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Phytoremediation of contaminated soils and groundwater: lessons from the field

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Abstract

Background, aim, and scope The use of plants and associated microorganisms to remove, contain, inactivate, or degrade harmful environmental contaminants (generally termed phytoremediation) and to revitalize contaminated sites is gaining more and more attention. In this review, prerequisites for a successful remediation will be discussed. The performance of phytoremediation as an environmental remediation technology indeed depends on several factors including the extent of soil contamination, the availability and accessibility of contaminants for rhizosphere microorganisms and uptake into roots (bioavailability), and the ability of the plant and its associated microorganisms to intercept, absorb, accumulate, and/or degrade the contaminants. The main aim is to provide an overview of existing field experience in Europe concerning the use of plants and their associated microorganisms whether or not combined with amendments for the revitalization or remediation of contaminated soils and undeep groundwater. Contaminations with trace elements (except radionuclides) and organics will be considered. Because remediation with transgenic organisms is largely untested in the field, this topic is not covered in this review. Brief attention will be paid to the economical aspects, use, and processing of the biomass.

Conclusions and perspectives It is clear that in spite of a growing public and commercial interest and the success of several pilot studies and field scale applications more fundamental research still is needed to better exploit the metabolic diversity of the plants themselves, but also to

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better understand the complex interactions between contaminants, soil, plant roots, and microorganisms (bacteria and mycorrhiza) in the rhizosphere. Further, more data are still needed to quantify the underlying economics, as a support for public acceptance and last but not least to convince policy makers and stakeholders (who are not very familiar with such techniques).

Keywords Field experiments · Metals · Organic contaminants · Phytodegradation · Phytoextraction · Phytoremediation · Phytostabilization · Rhizodegradation · Trace elements

1 Background, aim, and scope

There are many remediation techniques available for contaminated soils, but relatively few are applicable to soils contaminated with trace elements. Many soils contaminated with organics can be decontaminated by methods that destroy organics in place. Trace elements, on the contrary, are immutable and relatively immobile, and so many of the low cost options available for the remediation of organic contaminants (i.e., thermal volatilization, biodegradation, rhizodegradation, and phytodegradation) are not available for metal contaminated soils. Due to cost, time, and logistical concerns, relatively few options remain open.

In general, remediation technologies, whether in place or ex situ, do one of two things: they either remove the contaminants from the substratum ("site decontamination or clean-up techniques") or reduce the risk posed by the contaminants by reducing exposure ("site stabilization techniques").

One "gentle" plant-based site stabilization approach, suitable for heavily contaminated sites, is *phytostabilization* aimed to decrease soil metal bioavailability using a combination of plants and soil amendments (Vangronsveld and Cunningham 1998). Another approach directed towards real decontamination is trace element *phytoextraction*, representing use of plants for trace element removal from the soil by concentrating them in the harvestable parts (Vassilev et al. 2004a). An opinion exists that trace element phytoextraction will be more economically feasible if, in addition to metal removal, plants produce biomass with an added economical value (Vassilev et al. 2004a). In contrast to metals, organic contaminants can efficiently be degraded by the cooperation of plants and their associated microorganisms, generally termed *rhizo-* and *phytodegradation*.

The present paper aims give an overview of existing field experiments in Europe and to summarize available information concerning the development, economical aspects, and research needs of phytoremediation.

2 Phytostabilization

2.1 Introduction

Plant-based in situ stabilization, termed "phytostabilization", is not a technology for real clean-up of contaminated soils, but a management strategy for stabilizing (inactivating) contaminants which are potentially toxic. It reduces the risks presented by a contaminated soil by decreasing contaminants' bioavailability using plants, eventually in combination with soil amendments (Vangronsveld et al. 1995a, 1996; Vangronsveld and Cunningham 1998). Using these soil amendments strongly reduces the availability of the pollutants to plant uptake and thus limits eventual toxicity to plants, allowing revegetation of contaminated sites. Establishment of a vegetative cover markedly decreases pollutants' leaching to groundwater and prevents the dispersal of polluted dusts through wind and water erosion from formerly bare or sparsely vegetated sites (Vangronsveld et al. 1995a, b, 1996). This should lead to an attenuation of the impact on site and to adjacent ecosystems. Contamination is "inactivated" in place preventing further spreading and transfer into food chains. Therefore, long-term monitoring of the contaminants will be part of any successful management scheme which uses phytostabilization as a remediation tool.

Phytostabilization not necessarily must be considered as the final solution for a contaminated site. In any case, the development of a well-developed vegetation cover strongly reduces further horizontal and vertical spreading of the contaminants. Therefore, it may also be used as a temporary measure while attending a more definitive remediation.

It is clear that in situ inactivation whether or not combined with phytostabilization is simple, non-invasive, and cost-effective and has the potential to become a valuable strategy for a wide range of contaminated sites.

The amendments used for stabilizing trace elements in contaminated soils (for reviews see Mench et al. 1998; Vangronsveld et al. 2000a, b; Adriano et al. 2004) commonly include liming agents, phosphates (H_3PO_4, triple calcium phosphate, hydroxyapatite, phosphate rock), trace element (Fe/Mn) oxyhydroxides, organic materials (e.g., biosolids, sludge, or composts), natural and synthetic zeolites, cyclonic and fly ashes, and steel shots.

Plants may also help to stabilize contaminants by accumulating and precipitating toxic trace elements in the roots (or root zone) or by adsorption on root surfaces. Plants may assist in altering the chemical form of the contaminants by changing the soil environment (e.g., pH, redox potential) around plant roots. The microorganisms (bacteria and mycorrhiza) living in the rhizosphere of these plants also have an important role in these processes: not only can they actively contribute to change the trace element speciation, but they can also assist the plant in overcoming phytotoxicity, thus assisting in the revegetation process (van der Lelie et al. 1999; Mastretta et al. 2006).

The basis used for evaluating and classifying the effectiveness of soil treatment is not standardized and has to be evaluated on a case to case basis. For screening the most suitable amendments, different approaches are used in different batch, pot, and lysimeter experiments. The effectiveness of the amendments has been assessed in several different ways including chemical methods (e.g., selective or sequential chemical extractions, isotopic dilution techniques, adsorption–desorption isotherms, long-term leaching, and weathering simulations) and biological (e.g., plant growth and dry-matter yield, plant metabolism, ecotoxicological assays on soil invertebrates, and bacteria and microbial populations). One of the lessons learned was the evaluation of the amendments with unpolluted control soils, as some amendments may show undesirable side effects like matrix effects (f.i. zeolites with high sodium content destroying soil structure) or immobilization of essential nutrients (Mn, Mg, etc).

2.2 Field applications

To the best of our knowledge, phytostabilization in the field was only investigated for trace element-contaminated soils. Different soil amendments have been used to immobilize the toxic trace elements. An overview of known field experiments is presented in Table 1. A description of field cases is given according to the (main) soil amendment used.

Liming Application of chalk or limestone ($CaCO_3$), quicklime (CaO), or hydrated lime ($Ca(OH)_2$) to increase soil pH has been commonly used for centuries (Goulding and Blake 1998). Amendments containing Mg such as dolomitic limestone are often used to obtain an adequate Mg nutrition to crops. Rothamsted Experimental Station (UK) has a set of long-term experiments (e.g., Park Grass Experiment) established in the mid-nineteenth century that are particularly useful in understanding the relationship between land use, acidification, lime use, and trace element mobilization (Goulding and Blake 1998). The fact that immobilization of low Cd contents by liming is sometimes ineffective was illustrated by increased Cd concentrations in potato tubers at three sites in South Australia (McLaughlin et al. 2000).

The practice of liming to remediate contaminated soils and mine tailings has the potential to mobilize arsenic due to the pH dependence of As sorption reactions on oxide minerals and layer silicates (Jones et al. 1997). It may also be of concern for other trace elements such as Cu, Mo, U, V, and Se.

Biosolid compost and liming The use of biosolids and similar organic wastes such as paper mill sludge, alone and in combination with other materials, was used for restoration of many sites (Sopper 1993). These amendments provide organic matter improve soil physical properties, water infiltration, water holding capacity, and microbial activity. They further contain essential micro- and macro-nutrients for plant growth and also decrease bulk density. Biosolids, despite their trace element contents, can be combined with other materials that have a high calcium carbonate equivalent to restore trace element affected ecosystems, despite trace element concentrations in these biosolids (Brown et al. 2000). Combined application of biosolids and lime should reduce trace element availability and increase pH of the soil. Experiments in the USA at Palmerton, PA; Leadville, CO; Bunker Hill, ID; Baltimore, MD; and Pronto Mine, Ontario investigated the efficacy of biosolids and high calcium carbonate to restore a vegetative cover and/or to decrease human exposure at trace element-contaminated sites (Adriano et al. 2004). In Europe, biosolid compost has been used in the Aznalcollar region in Spain (Madejon et al. 2006).

Cyclonic ashes Mixing of cyclonic ashes (formerly called “beringite”) in the upper 30 cm of heavily Zn-, Cd-, and Pb-contaminated soils was shown to reduce plant exposure to trace elements and to restore a vegetation cover at several different sites. In the case of the Lommel-Maatheide (an old pyrometallurgical zinc smelter site in Belgium) even 12 years after the treatment, vegetation on the 3-ha pilot plot was healthy and regenerating by vegetative means and by seed. Both physico-chemical and biological evaluations performed at different times clearly indicate a very strong immobilization of trace elements and by consequence a strongly reduced availability for biota (Vangronsveld et al. 2000a, b). Five, 7, 10, and 12 years later, evaluations were made of soil physico-chemical parameters, potential phytotoxicity, floristic and fungal diversity, mycorrhizal infection of the plant community, and total numbers and diversity of nematodes (Vangronsveld et al. 1996; Bouwman et al. 2001; Bouwman and Vangronsveld 2004). Three other field experiments in the same region in Belgium (Overpelt en Balen) were successfully established on heavily contaminated soils in Belgium (Overpelt en Balen).

Iron oxides Iron, Al, and Mn oxides commonly occur in soils and react with trace elements (Knox et al. 2001). The OH–OH distance in Fe, Mn, and Al oxides matches well with the coordination polyhedra of many trace elements. Such hydroxyl groups form an ideal template for bridging trace elements (Manceau et al. 2002). Reactions with trace elements can be promoted when these (hydr)oxides are combined with alkaline materials (Mench et al. 1998).

Table 1 Inventory of field experiments on phytostabilization and metal inactivation in Europe

Site	Contaminant source	Amendment	Type of site	Plant species	Reference
Lommel, Belgium	Cd, Pb, Zn Zn smelter	Beringite, compost	Industrial area	*Festuca rubra* *Agrostis capillaris*	Vangronsveld et al. (1995a, b, 1996, 2000a, b)
				Lolium perenne	
			Domestic gardens	Vegetable crops	Vangronsveld (1998)
Rothamsted, UK	Al, Cd, Cu, Pb, Zn	Lime		Grasses	Goulding and Blake (1998)
Couhins, France	Cd, Ni	Beringite, steel shots	Agricultural land	*Zea mays*	Boisson et al. (1998)
	Sewage sludge			*Lactuca sativa*	Mench et al. (2000a, b)
Dottikon, Rafz, Giornico, Switzerland	Cd, Cu, Zn Sewage sludge	Gravel sludge		*Lactuca sativa* *Lolium perenne*	Krebs et al. (1999)
Walsall, UK	Cu metal refinery	Sugar beet washings, liming	Dumping site	*Betula pendula*, *Alnus cordata* *Alnus incana*	Dickinson (2000)
Mersey Forest, UK			Landfill (urban)	*Alnus glutinosa*	
Manchester, UK			Sewage sludge	*Crataegus monogyna*	
				Salix caprea	
Czech Republic	Cd, Zn floodwater	Dolomitic limestone, synthetic zeolites, organic loamy shales, muck, acid peat	Agricultural soil	Mustard Rye	Vacha et al. (2000)
				Oat	
Cornwall, Northampton, St Helens, UK	As As smelter	Iron oxides	Agricultural land, domestic garden	Vegetable crops	Warren et al. (2003)
	Sewage sludge				
Northampton, UK	Cd	Zeolites		Vegetable crops	Lepp et al. (2000)
Staffordshire, UK	Sewage sludge Pigment manufacture				Rebedea (1997)
Prescott, UK	Rod and wire plant	Zeolites		*Agrostis*	
Jales, Portugal	As, Cd, Cu, Pb, Zn	Beringite and steel shots	Mine spoil	*Agrostis castellana*	Bleeker et al. (2002)
	Gold mine			*Cytisus*	Mench et al. (2003)
Aznalcollar, Spain	As, Bi, Cd, Cu, Pb, Sb, Tl, Zn Mine spill		Agricultural land	Barley	Soriano and Fereres (2003)
				Triticale	
				Brassica sp.	
Skouriotissa, Cyprus	Cu Cu mine	Vinassa (wine waste product) Chicken fertilizer	Mine tailings	*Pistacia terebinthus* *Cistus creticus*	Johansson et al. (2005)
				Pinus brutia	
				Bosea cypria	
Arnoldstein, Austria	As, Cd, Pb, Zn Pb–Zn smelter	Red mud, gravel sludge	Industrial area	Barley	Friesl et al. (2006)
Sugar Brook, UK Fazakerley, UK	As, Cd, Cu, Ni, Pb, Zn		Urban area Industrial area	*Betula* *Alnus*	French et al. (2006)
Kirby Moss, UK			Agricultural land	*Salix*	
Merton Bank, UK			Urban area	*Populus*	
Cromdale Grove, UK			Industrial area	*Larix*	

