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Tobacco (*Nicotiana tabacum*) a Potent Phytoremediator

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ABSTRACT

The low-cost, plant-based phytoremediation technique has often been described as a promising technique to remediate agricultural land contaminated with organic and inorganic pollutants. The plants used, have to meet certain requirements, which are fulfilled by tobacco (*Nicotiana tabacum*). It is a fast growing plant with a high biomass, which is easily harvested. Its propagation is simple, as each plant generates thousands of seeds. It can prosper everywhere between the 50th latitude north and the 40th latitude south and has no demanding requirements on temperature, humidity and soil conditions. Tobacco has also revealed a high tolerance for various organic and inorganic pollutants. It can accumulate heavy metals in relatively high-levels, especially Cd, in comparison to other species and has also shown not be susceptible to various organic pollutants, such as polychlorinated biphenyls (PCB) and trinitrotoluene (TNT). Its rapid growth, high leave biomass and its high disposition for transformation has made tobacco an optimal plant for genetic engineering. It has not only been applied in the field of medicine, e.g. production of antibodies, but also in the area of phytoremediation. Metal chelator, metal transporter, metallothionein (MT), and phytochelatin (PC) genes have been transferred to plants for improved metal uptake and sequestration. Also the expression of bacterial enzymes has enabled the reduction of phytotoxicty or the concentration reduction of various organic pollutants.

Keywords: heavy metals, organic pollutants, phytoremediation
Abbreviations: EDDS, ethylene diamine disuccinate; **EDTA**, ethylene diamine tetraacetate; **MT**, metallothionein; **PCP**, pentachlorphenol; **PETN**, pentaerythritol tetranitrate; **PC**, phytochelatin; **PCB**, polychlorinated biphenyls; **TF**, translocation factor; **TNT**, trinitrotoluene

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INTRODUCTION

Over the centuries, human activities, such as extensive mining, agriculture, industry and military operations have released huge amounts of toxic compounds, contaminating large areas in both developed and developing countries. These contaminants have negative effects on the ecosystems and other natural resources, and, moreover pose great danger to public health, as pollutants can enter food via agricultural products or leach out into drinking water (Commission of the European Communities 2002; European Environmental Agency 2003). The European Environment Agency has estimated the total costs for the clean-up of contaminated sites in Europe to be between EUR 59 and 109 billion (Commission of the European Communities 2002). World wide about 2 000 million ha of soil, equivalent to 15 per cent of the Earth's land area (an area larger than the United States and Mexico combined), have been degraded (i.e. erosion, contamination) through human activities (UNEP 2002).

There are two major classes of contaminants: inorganic and organic. Inorganic compounds include heavy metals, such as cadmium (Cd), lead (Pb) and mercury (Hg), and non-metallic compounds, such as arsenic (As), and radio nuclides, such as uranium. Organic contaminants include different compounds, such as petroleum hydrocarbons, chlorinated solvents, halogenated hydrocarbons, such as trichloroethylene, and explosives, such as trinitrotoluene (TNT).

The clean up of contaminated land by traditional physiochemical methods, including soil excavation and land filling, soil washing and immobilisation or extraction can be very costly, and, in addition, destructive to the soil. Phytoremediation, the use of green plants to remove pollutants from the environment or to render them harmless (Raskin *et al*. 1997), with its lower cost and environmental friendly nature, has received increasing attention in the last decades (Salt *et al*. 1998; Garbisu and Alkorta 2001). This emerging low-cost technology can be applied to both inorganic and organic pollutants, present in solid substrates (e.g. soil), liquid substrates (e.g. water), and the air.

The area of phytoremediation focusing on the removal

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Invited Review

of inorganic pollutants from soil (phytoextraction) is based on a natural propensity of plants to take up metals. Certain plant species, known as hyperaccumulators, such as *Thlaspi*, *Urtica*, *Chenopodium*, *Polygonum sachalase* and *Alyssum* have shown the ability to extract, accumulate and tolerate high levels of heavy metals. By definition, a hyperaccumulator must accumulate at least 100 mg kg^{-1} (0.01% dry wt.) Cd, As and some other trace metals, 1000 mg kg^{-1} (0.1% dry wt.) cobalt (Co), copper (Cu), chrome (Cr), nickel (Ni) and Pb and 10,000 mg kg^{-1} (1% dry wt.) manganese (Mn) and Ni (Watanabe 1997; Reeves and Baker 2000). However, their potential for application in bioremediation is limited by the fact that they are slow growing and have a small biomass (Mulligan *et al*. 2001; Puschenreiter *et al*. 2001). Another option is the use of high biomass plants which are usually not considered to have metal-specific affinity and contain only low to average heavy metal concentrations, but which compensate for this by their high biomass, as for example tobacco (Keller *et al*. 2003).

The concept of using plants to remediate soils contaminated with organic pollutants is a recent development, based on observations that the disappearance of organic chemicals is accelerated in vegetated soils as compared with that of surrounding non vegetated bulk soils. In addition to the direct uptake and metabolism of organics, plants release exudates from their roots that enhance microbial bioremediation in the rhizosphere, which has been termed phytoremediation *ex planta* (Salt *et al*. 1995).

The remedial capacity of plants can be significantly improved by genetic manipulation and plant transformation technologies (Cherian and Oliveira 2005). Over the past years, a range of different plant systems such as *Arabidopsis thaliana*, Indian mustard (*Brassica juncea*), canola (*B. napus*) and tomato (*Lycopersicon esculentum* Mill. cv. 'Pera') have been developed to increase the uptake and the detoxification of inorganic and organic pollutants (Oller *et al*. 2005; Farwell *et al*. 2006; Li *et al*. 2006; Banuelos *et al*. 2007). The choice of system depends on many factors, but the intrinsic efficiency and the suitability for scale-up, storage and downstream processing are particularly important. Tobacco (*Nicotiana tabacum*) is a leafy crop which generates a large amount of biomass and allows rapid scale up due to the immense number of seeds produced in each generation. In addition, tobacco is long established as a model system for plant transformation, and therefore benefits from simple gene transfer, regeneration procedures and optimised vector systems. These characteristics make tobacco an excellent candidate for the production of transgenic plants in the field of phytoremediation (Stoger *et al*. 2005). However, the potential for using these genetic resources in transgenic phytoremediation approaches has been poorly explored. Inadequate data is available on the performance of these transgenic plants on soil substrate, or under field conditions. Most transgenic plants have so far only been tested only in hydroponic or agar-based media containing trace elements. In this medium, the concentration of competing ions and the speciation of trace element ions are highly reproducible and the plant exposure to elements is high. On contaminated field sites, the soil composition, trace element bioavailability and the chemical speciation are site-specific and subject to considerable spatial and temporal variations within one site (Sappin-Didier *et al*. 2005).

