

Agronomic Practices for Improving Gentle Remediation of Trace  
Element-Contaminated Soils

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### Agronomic practices for improving gentle remediation of trace element-contaminated soils

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REVIEW ARTICLE

## AGRONOMIC PRACTICES FOR IMPROVING GENTLE REMEDIATION OF TRACE ELEMENT-CONTAMINATED SOILS

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**KEY WORDS:** crop rotation, ecosystem services, fertilization, harvest management, phytomanagement, phytoremediation, soil contamination.

## ABSTRACT

The last few decades have seen the rise of Gentle soil Remediation Options (GRO), which notably include *in situ* contaminant stabilization (öinactivationö) and plant-based (generally termed öphytoremediationö) options. For trace element (TE)-contaminated sites, GRO aim to either decrease their labile pool and/or total content in the soil, thereby reducing related pollutant linkages. Much research has been dedicated to the screening and selection of TE-tolerant plant

species and genotypes for application in GRO. However, the number of field trials demonstrating successful GRO remains well below the number of studies carried out at a greenhouse level. The move from greenhouse to field conditions requires incorporating agronomical knowledge into the remediation process and the ecological restoration of ecosystem services. This review summarizes agronomic practices against their demonstrated or potential positive effect on GRO performance, including plant selection, soil management practices, crop rotation, short rotation coppice, intercropping/row cropping, planting methods and plant densities, harvest and fertilization management, pest and weed control and irrigation management. Potentially negative effects of GRO, e.g. the introduction of potentially invasive species, are also discussed. Lessons learnt from long-term European field case sites for aiding the choice of appropriate management practices and plant species.

## 1. INTRODUCTION

Worldwide environmental pollution by trace elements (TE), both metals and metalloids (with common concentrations of <100 mg/kg dry weight (DW) in living organisms), along with organic pollutants has led to severe and diffuse contamination of soils with significant environmental effects and health risks, e.g. soil erosion, loss of biodiversity, water pollution, food safety risks, etc. (Adriano 2001; Bhargava *et al.* 2012). Metal(loid)s are amongst the most frequently occurring soil contaminants at polluted sites across Europe (European Environment Agency (EEA), 2014), and their presence in elevated concentrations has been identified by the European Commission (EC) as one of the eight major threats to European soils (COM(2002) 179 final). The last few decades have seen the rise of *Gentle soil Remediation Options* (GRO), which notably include *in situ* contaminant stabilization (öinactivationö) and plant-based (öphytoremediationö) options. GRO are mainly based on the combined use of plants and their associated microorganisms, partly aided through the use of organic and inorganic amendments, and soil management practices which decrease the labile pool and/or total TE content. In addition to potentially restoring soil structure, functions and quality, and positively influencing other ecosystem services (e.g. increased biodiversity, improved surface and ground water quality, carbon (C)-storage, soil erosion, temperature regulation, etc.) these methods may also provide valuable sources of renewable biomass for the bio-based-economy (e.g. bioenergy, biocatalysis and platform molecules for green chemicals, and ecomaterials), thus contributing towards achieving European Union (EU) targets for renewable energy sources (EU directive



2009/28/EC) and avoiding the diversion of EU croplands to biofuel production and other non-food crops.

Several GRO have been developed for targeting distinct contaminant scenarios and post-treatment site uses (Bañuelos and Dhillon 2011; Friesl-Hanl *et al.* 2009; Mench *et al.* 2006; Singh *et al.* 2011; Vangronsveld *et al.* 1995a, 2009; Vangronsveld, Van Assche and Clijsters 1995b; Vangronsveld, Colpaert and Van Tichelen 1996). For TE-contaminated soils (TECS), the objective is to decrease the labile (bioavailable) pool and/or total contents of TE in the soil, or to reduce their entrance in excess into edible plant parts (thereby meeting with guideline values for contaminant levels in food or fodder crops) and any related pollutant linkages (e.g. leaching from the root zone, soil erosion and water runoff, etc.). Relevant GRO for TECS include phytoextraction, phytostabilization and *in situ* TE stabilization combined with phytoexclusion (using excluder phenotype crops).

Decades of research have been dedicated to the screening and selection of TE-tolerant plant species and genotypes. Within the field of phytoremediation, the number of studies carried out at a greenhouse level far outreaches the number of field evaluations (at the time of writing this review a search carried out in the ISI Web of Science generated 799 hits related to TE phytoremediation trials at a greenhouse or pot level and 319 hits at a field level). However, while plant selection is crucial for successful implementation of phytoremediation strategies, upscaling from greenhouse to field conditions also requires incorporating agronomical knowledge into the remediation process and the ecological restoration of ecosystem services. GROs are essentially based on soil and plant sciences, ecology, microbiology, ecotoxicology, and biogeochemistry, and their success will inevitably depend upon the careful implementation of effective agronomic

practices such as crop selection, crop rotations, intercropping, planting density, fertilization, irrigation schemes, bioaugmentation, weed, pest and herbivory management (McLaughlin, Parker and Clarke 1999; Claus *et al.* 2007; ITRC 2009; Vangronsveld *et al.* 2009; Vamerali *et al.* 2012). Conventional agricultural methods can be modified so as to suit both the characteristics of contaminated soils, and to meet the requirements of effective phytoremediating crops.

This review summarizes existing practical knowledge for enabling the move from greenhouse/pot experimentation to real-life *in situ* scenarios. Here we will focus on agronomical practices geared at increasing TE uptake and removal (phytoextraction) or reducing TE mobility and plant uptake (phytostabilization, *in situ* stabilization and phytoexclusion). Although the implementation of these GRO is known to be highly site-specific, the lessons learnt from long-term field applications will be useful when choosing appropriate management components and plant species. In addition to the available literature, recommendations and examples will be given from long-term European field case sites which are included in the EU FP7 GREENLAND project (No. 266124) and are described in Table 1.

## 2. PLANT SELECTION FOR IMPLEMENTING GRO

Phytotoxicity and other stress factors can severely limit the performance and establishment of the plant species used in the remediation process. The careful selection of plant species and optimization of growth are therefore key elements in successful phytoremediation of TE-contaminated soils under different pedo-climatic conditions. Plants must not only show tolerance to the contaminant(s) present but at the same time they may also require tolerance to numerous additional abiotic and biotic factors, such as water stress, soil acidity or salinity,

nutrient deficiency, frost, soil erosion or compaction, herbivory, pests, etc. (Petcu *et al.* 2001). An important source of TE-tolerant plant genotypes is the pioneer vegetation colonizing contaminated sites or present in surrounding areas. Within the same plant species various ecotypes, cultivars, varieties or clones can differ greatly in their response to the presence of contaminants (Vysloufilová, Tlusto– and Száková 2003; Marmioli *et al.* 2011; Ruttens *et al.* 2011). While tolerance to the contaminant in question will always be vital, at other times the selected plant will depend on the remediation option to be used e.g. TE-accumulating plants (phytoextraction) or TE-excluding plants or crop species (phytostabilization/*in situ* metal immobilization with phytoexclusion).