Avonmouth, UK	Cd, Cu, Cr, Ni, Pb, Zn Pb–Zn smelter	Red mud, lime		*Festuca rubra*	Gray et al. (2006)
Warrington, UK	Cd, Cu, Zn Sludge		Industrial area	*Salix, Populus Alnus*	King et al. (2006)
Northern France	Cd, Pb, Zn Pb smelter		Agricultural land	*Trifolium repens Lolium perenne*	Bidar et al. (2007)
Piekary Slaskie, Silesia, Poland	Zn, Cd, Pb Zn and Pb smelter waste	Biosolid compost, lime	Industrial area	*Festuca rubra, Poa pratensis, Festuca arundinacea, Festuca ovina*	Stuczynski et al. (2007)
Aznalcollar, Spain	As, Bi, Cd, Cu, Pb, Sb, Tl, Zn Mine spill	Biosolid compost, sugar beet lime, leonardite	River banks	*Helianthus annuus*	Madejon et al. (2003, 2006)
				Agrostis stolonifera	Perez-de-Mora et al. (2006)
Aznalcollar, Spain	As, Bi, Cd, Cu, Pb, Sb, Tl, Zn Mine spill		River banks	*Quercus ilex*	Dominguez et al. (2008)
				Olea europea	
				Populus alba	
				Mediteranean shrubs	
St. Médard, France	As, Cu, Cr	Iron grit, lime, compost		*Agrostis capillaris A. gigantea*	Bes and Mench (2008)

Three field trials in UK (Cornwall, Northampton, and St. Helens) were performed to investigate the efficacy of iron oxides as immobilising agents for As (Warren et al. 2003). The sites were (1) an agricultural field, adjacent to a derelict As smelter in Cornwall; (2) long-term sludge-treated plots at a sewage works in Northampton; and (3) a domestic garden in St Helens, Merseyside. Sources and soil concentrations of As differed between sites. Ferrous sulfate (commercial grade) was applied in solution at St Helens (both treatments), Cornwall, and Northampton (0.2% treatment) and as a solid at Cornwall (0.5% and 1% treatments). Because ferrous sulfate acidifies the soil, it was applied together with lime at Northampton. Lime was also applied to the Fe oxide treatments at St Helens.

Only at the Cornish site general reductions of transfer coefficients of As to plants were observed, although they were only significant for calabrese leaf, cauliflower, and radish. The As transfer coefficients were very variable for tomato, but values were in general very low for this crop.

Cyclonic ashes and zerovalent iron grit On an agricultural soil in the vicinity of Bordeaux (Couhins, France), sewage sludges were applied to a coarse sandy soil from 1976 to 1980. In 1995, one block was amended with cyclonic ashes (formerly called "beringite" (5%, *w/w*) and another one with zerovalent iron grit (1%; (Mench et al. 2000a). Based on corn grain yield, there were no differences between Fe^0-treated and untreated plots, but a continuous decrease was observed for the cyclonic ash-treated plot (Boisson et al. 1998). Corn ears were better filled in the Fe^0 plot, while those from the cyclonic ash-treated plots showed poor filling due to Mn deficiency, which occurred due to Mn immobilization by cyclonic ash in the sandy soil. At moderate rates of soil Cd contamination, Fe^0 addition appeared to result in a sustainable decrease in Cd bioavailability to maize and reduced grain Cd content.

Previous attempts to establish a vegetation cover on the fine-grained spoil at the former Jales Gold mine (Portugal) were unsuccessful. Colonization by vegetation was limited to a few isolated spots. The grasses *Holcus lanatus*, *Agrostis castellana*, and *Agrostis delicatula* were the sole colonisers, growing in small isolated tufts (De Koe 1994). Consequently, erosion of the spoil heaps by wind and water resulted in a permanent pollution of surrounding terrestrial and aquatic ecosystems. A small-scale semi-field experiment was established at Bordeaux, France (Vangronsveld et al. 2000b), and a field experiment was set up in situ on the spoil pile at Jales (Bleeker et al. 2002; Mench et al. 2003).

The arsenic transfer to the river (mainly by superficial runoff) from the site La Combe du Saut (near the former arsenic and goldmine site in Salsigne, south of France) leads to unacceptable risks for human health and the environment (Jacquemin 2006). The highly concentrated sources of pollution were treated with a combination of traditional methods (excavation, physico-chemical stabilization, and confinement) which will strongly decrease the impacts of the site on the river. However, the impacts of the residual pollution after excavation and of the diffuse pollution sources present at large areas need to be controlled as well. One of the scenarios that was implemented was the phytostabilization of the bare polluted soils.

Initially, laboratory experiments have been performed in order to evaluate the phytotoxicity of the soil and the necessity to add arsenic-immobilizing agents (Fe^0). Secondly, an inventory of the natural plant species present at and around the site was made. Germination and growth of commercially available seeds of these species on polluted soils from the site (with and without the addition of SS) were tested in greenhouse experiments in order to select species for in situ testing. In a first phase, five field plots (100 m^2) have been implemented, and the development of the selected plant species is followed up since the beginning of 2004. The results allowed for the final selection of the species used in the full-scale phytostabilization (in 2006).

A study concerning the decrease in arsenic transfer after phytostabilization is ongoing. A special measuring device has been developed, being able to measure continuously water flow rates (1 till 3,000 l h^{-1}). Besides, the arsenic concentration in the runoff water is determined. The benefits of phytostabilization will thus be made evident.

Zeolites and other amendments A field trial using two synthetic zeolites, 4A and P, was established in an area by atmospheric deposition copper-contaminated (as copper oxides) grassland at the Rod and Wire plant, Prescot (Merseyside, UK). Soil analyses performed 18 months after the zeolite application demonstrated that the amendments had reduced water-extractable Cu fractions by up to 75% of the initial value. The results were considered as a good indication of the durability of zeolite effects, even under conditions of ongoing copper input to soil. Qualitative evaluation of root growth in *Agrostis* indicated a much greater proportion of new root growth in the two zeolite plots in comparison to the control plots (Rebedea 1997).

Red muds Red mud is a by-product of the alumina industry that is alkaline and rich in Al/Fe oxides. Red mud performed well in a 15-month pot study carried out on a soil from Arnoldstein (Austria) by Friesl et al (2006). Red mud reduced Zn, Cd, and Ni extractability by 63%, 42%, and 50% as compared to the control. Zinc, Cd, and Ni uptake in *Amaranthus hybridus* were reduced by 53%, 40%, and 59%. Field experiments were performed. Important conclusions were that field experiments did not support data from the pot trials and that depth of treatment can be an important factor (Friesl et al. 2006).

Phosphates Phosphates react with many trace elements. Precipitates formed can be stable over a wide range of geochemical conditions. A range of phosphate (containing) compounds has been evaluated including mineral apatite, synthetic hydroxyapatite, and diammonium phosphate materials. These materials were shown to be effective at attenuating trace element exposure through the soil solution and incidental ingestion, especially for Pb (converted to pyromorphite, a lead phosphate [$Pb_5(PO_4)_3(OH, Cl, F,\ldots)$]), but also for Zn, Cd, Ni, U, and Mn through formation of trace element-phosphate minerals (Ma 1996; McGowen et al. 2001; Seaman et al. 2001a, b; Geebelen et al. 2003, 2006). Arsenic mobilization due to addition of phosphates has been reported (Peryea and Kammereck 1997; Boisson et al. 1999). This can increase the size of the mobile As fraction and As uptake by higher plants (Gulz and Gupta 2001).

Phosphates have been applied in Joplin (MO) and Jacksonville (FL), USA. Results were very promising, but to the best of our knowledge, until now no field experiments with phosphates have been performed in Europe.

Clays Clay addition to soil can change physical and chemical properties that could affect contaminant fate and transport. These include increase of cation exchange capacity, increase of mineral surface areas, and sorption within the clay interlayer. Montmorillonites modified by aluminum were found to cause a preferential sorption of trace elements compared to other bivalent cations and have been proposed as immobilizing agents (Mench et al. 1998; Lothenbach et al. 1997, 1999). Trace element immobilization by clay–aluminum complexes is higher than immobilization by clay or aluminum alone.

Gravel sludge, a waste product of the gravel industry, contains illite (29%), calcite (30%), and quartz (18%). Its efficacy as a trace element immobilizing additive was investigated at two application rates in three field trials with sandy loam soils at Dottikon, Rafz, and Giornico in Switzerland, contaminated by Zn, Cu, and Cd (Krebs et al. 1999). Gravel sludge application increased pH in all three topsoils by up to 0.6 units and reduced $NaNO_3$-extractable Zn concentration by more than 65%. No effect was found for the $NaNO_3$-extractable Cu concentration at Rafz, and an increase in this Cu fraction was evident at Giornico. In the Dottikon soil, $NaNO_3$-extractable Cu concentration slightly decreased, while Zn and Cu concentrations in ryegrass were reduced by more than 35%.

Lettuce Zn and Cd tissue concentrations were decreased by 22% to 48% at Giornico and Dottikon, whereas no effect was found at Rafz. Gravel sludge efficacy was highest in soils with high $NaNO_3$-extractable trace elements and higher for ryegrass than for lettuce.

Comparison of different additives in field experiments In Northampton and Staffordshire (UK), field trials were established to evaluate the efficacy of soil amendments in reducing Cd transfer from soil to vegetables (Lepp et al. 2000). Increased soil Cd content at the Northampton site (mean total Cd content 47 mg kg^{-1}) resulted from the application of sewage sludge, while at the Staffordshire site (mean total Cd content 16 mg kg^{-1}), deposition of Cd oxide particles from an adjacent pigment manufacturer had contaminated domestic gardens. In Staffordshire, Cd was the only contaminant, while at the Northampton site also elevated contents of other trace elements were found. Plots were treated in 1998 with: $FeSO_4$, iron grit, lime, and zeolite 4A. The treatments were added to the top 10 cm of soil.

Results were not very conclusive. There were no significant reductions in plant Cd at either site following the incorporation of in situ soil amendments. Both lime and zeolite treatments achieved some reductions in soil–plant Cd transfer, but samples were too variable within each site to be considered as significant at the replication level employed. Cadmium uptake by the same crop showed well-marked cultivar differences in successive growing seasons.

In another field experiment in the Czech Republic (Vacha et al. 2000), efficacy of dolomitic limestone was compared with that of other amendments, i.e., synthetic zeolites prepared from fly ashes, organic loamy shales (top layer of coal beds), muck (material of sapric Histosols), and acid peat in a soil contaminated by flood water (15 mg Cd, 1,900 mg Zn, and 1,200 mg Pb kg^{-1} soil). Amendments were mixed in the 0–20-cm top soil layer, and mustard, rye, and oats were cultivated. With the exception of acid peat, amendments improved plant yield. Best results were found with muck and dolomitic limestone. Both amendments decreased Cd and Zn concentrations in plant tissues, whereas their influence was marginal for Pb. Synthetic zeolites and organic loamy shales were less effective.

2.3 Monitoring the efficacy of trace element phytostabilization: lessons from the field and research needs

Both successes and failures have been reported. A thorough evaluation of the overall effect of ameliorants and the developing ecosystem and the sustainability (durability) of trace element immobilization in contaminated soils is crucial for the acceptance of inactivation/stabilization strategies. This evaluation should combine physico-chemical and biological methods. Trace element speciation work with X-ray diffraction or X-ray absorption techniques can be used to support selective or sequential extraction methods, providing valuable data on the chemical forms of trace elements in the soil. An understanding of the forms of contaminants present in the soil can be used to make reliable predications about sustainability of the in situ inactivation.

