sampled trees, while no significant correlations were evidenced with the other metals (Watmough & Hutchinson 1998). In *Acer pseudoplatanus* callus culture, Cu-, Zn- and Cd-resistance traits were identified in cell lines originating from trees at a site contaminated by these metals (Watmough & Dickinson 1995). *In vitro* screenings were also used to investigate how several heavy metals affect pollen germination and tube elongation in *Pinus resinosa* (Chaney & Strickland 1984) and to test the tolerance for Zn and Cu in mycorrhyzal isolates collected in an abandoned Cu mines, in view of their inoculation into *Pinus sylvestris* seedlings (Adriaensen et al. 2005). Combined micropropagation and hydroponic culture were used to study tolerance to copper and zinc in *Betula pendula*, finding that a seed-derived clone from a Pb/Zn-contaminated site showed more tolerance to Cu and Zn than budderived clones from a Cu/Ni-contaminated site or from an uncontaminated area (Utriainen et al. 1997).

Role of endophytic bacteria and mychorrhizas in phytoremediation

Bacteria living within plant tissues without causing disease are referred to as endophytes. Some of these have shown the capacity to enhance plant growth and resistance to biotic and abiotic stresses by various mechanisms (*e.g.*, nitrogen fixation, production of phytohormones, solubilisation of minerals, etc.), therefore, recently attention has been focusing on the role of endophytic bacteria in phytoremediation (Selosse et al. 2004, Newman & Reynolds 2005). Endophytes have been inoculated and studied, *e.g.*, in hybrid spruce (Chanway et al. 2000), lodgepole pine (Chanway & Holl 1994a), Douglas-fir (Chanway & Holl 1994b), poplar and willow. Based on their potential for remediation, three *Pseudomonas* strains were identified and tested in a clone of hybrid cottonwood (*Populus trichocarpa* x *P. deltoides*) (Germaine et al. 2004). A large part of the research on this subject has been dealt with the activity of endophytes on hydrocarbons. For instance, in hybrid cottonwood a strain of the endophyte *Rhizobium tropici* was found active in the degradation of explosives (Doty et al. 2005), as well as a *Methylobacterium* strain isolated from hybrid poplar (*Populus deltoides* x *P. nigra* - Van Aken et al. 2004a, 2004b); poplar endophytic bacteria have been engineered for enhancing thrichloroethylene degradation (Shim et al. 2000) and Taghavi et al. 2005 observed a horizontal gene transfer of a plasmid conferring toluene degradation.

Concerning specifically heavy metals, it has been observed that heavy metal resistant endophytes are present in various hyperaccumulator plants growing on heavy-metal contaminated soil (Rajkumar et al. 2009). Among herbaceous plants, *e.g.*, shoot endophytes of *Thlaspi goesingense* were found more tolerant to high nickel concentration than the correspondent rhizospheric bacteria (Idris et al. 2004), endophytic bacteria of *Nicotiana tabacum* could reduce cadmium phytotoxicity (Mastretta et al. 2009), recombinant heavy-metal resistant endophytic bacteria were studied in *Lolium perenne* and *Lupinus luteus* (Lodewyckx et al. 2001). Among woody species, some isolates of hybrid cottonwoods have demonstrated tolerance to heavy metals (Moore et al. 2006); bacteria associated with Zn/Cd-accumulating *Salix caprea* have been studied regarding their potential to support heavy metal phytoextraction (Kuffner et al. 2010).

Arbuscular mychorrhizas are also well known to be involved in the metal uptake; their presence in the soil may significantly affect the plant response to metal stress (Pawloska & Charvat 2004). A vast amount of literature is available on the effects of mycorrhizal colonisation of plants living in heavy metal-polluted soils (Göhre & Paszkowski 2006), on the protective role of mycorrhizas against heavy-metal induced oxidative stress (Schützendübel & Polle 2002), and on their possible role in remediation (Khan et al. 2000, Audet & Charest 2007b). In the hyperaccumulating fern *Pteris vittata*, for instance, they have been found to increase arsenic uptake (Trotta et al. 2006). Adriaensen et al. (2006) found that *Pinus sylvestris* seedlings colonized by a Zn-tolerant isolate of *Suillus bovinus* grew much better and remained physiologically healthier when exposed to elevated Zn concentration than seedlings not inoculated or colonized by a Zn-sensitive isolate. The response to high copper (Todeschini et al. 2007) and zinc (Lingua et al. 2008) concentration was studied on poplar clones inoculated with arbuscular mycorrhizal fungi, while in mycorrhyzed *Betula* spp. tolerance to zinc (Denny & Wilkins 1987) and Cu and Pb accumulation (Bojarczuk & Kieliszewska-Rokicka 2010) were studied. By X-ray microanalysis of heavy metals, it was found that, in mycorrhized *Picea abies* seedlings, extracellular complexation of Cd occurred predominantly in the Hartig net hyphae and both extracellular complexation and cytosolic sequestration of Zn occurred in the fungal tissue (Frey et al. 2000). The potential benefits of ectomycorrhizal fungi in protecting their host plants from metal contamination were also investigated by Blaudez et al. (2000) after testing thirty-nine ectomycorrhizal isolates for their tolerance to cadmium, copper, nickel and zinc. The potential of *Salix viminalis* and *Populus* x *generosa* for the phytoextraction of heavy metals, inoculated or not with the fungus *Glomus intraradices*, was recently assessed (Bissonnette et al. 2010), while in *Eucalyptus globulus* grown in Zn-contaminated soil, the improving potential of interactions between saprophytic and arbuscular micorrhizal fungi was investigated (Arriagada et al. 2010).