INORGANIC CONTAMINANTS

Phytoextraction

The uptake capability can vary greatly among the tobacco species (Doroszewska and Berbec 2004). The study of Mench *et al*. (1989) revealed that *N. tabacum* extracted a significant higher Cd amount than *N. rustica* as verified in the higher Cd concentration in the leaves and greater biomass. Such differences have been reported even amongst *N. tabacum* varieties, although these differences appear less important than external factors, such as soil characteristics (Lugon-Moulin *et al*. 2004). Tobacco (*N. tabacum*) can accumulate Cd at relatively high levels compared to other species (Davis 1984; Mench *et al*. 1989; Wagner 1993; Kayser *et al*. 2000; Wenger *et al*. 2002; Keller *et al*. 2003; Evangelou *et al*. 2004, 2007b) as summarised and compared to other crop plants in **Table 1**. Concentrations in field-grown tobacco leaves usually range from <0.5 to 5 mg Cd kg^{-1}, although higher values can also be found (Lugon-Moulin *et al*. 2004). In studies by Kayser *et al*. (2000), Cd concentrations in *N. tabacum* were only 50% lower than in the hyperaccumulators, *T. caerulescens* and *A. murale* and were respectively 6-, 7- and 2.5-fold higher than in the crop plants, corn (*Zea mays*) sunflower (*Helianthus annuus*) and Indian mustard (*B. juncea*). In the same study, in the case of zinc (Zn) and Cu the concentrations in tobacco showed no significant difference to the crop plants. The hyperaccumulators, *T. caerulescens* and *A. murale* however reached Zn and Cu concentrations in shoots which were 20- and 2.5-fold respectively, higher compared to the concentrations achieved by *N. tabacum*. The findings of Mench *et al*. (1989) support the results of Kayser *et al*. (2000), as *N. tabacum* reached significantly higher Cd (164 mg kg^{-1}) concentrations than *Z. mays* (21.7 mg kg^{-1}). The studies of Jiang *et al*. (2003) and Quartacci *et al*. (2003) display a Cd shoot concentration of 200 mg kg^{-1} in *B. juncea*. In these studies however the Cd soil concentration was 200 mg kg^{-1} which is 10-fold higher than any study conducted with *N. tabacum*. *N. tabacum* as seen in **Table 1** can achieve high concentrations in the case of Zn whereas the uptake of Cu and Pb is in the same order of magnitude as the other listed crop plants.

The ability of tobacco to achieve high heavy metal concentration in the shoots, combined with high biomass, makes it possible to reach high heavy metal outputs. In Keller *et al*. (2003) *N. tabacum* had significantly higher Cd outputs (g ha^{-1}) than *B. juncea* and *Z. mays*, and similar outputs compared to willow (*Salix viminalis*), but lower than *T. caerulescens*. In the case of Cu *N. tabacum* achieved significantly higher outputs than all the other plants mentioned above, with the exception of *Z. mays*. In Wenger *et al*. (2002), although *N. tabacum* reached higher Zn concentrations than *Z. mays*, the biomass was lower and, therefore, the removal of Zn was higher by *Z. mays* than by *N. tabacum*.

In this review the effect of added chelating agents and their effect on the uptake of heavy metals will not be examined as it has been recently elsewhere reviewed by Evangelou *et al*. (2007). However, the tolerance or susceptibility of *N. tabacum* to various chelating agents applied to soil will be briefly discussed. Ethylene diamine disuccinate (EDDS) displayed a similar degree of toxicity towards *H. annuus* (Meers *et al*. 2005) and a higher one towards *Z. mays* and white bean (*Phaseolus vulgaris*) (Luo *et al*. 2005) compared to *N. tabacum*. Meers *et al*. (2005) applied 1.77 mmol kg^{-1} EDDS and observed no toxicity symptoms, whereas Luo *et al*. (2005) applied 5 mmol kg^{-1} EDDS and the dry weight decreased by approximately 55% in comparison to the control. With respect to ethylene diamine tetraacetate (EDTA), it showed a similar degree of toxicity towards *B. juncea* (Epstein *et al*. 1999; Wu *et al*. 2004) and *H. annuus* (Meers *et al*. 2005). Regarding EDDS, this was not the case with *N. tabacum*. It was no more susceptible to chelating agents than other plant species. The degree of toxicity is in concurrence with present literature on the subject.

Besides achieving a high heavy metal concentration in the shoots, the translocation factor (TF), the ratio of metal (loid) concentration in shoots to that in roots, is very important. The TF can be used to evaluate the capacity of a plant to transfer metals from roots to shoots (TF is usually >1 (or ≥1) in (hyper)accumulators and <1 in excluders (McGrath and Zhao 2003), thus revealing if the harvestable part of the plant is the component with the highest metal concentration. In the study of Keller *et al*. (2003), *N. tabacum* was the only species which showed TF >1 for Cd, Cu and Zn. *Zea mays*

Table 1 Dry matter yields, heavy metal concentrations, and heavy metal yields of various crops. No differentiation was made between pot and field experiments.

Metal	Crop	DM yield t ha^{-1}	Heavy metal concentration mg kg^{-1}	Heavy metal yield g ha^{-1} yr^{-1}	Reference
Cd	*N. tabacum*	13	3.5-164.5 (leaf)	45	Davis *et al.* 1984; Mench *et al.* 1989; Kayser *et al.* 2000; Keller *et al.* 2003; Evangelou *et al.* 2004, 2007b
	S. vinimalis	13	3.8-7	17-49	Kayser *et al.* 2000; Keller *et al.* 2003; Klang-Westin and Eriksson 2003
	B. juncea	7	1 (200)	7	Kayser *et al.* 2000; Jiang *et al.* 2003; Keller *et al.* 2003; Quartacci *et al.* 2003
	B. rapa		1.5-2.1		Grčman *et al*. 2001; Shen *et al.* 2002; Grčman *et al*. 2003
	H. annuus	28	0.6-1.5	11-20	Kayser *et al.* 2000; Keller *et al.* 2003; Liphadzi *et al.* 2003; Meers *et al.* 2005
	Z. mays	16	0.6-21.7 (leaf)	10	Mench *et al*. 1989; Kayser *et al.* 2000; Keller *et al.* 2003; Luo *et al.* 2005
	A. murale	0.8	7	5.6	Kayser *et al.* 2000; Keller *et al.* 2003
	T. caerulescens	0.5	7	3.5	Kayser *et al.* 2000; Keller *et al.* 2003
	Populus sp.		3-75		Robinson *et al*. 2000; Liphadzi *et al.* 2003
Zn	*N. tabacum*	2-13	150-1900	1950	Kayser *et al.* 2000; Wenger *et al.* 2002; Keller *et al.* 2003
	S. vinimalis	13	300	3900	Kayser *et al.* 2000; Keller *et al.* 2003
	B. juncea	7	120	840	Kayser *et al.* 2000; Keller *et al.* 2003
	B. rapa		50-450		Grčman *et al*. 2001; Shen *et al.* 2002; Grčman *et al*. 2003
	H. annuus	28	10-110	120-3080	Kayser *et al.* 2000; Keller *et al.* 2003; Liphadzi *et al.* 2003; Meers *et al.* 2005
	Z. mays	4.5-16	24-140-1365	2240-4700	Cooper *et al.* 1999; Wu *et al.* 1999; Kayser *et al.* 2000; Wenger *et al.* 2002; Keller *et al.* 2003; Luo *et al.* 2005
	A. murale	0.8	1000	800	Kayser *et al.* 2000; Keller *et al.* 2003
	T. caerulescens	0.5	2000	1000	Kayser *et al.* 2000; Keller *et al.* 2003
	P. sativum		47		Cooper *et al.* 1999
	Populus sp.		50		Liphadzi *et al.* 2003
Cu	*N. tabacum*	13	18-38	490	Kayser *et al.* 2000; Keller *et al.* 2003; Evangelou *et al*. 2007
	S. vinimalis	13	14	190	Kayser *et al.* 2000; Keller *et al.* 2003
	B. juncea	7	20	140	Kayser *et al.* 2000; Keller *et al.* 2003
	B. rapa		36		Shen *et al.* 2002
	H. annuus	28	9-80	80-560	Kayser *et al.* 2000; Keller *et al.* 2003; Liphadzi *et al.* 2003; Meers *et al.* 2005
	Z. mays	16	10-57	160	Cooper *et al.* 1999; Wu *et al.* 1999; Kayser *et al.* 2000; Keller *et al.* 2003; Luo *et al*. 2005
	A. murale	0.8	70	56	Kayser *et al.* 2000; Keller *et al.* 2003
	T. caerulescens	0.5	80	40	Kayser *et al.* 2000; Keller *et al.* 2003
	P. sativum		14		Cooper *et al.* 1999
	Populus sp.		6		Liphadzi *et al.* 2003
Pb	*N. tabacum*		16-40		Evangelou *et al.* 2006; Sudová *et al.* 2007
	B. rapa		2-120		Grčman *et al*. 2001; Shen *et al.* 2002; Grčman *et al*. 2003; Kos *et al.* 2003
	H. annuus		4-50	6	Huang *et al.* 1997; Liphadzi *et al.* 2003
	Z. mays		2-90		Huang *et al.* 1997; Cooper *et al.* 1999; Wu *et al*. 1999; Luo *et al.* 2005
	P. sativum		12-52		Huang *et al.* 1997; Cooper *et al.* 1999
	Populus sp.		10		Liphadzi *et al*. 2003