Biological methods complement physico-chemical evaluation methods, which do not directly address biological availability or toxicity. Several case studies demonstrated that amendments decreased the soluble and exchangeable trace element fractions, but that changes in trace element uptake by plant were not significant. Not only toxicity but also possible soil additive-induced deficiencies of essential elements must be considered.

Biological evaluation should be performed using various living organisms from different trophic levels; existing ecotoxicity tests can be used, but new ones may need to be developed. Some microbial assays can detect specific categories of toxicants such as trace elements.

Another important parameter to monitor is the evolution of biodiversity. Increasing biodiversity (plant species, mycorrhiza, soil bacteria, and invertebrates) is a good indicator of the quality and durability of trace element immobilization. Nevertheless, food chain contamination must be monitored. When earthworm survival rate increased, despite decrease in trace element labile pool for biological action, depurate earthworms may have high trace element content. The establishment of a mycorrhizal network in revegetated areas is thought to be essential for the development of a sustainable ecosystem, while highly mycotrophic plants are characteristic of stable, sustainable ecosystems.

Other aspects of monitoring may include in situ plant sampling to determine plant tissue concentrations of trace element contaminants. Plant sampling may indicate whether or not treatment is effective and if a hazard exists due to human or animal ingestion of plants on the site. Along with contaminant monitoring, soil sampling for fertility purposes should be conducted on a regular basis (every 3 to 5 years) to indicate fertilizer and other requirements. Indeed, deficiencies for some essential trace elements (f.i. Mn in case of cyclonic ash addition) have been reported. On the other hand, the use of soil additives immobilizing toxic elements may lead to mobilization of others. Like mentioned before, phosphate amendments are efficient in immobilizing Pb, Zn, Cd, etc., but may mobilize As (Boisson et al. 1999).

Geebelen et al. (2003, 2006) underline the necessity of a preliminary laboratory study before setup of large-scale in situ field treatment. The intended land use of post-treated sites should be taken into consideration in any remedial undertaking. Addition of some amendments may be very efficient in case it is the intention to develop a new landscape. However, in establishing playgrounds for young children where oral intake is of greater concern, another amendment might be the option. It is paramount to demonstrate the long-term stability of the amendments using field plot experiments since aging of additives can change their effectiveness. Several studies indicate that combining certain industrial by-products might enhance the efficacy than where only one is applied (Vangronsveld et al. 2000a, b; Geebelen et al. 2003). For example, Fe^0 can immobilize arsenic and might be applied together with apatite. Application of such mixed additives can plausibly increase immobilization on more soil types, thereby enhancing their ecological values.

3 Phytoextraction

3.1 Introduction

Phytoextraction is one of the phytoremediation's sub-areas based on the use of pollutant-accumulating plants for trace elements and organics removal from soil by concentrating them in the harvestable parts (Salt et al. 1998).

An ideal plant for trace element phytoextraction should possess the following characteristics: (a) tolerance to the trace element concentrations accumulated, (b) fast growth and highly effective trace element accumulating biomass, (c) accumulation of trace elements in the above ground parts, and (d) easy to harvest.

In general, a trace element phytoextraction protocol consists of the following elements: (a) cultivation of the appropriate plant/crop species on the contaminated site; (b) removal of harvestable trace element-enriched biomass from the site; and (c) post-harvest treatments (i.e., composting, compacting, thermal treatments) to reduce volume and/or weight of biomass for disposal as a hazardous waste or for its recycling to reclaim the trace elements that may have an economic value.

Trace element phytoextraction, as any other technology, has both its advantages and limitations. The main advantage of this technology, as often mentioned, is its lower cost as compared to the other known remediation techniques, which is due to plant's ability to work as a solar-driven pump, extracting and concentrating particular elements from the environment. Direct comparison of the costs associated with landfill excavation and phytoextraction revealed that the cost of the latter should be significantly cheaper (see Section 5). The possible trace element recycling should provide further economic advantage as the ash of some hyperaccumulators consists of significant amounts of trace elements (20–40% Zn for *Thlaspi caerulescens*) and there is no need to pay for safe disposal (Chaney et al. 1997). Another advantage is that phytoextraction can work without further disturbing the site, which is believed to be of great importance for its public acceptance.

Important limitations of trace element phytoextraction are: (1) it can only be used for low to moderately contaminated soils; and (2) its applicability is limited to surface soils (at rooting depth) which varies with the species used, but on average is less than 50 cm. A remarkable exception is the case for some trees, where the target zone is in the range of one to several meters. The application of fast growing trees, such as *Salix* sp., also offers the possibility to combine trace element extraction with the production of biomass for bioenergy production (Schröder et al. 2008). The options of trace element extraction and bioenergy production should, among many other factors, be part of an integrated concept that decides on the feasibility to apply phytoextraction as a remediation technique (see also further in Section 5).

If cost is the main advantage, time is the greatest disadvantage of trace element phytoextraction. It is known that this process is not fast, but (to be realistic for the practical purpose) time should preferably not exceed 10 years or even shorter (Robinson et al. 1998; Blaylock and Huang 2000). Another disadvantage is that (as any biological approach) this technology is not capable for full decontamination because it is limited to the plant available fraction of the trace elements. This probably is not a very strong limitation, as contaminated soil has to be cleaned to some degree: for agricultural soils it should be to levels below the threshold value and for industrial or non-residential soils to the legislative cleanup criteria which can vary per country. If remedial action aims at removing only the trace element fractions readily available to plants, the time required is also significantly reduced from decades to only a few years time span. More details are discussed below (Section 3.6.2).

Trace element phytoextraction technology is still at the development stage. Small companies and universities are driving much of its innovation and research, whereas environmental engineering firms are involved in application projects. The available data from finished, full-scale projects are still limited. According to the USA Environmental Protection Agency (EPA 2000), more data should become available in the next few years. On the other hand, there is evidence that the trace element phytoextraction market is continuously increasing. It was evaluated to grow from 15–25 million USD in the year 2000 to 70–100 million USD by the year 2005 (Glass 2000).

There is a need for enhancement of natural phytoextraction potential and several studies have addressed this problem (Vassilev et al. 2004a).

The plant–rhizosphere interactions controlling trace element uptake by roots are of primary interest. To what extent root exudates can mobilize trace elements (as was shown for Fe and possibly Zn; Marschner 1995) or if microbial rhizosphere communities stimulated by these root exudates (Anderson 1997) can contribute to trace element phytoavailability remains to be further examined. As certain plants can use microbial siderophores to improve their iron uptake, it has been hypothesized that bacterial trace element chelators, such as siderophores, can eventually improve the uptake of heavy trace elements by plants (van der Lelie 1998; van der Lelie et al. 1999).

At present, there are three basic strategies of trace element phytoextraction being developed: (1) continuous or natural phytoextraction using hyperaccumulators; (2) continuous or natural phytoextraction using fast-growing plants (e.g., *Salix* or *Populus* sp.) for trace element removal; (3) induced or chemically assisted phytoextraction introducing soil amendments (e.g., chelators or acidifying amendments) to increase trace element mobility in the soil. The maximum trace element uptake in all these approaches depends on two main variables: trace element concentration in harvestable plant parts and biomass yield. Several other important facts should also be considered when phytoextraction potential is calculated: the plant-available fraction of the trace element in the soil, the number of consecutive crops per annum as well as the trace element decrease during the process of extraction.

3.2 Choice of suitable approach and crop

From the very beginning of the introduction of the trace element phytoextraction concept, one key question is still in debate: “What is preferable—to use trace element hyperaccumulator plants or to use high biomass producing crop species?” Chaney et al. (1997) considered that trace element hyperaccumulation is a more important trait than dry biomass yield. In support to this assumption, they hypothetically calculated Zn removal by hyperaccumulator and high biomass plants and concluded that in any case the use of hyperaccumulators resulted in higher trace element removal. The opposite opinion also exists. For example, Kayser et al. (2000) reported that the trace element removal capacity of *T. caerulescens* was not very different from that of crop species used, this due to poor growth and weak resistance to hot environments, resulting in maximum DM yield of about 1 t ha^{-1}. Ebbs et al. (1997) came to the same conclusion after observing ten times higher Cd concentrations in *T. caerulescens*, but also ten times less biomass production as compared to the crops used.

Obviously, the choice of the phytoextractor depends on the site characteristics: if crops would suffer from toxicity problems, hyperaccumulators, which in general possess a higher trace element tolerance, should have an obvious advantage. Another argument that favors hyperaccumulators is possible reclaiming of Zn from Zn-rich biomass, but Ernst (1998, 2000) pointed out that the real recycling of trace elements from trace element-loaded plants has not been proven up to now, and without this operation the option of hyperaccumulators may be overestimated. Moreover, the zinc price at the world market is actually too low to make “zinc-recycling” from trace element-contaminated soil economically feasible. However, phytomining of nickel was proven to be economically feasible in the USA (Chaney et al. 1999, 2005, 2007). Also in Europe (Albania), successful field experiments using a nickel hyperaccumulator (*Alyssum murale*) have been performed (Bani et al. 2007).

On the other hand, if high biomass crops are chosen, which one is the most suitable? Obviously, no general answer exists to this question, as there should be different choices for different cases, but several suggestions should be mentioned. If trace element contamination is deeper than 20–30 cm, the choice of deep rooting *Salix* or *Populus* will have an obvious advantage. If Cd is the target trace element, the choice of tobacco over maize and sunflower would be preferable as it is known that tobacco is relatively resistant (Davies and Carlton-Smith 1980), while cereals are semi-resistant, while dicotyledons are more sensitive to this trace element (Kuboi et al. 1986). Additionally, the opinion that trace element phytoextraction would only be economically feasible if, in addition to the plant role in phytoextraction, the used crops produce biomass with an added value (Vassilev et al. 2004a). For example, energy crops (oilseed and wood), fibers, and fragrance-producing plants could be used to recover these valuable products (Schwitzguébel et al. 2002). A reasonable gain of a factor of 4.4 in trace element extraction efficiency could be reached by a field-based screening of commercial sunflower cultivars followed from an appropriate ammonium-sulfate fertilization (Nehnevajova et al. 2005). An overview of field experiments in Europe is given in Table 2.

3.3 Natural trace element phytoextraction using hyperaccumulators

Introduction At present, more than 400 trace element-accumulating taxa, belonging to at least 45 plant families, have been identified (Reeves and Baker 2000). Most of the hyperaccumulator plant species are able to accumulate just one trace element, but there are also multitrace element accumulators. Some populations of *T. caerulescens* are found to have not only high levels of Zn, but also of Cd,

Table 2 Inventory of field experiments on phytoextraction in Europe

Site	Origin of pollution contaminant	Amendment	Type of site	Plant species	Reference
Woburn, UK	Cd, Zn, Ni, Cu, Pb sewage sludge	EDTA, NTA, citric acid		*Thlaspi caerulescens* *Arabidopsis halleri*	McGrath et al. (1993, 2006)
Aznalcollar, Spain	As, Bi, Cd, Cu, Pb, Sb, Tl, Zn mine spill		Agricultural land	*Brassica carinata* *Brassica juncea*	del Rio et al. (2000)
Walsall, UK Mersey Forest, UK	Cu metal refinery	Sugar beet washings, liming	Dumping site Landfill (urban)	*Betula pendula, Alnus cordata* *Alnus incana*	Dickinson (2000)
Manchester, UK			Sewage sludge	*Alnus glutinosa*	
				Crataegus monogyna	
				Salix caprea	
Dornach, Switzerland	Cd, Cu, Zn metal smelter	NTA, elemental sulfur		*Salix viminalis* *Nicotiana tabacum*	Kayser et al. (2000)
				Helianthus anuus	
Caslano, Switzerland	Cd, Cu, Zn Sludge	Fertilizer	Meadow	*Salix viminalis*	Hammer et al. (2003)
Dornach, Switzerland	Metal smelter				
Caslano, Switzerland	Cd, Cu, Zn Sludge		Meadow	*Thlaspi caerulescens*	Hammer and Keller 2003
Dornach, Switzerland	Metal smelter				
Dornach, Switzerland	Cd, Cu, Zn Metal smelter			*Salix viminalis* *Nicotiana tabacum*	Keller et al. (2003)
				Helianthus anuus	
				Brassica juncea	
				Thlaspi caerulescens	
				Alyssum murale	
				Zea mays	
Uppsala, Sweden lake Malaren, Sweden	Cd Sewage sludge	N fertilizers	Agricultural land	*Salix viminalis*	Klang-Westin and Eriksson (2003)
Le Locle, Switzerland	Cd, Cu, Zn Sewage sludge		Landfill	*Betula pendula* *Salix viminalis*	Rosselli et al. (2003)
				Alnus incana	
				Fraxinus excelsior	
				Sorbus mougeotii	
La Bouzule, France	Cd, Zn		Brown soil	*Thlaspi caerulescens*	Schwartz et al. (2003)
Gyöngyösoroszi, Hungary	Cd, Cu, Zn, Pb		Agricultural soil	Rape, horseradish, willow, maize, orache, golden-rod, amarant, robinia, rye-grass	Máthé-Gáspár and Anton (2005)
Mártély, Hungary	Cd, Cu, Cr, Zn Dredged sediment		Dredged sediment	*Salix alba*, *Salix caprea*, *Salix viminalis*	Vashegyi et al. (2005)
Czech Republic	Cd, Zn, Pb, As Mining and metallurgical activities		Agricultural soil	*Melilotus alba* *Trifolium pratense*	Tlustoš et al. (2006)
				Malva verticillata	
				Carthamus tinctorius	