Molecular biology and genetic engineering for phytoremediation

Molecular biology and genetic engineering are being increasingly considered as effective tools for better understanding and improving the phytoremediation capability of plants, whose biological functions can now be analyzed in detail and partly modified. The metal resistance systems are better known in microorganisms (Silver 1996, Hall 2002, Silver & Phung 2005); in plants only a few systems of metal tolerance and/or sequestration are sufficiently characterized (Kärenlampi et al. 2000). In recent years, several key steps have been identified at the molecular level, allowing an increasing application of molecular-genetic technologies and a transgenic approach to a better understanding of mechanisms involved in heavy metal tolerance and accumulation in plants (Clemens et al. 2002, Yang et al. 2005). It has been demonstrated in classic genetic studies that only a few genes are responsible for metal tolerance (Macnair et al. 2000). Transfer and/or overexpression of genes responsible for metal uptake, translocation and sequestration may allow for the production of plants which, depending on the strategy, can be successfully exploited in phytoremediation (Krämer & Chardonnens 2001, Pilon-Smits & Pilon 2002, Clemens et al. 2002, Rugh 2004, Eapen & D'Souza 2005, Cherian & Oliveira 2005, Doty 2008). In this case, special attention must be paid to problems related to the introduction of genetically modified trees, particularly concerning their legal and social acceptance (Knowles & Adair 2007). Promotion of growth and biomass production is a correlated task accomplished, for instance, by increased gibberellin biosynthesis (Eriksson et al. 2000).

The improvement of the phytoremediation properties of plants can be achieved by the modification of their primary and secondary metabolism and the introduction of new phenotypic and genotypic characters (Davison 2005). Even if most of the genes involved in metal uptake, transport and sequestration have been studied on the herbaceous model plant *Arabidopsis*, it must be considered that this species, as well as the most common species defined as hyperaccumulators, have a limited phytoremediation capacity due to their small size or slow growth rate. On the contrary, large, fast-growing plants, like some woody plants, are an important tool for this purpose; on poplars, for instance, reasonable transformation frequencies have been achieved (Han et al. 2000).

Studies on *Arabidopsis* and other species (hyperaccumulators), however, open the way to a transfer and application to high-biomass plants. Among these, *Populus* species (poplars, cottonwoods, aspens) and hybrids have become a model system in forest tree biotechnology (Bradshaw et al. 2000), due to several useful features: short rotation cycle, rapid growth rate and ease of vegetative propagation and *in vitro* multiplication (Confalonieri et al. 2003). Moreover, the poplar genome has been entirely sequenced (Tuskan et al. 2006). It is always important, however, to take into account the risks associated with the biotechnological applications and carefully evaluate the field performances of transgenic plants (Confalonieri et al. 2003).

Hairy roots induction by *Agrobacterium rhizogenes* is probably the easiest method for enhancing the root biomass and, consequently, improving metal uptake. This has been demonstrated for some hyperaccumulator plants (Maitani et al. 1996, Nedelkoska & Doran 2000, Eapen et al. 2003, Eapen & D'Souza 2005).

Transgenic white poplar has been recently obtained expressing the *PsMTa1* gene from *Pisum sativum* for a metallothionein-like protein. Transformed plants showed enhanced resistance to heavy metal, surviving high concentrations of $CuCl_2$ in *in vitro* culture, which strongly affected the nontransgenic plants. Rooting capacity of microshoots was maintained in transgenic lines exposed to 0.1 mM $CuCl_2$, while it was totally destroyed in nontransgenic shoots. In the presence of 1 mM ZnSO4, nontransformed shoots rooted abundantly, while different rooting rates were observed in transgenic lines (Balestrazzi et al. 2009).