showed a TF >1 only for Zn, while *B*. *juncea* showed a TF >1 for Cd and Zn as did *T*. *caerulescens*. Although Chiang *et al*. (2006) in their study of *N*. *benthamiana* and *H*. *annuus* reported a TF <1 for Cd, this does not contradict the results of Keller *et al*. (2003), as another tobacco species was used.

Transgenic tobacco and phytoextraction

A large number of genes involved in acquisition, allocation and detoxification of trace elements have been identified and characterised from a variety of organisms. Genetic engineering methodologies have made it possible to transfer such appropriate genes into high biomass plants. An acquisition strategy would entail in using genes involved in the biosynthesis of metal chelators, such as citrate, malate, histidine or in root exudation of protons (Hall 2002). An allocation strategy would examine the modification of the metal transport across the plasma membrane. Several transporters (i.e., the heavy metal (CPx-type) ATPases, the natural resistance-associated macrophage protein family, members of the cation diffusion facilitator family) (Williams *et al*. 2000) and *N*. *tabacum* calmodulin-binding protein (Arazi *et al*. 1999) involved in the transport of metals could be over expressed in order to increase metal uptake and detoxification. A detoxification approach could be the over expression of peptides involved in the metal homeostasis of plants such as metallothioneins (MT), phytochelatines (PC) and glutathione (Cobbett and Goldsbrough 2002), the reduction of free metal concentration in the cytoplasma by chemical transformation (Bizily *et al*. 1999; Rugh *et al*. 1999) or the over expression of superoxide dismutase to reduce active oxygen species (Boominathan and Doran 2006). An overview of the methods applied to transform tobacco and their uptake and tolerance enhancement are displayed in **Table 2**.

Most of the studies have focused on Cd as tobacco has a natural propensity to accumulate Cd. In order to confer resistance to toxic levels of heavy metals, Misra *et al*. (1989) introduced a chimeric human MT gene into *N*. *tabacum* cells. The transgenic plants displayed a 6-fold higher tolerance to Cd than the control plants. The dry weight of the control dropped from 28.6 g to 5 g owing to the Cd treatment (0.1 mM $CdCl_2$), whereas the dry weight of the transformed *N*. *tabacum* plants remained at approximately 28 g. de Borne *et al*. (1998) used tobacco plants which expressed the mammalian MT gene, and observed that the leaf Cd levels were decreased by 73% compared to the control plants. The Cd concentration in control leaf lamina, 4.44 mg kg^{-1} was reduced to 1.8 mg kg^{-1}. However, the decrease in leaf Cd was correlated with an increase in Cd in the roots and stems, thus leaving a large part of the Cd in the non harvestable part of the plant. In controls, 70-80% of the Cd was translocated to the leaves, whereas 40-50% was translocated in MT1 plants. Significantly Macek *et al*. (2002) introduced an additional small metal binding domain, a poly-

Table 2 Genes and methods used for the development of transgenic tobacco plants to cope with inorganic pollutants, as well as the effect resulting from the transformation.

Targeted metal	Gene	Method	Effect (compared to control)	Reference
Cd	Human MT-II processed gene (*hMT-II*) with CaMV 35S promoter	*Agrobacterium tumefaciens* (Ti-plasmid)	6-fold better tolerance	Misra *et al.* 1989
	hMT-II with CaMV 35S promoter	plant transformation vector (pKYLX7135S^2)	73% lower Cd levels in the leaves	de Borne *et al.* 1998
	CUP1 MT gene (*Saccharomyces cerevisae*) with CaMV 35S promoter + HISCUP	*A. tumefaciens* (plamid PBI121)	90% higher Cd accumulation in leaves and 50% lower in the roots	Macek *et al*. 2002
	CUP1 MT gene (*Saccharomyces cerevisae*) with CaMV 35S promoter + HISCUP	*A. tumefaciens* (plasmid PBI121)	110% higher Cd accumulation in leaves	Pavlíková *et al.* 2004b
	Wheat gene encoding phytochelatin synthase (TaPCS1)	*A. tumefaciens* (Ti-plasmid)	160% longer roots	Gisbert *et al.* 2003
	CUP1 MT gene (*Saccharomyces cerevisae*) with CaMV 35S promoter	*A. tumefaciens* (plasmid pRSGCUP 1)	Up to 3-fold higher Cd concentration	Thomas *et al.* 2003
	Bacterial arsenate reductase gene (*arsC*)	*A. tumefaciens*	Higher fresh weight and 50% higher Cd concentration	Dhankher *et al.* 2003
	Cysteine synthase	Cross fertilisation		Kawashima *et al.* 2004
	CAX2 (calcium exchanger 2) with CaMV 35S promoter	*A. tumefaciens* (plasmid pBIN19)	15% higher Cd concentration	Hirschi *et al.* 2000
	Human multidrug resistance-associated protein (hMRP1) – ABC-transporter with CaMV 35S promoter	*A. tumefaciens* (plasmid pJ3Ω-MRP)	Up to 50% better growth, 1.5-fold higher Cd uptake	Yazaki *et al.* 2006
	MThis from *S. vulgaris*	*A. tumefaciens* (pCAMBIA 2301 vector)	Up to 2-fold higher dry weight, 50% higher Cd accumulation but no effect on photosynthesis	Gorinova *et al.* 2007
Pb	Wheat gene encoding phytochelatin synthase (TaPCS1)	*A. tumefaciens* (Ti-plasmid)	50% higher Pb content	Gisbert *et al.* 2003
Cu	CUP1 MT gene (*Saccharomyces cerevisae*) with CaMV 35S promoter	*A. tumefaciens* (plasmid pRSGCUP 1)	Up to 3-fold higher Cu concentration	Thomas *et al.* 2003
Hg	Bacterial *ppk* (polyphosphate kinase) gene with E12 (CaMV 35S + 2 enhancers) promoter	*A. tumefaciens* (plasmid pPKT116)	Up to 2-fold higher biomass and root lenght	Nakata *et al.* 2006
	Bacterial *merA/merB* gene		Up to 3.5-fold higher biomass and up to 80% less Hg in leaves	Heaton *et al.* 1998
	Bacterial *merA* gene with CaMV 35 S promoter	*A. tumefaciens*	Up to 2-fold higher transpiration and 75% less Hg in leaves	Heaton *et al.* 2005
	Bacterial *merA/merB* gene	Particle bombardement	Up to 2-fold higher biomass and up to 2-fold higher chlorophyll content	Ruiz *et al.* 2003
	Bacterial *merA* gene with CaMV 35S promoter	*A. tumefaciens* (plasmid pVSTImerApe9)	5-fold higher Hg release from the leaves	He *et al.* 2001
Zn	Polyhistidine-tagged Rubisco	Particle bombardment	30% higher Zn content	Rumeau *et al.* 2004