Rafz, CH	Cd, Cu, Cr, Zn, Pb Industrial sewage sludge	N fertilizer, NTA, citric acid, elemental sulfur	Agricultural land	Non-GM mutants of *Helianthus annuus*, *Nicotiana tabacum*	Nehnevajova et al. (2005, 2009)
Bettwiesen, CH	Bioavailable Zn Galvanic plant	N fertilizer	Agricultural land Landfill	Non-GM mutants of *Helianthus annuus*, *Nicotiana tabacum*	Herzig et al. (2008, 2009)
Menen, Belgium	Cd, Cr, Cu, Ni, Pb, Zn		River bank	*Salix viminalis*	Vervaeke et al. (2003)
	Dredged sediment				Meers et al. (2005)
Uppsala, Sweden Enköping, Sweden	Cd, Cr, Cu, Ni, Pb, Zn		Tree plantation	*Salix viminalis*	Dimitriou et al. (2006)
Sugar Brook, UK Fazakerley, UK	As, Cd, Cu, Ni, Pb, Zn		Urban area Industrial area	*Betula* *Alnus*	French et al. (2006)
Kirby Moss, UK			Agricultural land	*Salix*	
Merton Bank, UK			Urban area	*Populus*	
Cromdale Grove, UK			Industrial area	*Larix*	
Lommel, Belgium Budel, Nl	Cd, Zn Zn smelter			*Brassica napus*	Grispen et al. (2006)
Warrington, UK	Sludge		Industrial area	*Salix*, *Populus* *Alnus*	King et al. (2006)
Torviscosa, Italy	As, Cu, Cd, Co, Pb, Zn Industrial waste	Mineral fertilization Cow manure		*Helianthus annuus* *Sorghum bicolor*	Marchiol et al. (2007)
Pojske, Albania	Ni Natural soil		Ultramafic soil	*Alyssum murale*	Bani et al. (2007)
Nottingham, UK	Cd, Zn Sewage sludge		Agricultural land	*Salix* *Thlaspi caerulescens*	Maxted et al. (2007a, b)
Bazoches, France Toulouse, France	Pb Atmospheric fallout		Industrial site	*Pelargonium* cv	Arshad et al. (2008)
Aznalcollar, Spain	Mine spill		River banks	*Quercus ilex* *Olea europea* *Populus alba* Mediteranean shrubs	Dominguez et al. (2008)
Pribram, Czech Republic	Cd, Pb Metal smelting and mining activities	Mineral fertilization Manure EDTA	Agricultural land	*Zea mays*	Neugschwandtner et al. (2008)
Lommel (Belgium)	Cd, Zn Zn smelter		Agricultural land	*Zea mays* *Brassica napus* *Nicotiana tabacum* *Salix* species Hybrid poplar	Vangronsveld et al. 2009 (this publication)

Co, and some other trace elements (Baker et al. 1994), whereas others do not express this ability (Lombi et al. 2000). Some families and genera are known as a source of specific trace element hyperaccumulators: Ni (Brassicaceae: *Alyssum* and *Thlaspi*; Euphorbiaceae: *Phyllanthus*, *Leucocroton*), Zn (Brassicaceae: *Thlaspi*), and Cu and Co (Lamiaceae, *Scrophulariaceae*). For more detailed information on hyperaccumulators, see Baker and Brooks (1989) and Reeves and Baker (2000). Recently selected cultivars of *Pelargonium* ssp., i.e., *Pelargonium atomic* were found on calcareous and acidic soils to be real hyperaccumulators for Pb with a relatively good yield performance that make them interesting for Pb phytoextraction (Arshad et al. 2008).

Pteris vittata (brake fern) was proposed for As decontamination. The levels of As in plants are generally less than 12 mg kg^{-1} DW, but *P. vittata* was found to accumulate As at levels of more than 7,000 mg kg^{-1} DW in its fronds, which is hundred times more than any other plant species tested (Ma et al. 2001). High capacity for As accumulation was also reported for asparagus fern (Bagga and Peterson 2001). The capacity for As accumulation of brake fern together with its ability to cope and survive in many areas with a mild climate as well as its considerable biomass, fast growing, etc. has opened a possibility to be used for As phytoextraction.

In general, the prevailing number of reports assessing trace element phytoextraction potential is based on pot experiments, where compared to field experiments, higher trace element extracting values have been observed: these are due to both higher solubility of trace elements, the effects of amendments aiming at mobilizing the trace elements, etc. Some European field trial based data became available (see Table 2), but as this database is still limited, also results from non-European field experiments and even pot experiments were included in the discussion.

Field experiments The first field-based experiment using natural hyperaccumulator plants is conducted in 1991–1992 in sewage sludge-treated plot at Woburn, UK (McGrath et al. 1993). The highest Zn uptake was observed in *T. caerulescens* accumulating 2,000 to 8,000 mg Zn kg^{-1} DW shoots when growing on soil containing total Zn of 150–450 mg kg^{-1}. From these data, the total Zn uptake was calculated to be 40 kg ha^{-1} in a single growing season. With this extraction rate, it was concluded that it would take nine crops of *T. caerulescens* to reduce total Zn from 440 to 300 mg kg^{-1}—the threshold value established by the Commission of the European Community (CEC 1986). In a field trial supervised by Chaney and his collaborators at Pig's Eye landfill site in St-Paul (Minnesota, USA), it was found that under optimum growth conditions *T. caerulescens* could take in Zn at a rate of 125 kg ha^{-1} year^{-1} and Cd at 2 kg ha^{-1} year^{-1} (Saxena et al. 1999). Robinson et al. (1998), on the basis of both field observations and pot-soil experiments, concluded that the potential of *T. caerulescens* for Zn and Cd extraction is rather different. They reported Zn removal values very close to that observed by McGrath et al. (1993) and suggested that it will be not feasible to remediate the Zn-contaminated mine wastes because of both their high Zn content and low Zn bioaccumulation factor. They considered the case of Cd as different due to very high Cd accumulation in *T. caerulescens* leaves (0.16%) and comparatively lower Cd contamination, especially in some agricultural soils, where phosphate fertilizers have been applied for a long period.

Due to high mobility of Cd in the plant–soil system, values exceeding the established food standard (0.1 mg Cd kg^{-1}) could appear quite often in contaminated regions all over Europe. Thus, there is a need to solve this problem and it seems that it would be entirely feasible by Cd phytoextraction. According to Robinson et al. (1998) a single cropping of *T. caerulescens* would reduce 10 mg Cd kg^{-1} soil by nearly a half after 1 to 2 years only. More realistic data concerning Cd extraction by *T. caerulescens* have been obtained by Schwartz et al. (2003) during the work on EU research project PHYTOREM. The authors measured Cd uptake and mass balances after several years of experimentation on agricultural soil amended with heavy trace element rich urban sludge and found that two crops of *T. caerulescens* extracted about 9% of the total Cd and 7% of the total Zn.

If the high trace element concentration (Zn, Cd) of *T. caerulescens* is an advantage, its slow growth rate, low dry mass yield, and rosette characteristics are the main limitations (Ernst 1998; Assunçao et al. 2003). Field observations and measurements on natural populations of *T. caerulescens* have shown that these plants have an annual biomass production of 2.6 tha^{-1} (Robinson et al. 1998). Kayser et al. (2000) reported a maximum yield from *T. caerulescens* of about 1 tha^{-1} under field trails due to poor growth and weak resistance to hot environment. On the other hand, Bennett et al. (1998) showed that the yield of fertilized crop of *T. caerulescens* could be easily increased by a factor of 2–3 without significant reduction in Zn and Cd tissue concentrations. Schwartz et al. (2003) showed evidence for this statement observing that Zn and Cd extraction by *T. caerulescens* has been improved significantly by nitrogen fertilization (80–200 mg N kg soil^{-1}). Zhao et al. (2003) suggested that an average *T. caerulescens* biomass of 5 tha^{-1} should be achieved with optimized agronomic inputs. Further on, they suggested that it is possible to double this yield in the future by successful screening and plant breeding. Using the target biomass yields (5 and 10 t ha^{-1}) and assuming that soil trace element contamination occurs only in the active rooting zone (0–20 cm), these authors did some model calculations for Zn and Cd extraction by *T. caerulescens.* For initial concentration of soil Zn of 500 mg kg^{-1}, it would take 18 to 35 crops of *T. caerulescens* to reduce soil Zn to 300 mg kg^{-1} with 10 and 5 tha^{-1} biomass, respectively. In the case of Cd, five to nine crops would be required to reduce soil Cd concentration of 5 to 3 mg kg^{-1}. If the aim of the phytoextraction is only to strip bioavailable Cd from soil, the time will be much shorter. For example, Schwartz et al. (2003) reported that the availability of Cd and Zn (assessed by NH_4NO_3 extraction and by growing lettuce as the next crop) decreased significantly, more than 70% in the case of Zn.

3.4 Natural phytoextraction using high biomass-producing non-hyperaccumulators

Introduction The ideal plant for trace element phytoextraction has to be highly productive in biomass and to uptake and translocate to its shoots a significant part of the trace elements of concern. Additional favorable traits are fast growth, easy propagation, and a deep root system. Some tree species, mainly willows (*Salix*) and poplars (*Populus*), exhibit these traits and are already used in phytoremediation programs, primarily for rhizofiltration and phytodegradation of organics in contaminated groundwater (Dietz and Schnoor 2001), but also for Cd phytoextraction from lightly polluted agricultural soils (Landberg and Greger 1994). Greger and Landberg (1999) demonstrated the rationale of this option in Sweden, namely: (1) willows are currently being grown on about 15,000 ha in this country as bioenergy source; (2) high Cd accumulators are identified among the *Salix* species (mainly from *Salix viminalis*); (3) the ashes contain ten times more Cd than the ashes from other forest trees; and (4) a method for Cd removal from the ashes is available (Westberg and Gromulski 1996).

In fact, *Salix* species are not trace element hyperaccumulators, but it was shown that among different clones there are high accumulators of Cd and Zn. Up to 150 clones of different *Salix* species (mainly *S. viminalis*) have been screened for uptake, transport of trace elements to shoots, and tolerance to Cd, Zn, and Cu (Landberg and Greger 1994, 1996; Vervaeke et al. 2003; Meers et al. 2005). Some Cd accumulators were found to contain up to 70 mg kg^{-1} DW in leaves, which is close to Cd hyperaccumulation criteria of 100 mg kg^{-1} (Greger and Landberg 1999).

Due to large variation in shoot Cd concentrations (5–70 mg kg^{-1}) found in different *Salix* clones, very different calculated values of Cd removal are given in the literature. With mean leaf concentration of 20 mg Cd kg^{-1} and yield of 10 tha^{-1}, Felix (1997) calculated that Cd removal rate by *Salix* is 0.222 kg Cd ha^{-1} $year^{-1}$. Greger and Landberg (1999) reported that the cultivation of a high-accumulating clone of *S. viminalis* results in 16% removal of total Cd from soil containing 6 mg Cd kg^{-1} soil, which after recalculation gives at least ten times more Cd removal than shown by the previous author. Klang-Westin and Eriksson (2003) estimated the long-term Cd removal by *Salix* using commercial *Salix* stands grown on different soil types. The net removal of Cd from the plough layer by *Salix* crop varied between 2.6 and 16.5 g Cd ha^{-1} $year^{-1}$ using 8 tha^{-1} as the highest *Salix* biomass value in the models. The authors concluded that *Salix* has a high potential for Cd removal as for a long-term perspective (6–7 cutting cycles = 25 years), and it would be possible to extract theoretically a maximum of 413 g Cd ha^{-1}. Under optimal conditions, the yield of *Salix* can be much higher, up to 30 tha^{-1}, so the resulted Cd phytoextraction would also be higher (Robinson et al. 2000).