## 2.1 Phytoextraction and phytostabilization

For phytoextraction, the plants must be able to accumulate high concentrations of TEs in their harvested parts (e.g. shoots) and have a reasonably high biomass production. One relevant option is using TE-hyperaccumulators which are able to accumulate extreme concentrations of metal(loid)s (e.g. cadmium (Cd), nickel (Ni), zinc (Zn), selenium (Se), and arsenic (As)) in their above-ground biomass (often endemic to metal-enriched substrates, such as ultramafic or calamine soils) and at the same time possess some economic added value (renewable biomass for bio-economy and/or bio-ores (van der Ent *et al.* 2013a, b; Escande *et al.* 2014). Great variation in both biomass production and metal accumulation between ecotypes or populations of hyperaccumulating plant species, such as *Noccaea caerulescens*, *Alyssum murale*, *A. bertolonii* and *A. corsicum*, allows for the selection and breeding of improved phytoextractor plants (Chaney *et al.* 2007; Bhargava *et al.* 2012). An argument in favour of hyperaccumulators is the

possible recuperation of TE from TE-rich biomass, but as Ernst (2000) pointed out effective TE recycling from TE-loaded plants has not yet been proven, and without this the option of hyperaccumulators may be overestimated. Indeed, the price of Zn in the world market is currently too low to make Zn-recycling from TE-contaminated soil economically feasible (Vangronsveld *et al.*, 2009). However, Ni phytomining was proven to be economically feasible in the USA (Chaney *et al.* 2007). Also in Europe (Albania) successful field experiments using a Ni hyperaccumulator (*Alyssum murale*) were reported (Bani *et al.* 2007). Moreover, the REACH legislation and specific reactivity are driving force for using hyperaccumulator biomasses in biocatalysis (Escande *et al.* 2014). Nonetheless, the main bottleneck limiting the practical application of hyperaccumulators is the low biomass production of most of these species (except some of the Ni-hyperaccumulators) and the high number of cropping cycles required for clean-up (if the objective is to reduce total TE concentrations in soils). Additional limiting factors include the absence of commercially available seeds/seedlings, their sensibility to the presence of contaminants other than the hyperaccumulated TE, a lack of knowledge related to their cultivation, climate needs or competition with other TE-tolerant plants.

As a result, high-biomass crops (annuals or perennials) and woody plants are recognized as viable alternatives to hyperaccumulators for phytoextraction of TEs (particularly Cd, Se and Zn) if they also show relevant shoot TE removals (i.e. moderate-high bioconcentration factor (BCF) and high shoot yield). For example, the capacity of poplar and willow to colonize hostile environments such as mine wastes has long since been known (Klubek *et al.* 1992). Over the last two decades, both high yielding crop species, such as tobacco (*Nicotiana tabacum*) and sunflower (*Helianthus annuus*), and specific clones of several members of the *Salicaceae* family

have been assessed for their suitability within GRO. A large number of *Salix* and *Populus* clones have been screened, and show great variation in biomass production, TE tolerance and accumulation patterns in roots and leaves between clones (Landberg and Greger 1994; Pulford, Riddell-Black and Stewart 2002; Migeon *et al.* 2009; Gaudet *et al.* 2011; Ruttens *et al.* 2011; Van Slycken *et al.* 2013). Examples of woody crops which have been evaluated for their potential application in distinct GRO are given in Table 2. Some species or clones of willow (*Salix* sp.) have high BCFs for Cd (up to 27) and Zn (up to 3) (Dickinson and Pulford 2005; Wieshammer *et al.* 2007). Given the ample variation in metal accumulation it is possible to select the best-performing clones based on their TE tolerance, uptake efficiency (accumulating clones for phytoextraction vs. excluding clones for phytostabilization), TE translocation from roots to shoots, and biomass production (Pulford and Dickinson 2005; Unterbrunner *et al.* 2007; Wieshammer *et al.* 2007; Pourrut *et al.* 2011). Clones can also be selected for their ability to accumulate selected TEs (e.g. Cd and Zn) while at the same time immobilizing elements such as copper (Cu) or lead (Pb) (French, Dickinson and Putwain 2006). Several long-term field experiments show the successful and sustainable application of SRC within GRO (French, Dickinson and Putwain 2006; Hartley *et al.* 2011; Dimitriou *et al.* 2006, 2012). Additional factors influencing clone selection include their tolerance to abiotic and biotic factors other than soil contaminants, such as fungal and insect infection (e.g. leaf rust (*Melampsora* sp.) and lace bug (*Monosteira unicastata*)), cold and drought adaptation (Fernandez-Martinez *et al.* 2013).

Phytostabilization can be combined with excluder-based SRC for bioenergy purposes. In this case the selection of genotypes can also be based on their characteristics in relation to

conversion processes, e.g. calorific value, bulk density, moisture content, ash and extractive content (Demirbas and Demirbas 2009; Chalot *et al.* 2012).

*In vitro* breeding (cell and callus tissue culturing on metal spiked media) and chemical mutagenesis can significantly improve the metal tolerance and phytoextraction capacity of high-yielding annual crops such as tobacco (Guadagnini *et al.* 1999; Guadagnini 2000; Herzig *et al.* 2005; Nehnevajova *et al.* 2007, 2009). These non-genetically modified plants can be directly tested for their metal extraction potential under real field conditions without any legal restrictions (Herzig *et al.* 2014). Tobacco for example is a well-known and efficient accumulator of trace elements, especially for Cd (Mench *et al.* 1989). Several authors have bred and selected best-performing tobacco *in vitro* cultivars showing enhanced metal-extraction for Cd, chromium (Cr), Pb and Zn (Guadagnini *et al.* 1999; Guadagnini 2000; Herzig *et al.* 2003, 2005). Based on pot and field-scale experiments with metal-contaminated soils, 17 somaclonal tobacco variants displayed improved shoot metal removals (Guadagnini *et al.* 1999; Guadagnini 2000). Field trials within the EU FP5 project PHYTAC (2005) confirmed enhanced shoot metal removals of up to 1.8- (Cd), 3.2- (Zn) and 2.0-fold (Pb) higher than that of mother lines at the Swiss Rafz site (soil contaminated by industrial sewage sludge). At the Belgian Lommel site (acid sandy soil contaminated by deposits from a Zn smelter; Table 1) shoot removals were up to 12.4- (Cd), 13.7- (Zn) and 13.5-fold (Pb) higher for best-performing tobacco variants (NBCu 10-8F1 and NBCu 10-4F1) than mother lines (Herzig *et al.* 2003, 2005; Vangronsveld *et al.* 2009).

Commercial sunflower cultivars accumulate only moderate metal concentrations, but their high biomass production makes them interesting for phytoextraction (Lombi, Gerzabek and Horak 1998; Madejón *et al.* 2003; Nehnevajova *et al.* 2005; Mench *et al.* 2010; Kolbas *et al.*

2011). Some oleic cultivars can provide both relevant oilseed yield and shoot Cu removal (Kolbas *et al.* 2011, 2012). Chemical mutagenesis (EMS) was used to improve shoot metal concentrations and biomass production of a sunflower inbred line IBL04 (Nehnevajova *et al.* 2007, 2009). At the Rafz site (Switzerland), using the second mutant generation of sunflowers (F2) with improved metal extraction, shoot metal removals were up to 7.5-, 9.2- and 8.2-fold higher for Cd, Zn and Pb than the inbred line, respectively. The genetic changes of the mutant inbred lines of sunflower were demonstrated to be stable and inherent (Nehnevajova *et al.* 2009). These sunflower mutant lines and tobacco variants have been positively assessed in several open-field experiments in Europe (Kolbas *et al.* 2012; Mench *et al.* 2012, 2013; Herzig *et al.* 2014; and Table 1).