histidine chain, to the metal binding protein, yeast MT, and observed a 90% increased Cd accumulation (27.0 mg kg^{-1}) combined with a reduction of Cd content in the roots, by 55% (9.0 mg kg^{-1}), compared to the control which accumulated 15.8 and 9.0 mg kg^{-1} Cd respectively. With the same construct Pavlíková *et al*. (2004b) investigated transgenic *N. tobacco* and their study indicated 110% higher Cd levels than the control. However, the TF decreased as the Cd content in the soil increased (from a TF >1 at a Cd soil content of <0.6 mg Cd L^{-1} to <1 for a Cd soil content >0.6 mg Cd L^{-1}). Additionally, the construct had no effect on the uptake of Zn and Ni. Gisbert *et al*. (2003), demonstrated in their study of *N. glauca* R. Graham (shrub tobacco) that overexpressing a wheat gene encoding phytochelatin synthase (*TaPCS1*) greatly increased its tolerance to metals such as Pb and Cd, displayed by the by 160% higher root length compared to the control. In addition, seedlings of transformed plants grown in mining soils containing high levels of Pb (1572 ppm) accumulated this heavy metal at concentration of 50% higher (52 mg kg^{-1}) compared to those of the wild type. In a study by Thomas *et al*. (2003) pooled leaves of transgenic *N. tabacum* containing yeast MT (CUP 1) grown in soils from Cu stamp-sands contained two to three times the Cu content of those of the control plants.

In order to increase the tolerance of tobacco to heavy metals other strategies have been also applied. Dhankher *et al*. (2003) showed that *N. tabacum* which over expressed bacterial arsenate reductase gene had an 2-3-fold higher fresh weight and contained a 50% higher Cd concentration compared to the control plants, while Nagata *et al*. (2006) increased the tolerance of *N. tabacum* to Hg (2-fold higher biomass and root length) by over expressing the bacterial polyphosphate kinase. Rumeau *et al*. (2004) increased Zn content by 30% in transplastomic tobacco plants expressing a polyhistidine-tagged Rubisco large subunit however it had no effect on the uptake of Cu and Fe. Kawashima *et al*. (2004) increased Cd, Se and Ni tolerance by the over expression of cysteine synthase in the cytosol and chloroplast of *N. tabacum*. *N. tabacum* plants expressing *Arabidopsis* antiporter CAX2 (calcium exchanger 2) were not only more tolerant to elevated Mn^{2+} levels but also accumulated more Ca^{2+} (20%), Cd^{2+} (15%), and Mn^{2+} (20%) (Hirschi *et al*. 2000). Yazaki *et al*. (2006) inserted the human multidrug resistance-associated protein, one of the most intensively studied ABC transporters, into *N. tabacum* cv. 'Samsun NN' and achieved a higher Cd tolerance compared to the control plants. The effect of a transformation is not only correlated to the construct introduced but also to where the construct is over expressed (Sappin-Didier *et al*. 2005) and to the target metals as displayed by Pavlíková *et al*. (2004). In the case of Sappin-Didier *et al*. (2005) the constructs (ferritin-over-expression) inserted in the plasmid had 10-30% higher accumulation of Mn, Zn, Fe, Cd, Cu and Pb compared to the construct inserted in the cytoplasma, while in the case of Pavlíková *et al*. (2004) the introduced construct increased the uptake of Cd but did not have any effect on the uptake of Zn and Ni.

merA and *merB* genes have been isolated from bacteria,

and when combined may produce elementary Hg from methyl-Hg, which can be introduced into plants. *A. thaliana* was first transformed with modified *merA* and *merB* genes, and produced transgenic plants Hg-resistant and volatile (Rugh *et al*. 1996; Bizily *et al*. 1999). Since then several plants including tobacco, have been transformed. Rugh *et al*. (2000) showed that *merA*/*merB* tobacco plants displayed an enhanced Hg-tolerance, while *merA* tobacco plants removed 3- to 4-fold more Hg from hydroponic medium than untransformed controls (Meagher *et al*. 2000). Heaton *et al*. (1998), He *et al*. (2001), Ruiz *et al*. (2003) and Heaton *et al*. (2005) have also worked with similar constructs and have achieved encouraging results. In Detail: In Heaton *et al*. (1998) the tranformed tobacco plants displayed up to 3.5-fold higher fresh weight and up to 80% lower Hg concentration, owing to the volatilisation of Hg, compared to control. He *et al*. (2001) observed a higher germination rate and a 5-fold higher Hg release from the leaves. Ruiz *et al*. (2003) achieved 2-fold higher biomass and 2-fold higher chlorophyll content with the introduction of the construct. The *merA* construct introduced by Heaton *et al*. (2005) increased the transpiration of the transformed tobacco plants by 2-fold and reduced the Hg concentration by 75% in comparison to the wild type tobacco plants.

ORGANIC CONTAMINANTS

Phytodegradation

As organic compounds are usually man-made and xenobiotic in plants, there are no transporters for their uptake. The usual mechanism of uptake is by simple diffusion (passive uptake). When organic contaminants come into contact with roots, they may be sorbed to the root structure. The hydrophobic or hydrophilic nature of the organic compounds also determines their possible uptake (Cherian and Oliveira 2005).

Organic pollutants can be degraded or mineralised by plants, either independently or in association with microorganisms. According to a study by Ryslava *et al*. (2003), the degradation rate of organic pollutants, such as polychlorinated biphenyls (PCB) by rhizospheric microbial activity depends on the plants used. *N. tabacum* sufficiently degraded PCB, whereas black nightshade (*Solanum nigrum*) and alfalfa (*Medicago sativa*) owing to their rhizospheric microbial community displayed significantly lower degradation rates. Moreover, *N. tabacum* showed significantly higher PCB contents in its shoots compared to the other two species used.

Plants have significant metabolic activity in both roots and shoots, and some of the enzymes involved in these metabolic processes, namely nitroreductases, dehalogenases, laccases, peroxidases, etc., are useful in the remediation process. The process though, in native plants is often incomplete and inefficient for the vast amount of different organic pollutants in the environment, and thus genetic engineering can be viewed as a useful tool in coping with these challenges.