Field experiments In preliminary experiments on a former maize field in Lommel (Belgium), different plant species and clones were compared in a small-scale experiment; the soil was polluted by aerial deposition from a zinc smelter and considered as moderately contaminated (mean of about 5 mg Cd kg^{-1} soil and about 220 mg Zn kg^{-1}). From a phytoextraction point of view, willow and tobacco proved to be the most promising species (Table 3). A simple calculation shows that even supposing that a linear decrease of metal contents should be possible, time needed to reduce the Cd concentration in the upper 25 cm from 5 to 2 mg kg^{-1} soil should be at least 58 years until more than a century, depending on the various tobacco cultivars and specific fertilization treatments used. When most efficient tobacco in vitro selections and selected commercial sunflower traits were used in multicropping techniques with most appropriate fertilization treatments, the expected cleaning up time for the Lommel soil decontamination from 5 to 2 mg kg^{-1} should be reduced to 29 years (Herzig et al. 2005).

In a large-scale field experiment on the same site, several willow and poplar clones proved to be capable of annually removing up to 120 g Cd ha^{-1} in case only wood was harvested and up to 240 g Cd ha^{-1} if also leaves should be harvested. This corresponds with a projected annual removal of respectively 0.04 Cd mg.kg^{-1} and 0.08 Cd mg kg^{-1} from the topsoil layer (upper 25 cm). Therefore, in the most optimistic scenario, a reduction of the total Cd content of a soil with 1 mg kg^{-1} will last at least 12.5–25 years. The extraction efficiency of energy maize even is lower. These data illustrate that economic valorization of the produced biomass is needed.

Table 3 Phytoextraction potential of different species in a field experiment in Lommel (Belgium)

Species	Cd (mgkg^{-1} DW)	BCF	Biomass (t ha^{-1})	Cd removal (kg ha^{-1} year^{-1})	Cleanup time (year)
Maize	3	0.6	20	0.06	188
Rapeseed	6	1.2	8	0.05	234
Sunflower	12	2.4	10	0.1	117
Tobacco	24	4.8	8	0.19	58
Poplar − twigs	11	2.2	8	0.09	255
Poplar – leaves	28	5.6	2.4	0.07	
Poplar twigs + leaves				0.16	144
Willow − twigs	24	4.8	8	0.19	117
Willow − leaves	60	12	2.4	0.14	
Willow twigs + leaves				0.34	67

Given are Cd contents in aerial part (mg kg^{-1} DW), bioconcentration factor (BCF), biomass production (t ha^{-1}), Cd removal (kg ha^{-1} year^{-1}) and predicted cleanup time supposing a linear extraction

[a] Calculation based on 25 cm soil depth for agricultural crops; 50 cm for willow and poplar and linear extrapolation

In 2008, the yield of the energy maize on this field in Lommel amounted up to 20±3 t dry biomass ha^{-1}. Differences in total fresh and dry biomass production between the different cultivars were limited. The fresh biomass was mainly situated in the stems and the leaves (57±7%). The main portion of the dry biomass was located in the grains (42±5%). The concentration of Cd in the plant parts was decreasing from stem > leaves > bract > rachis > grain. Because no significant differences in Cd concentration between the cultivars were observed for each plant compartment, it was concluded that the trace element extraction potential is not depending on the cultivar. The Cd removal rate with energy maize reached a level of 18± 3 g ha^{-1}. The Cd concentrations in the harvested biomass are exceeding the limits for fodder crops (1.1 mg Cd kg^{-1} dry matter). Therefore, the biomass cannot be introduced in the food chain and should be used for industrial non-food purposes such as energy generation. Batch tests for anaerobic digestion, performed by Organic Waste Systems (Belgium) showed no difference in biogas potential of the silage of the Cd-enriched maize compared with an uncontaminated material. This offers good perspectives for the use of energy maize as an alternative crop, but further research on trace element balance in this process and the disposal of the digestate is still ongoing.

In a willow screening on the Belgian site, Zwarte Driebast, followed by Loden and Belders, delivered the highest biomass production. The concentration of Cd in the leaves was higher than the concentration in the bark and wood. The clones Loden and Tora contained higher concentrations than the other clones. Therefore, the clones Loden, Zwarte Driebast, and Tora will perform best in Cd removal. Among the poplar clones, Grimminge and Koster are combining a better biomass production with a higher Cd concentration, resulting in a higher Cd extraction potential in comparison to the other poplar clones.

Results of short rotation coppice are however provisional, since they are based on measurements of only 2 years of growth instead of a complete rotation cycle of 3 years. Conclusions concerning phytoextraction potential can therefore only be obtained after a full rotation cycle and after regrowth measurements following on the first harvest. The effect of clonal selection on extraction potential is obvious. As the leaves represent the highest extraction potential, techniques for harvest of the leaves need to be explored. After 2 years, the removal of Cd can be enhanced with 45±3% for willow and 43±8% for poplar if the removal of the leaves is included.

Biomass conversion, for instance wood gasification, allows for the production of 1.25 MWh t^{-1} electricity and 9 GJ t^{-1} thermal energy (Vervaeke et al. 2003). Energy production in this manner is considered CO_2 neutral since the amount released during energy production is absorbed by the growing energy crop. Substitution of fossil resources by plant biomass would result in a reduction of greenhouse gas emission by 18–32 t CO_2 per hectare of energy crops. Also pyrolysis of biomass is considered as an alternative. Besides energy, this process can deliver highly valuable products for chemical industry.

As an added incentive for regions with intensive animal production, such as Flanders and the Netherlands, biomass cropping is a nutrient requiring process and could therefore serve as an additional sink for excesses in animal manure production.

From the results that are available until now, it can be concluded that for trace element-enriched agricultural soils the use of fast-growing crops with a high biomass potential is appropriate for sustainable use of these soils. No significant differences in biomass production and extraction potential were found between different energy maize cultivars. However, consumption as fodder can be excluded since the threshold value for Cd is exceeded. Provisional results for use in energy generation by anaerobic digestion are promising. After 2 years of growth, certain willow and poplar clones are showing good potential for phytoextraction purposes, combining elevated biomass production with a relatively high Cd removal potential. The removal of the leaves could enhance phytoextraction efficiency with 45%. Final conclusions, however, will only be obtained after a full rotation cycle of 3 years and after a thorough evaluation of continued growth. Additional investigations on environmental risks, trace element behavior, and balances during subsequent processing of the biomass and on economical aspects are ongoing. All of this information will allow to fully evaluate the feasibility of the various phytoremediation approaches for a safe management of trace element-enriched agricultural soils.

Another approach in this context is the so-called adaptable agriculture implemented in some industrial regions in Bulgaria and in the vicinity of Liverpool (N. Lepp, personal communication). Near the city of Plovdiv, about 2,100 ha of arable lands has been polluted by heavy trace elements through dust spreading from a non-ferrous trace element-producing smelter. Food or feed production on these soils is not recommendable. The first experiments have been successfully conducted with some aromatic and medicinal plant species, such as peppermint (*Mentha piperita* L.) and lavender (*Lavandula angustifolia* Mill.; Zheljazkov and Nielsen 1996a, b). The oil as a final economic product was not contaminated by heavy trace elements. Finally, Zheljazkov et al. (1999) found that peppermint and cornmint plants removed moderate amounts of heavy trace elements from the soil by the harvested biomass, thus in long-term perspective, the cultivation of these crops would contribute to the soil remediation.

Yankov et al. (2000) and Yankov and Tahsin (2001) studied growth, development, yield, and quality of cotton plants, grown on polluted soil from the same region. They found that although cotton accumulated significant amounts of trace elements in the harvested biomass, the processing of fiber with boiling water reduced the contents of Cu, Zn, Cd, and Pb to levels found for the plants grown in non-contaminated soil, and they concluded that cotton is suitable for growing on trace element polluted soils in this region. These studies as well as other reports (Grant and Bailey 1997; Griga et al. 2003) showed that crops for fiber or oil production could be used for profitable crop production accompanied by phytoextraction of trace element from polluted soils.

3.5 Chemically assisted trace element phytoextraction

Chemically assisted phytoextraction is based on the use of non-accumulator plants (f.i. maize or *Brassica*) with trace element accumulation levels far below than those of hyperaccumulators, but with high biomass potential. In general, this approach is aimed to overcome the main limitations of natural phytoextraction of a very limited number of suitable hyperaccumulators for some important trace element pollutants such as Pb (Huang et al. 1997; Lasat 2000), several radionuclides (Salt et al. 1998) as well as their low biomass.

In general, only a part of total trace element content is phytoavailable, mainly the one that is present as free ions, soluble forms, and absorbed to inorganic constituents at ion exchange sites. Some trace elements such as Zn and Cd occur in exchangeable forms, while others as Pb are less bioavailable and are mainly being precipitated (Puschenreiter et al. 2001). In any case, to achieve the requested trace element uptake value, the concentrations of soluble trace elements in soil must be enhanced. It has been identified that it is possible by rhizosphere manipulation based on the application of chemical agents. For this purpose, chelators like ethylene-diamine tetraacetic acid (EDTA), hydroxyethyl-ethylenediamine triacetic acid, ethylene-bis (oxyethylenetrinitrilo) tetraacetic acid, diethylene-triamine pentaacetic acid, EDDS, and soil acidification using organic acids have been investigated at laboratory scale.

Restrictions apply, however, to both the use of complexing agents and artificial soil acidification. It was found that EDTA and EDTA–trace element complexes are toxic for some plants and that high dose of EDTA inhibited the development of arbuscular mycorrhiza (Dirilgen 1998; Crčman et al. 2001; Geebelen 2002). Furthermore, EDTA is poorly photo-, chemo-, and biodegradable (Nörtemann 1999). In situ application of chelating agents can cause groundwater pollution by uncontrolled trace element dissolution and leaching. Some evidence supporting this apprehension has been found (Crčman et al. 2001; Sun et al. 2001), thus mass balances to confirm that trace elements are not leached to groundwater have been recommended (Schwitzguébel et al. 2002). Wenzel et al. (2003) used outdoor pot and lysimeter experiments to provide information that supported the presumed risk of EDTA application. They confirmed that EDTA considerably increased trace element liability in soil, but also observed enormously increased trace element concentrations in the leachates collected below the root zone. Furthermore, they found that the enhanced trace element liabilities and leachate concentrations persisted for more than 1 year after harvest. Although the problems linked to the EDTA application might be overcome by using other chelating agents, such as nitrilotriacetate (NTA) or citric acid, we will not consider this possibility due to the still existing inbalance between mobilized trace elements and the amount taken up by plants.

It was shown that slight acidification of the soil can be induced by applying elemental sulfur or physiologically acid fertilizers, such as NH_4SO_4 (Kayser et al. 2000; Puschenreiter et al. 2001). Chaney et al. (1999) noted that there might be some negative effects associated with soil acidification. Kayser et al. (2000) found that the application of elemental sulfur on carbonate rich soils is a useful approach creating minimum risk as it is gradually oxidized by sulfur-oxidizing bacteria. Furthermore, we might suppose that sulfur application could improve trace element phytoextraction, such as that of Cd, in two ways: (1) by enhanced Cd solubility in the soil, followed by higher plant Cd uptake; and (2) by improved plant S status, allowing an adequate plant defence response to enhanced Cd loading as well as preventing S deficiency onset. Within the PHYTAC project, several metal-mobilizing fertilizers (ammonium- and potassium-sulfate, ammonium-nitrate) and other amendments (NTA, elemental sulfur, EDTA) were assessed at the Swiss Rafz site for the tobacco and sunflower traits. For the sunflower traits, an enhanced cumulative metal uptake (Cd, Zn, Pb) and yield was found for the ammonium-sulfate fertilization, whereas the ammonium-nitrate fertilization was for some tobacco mutants (NBZn7-51F1, NFCu7-19F1) the more efficient treatment. The direct field-based comparison of three concentrations (one, two, and three times, as normal load) of ammonium-nitrate, ammonium-sulfate (amendments followed the standard procedure of the Landwirtschaftliche Beratungszentrale (2004), 1 AN (ammonium nitrate) = 170 kg ha^{-1}; 1 AS (ammonium sulfate)=170 kg ha^{-1}; 2 AN; 2 AS; 1.5 AN; 1.5 AS were the respective multiples of the basis fertilization), and 2, 10, and 25 mM of citric acid including a single concentration of NTA (10 mM) showed for the tobacco mutants NFCu7-19F1 and NBZn7-51F1 a more efficient Cd, Zn, and Pb extraction for the ammonium-nitrate fertilization compared to citric acid and NTA, in the case of NFCu7-19F1 (Herzig et al. 2005). These results show that commercial fertilizers can be used for the slight and well-controlled metal mobilization of metals for stimulating the efficiency of metal extraction by plants in field experiments. In contrary, metal-mobilizing amendments/chelators such as NTA and EDTA are not allowed for field applications in Switzerland and many EC countries.