Perennial herbaceous crops, such as switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* spp.) and giant reed (*Arundo donax*) are good examples of grass crops which are being adopted as bioenergy crops in Europe and North America (Zegada-Lizarazu *et al.* 2010; Nsanganwimana *et al.* 2013). The attraction lies in their wide climatic adaptability, low production costs, suitability to marginal lands, relatively low water requirements, low nutrient and agrochemical needs, and possible environmental benefits such as the potential for C storage through their deep and well-developed root system (Zegada-Lizarazu *et al.* 2010). *Miscanthus sinensis* x *giganteus* has been cultivated in field trials on a polluted soil near Copsa Mica (Romania) since 2008, and show low metal(loid) concentrations in aerial tissues (Barbu *et al.* 2013). The low TE uptake and transfer from soil to shoots, combined with a potential use in bioenergy, make these species attractive candidates for phytostabilization options. Other lesser-known potential renewable bioenergy resources include crops such as foxtail millet (*Setaria*

*italica*), biomass sorghum (*Sorghum* spp.), fibre hemp (*Cannabis sativa*), kenaf (*Hibiscus cannabinus*), vetiver (*Vetiveria zizanioides*) or bamboo (Rodriguez-Echeverria *et al.* 2003; Hego, Mench and Bes 2009; Hong *et al.* 2011; Zegada-Lizarazu and Monti 2011; Finnan and Styles 2013; Kocar and Civas 2013; Lata, Gupta and Prasad 2013; Rehman *et al.* 2013; Al Chami *et al.* 2014). However, the invasiveness of some of these species (e.g. *Miscanthus* and giant reed) is a controversial topic, and is discussed in the following sections.

**2.2 Metal(loid)-excluding staple crop cultivars and *in situ* metal immobilization** Cultivars within species from major staple crops such as wheat, barley, rice, potato or maize differ widely in their ability to accumulate metal(loid)s. Selection of pollutant-excluding cultivars for cultivation on contaminated and/or remediated land contributes towards reducing the entrance of harmful trace elements into the human food chain (Yu *et al.* 2006). Such cultivars can be obtained through the selection of excluder-phenotype commercial cultivars and their incorporation into plant breeding programs.

Major staple crops have been screened for their TE uptake ability: including, wheat (Stolt, Asp and Hultin 2006), barley (Chen *et al.* 2007), rice (Yu *et al.* 2006), potato (McLaughlin *et al.* 1994) and maize (Kurz, Schulz and Romheld 1999). Cd is one element of most concern regarding metal uptake into the food chain (Grant 1999; Grant *et al.* 2008). Wheat cultivars differed in Cd concentration due to various translocation rates from the roots to the shoot and within the shoot, rather than to differences in root uptake (Greger and Lofstedt 2004). Low-Cd cultivars (õexcluder-typeö) tended to have similar pedigrees, indicating potential for selecting low-Cd concentration lines. Sorghum cultivars (Biomass and Sucro) were assessed at



the Biogeco site (France, Table 1) but both were sensitive to excess Cu (Kolbas 2012). Selection of the most appropriate cultivar for use in contaminated sites can ensure that food and forage production is in compliance with the respective regulations on threshold TE contents. However, in many countries, farmers often have limited access to excluder type cultivars on a regional base due to the lack of information about the uptake properties of available cultivars. A list of Cd-excluding crop cultivars is provided in Table 3.

In Austria, information on the TE uptake properties of several barley (*Hordeum vulgare*) cultivars was not available to farmers and studies carried out in nutrient solution cultures allowed the selection of appropriate pollution-safe cultivars for a contaminated area (Friesl *et al.* 2006). Application of the excluder-phenotype Bodega vs. accumulator-phenotype Hellana reduced barley grain Cd by >40 %. In combination with the incorporation of gravel sludge and red mud into the contaminated soil, a further >30 % Cd uptake could be avoided. Five years after soil treatment in the field (Arnoldstein site (Austria), Table 1), the amendments were still effective immobilizing agents showing the combination of soil treatment with cultivar selection to be an easy and cost-effective GRO for farmers to improve their situation (Friesl-Hanl *et al.* 2009). The Austrian Agency for Health and Safety has taken initiatives to overcome this barrier, and investigated 12 varieties of winter wheat, 6 of spring durum wheat, 5 of winter durum wheat, 7 of rye, 5 of spring barley and 5 of potatoes (Spiegel *et al.* 2009). The study confirmed the barley cv. Bodega as an excluder phenotype and recommended Cd excluder cultivars for the above mentioned crops for cultivation in Austria.

In Saxony (Germany) an area of several hundred km<sup>2</sup> of land is affected by elevated Cd concentrations in agricultural soils (Freiberg site, Table 1 and Figure 1). The main staple crop in

the region is winter wheat. Since 2002 the Operating Company for Environment and Agriculture of the Saxon State (Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Germany) has been screening cultivars in use and providing data regarding their Cd uptake properties to local farmers. Accordingly, the use of TE-excluding cultivars of annual crops such as winter wheat and summer barley can be an effective option for coping with soil contamination on agricultural land (Serfling and Klose 2008; Klose 2013). Recommended winter wheat cultivars were Batis, Skagen and Tommi for 2009/2010, and Batis and Orkas for 2011/2012 (Klose 2013). Since the availability of certain cultivars changes rapidly the data for current cultivars has to be frequently updated to allow adequate selection of cultivars appropriate for contaminated land. Since the farmers' choice of cultivar does not always focus sufficiently on its metal exclusion capacity, additional management practices are recommended by the authorities to the owners of contaminated land, such as increasing soil pH to values of 5.8 to 6.5, moderate phosphorus (P)-fertilization at the beginning of the growing season, increase of redox potential, and appropriate harvesting methods which minimize contamination of grass forage by soil particles.

### 3. CROP MANAGEMENT PRACTICES

Crop patterns can be designed so as to improve plant biomass and nutrition, and/or enhance or mitigate TE availability and uptake and accumulation. Management practices will also influence microbial communities and as a result soil quality and function. Habitats and biodiversity can be enhanced through, for example, the incorporation of hedgerows and woodlots. For example, *Miscanthus* plantations increase densities and diversity of soil invertebrates compared to annual cropping systems (Hedde *et al.* 2013).

### 3.1 Crop rotation

Most plant species selected as relevant candidates for phytoremediation have been studied as monocultures. However, alternative cropping patterns can significantly influence the phytoremediation process and the extraction or immobilization of contaminants, as well as soil protection and quality. Moreover, they can be of high importance in terms of plant productivity and nutrition, enhancing biodiversity, or even in pest control. Monocultures can lead to a decline in biomass yield due to the depletion of nutrients, occurrence of diseases, pests, and weeds, and have a negative effect on soil fertility (Facknath and Lalljee 2000; Lasat 2000; Mench *et al.* 2010). Any reduction in yield can induce a consequent drop in the plants' phytoextraction capacity. Considering that GRO processes are generally expected to be of a long duration, clean-up based on single species cultivated in monoculture is unlikely to be effective. Bañuelos (2000) recommended rotating the TE-accumulating plant species with agronomic crops, thereby improving the economic balance of the process and maintaining adequate growth and yield of the phytoremediating species. Certain annual crops can be fitted into crop rotations where they serve to control weeds, diseases and pests. Incorporating crops with a deep root system into rotations favors a more complete and deeper use of soil resources, or targets deeper contaminants. In a field study in Sweden, growing *Salix* prior to a wheat crop decreased the Cd concentration significantly in the soil as well as in the wheat grain (Greger and Landberg 2008). However, in short-term phytoextraction experiments the opposite effect may also occur: in a pot experiment, Puschenreiter *et al.* (2013) found that after two years of phytoextraction with *Salix smithiana* the shoot uptake of Cd and Zn by a subsequent crop of barley was increased significantly on four

different soils out of seven. This suggests that during the early phytoextraction period root exudates may solubilize more metals compared to the amount removed from the labile pool. In such a case, longer periods for crop rotation would be needed.