Transgenic tobacco and phytodegradation

Genetic engineering has several targets in the process of phytotransfomation where it can be introduced and used to increase its effect. Phytotransformation is a process by which plants uptake organic pollutants and, subsequently, metabolise or transform them into less toxic metabolites. Once taken up and translocated the organic chemicals generally undergo three transformation stages: (a) chemical modification (oxidations, reductions, hydrolysis); (b) conjugation (with glutathione, sugars, amino acids); and (c) sequestration or compartimentation (conjugates are converted to other conjugates and deposited in plant vacuoles or bound to the cell wall and lignin) (Cherian and Oliveira 2005). An overview of the methods applied to transform tobacco and their effectiveness are displayed in **Table 3**.

Typical plant enzymes which catalyse the first phase of the reactions are P450 monoxygenases, and these are a good candidate for enhancing the phytoremediation potential. Bode *et al*. (2004) introduced two species of human P450 cDNA in tobacco cells by *Agrobacterium*-mediated transformation, and these were tested against atrazine metabolism. Transgenic cultures were able to produce larger amounts of nonphytotoxic (primary oxidised) metabolites than non-transformed cultures. In another study, Didierjean *et al*. (2002), introduced Jerusalem artichoke (*H. tuberosus*) xenobiotic inducible cytochrome P450, CYP76B1, into *N. tabacum* and reported an increase in the tolerance to various herbicides, such as linuron (20-fold), isoproturon and chlortoluron (10-fold). Transgenic tobacco expressing mammalian cytochrome P450 monooxygenase has been shown to harbor oxygenating activity for organic compounds, such as the herbicide chlortoluron, 7-ethoxycoumarin, benzo[a] pyrene, and halogenated hydrocarbons (Shiota *et al*. 1994; Doty *et al*. 2000).

Table 3 Genes and methods used for the development of transgenic tobacco plants to cope with various organic pollutants; effect resulting from the transformation.

Targeted organic pollutant	Gene	Method	Effect (compared to control)	Reference
Atrazine	Human CYP1A1 and human CYP1A2 with CaMV 35 S promoter	*Agrobacterium tumefaciens*	transgenic plants transformed 100% of atrazine to metabolites, control plants only 20%	Bode *et al.* 2004
Linuron, Isoproturon, Chlortoluron	CYP76B1 from *Helianthus tuberosus* with GAL10-CYC1 promoter	*A. tumefaciens* (plamid RK2013)	20-fold tolerance increase to linuron and 10-fold to isoproturon and chlortoluron	Didierjan *et al.* 2002
7-ethoxycoumarin benzo[α]pyrene	Fusion of rat CYPlAl and yeast NADPH-cytochrome P450 oxidoreductase	*A. tumefaciens* (plamid pAFCRl and plasmid pTF2)	10-fold higher activity toward both 7-ethoxycoumarin and benzo[α]pyrene	Shiota *et al.* 1994
TNT	Nitroreductase (*nfsI*) from E. cloacae	*A. tumefaciens*	Up to 2-fold higher dry weight, higher TNT removal	Hannink *et al.* 2001
GTN	PETN-reductase (*onr1*) with CaMV 35 S promoter	*A. tumefaciens*	2-fold faster GTN metabolism	Gisbert *et al.* 2003
Atrazine	modified bacterial *atzA* gene with CsVMV promoter	*A. tumefaciens* (plasmid pPW1)	38-fold higher atrazine tolearance	Wang *et al.* 2005
PCP	Mn-peroxidase of *Coriolus versicolor* with CaMV 35 S promoter	*A. tumefaciens*	2-fold higher PCP removal	Iimura *et al.* 2002
PCP, BCP	laccase of *Coriolus versicolor* with CaMV 35 S promoter	*A. tumefaciens*	Up to 6-fold higher reomoval	Sonoki *et al.* 2005
PCB	Bacterial gene *bphC* with CaMV 35 S promoter	*A. tumefaciens*	2-fold higher germination capability	Macek *et al.* 2005

Besides assisting the natural phytotransformation, new compounds from various organisms can be introduced into plants to increase their phytoremediation potential for organic pollutants. For instance, as 2,4,6-trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN) and nitroglycerin, are useful because as stated by Meagher (2006) mineralisation of TNT by native (that is, non genetically engineered) plants is inefficient and generally incomplete. To engineer plant tolerance to TNT, two bacterial enzymes (PETN reductase and nitroreductase), able to reduce TNT into less harmful compounds, were over expressed in *N. tabaccum* cv. 'Xanthi'. The two genes, *onr* (encoding PETN reductase) and *nfsl* (encoding nitroreductase), under the control of a constitutive promotor provided the transgenic plants with increased tolerance to TNT at concentrations that severely affected the development of wild type plants (French *et al*. 1999; Hannink *et al*. 2001, 2003).

Other organic compounds, such as atrazine, pentachlorphenol (PCP), bisphenol A (BPA) and PCBs have also become targets for genetic engineering in the field of phytoremediation.

The biodegradation of atrazine in transgenic *N. tabacum* expressing a modified bacterial atrazine chlorohydrolase (*atzA*) gene was increased, and was accompanied by an increased atrazine tolerance compared to the control plants (Wang *et al*. 2005). In 2002 Iimura *et al*. reported that manganese peroxidase-expressing transgenic tobacco was able to remove PCP effectively. In a study by Sonoki *et al*. (2005) the laccase of *Coriolus versicolor*, an extracellular fungal enzyme, was introduced into *N. tabacum* cv. 'Samsun NN' and the transgenic plants were able to remove BPA and PCP from an aqueous solution, by secreting laccase into rhizosphere. Tobacco plants expressing the barterial enzyme responsible for cleaving PCBs, coded by the gene *bphC* from the bacterial biphenyl operon, displayed a higher PCB tolerance than the control plants (Macek *et al*. 2005).

As in the case for genetic engineering in the field of heavy metal phytoextraction the effect of a transformation is correlated to the over expression of the construct introduced. In a study by Uchida *et al*. (2005), crude leaf extracts of transgenic plants expressing tobacco expressing haloalkane dehalogenase, which catalyses hydrolytic dechlorination of 1-chlorobutane, cytoplasmic enzymes showed 76.4-fold higher xenobiotic-degrading activity than did leaves expressing apoplast-targeted enzymes.

ROOTS AND DEPTH OF CONTAMINATION

Before selecting the plant to be applied on the contaminated field different parameters have to be taken into account. These include the initial level of contamination, the type and the use of the soil to remediate, the area and depth of soil concerned. Indeed, plants have to be able to reach the metal to be removed, which means that their root system must develop within the contaminated zone. According to Keller *et al*. (2003) *N. tabacum* is able to extract metals only from the upper part of the contaminated layer (0.2 m) unlike *Z. mays* and *S. viminalis* which could extract metals from depths up to 0.75 m). Consequently, tobacco is suitable only for the phytoextraction of areas where the contaminated soil does not have a great depth.

FUTURE OF TRANSGENIC AND WILD TYPE TOBACCO IN THE FIELD OF PHYTOREMEDIATION

In the last decades transgenic tobacco plants with enhanced capacities to chelate or metabolise toxic metals have been tested in the field (Yeargan *et al*. 1992; Brandle *et al*. 1993). Although the future is promising for transgenic plants, there is much concern about their use. Questions not dissimilar to those surrounding genetically modified food plants have been raised: Will they spread uncontrollably? Will they decrease genetic variability by interbreeding with wild plants? Will they rob the soil of its nutrients as well the toxin? Will they somehow find their way into the food chain and harm human and animal health (Watanabe 2001)? The last question, though, is not applicable to tobacco, as it is a non-food/non-feed crop. However, transgenic plants will probably not be applicable in the near future owing to restrictive laws and low public acceptance in several countries.