From a field study, McGrath et al. (2006) reported that none of the metal-mobilizing chemicals used were able to enhance Cd and Zn hyperaccumulation by *T. caerulescens*. In fact, EDTA actually decreased the concentrations of Cd and Zn in plants. On the other hand, the application of EDTA to soil did increase the accumulation of both Cu and Al in *T. caerulescens*. Neugschwandtner et al. (2008) concluded that the low phytoremediation efficiency in the field and the mobilization of high amounts of Pb and Cd down the soil profile may make the use of EDTA and *Zea mays* not suitable for the remediation of severely trace element-contaminated soils in a reasonable time frame and may result in substantial groundwater pollution under used crop management.

3.6 Trace element phytoextraction: lessons from the field and research needs

The success of phytoextraction depends on several factors including the extent of soils contamination, metal availability to the roots, and the ability of the plant to intercept, absorb, and accumulate trace elements in shoots (Ernst 2000). The two main bottlenecks are trace element availability in the soil and trace element uptake by the plants. Ultimately, the potential for phytoextraction depends on the interaction between soil, trace elements, and plants. The complexity of this interaction that is taking place under site-specific conditions requires that phytoextraction of trace elements must be approached as a multidisciplinary research effort (Lasat 2002).

3.6.1 Phytoextraction of the total metal content contamination

The information presented so far has led to the conclusion that there is remediation potential, but it is clear that using non-hyperaccumulators even in the most optimistic scenario at least 60 to 70 years is needed to reduce the total Cd content from 5 to 2 mg kg^{-1}. This shows that economical valorization of harvested plant material is a must. Therefore, there still is a high need for improvement of the extraction efficiency. Salt et al. (1998) suggested two different strategies: (1) in a short-time perspective, improvement could be achieved by optimization of agronomic practices; and (2) in a long-term view, by the use of genetically modified organisms (GMOs). Non-GM fast-track breeding (such as mutagenesis or in vitro breeding) might be a promising alternative to genetic transformation for the improvement of the metal extraction characteristics of high yielding crops (Herzig et al. 1997; Guadagnini 2000; Nehnevajova et al. 2007; Schröder et al. 2008).

In the case of natural phytoextraction, the lack of any protocol with respect to cultivation, pest management, and harvesting practices limits more successful implementation, so it has to be developed. On the other hand, screening for suitable ecotypes among known hyperaccumulators as well as a search for new ones should continue. Great differences have been observed among the diverse genotypes of *T. caerulescens* in Zn and Cd tolerance and Cd uptake (Li et al. 1996; Lombi et al. 2000). Barcelo et al. (2001) have stressed the need of hyperaccumulators that not only exhibit extraordinary levels of trace element accumulation in their harvestable biomass, but also to develop better survival strategies at different climatic conditions. For example, *T. caerulescens* is not the best candidate in Mediterranean area because of its sensitivity to heat and drought. Chaney et al. (1995) proposed the development of breeding programs for improved cultivars of hyperaccumulators. A partial success from breeding activities has been reported by Brewer et al. (1999) who generated somatic hybrids between *T. caerulescens* and *Brassica napus* and recovered high biomass hybrids with superior Zn tolerance.

The chemically assisted phytoextraction, if applicable (high environmental risks), needs strong technological optimization. It seems that by appropriate mineral nutrition it could be possible to significantly increase trace element removal. Huang et al. (1997) achieved a twofold increase in Pb removal by goldenrod plants just by foliar phosphorus application. Other options include screening programs for genotypes with high trace element accumulation potential together with better resistance abilities to excess trace elements. Greater than tenfold difference in shoot Pb concentration among 50 species/cultivars screened has been observed (Huang et al. 1997). Significant differences in shoot Cd accumulation among maize genotypes have also been reported (Hinesly et al. 1978). Well-expressed cultivar-dependent Cd accumulation and resistance has been shown in barley, but it was concluded that Cd phytoextraction capacity of this crop was not sufficient for practical implementation (Vassilev et al. 2004b). Since in many cases trace element absorption in roots is limited by low solubility in soil solution, it is necessary that the efforts for selection of appropriate rhizosphere manipulation be continued. There is a need to find cheaper, environmentally benign chemical compounds with chelating properties (Lasat 2000) as well as to better understand the role of rhizospheric bacteria in trace element solubility, plant uptake, and tolerance (Shilev et al. 2001; van der Lelie et al. 1999).

Another possibility that should be considered is the use of plant growth-promoting bacteria that stimulate root formation by plants and also produce siderophores (Lebeau et al. 2008; Weyens et al. 2009b). These siderophores can interact with trace elements, in certain cases reducing their toxicity and increasing their bioavailability and uptake by plants. Endophytic bacteria can be engineered for increased trace element sequestration (Lodewyckx et al. 2001, 2002). The (combined) activities of these new bacterial strains will be used to enhance trace element uptake and translocation by the host plants. These bacterial siderophores can be considered as natural chelators and the bacterial production of which is in tight equilibrium with plant activity, thus improving trace element uptake and translocation as part of the phytoextraction process. Zimmer et al. (2009) obtained results that bacterial support of root growth-promoting ectomycorrhizal fungi may be a promising approach to improve remediation of metal-contaminated soils by using willows.

Of course, there are also needs to optimize technology elements like plant density per area, number, and alternations of appropriate successive crops, time of harvest as well as pest control, irrigation, etc.

GMOs for improved phytoextraction GMOs are expected to greatly contribute to trace element phytoextraction, but in several parts of Europe and in the USA there is still reluctance to accept their introduction (Dunwell 1999; Clemens et al. 2002; Kärenlampi et al. 2000). The most important achievement in that approach is a transgenic yellow poplar (*Liriodendron tulipifera*) expressing bacterial mercuric reductase gene and able to release elemental mercury ten times more than untransformed controls (Rugh et al. 1998). The research efforts are mainly aimed to increase metallothionein or phytochelatin concentrations in plant cells with the hope to improve resistance as well as trace element accumulation and translocation pattern in high biomass-producing species. There are several promising examples of successfully transformed plants exhibiting better phytoextraction capacity tested at a laboratory scale. For example, the expression of mammalian MTs in transformed tobacco plants resulted in improved Cd resistance (Pan et al. 1994). Transgenic *Brassica juncea* plants overexpressing bacterial glutathione synthetase gene were found to have both higher Cd uptake and enhanced Cd tolerance (Zhu et al. 1999). Arisi et al. (2000) reported that poplars overexpressing bacterial (γ-glutamylcysteine synthetase showed better Cd accumulation, but not improved Cd tolerance. However, the use of GMOs for phytoextraction still remains an open question as its answer strongly depends on public perception. More detailed information about the achievements in GMOs in view of trace element phytoextraction is provided by Krämer and Chardonnens (2001) and Mejare and Bülow (2001).

Non-GM fast-track breeding of plants With respect to legal and societal restrictions in Europe, conventional, non-GM fast-track breeding (such as mutagenesis or in vitro breeding) could be a promising alternative to genetic transformation for the improvement of the metal extraction characteristics of high yielding crops (Herzig et al. 1997; Guadagnini 2000; Nehnevajova et al. 2007; Schröder et al. 2008). Mutation and in vitro techniques (somaclonal variation—spontaneous mutations) had often been used to improve yield, oil quality, disease, drought, salt, and pest resistance in crops, or to increase the attractiveness of flowers and ornamental plants. In some economically important crops (e.g., barley, durum, wheat, and cotton), mutant varieties nowadays occupy the majority of cultivated areas in many countries (Maluszynski et al. 1995). In the last 40 years, mutagenesis has also played an important role in improving agronomic characteristics of *Helianthus annuus* L., one of the most important oilseed crops in the world. Osorio et al. (1995) have reported an increased variability in fatty acid composition of sunflower mutants, obtained from the seeds mutagenized with ethylmethanesulfonate (EMS). New sunflower mutants with an enhanced biomass production and oil content were obtained by Chandrappa (1982) after mutagenesis with EMS or DES. Kübler (1984) has obtained sunflower mutants of M_2 and M_3 generations with high linoleic acid content for diet food and mutants with high oleic acid content for special purposes like frying oils after EMS mutagenesis.

Chemical mutagenesis (EMS) was used to improve metal uptake and yield of sunflowers (Herzig et al. 2005; Nehnevajova et al. 2007, 2009). In the second mutant generation, M_2 sunflowers showed improved yield (six to nine times), metal accumulation (two to three times), and metal extraction efficiency (Cd 7.5 times, Zn 9.2 times, Pb 8.2 times) on a very dry and sewage sludge contaminated site Rafz (CH). Whereas the best sunflower mutant yielded 26 t of dry matter ha^{-1}, the control sunflower inbred lines produced only 3 t in the same experimental condition. EMS mutagenesis obviously led to enhanced biomass production with partially improved characteristics for metal uptake and drought resistance in sunflowers. Meanwhile, improved and genetically stable sunflower mutant lines exist up to the M_6 generation (Nehnevajova et al. 2009). Due to the fact that the initial screening of the mutagenized M_1 sunflower mutants at the extraordinary dry Rafz site (500–600 mm rainfall per year, 50% of normal) was performed in summer 2003, with the absolute drought anomaly of the last century without any drop of rain during the main vegetation period for 10 weeks, the existing sunflower genotypes also integrate a very good drought tolerance that can be further improved by specific selection. Whereas other commercial crops (corn, wheat, sunflower) dried up under this extreme drought, the sunflower mutants and tobacco in vitro selection resists and grew well. High yielding oilseed crops, such as sunflower (*H. annuus*) and tobacco (*Nicotiana tabacum*) are promising for a combined use in metal phytoremediation of degraded and contaminated soils and efficient biogas/oil and compost production (added value) and to ameliorate poor soil quality. Tobacco was selected for fast-track breeding due to its outstanding performances in drought resistance, in yield, metal tolerance and Cd uptake characteristics on contaminated soil and the fact that promising tobacco traits can easily be in vitro bred and micropropagated that strongly fastens the development and field assessment in a restricted period of time (Herzig et al. 1997, 2005; Guadagnini 2000). According to smoke prevention campaigns of the WHO and the EC, tobacco is nowadays an expiring cash crop, and therefore, new applications are highly welcome for farmers.

Within the frame of the PHYTAC project, the tobacco in vitro clones were simultaneously assessed in a comparative field experiment at Lommel (B) on an acid (pH_{KCL} 5–5.5), sandy soil with enhanced Cd and Zn contamination from a Zn smelter, and at Rafz (CH), on a sandy loam (haplic luvisol), (pH_{KCL} 6–6.8), contaminated with industrial sludge: Zn, Cd, Cr, and Pb. At the Rafz site, also the sunflower mutant screening of M_1–M_4 generation was done. Whereas the best results for in vitro bred tobaccos showed on the slightly alkaline Swiss soil of Rafz an improved metal extraction of a factor of 1.3–3.2 for Cd, Zn, and Pb, the most promising results were found on the slightly acid, sandy soil of Lommel in Belgium, with an enhanced Cd contamination. At the Lommel site, the relative gain in metal extraction of the most promising tobacco clone NBCu10-8F1 and an optimized ammonium-nitrate fertilization was found of a factor 12.4 for Cd, 14.6 for Zn, 13.7 for Cu, and 13.5 for Pb, compared to the non-modified mother-clone BaG. These results were used for the calculation of the “optimized cleaning up time” and showed a strong reduction of the expected cleaning up time for the total Cd topsoil contamination to reasonable 29 years, compared to 58 year until more than a century with commercial crops.

3.6.2 Phytoextraction of the bioavailable metal soil contamination

Whereas theoretical extrapolations of freeland data from phytoextraction experiments show some difficulties for a fast decontamination of the “total metal” concentrations in soil, especially for Zn and Pb, very promising prognosis of a short decontamination time of a few years only can be made for the fast and economically feasible phytoextraction of the “bioavailable metal” contamination in soil (Herzig et al. 2005, 2007, 2008) that is accessible for plants (plant-available). Moreover, the bioavailable metal contamination in soil is rightly regarded as the main risk of a possible contamination of both the food chain and the groundwater (Karlaganis 2001).