For both improved sunflower and tobacco mutant lines a crop rotation scheme is obligatory to avoid plant diseases over a longer period of time. Crop rotation with non-hosts, such as rapeseed, corn, and winter fodder pea is suggested. Although a minimum rotation period of four years is recommended between successive sunflower crops, good results have been obtained with three year crop rotations of sunflower ó tobacco ó corn, or a two year rotation of sunflower ó tobacco followed by winter fodder pea in Zn-contaminated agricultural soils which were polluted with deposits of a former hot dip Zn factory at the Bettwiesen site, Switzerland (Table 1) (Herzig *et al.* 2005, 2014; Nehnevajova *et al.* 2009). Winter fodder pea (green fertilizer) can still grow during winter and avoids periods of bare soil, thus reducing the risks of transfer of the contaminated soil material by wind or water erosion processes and the leaching of TE<sub>05</sub> during this time. Harvesting occurs prior to sunflower and tobacco sowing or plantation, and bioavailable (NaNO<sub>3</sub>-extractable) Zn concentrations at the Bettwiesen site were shown to remain stable over the winter time. After five years of the sunfloweró tobacco rotation scheme, bioavailable (labile) Zn concentrations were reduced by up to 70 % in top soils (Herzig *et al.* 2014). At the Biogeco site, with Cu-contaminated topsoils due to a wood preservation industry, sunfloweró tobacco rotations were also successfully implemented (Kolbas 2012 and Figure 2). A crop rotation with tobacco ó sunflower ó vetiver was implemented at the same site (Kolbas 2012). Fässler *et al.* (2010) carried out a three-year crop rotation of non-improved, commercial maizeó sunfloweró tobacco in a contaminated agricultural site in Switzerland. The experiment

was run for 6-years and the crop rotation was successfully applied as part of an aided-phytoextraction strategy. Accordingly, such crop rotation could be used to generate profitable crops, including the production of safe (low Cd) stock fodder fortified with Zn, green manure for micronutrient-deficient soils, or bioenergy.

Crop rotation is a stronger key-player for sunflower compared to tobacco. Depending on the climatic conditions, it is possible to harvest tobacco shoots two to three times per year before flowering (Kolbas 2012). Kolbas (2012) predicted that shoot re-growth from bottom suckers could increase annual shoot Cu removal (by 10 to 45%) in contaminated soils at the Biogeco site. Early topping avoided loss of dried leaves and promoted the development of bottom suckers. Recommendations on site-specific crop rotations especially for biogas production to avoid maize monoculture were given by Strauß *et al.* (2012).

The incorporation of cover crops into GRO based on annual crops (e.g. phytoextraction) can also bring additional benefits such as contributing to the maintenance of soil organic material in upper soil layers and an improved soil aggregation, or a promotion in biological soil tillage through root development, or weed and pest control. Legume cover crops can fix nitrogen (N), thereby improving soil N status for subsequent crops planted in the following growth season. At As-contaminated sites soil compaction induces elevated mobility and plant availability of As (Zhao *et al.* 2007). Since deep rooting cover crops were shown to reduce soil compaction (Birkás *et al.* 2004) and enhance aeration/redox potential, in Saxony (Germany) intercropping of i.e. *Sinapis alba* or *Raphanus sativus* are officially recommended by the Saxon State Office for Environment, Agriculture and Geology to the farmers (Serfling and Klose 2008).

### 3.2 Short rotation coppice

Short or very short rotation coppice (SRC and VSRC) of fast-growing tree species (e.g. *Salix* and *Populus* spp.) grown on agricultural soils for production of biomass for conversion to energy or as a feedstock for other chemical processes can also be implemented within GRO on metal-contaminated soils. These woody species show the ability to re-sprout from the stumps after harvests which are performed at short time intervals (i.e. 2 ó 6 years) (Dimitriou *et al.* 2012). Management practices for SRC e.g. weed control, planting, fertilization, and harvesting, are in between agriculture and forestry and the equipment used resembles more that of common agriculture, although they are specially designed for SRC (Dimitriou *et al.* 2012). Soil tillage is only carried out at stand establishment, and the soil is then typically left undisturbed until the plantation is terminated after 10-25 years. The trees are usually propagated through cuttings (approximately 20 cm long) but unrooted cuttings of 2 m long (particularly in the case of poplars) can also be directly planted. This last practice allows the growth of trees in height rather than in width and thus the preservation of a plant soil cover. It also minimizes the damage that can be caused by grazing animals during the first establishment year. Harvest of SRC is carried out in winter or early spring, plants are rooting deeper than annual crops and no agrochemicals are applied after the year of establishment.

#### 3.2.1 Phytoextraction options based on SRC

Willow or poplar SRC is primarily a commercial agricultural biomass production system that due to some plant characteristics (tolerance to TE and uptake of Cd/Zn, etc.) has also been used in several dedicated phytoremediation projects to reduce the effect of hazardous compounds

(Baum *et al.* 2009). For 14 commercial willow SRC plantations grown for long-periods (ca. 15 years) on agricultural soil in Sweden, topsoil total Cd concentrations decreased significantly (ca. 13% on average) compared to adjacent fields cultivated with cereals in common crop-rotations (Dimitriou *et al.* 2012). The biomass productions on these SRC fields were substantially lower than the indicative 10 t dry matter per hectare and year ( $\text{DM ha}^{-1} \text{ yr}^{-1}$ ) expected nowadays in well-managed fields. This was due to bad management carried out by the farmers, in line with the lack of experience in growing the at the time new crop, and rather beneficial incentives to the farmers in terms of subsidies that caused limited engagement throughout the process. If higher biomass from current commercial SRC fields is achieved, which is currently the case (Mola-Yudego 2010, 2011), Cd reductions in the topsoil of SRC compared to the most likely alternative agricultural land-uses are expected to be higher, as has been indicated in other field trials in Sweden (Greger and Landberg 2008). A long-term (now eight years) field experiment with a SRC plantation on contaminated agricultural land in Krummenhennersdorf (Saxony (Germany), Table 1 and Figure 1), shows an average shoot yield of  $16 \text{ t DM ha}^{-1} \text{ yr}^{-1}$  which is above the average of  $10\text{-}12 \text{ t DM ha}^{-1} \text{ yr}^{-1}$  but within the amplitude of  $6\text{-}16 \text{ t DM ha}^{-1} \text{ yr}^{-1}$  achieved in practice (Röhricht and Ruscher 2009). The selection of willow and poplar cultivars for SRC is not based on TE transfer criteria but on yield parameters and resistance against pests and diseases. There are presently 24 certified EU varieties of willows commercially available of which 10 are currently in mainstream use. Only a few of them are characterized for their tolerance to TEs and accumulation traits. Several ways of enhancing the ability of these woody tree species to treat TE-contaminated soils have been suggested, e.g. the use of a range of chelating agents such as ethylenediamine-N,N $\phi$ -disuccinic acid (EDDS), oxalic and citric acids,

and others, to increase the metal uptake rates by willow and poplar plants (Hooda *et al.* 1997; Robinson *et al.* 2000; Schmidt 2003; Komárek *et al.* 2008). However, the use of such mobilizing agents is generally not recommended due to the associated potential risks of groundwater contamination as a result of leaching of TEs. Other practices such as inoculation with mycorrhizal fungi and plant-associated bacteria (rhizobacteria and endophytes) have been reported to improve plant growth and/or modify soil metal mobility and their uptake/translocation by woody crops (Sell *et al.* 2005; Baum *et al.* 2006; Kuffner *et al.* 2008; Langer *et al.* 2012a). Pioneer studies already showed the positive influence of mycorrhizae on plant growth in TE-contaminated soils and mined lands. For example, mycorrhized plants were described on coal wastes from various areas by Daft and Nicolson (1974) and Daft and Hacskeylo (1976). More recently, the influence of bacterial and fungal activity on TE mobility and its application in phytoremediation has been discussed in several reviews (Gadd 2004; Haferburg and Kothe 2007; Lebeau, Braud and Jezequel 2008; Weyens *et al.* 2009; Sessitsch *et al.* 2013). The potential of, and mechanisms behind, these biologically-based improvements of phytoextraction efficiency are far from unravelled, though in some cases, and for some TEs, the results are very promising (Sessitsch *et al.* 2013). Biomass production and performance of poplars inoculated with ectomycorrhizae at the Biogeco site was significantly improved compared to non-inoculated trees (Figure 2).