Tobacco is a self pollinating plant; however it produces extensive amounts of pollen which could be distributed by wind. In order to avoid the transfer of transgenic pollen to wild populations various legislation organs have adopted several laws. In the case of transgenic tobacco in the field of phytoremediation, harvesting before bloom would minimize the risk of hybridisation. During bloom tobacco biomass increases only very slowly, thus it does not extract high amounts of various elements from the soil as a consequence the most effective part for phytoremediation has been completed. Harvesting in that point in time would therefore, not significantly reduce the phytoremediation efficiency.

Therefore, the use of the less efficient wild type tobacco plants could be combined with profit making operations, such as bioenergy. Depending on the family of *N. tabacum* 30-40% of the seed is in average oil (Umarov *et al*. 1991; Giannelos *et al*. 2002) and is composed of linoleic acid (71.63%), oleic acid (13.46%) and palmitic acid (8.72%) (Mukhtar *et al*. 2007). Although tobacco seed oil is a non-edible vegetable oil, it can be utilised for biodiesel production as a new renewable alternative diesel engine fuel (Usta 2005; Veljkovic *et al*. 2006). For this purpose research has to focus on the amounts of inorganic and organic pollutants accumulated in the seeds during the phytoremediation process. Tobacco is a plant, which fulfils all the characteristics for a suitable phytoremediation plant and combined with the production of bioenergy it could become one of the main plants used in the field of phytoremediation.

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REFERENCES

Arazi T, Sunkar R, Kaplan B, Fromm H (1999) A tobacco plasma membrane calmodulin-binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic plants. *Plant Journal* **20**, 171-182

Banuelos G, Leduc DL, Pilon-Smits EAH, Terry N (2007) Transgenic Indian mustard overexpressing selenocysteine lyase or selenocysteine methyltransferase exhibit enhanced potential for selenium phytoremediation under field conditions. *Environmental Science and Technology* **41**, 599-605

Bizily SP, Rugh CL, Summers AO, Meagher RB (1999) Phytoremediation of methylmercury pollution: merB expression in *Arabidopsis thaliana* confers resistance to organomercurials. *Proceedings of the National Academy of Sciences USA* **96**, 6808-6813

Bode M, Stobe P, Thiede B, Schuphan I, Schmidt B (2004) Biotransformation of atrazine in transgenic tobacco cell culture expressing human P450. *Pest Management Science* **60**, 49-58

Boominathan R, Doran PM (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator *Thlaspi caerulescens*. *Biotechnology and Bioengineering* **83**, 158-167

Brandle JE, Labbe H, Hattori J, Miki BL (1993) Field performance and heavy metal concentrations in transgenic flue-cured tobacco expressing a mammalian metallothionein-β-glucuronidase gene fusion. *Genome* **36**, 255-260

Cherian S, Oliveira MM (2005) Transgenic plants in phytoremediation: Recent advances and new possibilities. *Environmental Science and Technology* **39**, 9377-9390

Chiang PN, Wang MK, Chiu CY, Chou SY (2006) Effects of cadmium amendments on low-molecular-weight organic acid exudates in rhizosphere soils of tobacco and sunflower. *Environmental Toxicology* **21**, 479-488

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annual Review of Plant Biology* **53**, 159-182

Commission of the European Communities (2002) Communication from the Commission to the Council the European Parliament the Economic and Social Committee and the Committee of the Regions: Towards a Thematic Strategy for Soil Protection COM(2002) 179 final European Commision Brussels, Belgium, 9 pp

Cooper, EM, Sims, JT, Cunningham, SD, Huang, JW, Berti, WR, (1999) Chelate-assisted phytoextraction of lead from contaminated soils. *Journal of Environmetal Quality* **28**, 179-198

Davis RD (1984) Cadmium – a complex environmental problem Part II: Cadmium in sludges used as fertilizer. *Experimentia* **40**, 11-126

de Borne FD, Elmayan T, de Roton C, de Hys L, Tepfer M (1998) Cadmium partitioning in transgenic tobacco plants expressing a mammalian metallothionein gene. *Molecular Breeding* **4**, 83-90

Dhankher OP, Shasti NA, Rosen BP, Fuhrmann M, Meagher RB (2003) Increased cadmium tolerance and accumulation by plants expressing bacterial arsenate reductase. *New Phytologist* **159**, 431-441

Didierjean L, Gondet L, Perkins R, Lau SMC, Schaller H, O'Keefe DP, Werck-Reichhart D (2002) Engineering herbicide metabolism in tobacco and Arabidopsis with CYP76B1 a cytochrome P450 enzyme from Jerusalem artichoke. *Plant Physiology* **130**, 179-189

Doroszewska T, Berbec A (2004) Variation for cadmium uptake among *Nicotiana* species. *Genetic Resources and Crop Evolution* **51**, 323-333

Doty SL, Shang TQ, Wilson AM, Tangen J, Westergreen AD, Newman LA, Strand SE, Gordon MP (2000) Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1. *Proceedings of the National Academy of Sciences USA* **97**, 6287-6291

Epstein AL, Gussman CD, Blaylock MJ, Yermiyahu U, Huang JW, Kapulnik Y, Orser CS (1999) EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. *Plant and Soil* **208**, 87-94

European Environmental Agency (2003) Europe's environment: The third assessment Environmental assessment report No 10 Copenhagen Denmark: European Environmental Agency, pp 344

Evangelou MWH, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity and fate of chelating agents. *Chemosphere* **68**, 989-1003

Evangelou MWH, Ebel M, Schaeffer A (2007b) The influence of EDDS and EDTA on the uptake of heavy metals of Cd and Cu from soil with tobacco *Nicotiana tabacum*. *Chemosphere* **68**, 345-353

Evangelou MWH, Ebel M, Schaeffer A (2006) Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*. *Chemosphere* **63**, 996-1004

Evangelou MWH, Daghan H, Schaeffer A (2004) The influence of humic acids on the phytoextraction of cadmium from soil. *Chemosphere* **57**, 207-213

Farwell AJ, Vesely S, Nero V, Rodriguez H, Shah S, Dixon DG, Glick BR (2006) The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site. *Plant and Soil* **288**, 309-318

French CE, Rosser SJ, Davies GJ, Nicklin S, Bruce NC (1999) Biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase. *Nature Biotechnology* **17**, 491-494

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology* **77**, 229-236

Giannelos PN, Zannikos F, Stournas S, Lois E, Anastopoulos G (2002) Tobacco seed oil as an alternative diesel fuel: Physical and chemical properties. *Industrial Crops and Products* **16**, 1-9

Gisbert C, Ros R, De Haro A, Walker DJ, Bernal MP, Serrano R, Navarro-Avino J (2003) A plant genetically modified that accumulates Pb is especially promising for phytoremediation. *Biochemical and Biophysical Research Communications* **303**, 440-445