Since phytoextraction should preferably not exceed 10 years to become economically feasible, the group of Herzig and co-workers concentrated their follow-up field experiments with then non-GM mutants of tobacco and sunflower on the fast reduction of the soluble metal concentrations that are available to plants and thereby reduce the risk of groundwater contamination. The experimental plot is located on a zinc-contaminated site in the eastern part of Switzerland. The pseudo-total soil concentrations (2 M HNO_3, according Swiss Law) varied from (milligram per kilogram) 500 to 55,000 Zn, 0.25 to 15.4 Cd, 32 to 189 Cr, 28 to 48 Ni, 28 to 115 Cu, and 30 to 1920 Pb, whereas the soluble Zn concentrations (0.1 M $NaNO_3$) varied from 0.2 to 35 mg kg^{-1} and the pH_{KCL} value from 6.0 to 7.1. Our first year freeland results of 2006 on the phytoextraction of soluble zinc by a selected tobacco clone were nicely confirming these theoretical prognoses and revealed a reduction of the soluble zinc concentration of 40–60% with one tobacco cultivation only. Moreover, the phytoextraction with tobacco can lead to a highly welcomed enhanced pH and immobility of metals in soil (Herzig et al. 2007, 2008). Our second year freeland results are nicely confirming the theoretical prognosis, and the most promising traits of sunflower and tobacco mutants show a fasten decontamination time of 1–6 years (based on linear decay) for the phytoextraction of soluble 6–16 mg kg^{-1} Zinc from topsoil (Table 4). Because of these promising results, these field assessments at the Bettwiesen site (CH) are continued and enlarged on other metals, and on other promising mutants and cultivars for phytoremediation, such as tobacco (Herzig et al. 2003), energy maize, and sunflower (Nehnevajova et al. 2007) to assess the decontamination efficiency for soluble Zn, Cd, Ni, Cr, Cu, and Pb (Herzig et al. 2009).

4 Rhizo- and phytodegradation

4.1 Introduction

Phytoremediation of soils and groundwater contaminated with organic xenobiotics is becoming increasingly popular as a cost-effective remediation strategy (Glass 1999). Systems established seem to meet the expectations and removal of pollutants from various matrices with sufficient efficiency and at a comparatively low cost could be realized. However, a more profound knowledge of the fundamental mechanisms involved and a more systematic approach for selection of plants and optimization of the remediation process are needed.

Quite a lot of basic research aiming to unravel the metabolism of xenobiotics by plants and their associated

Table 4 Expected cleaning up time for the bioavailable zinc contamination (moderate, high, very high level) of topsoil by improved phytoextraction with efficient mutants of sunflower and tobacco and optimized fertilization treatment, based on field data of 2005 of the Bettwiesen site in Switzerland

Bioavailable zinc optimised phytoextraction Szenario Bettwiesen (CH)	Tobacco	Tobacco	Tobacco	Sunflower	Sunflower	Sunflower
Clone/mutant	NBZn7-51F1	NBCu10-8F1	BaG	8-185-04	41-190-04	57-19-S
Origin of clone	In vitro	In vitro	In vitro	Mutagenesis	Mutagenesis	Mutagenesis
Zn uptake (mg kg^{-1})	487	293	617	292	346	219
Fertilization	1.5 AS/AN	1 AS/AN	1.5 AS/AN	1.5 AS/AN	2 AS	2 AS
Biomass (t ha^{-1} DW)	24.7	37.5	32.5	24.4	26.8	19.7
Plant density (p ha^{-1})	40,000	40,000	40,000	70,000	70,000	70,000
Zn removal (kg ha^{-1} year^{-1})	12	11	20.1	7.1	9.3	4.3
Cleanup time 1 Bettwiesen 2005	Years	Years	Years	Years	Years	Years
6≥0.5 mg kg^{-1}						
Trigger value OIS CH						
0.5 mg kg^{-1}						
Linear decay[a]	2	2	1	3	2	5
First order decay[a]	4	5	3	8	6	12
Cleanup time 2 Bettwiesen 2005	Year	Year	Year	Year	Year	Year
10≥0.5 mg kg^{-1}						
Linear decay[a]	3	3	2	5	4	8
First order dacay[a]	9	10	5	15	12	25
Cleanup time 3 Bettwiesen 2005	Year	Year	Year	Year	Year	Year
16≥0.5 mg kg^{-1}						
Linear decay[a]	5	5	3	8	6	13
First order decay[a]	17	18	10	28	22	46

Cleaning up threshold for bioavailable zinc is the Swiss trigger value of 0.5 mg kg^{-1} (0.1 M $NaNO_3$ extraction, OIS-CH)

[a] Calculation based on 25 cm soil depth for selected crops, and linear and first order extrapolation of cleaning up time (decay time)

microorganisms is being performed. To protect themselves from phytotoxic effects, plants are equipped with a complex and versatile array of enzymes to combat natural products and man-made chemicals. The structure and function of many detoxifying enzymes have been revealed (Newman et al. 1998; Sandermann 1992; Schroeder et al. 2001). During the last decades, many valuable results have been reported, but most still seem far away from the practice of remediation of contaminated soils and waters. The big challenge is to translate and use this basic scientific knowledge to solve real world pollution problems.

Most of the plants used in the phytoremediation of xenobiotics are crops or weeds selected by agronomical practices. However, exploring and exploiting the natural biodiversity are important issues in the choice of appropriate species among agricultural plants (cultivation well known), hybrid poplars or willows (high water use), or wild plants growing in contaminated areas (Olson et al. 2001). Plant taxonomy and phytochemistry should be the first steps in the adequate use of the huge biochemical potential of plant species, with very specific metabolism: plants often produce natural chemicals whose structure is close to xenobiotic compounds (Siciliano and Germida 1998). Whereas natural biodiversity is not yet fully exploited, the use of transgenic plants is therefore not the only solution to improve the efficiency of phytoremediation.

Large root absorption area, big root tip mass, high enzyme activity, and increase of bioavailability using exudates are critical factors to the successful implementation of phytoremediation of soil-based organic pollutants (Schwitzguébel et al. 2002). Important tools to improve the removal of these pollutants could also be root biotechnology (using rhizogenic *Agrobacterium* to induce root proliferation), plant hairy root technology, and rhizosphere biotechnology (Pletsch et al. 1999).

A crucial limiting factor with organic pollutants in soils and groundwater often is neither the intensity of metabolism nor degradation capacity, but the transport of pollutants into the plant, which depends on the properties and bioavailability of the xenobiotic, as well as on the size and shape of the root system. Organic pollutants are often hydrophobic and bound to soil components, causing a severe problem to the uptake of the compound by plant roots. Once in the rhizosphere, microorganisms can perform part of the degradation; the remaining fraction of the pollutants migrate into the root, then become translocated into other tissues and organs of the plant, where detoxification and metabolism eventually take place. These uptake and translocation processes that involve plants as well as their associated bacteria and mycorrhiza are not yet well known and should be more carefully investigated (Schwitzguébel 2001; Siciliano and Germida 1998; Mehmannavaz et al. 2002).

One of the most striking features of many plants used in phytoremediation is the extensive evapotranspiration of water through the stomata. This high water consumption that may almost equal the amount of water added to an area via precipitation prevents or strongly reduces leaching of pollutants and retards the possible migration in the soil and into the groundwater. Furthermore, the evaporation stream will also transport soluble organic pollutants into the plants (Cunningham et al. 1996). The fate of organic contaminants in the rhizosphere–root system largely depends on their physical–chemical properties. Plants readily take up organics with a $\log K_{ow}$ between 0.5 and 3.5. These compounds seem to enter the xylem faster than the soil and rhizosphere microflora can degrade them, even if the latter is enriched with degradative bacteria (Trapp et al. 2000). Once these contaminants are taken up, plants may metabolize them, although some of them or their metabolites can be toxic (Doucette et al. 1998). For example, trichloroethylene (TCE) can be transformed into trichloroethane. Alternatively, some plants preferentially release volatile pollutants (such as TCE and ethyl-benzene and xylene (BTEX)) and/or their metabolites into the environment by evapotranspiration via the leaves. This raises questions about the merits of phytoremediation (Burken and Schnoor 1999; van der Lelie et al. 2001; Schwitzguébel et al. 2002; Ma and Burken 2003). The use of engineered endophytic bacteria, which complement the metabolic properties of their host, has the potential to overcome these limitations: while contaminants move through the plant's vascular system, endophytic bacteria, colonizing the xylem (Germaine et al. 2004; Weyens et al. 2009a, b), can promote their degradation. This may result in both decreased phytotoxicity and evapotranspiration, provided the bacteria possess the genetic information required for efficient degradation of the contaminants. These bacteria can be isolated, subsequently equipped with desirable characteristics and re-inoculated in the host plant to enhance their beneficial effects. Proof of concept was provided by inoculating yellow lupine plants (Barac et al. 2004) and hybrid poplar (Taghavi et al. 2005) with endophytic bacteria able to degrade toluene, which resulted in decreased toluene phytotoxicity and a significant decrease in toluene evapotranspiration.

In case of pollution with hydrophobic xenobiotics (i.e., those defined as having a $\log K_{ow}>4$), the associated microflora plays an important, if not the decisive role in the remediation. Hence, stimulation of microorganisms by plant exudates and leachates and by fluctuating oxygen regimes has also to be considered (Siciliano and Germida 1998; Mehmannavaz et al. 2002). Plant roots may excrete not only enzymes like peroxidases (favoring the formation of residues bound to the humic part of the soil), but also small soluble organic molecules, acting as biosurfactants, thus able to increase bioavailability and uptake of pollutants (Schwitzguébel et al. 2002).

4.2 Field experiments

Most examples on the successful application of phytoremediation of groundwater-based xenobiotics are found in the USA. Although many organic pollutants are metabolized in plants, xenobiotics—or their metabolites—can be toxic to plants, and this could limit the applicability of phytoremediation. Alternatively, in the case of volatile pollutants, plants can release the compounds, or their metabolites, through the stomata, which could question the merits of phytoremediation (Weyens et al. 2009a, b). This seems to be the case for the removal of benzene, toluene, BTEX, using hybrid poplar trees. A successful field experiment was performed remediating a BTEX-contaminated ground water plume on the site of a car-producing factory in Belgium (Barac et al. 2009). Hybrid poplar trees were planted on a field site near a car factory in order to install a bioscreen aimed to combine the biodegradation activities of poplar and its associated rhizosphere and endophytic microorganisms for containing a BTEX-contaminated groundwater plume. This BTEX plume occurred as the result of leaking solvent and fuel storage tanks. Monitoring, conducted over a 6-year period after the planting of the trees suggested that the poplar trees and their associated microorganisms had, once the plant roots reached the contaminated groundwater zone, an active role in the remediation of the BTEX plume, resulting in full containment of the contamination. Analysis of the microbial communities associated with poplar demonstrated that once the poplar roots got in contact with the BTEX contaminated groundwater an enrichment occurred of both rhizosphere and endophytic bacteria that were able to degrade toluene. Interestingly, once the BTEX plume was remediated, the numbers of toluene degrading rhizosphere and endophytic bacteria decreased below detection limits, indicating that their population resulted from selective enrichment by the presence of the contaminant.

In the case of solvents such as TCE, although preliminary work suggested phytovolatilization was the primary way plants deal with the compound, field studies showed that the majority of TCE is metabolized within the rhizosphere and/or in the plant (Shang et al. 2001; Collins et al. 2002; Weyens et al. 2009b). Weyens et al. (2009b) reported that along transects under a mixed woodland of English oak (*Quercus robur*) and common ash (*Fraxinus excelsior*) growing on a TCE-contaminated ground water plume, sharp decreases in TCE concentrations were observed, while transects outside the planted area did not show this remarkable decrease. This suggested a possible active role of the trees and their associated bacteria in the remediation process. The characterization of the isolates obtained in this study shows that the bacterial community associated with oak and ash on a TCE-contaminated site was strongly enriched with TCE-tolerant strains, but that this was not sufficient to degrade all TCE before it reaches the leaves. Significant TCE evapotranspiration was found. A possible strategy to overcome this evapotranspiration to the atmosphere is to enrich the plant-associated TCE degrading bacteria by in situ inoculation with endophytic strains capable of degrading TCE. This was successfully tested in the field (Weyens et al. 2009a).

A phytoremediation study on hydrocarbon-polluted agricultural soils was conducted successfully in Trecate in northern Italy (Schwitzguébel et al 2002). The soil, contaminated following the blow out of a land-based oil well, underwent on-site treatment in a biopile prior to being replaced in its original location. For a couple of years, the study compared the ability of 11 agricultural plants to facilitate hydrocarbon removal (via microbial degradation and/or plant uptake) with that of land farming and natural attenuation. Soil polyaromatic hydrocarbon and total petroleum hydrocarbon concentrations decreased in land-farmed parcels and weedy areas, but much less than in planted parcels, most notably in those planted with corn and sorghum.