Although the above-mentioned practices can enhance either TE availability and/or plant uptake, their use in SRC fields is limited due to the high costs and unknown potential negative side effects (e.g. mobilizing chelants). Consequently adjustments to the cropping management of SRC, which are considered less risky alternatives, have been suggested for achieving better



phytoremediation effectiveness. These include the leaf collection with nets (or something similar) to prevent leaf fall and therefore recirculation of contaminants back into the soil as foliar Cd and Zn concentrations are rather high compared to bark and wood (Vyslouffilová, Tlusto– and Száková 2003; Jensen *et al.* 2009; Kacalkova, Tlustos and Szakova 2009; Ruttens *et al.* 2011; Evangelou *et al.* 2013; Hu *et al.* 2013c). For example, on a contaminated soil, up to 83% Cd and 71% Zn of total removal was realized by willow leaves (Vyslouffilová, Tlusto– and Száková 2003). At the Krummenhennersdorf SRC site (Dietzsch 2011) the ratio of the three-years-average concentration in leaves compared to wood was about 2.6 and 2.7 for Cd and about 5.7 and 6.2 for Zn in poplar and willow, respectively. Delplanque *et al.* (2013) found also higher Zn and Cd concentrations in leaves than in stems of four years old willow, with a ratio of 1.62 and 1.44 for Cd and Zn, respectively. At the Lommel site, 816121 g Cd ha<sup>-1</sup> yr<sup>-1</sup> can be removed by poplars with only twigs and 1876240 if leaves are collected with twigs (Vangronsveld *et al.* 2009). Ruttens *et al.* (2011) calculated that if the leaves of SRC grown willow or poplar are assumed to be collected each year, calculated clean up times can be reduced by about 16% and up to 78%. As an alternative, Delplanque *et al.* (2013) calculated the potential of Cd phytoextraction of willow accounting for the number of possible harvests in a rotation and considered that wood and leaves were harvested only every three years. The collection of stems and leaves allowed a decrease in the number of harvests from 11 to 9, which could represent one SRC if nine cycles of three years were possible. As the harvest of willows before leaf fall is not a usual practice in SRC, it should be investigated whether this option is feasible. Mertens *et al.* (2006) pointed out seasonal changes of TE accumulation in willows and a decrease in accumulation with stand age, suggesting that the harvest should be performed at the end of the

growing season and before leaves fall to remove the highest TE amount. This option would have the advantage of minimizing the metal impact on food chain and ecosystem. Nonetheless (innovative) options to collect leaves of willow and poplar SRC should be further considered. More frequent SRC harvests than the currently conducted three to four year cycles could also represent a means of increasing the amount of Cd taken up in the willow SRC shoots more intensively (Dimitriou *et al.* 2006). Harvesting shoots can increase the willow root growth and rigorousness which might increase the uptake of TEs (e.g. Cd) (Rytter 1999). Additionally, early harvests imply harvesting of shoots with higher bark/wood ratio, which means an increase of the shoot concentration of elements such as Cd, compared to when harvesting is carried out at a later stage (Adler *et al.* 2008).

### ***3.2.2 Phytostabilization options based on SRC***

Many woody tree species are well suited for phytostabilization due to their deep root systems, high transpiration rate, high TE tolerance, and ability to grow on nutrient-poor soils. Trees can stabilize less mobile metals (e.g. Cu, Pb) in the soil by physically preventing migration via wind or water erosion, leaching, and soil dispersion; alternatively, they can immobilize the TEs through uptake and accumulation by the roots, adsorption on the root plaque, or precipitation in the rhizosphere (French, Dickinson and Putwain 2006; Van Nevel *et al.* 2007; Mendez and Maier 2008). French, Dickinson and Putwain (2006) initiated long-term phytostabilization trials of former landfill sites in the UK, which had received domestic, industrial and chemical wastes in the 1960s and 1970s. Soils were crawler ripped to 2 m, in an attempt to resolve perceived drainage problems. Weed control was carried out during the first

two years of cultivation using a combination of strimming and herbicides. *Salix* and *Populus* were manually cut back after 1 year; thereafter, the aboveground biomass of all trees (*Salix*, *Populus* and *Alnus*) was manually harvested after a further two years.

Both biological and chemical agents can also be used for phytostabilization purposes to reduce the transfer of metal(loid)s to aboveground parts of willows and poplars. Soil amendments like liming agents, phosphates and apatites, iron (Fe), aluminium (Al) and manganese (Mn) oxyhydroxides, organic amendments, and industrial waste products have been widely used in phytostabilization experiments. The formation of insoluble TE chemical species reduces leaching through the soil profile and the labile metal pool in the soil (Vangronsveld *et al.* 1995a; Vangronsveld, Van Assche and Clijsters 1995b; Vangronsveld, Colpaert and Van Tichelen 1996; Mench *et al.* 2000, 2003; Geebelen *et al.* 2003; Brown *et al.* 2005; Nachtegaal *et al.* 2005; Clemente, Escolar and Bernal 2006; Ruttens *et al.* 2006b; Lagomarsino *et al.* 2011; Bert *et al.* 2012). In the 1 ha Phytosed Ec 1 field site in France (Table 1), Thomas Basic Slag (TBS), an alkaline by-product of the steel industry, was applied at 9 t ha<sup>-1</sup> to a depth of 25 cm with a spreading farm machine. *Deschampsia cespitosa*, a commercial grass, was sown at a high density to cover the sediment during the autumn and winter seasons. Six months later, the grass was mechanically removed and replaced by a tarpaulin where the willows were to be planted. Two meter long unrooted cuttings of two commercial willow clones were manually planted in the top sediment following the common SRC plantation scheme (at a density of 12,000 willows per ha<sup>-1</sup>). Weed and grass control was performed mechanically twice during the first year. TBS is commercially available and used as a fertilizer by farmers to increase soil pH. At the Phytosed Ec 1 site, where the sediment landfill site is highly contaminated with metals (mainly Zn and Cd)

TBS is expected to decrease the metal shoot transfer towards both *Salix* clones and to decrease the labile pool of metals (Bert *et al.* 2012). The beneficial effects of TBS are expected to be reached by the pH increase that consequently decreases the metal mobility (short-term effect) and further chemical reactions (substitution, precipitation) that stabilize the metals in the long-term (Panfili *et al.* 2005; Bert *et al.* 2012). The TBS amount was chosen according to the maximum pH that willows can tolerate while achieving optimal development, i.e. the pH should not exceed 8. After one year, the pH reached 8.05 on average and did not exceed 8.25 at most places. The initial pH was 7.25 suggesting that the short-term effect occurred. The survival rate of the clones was 89 % and 93%, which is not far from the normal survival rate for a SRC (95% in Bullard *et al.* (2002); 97% in Walle *et al.* (2007)). Effects of TBS on soil metal mobility or the leaf metal concentrations were not yet determined.