Gorinova N, Nedkovska M, Todorovska E, Simova-Stoilova L, Stoyanova Z, Georgieva K, Demirevska-Kepova K, Atanassov A, Herzig R (2007) Improved phytoaccumulation of cadmium by genetically modified tobacco plants (*Nicotiana tabacum* L.). Physiological and biochemical response of the transformants to cadmium toxicity. *Environmental Pollution* **145**, 161-170

Grčman H, Vodnik D, Velikonja-Bolta S, Leštan D (2003) Ethylenediaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *Journal of Environmental Quality* **32**, 500-506

Grčman H, Velikonja-Bolta S, Vodnik D, Kos B, Leštan D (2001) EDTA enhanced heavy metal phytoextraction: Metal accumulation, leaching, and toxicity. *Plant and Soil* **235**, 105-114

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* **53**, 1-11

Hannink NK, Rosser SJ, Bruce NC (2002) Phytoremediation of explosives. *Critical Reviews in Plant Sciences* **21**, 511-538

Hannink N, Rosser SJ, French CE, Basran A, Murray JAH, Nicklin S, Bruce NC (2001) Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase. *Nature Biotechnology* **19**, 1168-1172

He YK, Sun JG, Feng XZ, Czako M, Marton L (2001) Differential mercury volatilization by tobacco organs expressing a modified bacterial *merA* gene. *Cell Research* **11**, 231-236

Heaton ACP, Rugh CL, Wang NJ, Meagher RB (2005) Physiological responses of transgenic *merA*-tobacco (*Nicotiana tabacum*) to foliar and root mercury exposure. *Water Air Soil Pollution* **161**, 137-155

Heaton ACP, Rugh CL, Wang NJ, Meagher RB (1998) Phytoremediation of mercury- and methylmercury-polluted soils using genetically engineered plants. *Journal of Soil Contamination* **7**, 497-509

Hirschi KD, Korenkov VD, Wilganowski NL, Wagner GJ (2000) Expression of *Arabidopsis* CAX2 in tobacco Altered metal accumulation and increased manganese tolerance. *Plant Physiology* **124**, 125-133

Huang JW, Chen J, Berti WB, Cunningham SD (1997) Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology* **31**, 800-805

Iimura Y, Ikeda S, Sonoki T, Hayakawa T, Kajita S, Kimbara K, Tatsumi K, Katayama Y (2002) Expression of a gene for Mn-peroxidase from *Coriolus versicolor* in transgenic tobacco generates potential tools for phytoremediation. *Applied Microbiology and Biotechnology* **59**, 246-251

Jiang XJ, Luo YM, Zhao QG, Baker AJM, Christie P, Wong MH (2003) Soil Cd availability to indian mustard and environmental risk following EDTA addition to Cd-contaminated soil. *Chemosphere* **50**, 813-818

Kawashima CG, Noji M, Nakamura M, Ogra Y, Suzuki KT, Saito K (2004) Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. *Biotechnology Letters* **26**, 153-157

Kayser A, Wenger K, Keller A, Attinger W, Felix HR, Gupta SK, Schulin R (2000) Enhancement of phytoextraction of Zn Cd and Cu from calcareous soil: The use of NTA and sulfur amendments. *Environmental Science and Technology* **34**, 1778-1783

Keller C, Hammer D, Kayser A, Richner W, Brodbeck M, Sennhauser M (2003) Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. *Plant and Soil* **249**, 67-81

Klang-Westin E, Eriksson J (2003) Potential of Salix as phytoextractor for Cd on moderately contaminated soils. *Plant and Soil* **249**, 127-137

Kos B, Leštan D (2004) Chelator induced phytoextraction and in situ soil washing of Cu. *Environmental Pollution* **134**, 333-339

Li YJ, Heaton ACP, Carreira L, Meagher RB (2006) Enhanced tolerance to and accumulation of mercury but not arsenic in plants overexpressing two enzymes required for thiol peptide synthesis. *Physiologia Plantarum* **128**, 48-57

Liphadzi MS, Kirkham MB, Mankin KR, Paulsen GM (2003) EDTA-assisted heavy-metal uptake by poplar and sunflower grown at a long-term sewage-sludge farm. *Plant and Soil* **257**, 171-182

Lugon-Moulin N, Zhang M, Gadani F, Rossi L, Koller D, Krauss M, Wagner GJ (2004) Critical review of the science and options for reducing cadmium in tobacco (*Nicotiana tabacum* L.) and other plants. *Advances in Agronomy* **83**, 111-180

Luo CL, Shen Z, Li X (2005) Enhanced phytoextraction of Cu Pb Zn and Cd with EDTA and EDDS. *Chemosphere* **59**, 1-11

Macek T, Surá M, Pavliková D, Francová K, Scouten WH, Szekeres M, Sylvestre M, Macková M (2005) Can tobacco have a potentially beneficial effect to our health? *Zeitschrift für Naturforschung C – A Journal of Bioscience* **60**, 292-299

Macek T, Macková M, Pavliková D, Szakova J, Truksa M, Cundy S, Kotrba P, Yancey N, Scouten WH (2002) Accumulation of cadmium by transgenic tobacco. *Acta Biotechnologica* **22**, 101-106

McGrath SP, Zhao FJ (2002) Phytoextraction of metals and metalloids from contaminated soil. *Current Opinion in Biotechnology* **14**, 277-282

Meagher RB (2006) Plants tackle explosive contamination. *Nature Biotechnology* **24**, 161-163

Meagher RB, Rugh CL, Kandasamy MK, Gragson G, Wang NJ (2000) Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. In: Terry N, Banuelos G (Eds) *Phytoremediation of Contaminated Soil and Water*, Lewis, Boca Raton, Florida, pp 201-221

Mench MJ, Tancogne J, Gomez A, Juste C (1989) Cadmium bioavailability to *Nicotiana tabacum* L., *Nicotiana rustica* L. and *Zea mays* L. grown in soil amended or not amended with cadmium nitrate. *Biology and Fertility of Soils* **8**, 48-53

Meers E, Ruttens A, Hopgood MJ, Samson D, Tack FMG (2005) Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. *Chemosphere* **58**, 1011-1022

Misra S, Gedamu L (1989) Heavy-metal tolerant transgenic *Brassica napus* L and *Nicotiana tabacum* L plants. *Theoretical and Applied Genetics* **78**, 161-168

Mukhtar A, Ullah H, Mukhtar H (2007) Fatty acid composition of tobacco seed oil and synthesis of alkyd resin. *Journal of Chinese Chemistry* **25**, 705-708

Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology* **60**, 193-207

Nagata T, Kiyono M, Pan-Hou H (2006) Engineering expression of bacterial polyphosphate kinase in tobacco for mercury remediation. *Applied Microbiology and Biotechnology* **72**, 777-782

Oller ALW, Agostini E, Talano MA, Capozucca C, Milrad SR, Tigier HA, Medina MI (2005) Over expression of a basic peroxidase in transgenic tomato (*Lycopersicon esculentum* Mill cv. Pera) hairy roots increases phytoremediation of phenol. *Plant Science* **169**, 1102-1111

Pavliková D, Macek T, Macková M, Surá M, Szaková J, Tlustos P (2004) The evaluation of cadmium zinc and nickel accumulation ability of transgenic tobacco bearing different transgenes. *Plant Soil and Environment* **50**, 513-517