Genes encoding enzymes changing the oxidation state of heavy metals can be introduced into plants (Rugh et al. 1996, Hansen et al. 1998). For instance, compared to wild type, transgenic *Populus deltoides* overexpressed *mer-A9* and *mer-A18* genes, when grown in soil with high mercury concentration, developing higher biomass and higher amount of Hg(0), which evaporates through the cell surface (Che et al. 2003). Increased tolerance to ionic mercury was first obtained in yellow poplar (*Lyriodendron tulipifera*) transformed with *mer-A* gene (Rugh et al. 1998). For the remediation of Hg, *Populus deltoides* has been engineered with the bacterial *mer-A* (mercuric ion reductase) and *mer-B* (organomercurial lyase - Che et al. 2003); transgenic trees expressing both genes showed tolerance up to 10 μM of phenylmercuric acetate (PMA - Lyyra et al. 2007). Significant results on tolerance to mercury and related remediation capacity were obtained also in *Oryza sativa* (Heaton et al. 2003) and in *Spartina alterniflora* (Czako et al. 2006). In *Salix* spp. it was proved that the majority of the mercury is accumulated and retained in the cell wall of the roots and only a very small part is transferred to the shoots (Wang & Greger 2004).

Genetic engineering for arsenate reduction, increased translocation from root to shoot, and volatilisation has been recently illustrated and discussed (Zhu & Rosen 2009). *Arabidopsis* has been transformed in order to better control the mobility and sequestration of arsenic (Dhankher et al. 2002). In *Pteris vittata,* genes have been identified that encode enzymes with arsenate reductive activity (Dhankher et al. 2002, Ellis et al. 2006, Rathinasabapathi et al. 2006).

For selenium, a strategy to protect protein synthesis from the activity of this metal was applied in transformed *Arabidopsis* by the expression of a mammalian selenocysteine lyase (Pilon et al. 2003) and could be now tested on woody species.

Transgenic poplar, with increased glutathione peroxidase activity, showed increased tolerance to zinc, probably due to an enhanced ability to detoxify the active oxygen species generated by the pollutant (Bittsanszky et al. 2005). Alterations in photosynthetic parameters and reduction in growth have been reported for a *Populus* x *euramericana* clone after treatment with high concentrations of zinc (Di Baccio et al. 2003).

Cadmium in the environment derives from industrial processes, urban pollution (heating systems and traffic), fertilizers and mineralization of rocks (Rauser & Muwly 1995). Sensitivity to and accumulation of cadmium in some woody species have been recently studied in Sweden (Österås et al. 2000). In a relatively new strategy, aimed to compartmentalize the metals, tolerance to lead and cadmium was enhanced in *Arabidopsis* by the overexpression of the yeast vacuolar transporter protein YCF1 (Song et al. 2003), demonstrating the possibility to engineer phytoremediators for increasing their ability to sequester heavy metals. Poplars overexpressing a bacterial glutamylcysteine synthetase in the cytosol reached a 30-fold increase in its foliar activity compared to untransformed controls; this allowed greater tissue cadmium accumulation but had only a marginal effect on cadmium tolerance (Arisi et al. 2001).

Plant roots are able to release into the rizosphere chelating agents with binding ability for metals (Kinnersely 1993). These metal chelators or other molecules within plant cells that have a high affinity for metals can help in the metal sequestering (Grill et al. 1985, Mehra & Tripathi 1999, Schat et al. 2002, Shah & Nongkynrih 2007, Fulekar et al. 2009). Plants may also be engineered to enhance the synthesis of metal chelators (Kärenlampi et al. 2000, Clemens et al. 2002). Metal chelators include phytochelatins, metallothioneins, organic acids and amino acids. *In vitro* experiments have shown that cadmium in the form of phytochelatin complex is much better tolerated by plant enzyme than its free radical ion (Kneer & Zenk 1992). In *Nicotiana glauca* transformed with a gene encoding a phytochelatin synthase, more metals were accumulated when grown in mine soils compared with non-transformed plants (Martinez et al. 2006). Attempts have been made to increase the formation of phytochelatins by overexpressing genes encoding enzymes stimulating the synthesis of cysteine and glutathione (Harada et al. 2001). Metallothioneins, a category of remarkable interest, are defined as low molecular mass cysteine-rich proteins that can bind heavy metals and may play a role in their intracellular sequestration. In the hybrid poplar genome, they form a multigene family and it has been hypothesised that they participate in the process of metal homeostasis and possibly in the process of tolerance (Kohler et al. 2004). Advances in understanding the regulation of phytochelatins biosynthesis and metallothioneins gene expression and their possible roles in heavy metal detoxification and homeostasis have been recently reviewed (Cobbett & Goldsbrough 2002).

Conclusions

Phytoremediation of metal-polluted soil by plant phytoextraction is a technique attracting the interest of an increasing scientific community and the use of woody species, in particular, presents some aspects of relevance. Biotechnologies are surely powerful tools allowing to investigate and evaluate the potential of phytoremediation. As described in this paper, many fields of study are contributing to a rapid increase of our knowledge on the mechanisms involved. However, despite of the intensive research carried out in the last years on this topic, very few field trials demonstrated the technical feasibility and economic workability of the described approaches (Van Nevel et al. 2007). Indeed, most of the literature rarely provides information on the practical application of phytoremediation techniques.

Specialisation and fragmentation of research is probably real, but it should not be seen as a limit: every progress can contribute and converge to increase the possibility of an advantageous exploitation of woody plants for phytoremediation.

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