Relevant ectomycorrhizal and endomycorrhizal strains can confer stress tolerance to tree species by promoting nutrient and water interception, providing them with macronutrients and/or plant growth hormones, and buffering TE accumulation by accumulation in the hyphae or increasing TE sorption through the production of glomalin, a glycoprotein produced by the symbiotic fungus *Glomus mossae* (Vodnik *et al.* 2008). Such beneficial effects are known for various symbioses e.g. the ectomycorrhizal fungi *Paxillus involutus*, *Hebeloma crustuliniforme*, and *Hebeloma salix* associated with *Populus nigra* and *Salix viminalis* in Cu-contaminated soils (Bes *et al.* 2007). In the BIOFILTREE project 2 m long unrooted cuttings of two poplar clones were mechanically planted in a metal contaminated sediment landfill site (60 cm depth) (BIOFILTREE, ANR project, 2011-2014, <http://dendroremediation.univ-fcomte.fr/spip.php?rubrique26>). Poplars were selected among 14 poplar cultivars grown on a

metal-contaminated soil as they showed the lowest Cd and Zn concentration in leaves and wood (Lacercat-Didier 2013). In total, 1536 poplars were planted on this site. Trees were protected from herbivores with wire netting of 60 cm height and weed control was mechanically carried out once a year. Trees were inoculated with mycorrhizal fungi by whitewashing the inoculum at the foot of the cutting. Inocula of mycorrhizal fungi are commercially available and used as fertilizers in plant and legume nurseries to increase the biomass production. In this project, mycorrhizal fungi are expected to decrease the metal shoot transfer towards the poplar clones, to decrease the metal labile pool through biological filtration and to increase the poplar biomass.

### 3.3 Intercropping/row cropping

Intercropping systems can promote positive below-ground, plant-soil and plant-plant interactions resulting in improved nutrient availability or increased crop yield. Nutrient deficiency is a common characteristic of contaminated soils, but intercropping with leguminous plants can supply nutrients (through N<sub>2</sub> fixation, transfer of fixed N and mobilization of P due to rhizosphere acidification) to phytoremediating crops. Intercropped species can access different nutrient pools and they may also alter soil TE bioavailability thereby altering their accumulation by phytoextracting crops. In summary, intercropping systems can be designed with the aim of (1) phytoprotecting the non-accumulating plant crop, (2) enhancing metal accumulation by the phytoextracting crop(s), or (3) improving plant biomass production (and nutrient status) and hence plant performance in phytoextraction or (aided)-phytostabilization.

Beneficial plants, such as leguminous species or alders (*Alnus* spp.), can lead to a reduced reliance on inorganic fertilizers. Legumes include economically important grain crops, oilseed

crops, forage crops, and agroforestry species. They are not only a rich source of quality protein for humans and animals but are known soil improvers, due to their ability to establish symbiotic interactions with N<sub>2</sub>-fixing bacteria. Moreover, most legume species as well as alders take profit from root symbiosis with mycorrhizal fungi (Escaray *et al.* 2012). Advantages due to the use of leguminous plants in the ecological restoration of N-deficient TE-contaminated soils or mine-spoils, was illustrated by Stroo and Jencks (1982) when re-vegetating coal strip mine-soils in West Virginia (USA). Amelioration of soil nutrient deficiency was attributed to the N fixing activity associated with leguminous plant roots. As part of a follow-up study, Stroo and Jencks (1985) evaluated the effects of liming, NPK fertilizers and sewage sludge amendments on tall fescue yields: the improvements in yield after addition of amendments were similar to the effects of incorporating leguminous plants. Alder is an important pioneer species and is recognized as a soil improving plant, through its symbiosis with N-fixing microorganisms (e.g. *Frankia*), ectomycorrhizae and arbuscular mycorrhizal fungi (Tarrant and Miller 1963; Taleshi *et al.* 2009). It can effectively colonize nutrient deficient and TE-contaminated soils and mine spoils characterized by low soil fertility, as pure stands or in crop rotations with other fast growing trees. Lohmus *et al.* (2006) studied the effects of *Alnus glutinosa* plantations of different ages on the remediation of oil shale mining areas in Northern Estonia. Survival and productivity of this species were high, confirming its reputation as a good survivor and highly productive species on degraded and marginal soils. Inter-cropping with alders could provide several benefits to phytoextracting and phytostabilization crops, e.g. *Salix* and *Populus*, such as an improvement in their N nutrition. Alders can also improve soil P availability, which might be a further benefit of co-cropping (Roy, Khasa and Greer 2007). Keleberda (1978) studied spoil heaps of a Mn quarry

in Ukraine planted with Scotch pine (*Pinus sylvestris*) and black alder (*Alnus glutinosa*). Tree growth was healthier in mixed rather than in monoculture stands, and the presence of alder much improved the soil fertility. The use of polyclonal stands of *Salix* and *Populus* clones rather than monocultures has also been recommended when implementing SRC in phytostabilization options as a means of reducing disease incidence, particularly from *Melampsora* rust (McCracken and Dawson 2003; French, Dickinson and Putwain 2006).

Nitrogen fixation can decrease soil pH due to nitric acid accumulation in the rhizosphere (Van Migroet and Cole 1984), which for phytoextraction purposes can in turn induce an increase in TE bioavailability to the co-cropped TE-accumulators. Inter-cropping the Cd/Zn-hyperaccumulator *Noccaea caerulea* with the legume *Lotus corniculatus* in a field experimental site in Piedrafita do Cebreiro (NW Spain) tended to increase Cd accumulation by the hyperaccumulator (Álvarez-López, unpublished results). Several species of *Lotus* show potential for incorporation into GRO due to their worldwide distribution and high adaptation to a number of abiotic stresses, such as metal contamination (Escaray *et al.* 2012). Candidates with good potential for cultivation in degraded or marginal soils include *Lotus corniculatus*, *L. uliginosus*, *L. tenuis* and *L. creticus* (Escaray *et al.* 2012; Safronova *et al.* 2012). *Ornithopus compressus*, *Medicago arabica* and *Trifolium pratense* develop well in the Cu-contaminated soils amended with compost and dolomitic limestone at the Biogeco site, particularly in the plots cultivated with vetiver. They have a slightly negative effect on similar plots cultivated with *Miscanthus* (Mench, unpublished results).

Another intercropping option has been suggested by Wieshammer *et al.* (2007), where two metal-accumulating species, *Salix caprea* and *Arabidopsis halleri*, were combined in a pot

experiment. The intention was to support the metal accumulation in the main phytoextraction crop, i.e. *S. caprea*, by a hyperaccumulating species. This intercropping reduced shoot biomass and shoot metal concentrations in both species due to competition for metal uptake and possibly also nutrients and water, but such effects of both species might be different in the field. However, the difficulties incurred in the management of *A. halleri* might be the more limiting factor of applying this plant combination.

A similar metal competition effect has been reported by Hu *et al.* (2013a), where the Zn and Cd hyperaccumulator *Sedum alfredii* tended to decrease the Cd accumulation in the co-cropped *Ipomoea aquatica*. The effect of intercropping varied according to the various metals present. *Noccaea caerulescens* increased the Cd accumulation but decreased the Zn accumulation in co-cropped *Hordeum vulgare* (Gove *et al.* 2002) Rhizosphere processes of metal solubilization induced by root exudation and metal depletion due to plant uptake may affect metals in a different way, which was reflected by the Zn and Cd accumulation behavior of the co-cropped plant species.

At the Biogeco site, with sandy Fluvisol, another intercropping strategy was tested. Sunflower was sown (35cm x 35cm) in April 2012 in field plots and tobacco seedlings were transplanted in May between the rows. Juvenile sunflower plants (before flowering) were harvested in mid-June, and tobacco plants were left to develop until the end of October. This management system allowed for successive harvest of the sunflower and tobacco Cu-rich shoot biomasses (Mench, unpublished results).