Pavliková D, Macek T, Macková M, Szaková J, Balik J (2004b) Cadmium

tolerance and accumulation in transgenic tobacco plants with a yeast metallothionein combined with a polyhistidine tail. *International Biodeterioration and Biodegradation* **54**, 233-237

Puschenreiter M, Stoger G, Lombi E, Horak O, Wenzel WW (2001) Phytoextraction of heavy metal contaminated soils with *Thlaspi goesingense* and *Amaranthus hybridus*: Rhizosphere manipulation using EDTA and ammonium sulphate. *Journal of Plant Nutrition and Soil Science* **164**, 615-621

Quartacci MF, Baker AJM, Navari-Izzo F (2005) Nitriloacetate- and citric acid-assisted phytoextraction of cadmium by Indian mustard (*Brassica juncea* (L.) Czernj, *Brassicaceae*). *Chemosphere* **59**, 1249-1255

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. *Current Opinion in Biotechnology* **8**, 221-226

Reeves RD, Baker AJM (2000) Metal-accumulating Plants In: Raskin I, Ensley BD (Eds) *Phytoremediation of Toxic Metals: Using Plants to Clean-Up the Environment*, John Wiley and Sons, New York, pp 193-230

Robinson BH, Millis TM, Petit D, Fung LE, Green SR, Clothier BE (2000) Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. *Plant and Soil* **227**, 301-306

Rugh CL, Bizily SP, Meagher RB (2000) Phytoreduction of environmental mercury pollution. In: Raskin I, Ensley BD (Eds) *Phytoremediation of Toxic Metals – Using Plants to Clean up the Environment*, John Wiley and Sons, New York, pp 151-171

Rugh CL, Wilde HD, Stack NM, Thompson DM, Summers AO, Meagher RB (1996) Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial *merA* gene. *Proceedings of the National Academy of Sciences USA* **93**, 3182-3187

Ruiz ON, Hussein HS, Terry N, Daniell H (2003) Phytoremediation of organomercurial compounds via chloroplast genetic engineering. *Plant Physiology* **132**, 1344-1352

Rumeau D, Becuwe-Linka N, Beyly A, Carrier P, Cuine S, Genty B, Medgyesy P, Horvath E, Peltier G (2004) Increased zinc content in transplastomic tobacco plants expressing a polyhistidine-tagged Rubisco large subunit. *Plant Biotechnology Journal* **2**, 389-399

Ryslava E, Krejcik Z, Macek T, Novaková H, Denmerová K, Macková M (2003) Study of PCB degradation in real contaminated soil. *Fresenius Environmental Bulletin* **12**, 296-301

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 643-668

Salt DE, Prince RC, Pickering IJ (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiology* **109**, 1427-1433

Sappin-Didier V, Vansuyts G, Mench M, Briat JF (2005) Cadmium availability at different soil pH to transgenic tobacco overexpressing ferritin. *Plant and Soil* **1-2**, 189-197

Shen ZG, Li XD, Wang CC, Chen HM, Chua H (2002) Lead phytoextraction from contaminated soils with high biomass plant species. *Journal of Environmental Quality* **31**, 1893-1900

Shiota N, Nagasawa A, Sakaki T, Yabusaki Y, Ohkawa H (1994) Herbicide-resistant tobacco plants expressing the fused enzyme between rat cytochrome P4501A1 (CYP1A1) and yeast NADPHcytochrome P450 oxidoreductase. *Plant Physiology* **106**, 17-23

Sonoki T, Kajita S, Ikeda S, Uesugi M, Tatsumi K, Katayama Y, Iimura Y (2005) Transgenic tobacco expressing fungal laccase promotes the detoxification of environmental pollutants. *Applied Microbiology and Biotechnology* **67**, 138-142

Stoger EM, Nicholson SL, Fischer R, Christou P (2005) Recent progress in plantibody. *Technology Current Pharmaceutical Design* **11**, 2439-2457

Sudová R, Pavliková D, Macek T, Vosatka M (2007) The effect of EDDS chelate and inoculation with the arbuscular mycorrhizal fungus *Glomus intraradices* on the efficacy of lead phytoextraction by two tobacco clones. *Applied Soil Ecology* **35**, 163-173

Thomas JC, Davies EC, Malick FK, Endreszl C, Williams CR, Abbas M, Petrella S, Swisher K, Perron M, Edwards R, Ostenkowski P, Urbanczyk N, Wiesend WN, Murray KS (2003) Yeast metallothionein in transgenic tobacco promotes copper uptake from contaminated soils. *Biotechnology Progress* **19**, 273-280

Uchida E, Ouchi T, Suzuki Y, Yoshida T, Habe H, Yamaguchi I, Omori T, Nojiri H (2005) Secretion of bacterial xenobiotic-degrading enzymes from transgenic plants by an apoplastic expressional system: An applicability for phytoremediation. *Environmental Science and Technology* **39**, 7671-7677

Umarov AU, Khasanov MKh, Teshabaeva KhT (1991) An investigation of the oil of *Nicotiana tabacum* seeds. *Chemistry of Natural Compounds* **26**, 706-707

UNEP (2002) Global Environment Outlook 3 (GEO-3)

Usta N (2005) An experimental study on performance and exhaust emissions of a diesel engine fuelled with tobacco seed oil methyl ester. *Energy Conversion and Management* **46**, 2373-2386

Veljkovic VB, Lakicevic SH, Stamenkovic OS, Todorovic ZB, Lazic ML (2006) Biodiesel production from tobacco (*Nicotiana tabacum* L.) seed oil with a high content of free fatty acids. *Fuel* **85**, 2671-2675

Wagner GJ (1993) Accumulation of cadmium in crop plants and its consequences to human health. *Advances in Agronomy* **51**, 173-212

Watanabe ME (2001) Can bioremediation bounce back? *Nature Biotechnology* **19**, 1111-1115

Watanabe ME (1997) Phytoremediation on the brink of commercialization. *Environmental Science and Technology* **31**, A343-A343

Wang L, Samac DA, Shapir N, Wackett LP, Vance CP, Olszewski NE, Sadowsky MJ (2005) Biodegradation of atrazine in transgenic plants expressing a modified bacterial atrazine chlorohydrolase (*atzA*) gene. *Plant Biotechnology Journal* **3**, 475-486

Wenger K, Gupta SK, Furrer G, Schulin R (2002) Zinc Extraction potential of two common crop plants *Nicotiana tabacum* and *Zea mays*. *Plant and Soil* **242**, 217-225

Williams LE, Pittman JK, Hall JL (2000) Emerging mechanisms for heavy metal transport in plants. *Biochimica et Biophysica Acta: Biomembranes* **1465**, 104-126

Wu J, Hsu F, Cunningham S (1999) Chelate-assisted Pb phytoextraction: Pb availability uptake and translocation constraints. *Environmental Science and Technology* **33**, 1898-1904

Yazaki K, Yamanaka N, Masuno T, Konagai S, Kaneko S, Ueda K, Sato F (2006) Heterologous expression of a mammalian ABC transporter in plant and its application to phytoremediation. *Plant Molecular Biology* **61**, 491-503

Yeargan R, Maiti IB, Nielsen MT, Hunt AG, Wagner GJ (1992) Tissue partitioning of cadmium in transgenic tobacco seedlings and field grown plants expressing the mouse metallothionein I gene. *Transgenic Research* **1**, 261-267