Other field projects on hydrocarbons were performed in Europe, particularly in Denmark (sites in Axelved and Holte; Trapp and Karlson 2001; Trapp et al. 2006) and Sweden (site in Husarviken and Bohus; Schwitzguébel et al. 2002).

Leigh et al. (2006) investigated the occurrence of culturable indigenous polychlorinated biphenyl (PCB)-degrading bacteria associated with five species of mature trees growing naturally on a contaminated site in Stare Mesto (Czech Republic). Isolates with broad congener specificity were found widespread at the site. The authors concluded that the apparent association of certain plant species with increased abundance of indigenous PCB degraders, including strains with outstanding degradation capacities, throughout the root zone supports the notion that rhizoremediation is a promising strategy for enhancing in situ PCB degradation.

Parts of an area of the derelict World War II ordinance plant Werk Tanne (Clausthal-Zellerfeld, Harz, Germany) are heavily contaminated by chemicals resulting from 2,4,6-trinitrotoluene (TNT) production and particularly by TNT itself. On this site, the TNT-degrading potential of the rhizosphere of the planted trees and shrubs was augmented by highly active mycorrhiza and white-rot fungi (Koehler et al. 2002). The effects of site preparation, mycorrhized rhizosphere, and white-rot fungi on the degradation of TNT were assessed over 1 year using a complex monitoring scheme, including a battery of five biotests and field investigations of selected indicators (soil mesofauna, decomposition). The results of the monitoring showed the great influence of the grading procedure for site preparation, a diversified sensitivity of the biotest battery and complex reactions of the field indicators. The grading procedure effectively reduced the contamination (almost 90% within the first 6 months regardless of the initial levels). The phytoremediation measure as a whole reduced hazards of transport of nitro-aromatics by dust or leachate, initiated a secondary succession of the soil ecosystem that could transform the remaining TNT and metabolites over a longer period of time, and thus proved to be an effective decontamination measure applicable in large-scale technology. Van Dillewijn et al. (2007) reported that in field experiments neither natural attenuation nor bioaugmentation with *Pseudomonas putida* JLR11 affected TNT levels to a significant degree. However, the extractable TNT content in rhizosphere soil associated to maize roots decreased by more than 96% in 60 days regardless of inoculation. They concluded that under these field conditions, the effect of phytoremediation by maize overshadowed any effect of rhizoremediation by *P. putida* JLR11.

A special phytoremediation concept is the use of constructed wetlands for cleanup of effluents and drainage waters. For example, a constructed wetland has been successfully operated and monitored in Portugal for the last 7 years to treat industrial effluents containing nitrogenous aromatic compounds from an aniline and nitrobenzene production plant. Using reed beds on a total planted area of 10,000 m^2, reductions in aromatic compounds up to 100% were obtained, depending on the acclimatization period for inlet effluent composition of 10–300 mg/L aniline, 10–100 mg/L nitrobenzene, and 10–30 mg/L nitrophenols (Martins Dias 2000).

4.3 Rhizo- and phytodegradation: lessons from the field and research needs

An important research question is to better understand and control the uptake and translocation of organic pollutants in green plants. Numerous pollutants are very hydrophobic, showing $\log K_{ow}$ values above 4. This characteristic and high chemical stability explains why such pollutants are persistent in the environment. A major limiting factor for phytoremediation of recalcitrant organic pollutants is often their low bioavailability. Therefore, there is an urgent need for research aiming at a better understanding of the subtle and complex interactions between pollutants, soil material, plant roots, and microorganisms in the rhizospheric zone. Of particular interest are the roles of root exudates, mycorrhizal fungi, and rhizospheric bacteria in the modification of the ability of plants to remove pollutants from contaminated soils. An extended knowledge of these mechanisms will contribute to optimize the phytoremediation process and make it more attractive in the future.

Inoculation of both rhizosphere and endophytic bacteria is a promising strategy for improving phytoremediation of organic contaminants. However, a number of obstacles should be overcome before engineered endophytes can be successfully applied in field scale phytoremediation projects (Newman and Reynolds 2005; van der Lelie et al. 2005) and the inoculated bacteria would have to compete against the endogenous microbial population, which is well adapted to the local conditions. Alternatively, horizontal gene transfer can play an important role in enhancing the metabolic capabilities of endogenous endophytes (Taghavi et al. 2005) rather than integrating a new bacterium in a stable community, and the degradation pathway is transferred among members of the community.

5 Economical aspects

Information on cost and performance of soil and groundwater reclamation is often available for treatment technologies that are considered “established” such as excavation with on- and off-site incineration, stabilization, soil vapor extraction, thermal desorption for source control, and pump and treat technologies for groundwater. Innovative technologies like phytoremediation are alternative treatment technologies with a limited number of applications and limited data on cost and performance. Additionally, phytoremediation costs are very dependent on the local situation w.r.t. the kind and depth of contamination, soil conditions, disposal of the biomass, etc. In 2001, the US Environmental Protection Agency (EPA) published an overview of completed soil remediation projects, among which a small number used phytoremediation. Table 5 gives an exemplary overview (median values over only a few cases) of the large spread in cost data (US dollars) of phytoremediation, together with a comparison with bioremediation, soil washing, and excavation.

From comparison with excavation (row So 7b), we indeed see that—apart from rare extreme cases—phytoremediation is at least 50% less expensive. It is also cheaper than bioremediation. From an economic point of view, the purpose of phytoremediation of polluted land can be threefold: (1) risk containment (phytostabilization); (2) phytoextraction of metals with market value like nickel, thallium, and gold; and (3) durable land management where phytoextraction gradually improves soil quality so that eventually crops with higher market value again can be cultivated.

Table 5 Exemplary cost data for phytoremediation

	In situ soil remediationtechnology	Source	Stage	Contaminant	Metric	Cost	
						Single value or min	Max
Soil							
(So1)	Phytoremediation, large site (USA)	a	Complex	Metals	$\$/m^3$	147	483
(So2)	In situ bioremediation (USA)	b	Complex	VOCs, heavy metals	$\$/m^3$	432	
(So3)	In situ bioremediation (USA)	b	Complex	BTEX, PHC	$\$/m^3$	226	
(So4)	Phytoremediation	c	Estimate	Metals	$\$/m^3$	13	131
(So5)	Soil metal washing	c	Estimate	Metals	$\$/m^3$	39	392
(So6)	Phytostabilization (France)	d	Running	Arsenic	$\$/m^3$	54	
(So7a)	Excavation (USA)	a	Median		\$/t	270	460
(So7b)	Excavation (USA) (assuming soil density 1.2 t/m^3)	a	Median		$\$/m^3$	324	552
Groundwater							
(Gw1)	Phytoremediation, large site	a	Complex	Metals	$\$/m^2$	4.8	6.9
(Gw2)	In situ bioremediation	b	Complex	VOCs	$\$/m^3$	381	

a US FRTR (2002); b US EPA 2001; c Watanabe (1997); d Jacquemin (2006)

5.1 Phytostabilization

Phytostabilization primarily aims at preventing the health risks emanating from the pollution. The economic aspect should be measured in terms of cost effectiveness, i.e., targeting at the lowest cost per unit of risk reduction. If the latter would be expressed in monetary terms—an ambitious undertaking—we arrive at cost–benefit analysis. Because of significant uncertainties (a) about the relation between the size and kind of the contamination and the emanating risks and (b) about monetizing health deficiencies, in practice the lowest cost per treated volume is aimed at (see row So6 in Table 5).

5.2 Phytoextraction

Phytomining the metal The main variables that control the economic feasibility of phytoming metals are the metal price, the plant biomass, and the highest achievable metal content of the plant (Brooks et al. 1998). Coupled with energy generation from the harvested biomass, the indicative profitability for a Ni phytomine in Australia is predicted to be 11,500 AU\$/ha/harvest. For Au, a profit of 26,000 AU\$/ha/harvest is predicted (Harris et al. 2009). Chaney et al. (2007) conclude that for Ni-contaminated or mineralized soils, the crop value of nickel phytomining could amount to 16,000 US\$/ha/harvest.

Durable land management and future higher land revenues after reclamation In the case "Lommel" (see Section 3.4), an area in the north-eastern part of Belgium, a modal farmer (36 ha) is confronted with on average a Cd pollution of 4.3 mg Cd kg^{-1} DW. Actually, his land is used to cultivate fodder maize for dairy cattle rearing. A durable land management scenario involves that from the start fodder maize is reduced to 10 ha and gradually evolves to 0 ha. To feed his cattle, the farmer will buy fodder maize from external uncontaminated areas. Land occupation is switched to three crops: short rotation coppice (willow), rape, and energy maize. One of the conditions in searching for valorization opportunities was that agricultural labor income would not decrease (probability of 90%). Following energy conversion, routes then are considered: anaerobic digestion of energy maize (Thewys et al. 2009), pyrolysis of the willow coppice (Thewys and Kuppens 2008), and rapeseed oil extraction. The respective prices for the biomass as a feedstock which could be paid to the farmer, taking into account a minimal profitability of the conversion installations, are: €31/t for energy maize, €28/t for willow, and €200/t for rape. Based on the uptake performance of the crops and according to the necessary rotation scheme, 34 ha reaches the remediation target level of 0.5 mg Cd kg^{-1} DW after 40 years. The contaminated volume treated is 68,000 m^3. Already after 18 years, lesser contaminated hectares reach the clean status and offer a 50% higher agronomic income because of the possibility to cultivate higher value crops like, e.g., vegetables. Over the 40-year period, the net present value of the yearly net income of the farmer on the 36 ha amounts to €459,000. Because the future higher incomes after reclamation outweigh the income loss (i.e., compared to the income before the remediation) during the reclamation period, phytoextraction does not entail a cost, but a net benefit (Vassilev et al. 2004a). In this case, the net benefit is calculated at €6.7/m^3 of soil treated, or \$7.9/m^3 (at the average dollar—Euro exchange rate in the period 1999–2007).

6 General conclusions

Phytoremediation undoubtedly has a high potential to enhance the degradation and/or removal of organic contaminants from soils, sediments, and (undeep ground) waters. However, based on often unrealistic extrapolations of data obtained from pot and hydroponical experiments, too enthusiastic interpretations and promises have been made concerning the possibilities of metal phytoextraction. Therefore, there is a risk that this technology will be dismissed without proper evaluation. Risk-managed phytostabilization and monitored natural attenuation seems to be one of the more realistic scenarios for brownfields, urban and industrial areas contaminated with metals (Dickinson et al. 2009), and recalcitrant organics.

It is clear that, in spite of a growing public and commercial interest and the success of several pilot studies and field scale applications, more fundamental research is still needed to better exploit the metabolic diversity of the plants themselves, and also to better understand the complex interactions between contaminants, soil, plant roots, and microorganisms (bacteria and mycorrhiza) in the rhizosphere.

A large amount of knowledge is now available on the biochemical processes involved in the detoxification of pollutants inside plant cells. One of the most important challenges is to use this basic scientific information to improve the efficiency of phytoremediation in the field. There indeed exist many reports of unsuccessful and inconclusive field trials. In the field, there are stressors that affect phytoremediation that are not encountered at laboratory or greenhouse scale: variations in temperature, nutrients, precipitation and moisture, plant pathogens and herbivory, uneven distribution of contaminants, soil type, soil pH, soil structure, etc. Agronomic techniques, such as (roto)tilling and addition of lime, nutrients, organic matter or other soil amendments, are often employed prior to planting or sowing to facilitate plant growth. However, these treatments can cause changes in soil pH, oxygen content, and bioavailability of the contaminants and hence affect degradation of contaminants in a positive or negative way.

A “simple” limiting factor is that in some cases the contaminated soil is deeper than the rooting zone. In this respect, choice of plant species can be very important.

On laboratory and greenhouse scale, promising results have been obtained using genetically modified organisms. However, regulatory restrictions for in situ applications have prevented any substantial accumulation of field data. There indeed is a risk for inserted genetic material to be transferred to indigenous species and populations.

More demonstration projects are urgently required to provide recommendations and convince regulators, decision-makers, and the general public of the applicability of a green approach for the treatment of soils, brownfields, groundwater, and wastewater contaminated by toxic metals, organic pollutants, and radionuclides. Further, more data are still needed to quantify the underlying economics as a support for public acceptance and last but not least to convince policy makers and stakeholders.

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