Wu *et al.* (2012) performed field trials on a Cd, Cu and polychlorinated biphenyls (PCBs) contaminated agricultural field in the Zhejiang province (China). Monocultures and



intercroppings of three species were established: the Cd/Zn-hyperaccumulator *Sedum plumbizincicola*, the Cu-tolerant and accumulating *Elsholtzia splendens*, and the legume *Medicago sativa*. Intercropping pattern had no effect on the plant uptake of trace metals or PCBs. However, greater reductions in soil Cd and Zn were observed in some combinations, and *S. plumbizincicola* intercropped with *M. sativa* together with liming was a suitable strategy for the remediation of this multi-contaminated soil.

Very little information is available on intercropping in (aided)-phytostabilization trials. In a field study, perennial grass mixtures which included the legume *Anthyllis vulneraria* achieved a higher coverage and biomass and improved nutrient status in highly polluted soils (Cd/Zn/Pb) compared to a control with grass only (Frérot *et al.* 2006).

### 3.4 Protection of phytoremediation crops

There is a general consensus around the idea that TE (hyper)accumulation in plants evolved as an elemental-based defense strategy against herbivores and pathogens (Poschenrieder, Tolrà and Barceló 2006). For example, a reduced infectivity of the hyperaccumulator *Noccaea caerulescens* by the *Pseudomonas syringae* pv. *maculicola* when grown in TE-enriched soils was reported (Fones *et al.* 2010). An important aspect in considering TE accumulating plants for TECS remediation is the possible interactive effects of both the pathogens on the host plants and the eventual TE-dependent resistance acquired by pathogens invading the selected crops. However, non-hyperaccumulating plants exposed to TE have proven to be generally more resistant to foliar or root pathogens than the same plants not previously exposed to TE, due to a general response to TE-induced oxidative stress, which is also effective against infection by plant

pathogens. In general, it is possible that in non-hyperaccumulating plants both redox-related molecules (e.g. glutathione, metallothioneins) and enzymes associated with oxidative stress (such as superoxide dismutase) may cooperatively protect the host plants from pathogens along with increased exposure to TE in the contaminated areas (Schutzendubel and Polle 2002).

For sunflower a crop rotation is required to avoid plant diseases, such as *Sclerotini* wilt (white mold) caused by the fungus *Sclerotinia sclerotiorum* which infects sunflower roots. Downy mildew is caused by the fungus *Plasmophora halstedii*, which is soil-borne, wind-borne and seed-borne. Sunflower plants are susceptible to infection for a very short period (2-3 weeks), depending on soil temperature and moisture. Instead a three-year crop rotation is recommended (CETIOM 2014).

Tobacco is less susceptible to diseases than sunflower. Nonetheless several diseases affect yield and quality of tobacco, and appropriate management practices (such as crop rotation of three to five years) are again the best protection against their proliferation. Selection of resistant cultivars is also a highly effective and inexpensive method of reducing yield loss and diseases. Bacterial wilt (caused by *Ralstonia solanacearum*) is a serious soil-borne disease, which infects tobacco roots by entering through wounds. Since these bacteria are very persistent in soil, rotations of approximately three years are needed. Some multipurpose chemicals can also help in control measures. Tobacco mosaic virus is caused by a virus which can be easily spread by hand or machinery at any time during the growth season. Appropriate practices for mosaic control include the rotation of tobacco fields, use of resistant varieties, avoiding use of tobacco products when working with tobacco in the plant beds and greenhouse, careful washing of hands with abrasive hand soap when manipulating seedlings, and removal of plants showing mosaic

symptoms before cultivation in the field. Black shank is caused by the soil-borne fungus *Phytophthora parasitica* pv. *nicotianae* which attacks the plants primarily through the roots (not necessarily via wounds). A sufficiently long rotation is considered the primary control strategy alongside the use of resistant varieties and some chemical treatment.

Commercial tobacco varieties show resistance against many diseases, such as bacterial wilt, tobacco mosaic, black shank and root-knot nematodes. Regarding the *in vitro* bred phytoextraction mutants of tobacco, pest resistance was not systematically tested, but in long-term field experiments the improved tobacco clones are sufficiently resistant to plant diseases and drought stress (Herzig *et al.* 2005; Kolbas 2012).

At the SRC-plantation in Krummenhennersdorf in Saxony, Germany (Table 1) frequent checks were made to determine the susceptibility of the different willow and poplar clones to insect and fungal infections (during the 3<sup>rd</sup> year of the 1<sup>st</sup> rotation). *Tuberolachnus salignus* (Gmelin) was observed throughout the summer months on trunks and branches of all of the willow clones but it does not cause economically relevant damages (Röhricht and Kiese-walter, 2008). Regarding fungal infections, *Ascomycota* such as *Pollaccia saliciperda* (anamorph) and *Venturia saliciperda* (teleomorph) affected all the clones, but the most susceptible clones were the willow öTordisö ((*S. viminalis* x *S. schwerinii*) x *S. viminalis*) and öGudrunö (*S. dasyclados*). *Melampsora* was detected in the poplar hybrids ö275ö and öMax 3ö. Willow (and poplar) leaves may be severely attacked by imported willow leaf beetle (*Plagioder a versicolora*), a small insect that feed the leaves and remove only a skeleton of leaf veins. Leaves may retain their common shape and remain on the trees but often browned leaves drop abundantly in late August and September. If the trees are not killed, they are obviously less vigorous. With cool weather, adults

hibernate for the winter in soil, sod, and under loose bark of trees. Larvae appear in April or May when leaves start to emerge and feed them, in general causing more damages than adults. Bifenthrin, chlorpyrifos or pyrethrin are insecticides registered for control of the imported willow leaf beetle. The insecticide should be applied immediately after the leaves have flattened out. A second spray application may be required to protect new growth from living adults that have moved in from surrounding unsprayed trees. At Phytosed Ec 1 field site (Table 1), both *Salix* cultivars -Ingerø and -Tordisø were susceptible to this insect. Such damage was not detected with native *Populus nigra*, *Salix viminalis*, and *S. caprea* at the Biogeco site. In contrast rust disease develops in early September in *Populus trichocarpa* x *deltoides* cv. Beaupré (sensitive cultivar) leading to early leaf fall.

While it is possible that increased metal tolerance by plant pathogens may enhance their ability to invade phytoremediation crops, there is still limited research on the evolution of TE tolerance or resistance in phytoremediation field trials. In particular, it will be important to assess the TE concentrations in the plant organs and fluids where pathogens act, so as to understand whether this factor may induce TE tolerance in plant pathogens.

#### **4. SOIL, PLANTING, IRRIGATION AND HARVEST MANAGEMENT**

##### **4.1 Soil management**

Few studies have been carried out to test the effect of tillage practices (i.e. conventional versus reduced or no tillage), or ploughing methods, on the mobility or uptake of TEs where GRO have been implemented, and information on these aspects is limited. Schröder, Stephan and Schulte-Karring (1985) studied the influence of some mechanical operations on technosols formed during

the re-vegetation of spoils from coal strip mining activities in the Rhine district (Germany). Spoils were levelled off and subjected, or not, to tillage (30-50 cm depth). Tillage led to the compaction of spoils thus hampering plant root growth and proliferation into deeper layers. Gilewska and Bender (1979) studied the re-vegetation of coal strip mine-spoils located in the Konin area (Poland), where ploughing (25 cm depth) was carried out in combination with various fertilization schemes. Ploughing resulted in good spoil structure and increased the soil biochemical activity and barley yields. For the re-vegetation of the lignite mine area of Meirama (Spain), spoils were subjected to ploughing and harrowing and to various amendment and fertilization regimes (e.g. liming, NPK) prior to seeding with mixed grass species (e.g. perennial ryegrass, red fescue, Kentucky bluegrass, and white clover) (Gonzalez-Sangregorio *et al.* 1991). The large increase in soil fertility and biochemical activity, in comparison to other experiments conducted on similar sites, was attributed to the adopted soil agronomical practices. Positive effect of ripping on ryegrass-clover yields on lignite mine-spoils was likely due to the reduction in the density of such fine textured spoils (Ross *et al.* 1992a). Vamerli *et al.* (2012) compared distinct tillage and ploughing methods on crop, TE concentration and growth on metal-polluted pyrite wastes. Tillage mode, either surface or sub-soiling tillage, was not found to be critical, although sub-soiling enhanced aboveground productivity. Ploughing was, however, shown to increase TE concentrations in plants. Overall, this mechanical activity can have variable effects on the performance of soil re-vegetation, and strongly depends on the properties of the parent material. Mechanical decompression of soil is of crucial importance prior to initiating remediation of industrial soils or mine-spoils, which are normally compacted due to the previous operations. Thereafter, tillage should primarily aim at developing a real soil profile with distinct

horizons, allowing optimal aeration, density and water drainage. Several field trials conducted on TE-contaminated sites or mine-spoils have clearly demonstrated that soil profile development can also be positively influenced by organic and inorganic fertilization.

#### 4.2 Planting methods and planting density

The establishment period is a critical phase for the successful development of a plant cover for GRO. Some phytoremediation crops can be directly sown (e.g. grass species, forage crops and sunflower) while in the case of others, pre-cultivation of seedlings and planting out after spring frosts (in temperate climate zones) is recommended for increasing plant survival (e.g. tobacco). During this plant establishment period, weed and pest control, reduced fertilization and supplemental irrigation may be necessary. It is recommended in some cases to use a seed density roughly double that of standard agricultural references, given the inhospitable conditions for plant germination in contaminated sites, and thinning of supplementary plants (Vamerali *et al.* 2012).

Pioneer studies already evaluated the importance of density of various woody plant species on TE-contaminated sites. In a floristic survey of the Pb mine-spoils at Grassington Moor (UK), plant density correlated well with soil biological and chemical fertility, indicating a more favorable soil development at higher plant density (Clark and Clark 1981). Li *et al.* (2009) compared the effects of two planting methods of the high-biomass tree, *Averrhoa carambola*, on a Cd-contaminated site. Plots were planted with seedlings germinated from seed at a high planting density and compared with grafted seedlings planted at a low density. It was concluded

that Cd phytoextraction using the high-density seed-seedling method was a feasible option to clean up agricultural soils slightly contaminated by Cd.

According to agronomical practices, sunflower kernel should be sown 60 cm apart in rows with a plant-to-plant spacing of 20 cm. For metal phytoextraction purposes, where maximum shoot metal removal and not kernel production is the main criterion, densities can be enhanced as follows: row distance of 30-50 cm and plant distance within rows of 20 or 10 cm, respectively, resulting in a plant density of 85,000 - 170,000 plants ha<sup>-1</sup>. This can be adapted according to the soil fertility and climate. Seeds should be sown at approx. 3-4 cm depth (depending on the soil type and water holding capacity) for better stand development, and shortly after sowing pest control is important (e.g. snails, rabbits and deer). After 10-12 days of germination, extra seedlings should be uprooted to provide a spacing of 20 cm between plants within rows. The sowing density is 10-15%, and for inbred lines up to 50% higher than the expected canopy density at harvesting (45,000-60,000 plants ha<sup>-1</sup> in rain-watered fields, 60,000-75,000 plants ha<sup>-1</sup> in irrigated fields). It is recommended to evaluate the survival and growth of sunflower in a contaminated soil prior to planting in the field so as to insure their tolerance.

Tobacco seeds are produced in abundance but are small, making direct sowing more difficult than for sunflowers. The most common method to obtain tobacco for phytoremediation purposes is a pre-growth of seedlings in pots prior to transplantation into the contaminated field. Another promising technique for fast production of tobacco seedlings for phytoextraction use is micropropagation (Herzig et al., 2003). Tobacco clones, developed by *in vitro* micropropagation, have shown sufficient growth on three contaminated sites in Rafz and Bettwiesen (Switzerland) and Lommel (Belgium) in field experiments. Pre-grown seedlings of tobacco (nine-week old)

can be planted in the field when there is no longer a risk of frost with an optimized plant density of up to 40,000 plants ha<sup>-1</sup> (with 0.5 m between rows and 0.5 m between individual plants in the same row, again depending on the soil fertility and climate). Prior to planting on site, the *in vitro* propagated tobacco seedlings (four to five weeks old) need to be transplanted for two weeks into soil in covered plates to permit rooting (under a 14 h photoperiod; at 25/22 °C in a growth chamber), followed by a two to three weeks outdoor conditioning (with initial protection from direct sunlight).

Soil preparation is of crucial importance for the successful installation of SRC. At the Lommel site, four weeks prior to planting, a glyphosate based herbicide (Roundup MAX™ 3%; 12 L ha<sup>-1</sup>) was used to eliminate existing weeds. Ploughing and careful harrowing 3 weeks later provided a smooth plant bed, permitting the cuttings to enter the soil sufficiently deep during mechanical planting. Current planting densities for commercial SRC plantations are up to 13,000 cuttings ha<sup>-1</sup>, and planting is carried out in such a way as to enable easy agricultural management, e.g. planting in double rows, for harvesting with special for SRC designed machines, or for fertilization with tractors and avoiding damage to trees (Tubby and Armstrong 2002).

A denser planting of SRC (than the conventional 13,000 cuttings ha<sup>-1</sup>) has been proposed to result in a higher biomass production in the case of more frequent harvests (e.g. after one or two years when plants have not yet started to outcompete each other and die-off due to inter-competition) and as a result potentially higher amounts of TE uptake from soils due to a denser and more extended root system (at least during the first couple of years when plant roots have not managed to spread widely as is the case in older SRC stands). At the Lommel site (Belgium)



commercial cuttings of 20 cm length are planted at a density of 18000 cuttings ha<sup>-1</sup> using an adapted leek planting machine with two seats. The planting design consists of twin rows as classically applied in SRC. In the twin row there is a distance of 0.75 m between the rows, between the twin rows there is a distance of 1.50 m. This planting design allows the harvest machine to harvest a twin row in one track without riding with its wheels on the stubs. Planting densities of up to 25,000 plants ha<sup>-1</sup> can produce higher yields in commercial SRC plantations (Wilkinson *et al.* 2007). The potential positive outcome of a denser planting in TE-contaminated sites would however mean that prevailing and ðworkingö current management practices for commercial SRC plantations (such as planting, fertilization, harvesting and removal) would have to be conducted with increased costs. However, higher planting densities may also lead to a plateau of biomass production (Hammer, Kayser and Keller 2003). Also, a higher risk of disease due to easier spreading and infection should not be underestimated. Such changes in management might on the one hand imply an increase in the ability to take up the targeted TEs but on the other hand, they may incur potential decreases, or the risk of long-term decreases, in the biomass produced. Additional research and development for methods towards increasing the phytoremediation efficiency are therefore needed and any suggested management adjustments need to consider all factors influencing the amounts of TEs phytoextracted, e.g. the combined results of biomass produced and the concentrations of metal(loid)s.

### 4.3 Irrigation management

Soil moisture affects both plant growth and TE transport in soil, and GRO management also needs water management, especially in sub-arctic, arid and semi-arid areas that undergo

relatively long periods of drought. Prolonged drought induces stress which enhances plants' sensitivity to pathogens and, more importantly, reduces plant growth with negative implications on the phytoremediation success. Additional site-specific problems concern mining areas where soils are often characterized by a low water retention capacity. The importance of irrigation for spoil re-vegetation was illustrated by Paunescu and Stefanic (1989). At a field site targeting the phytoextraction of metal-contaminated sludge, a surface area of 27000 m<sup>2</sup> was planted with *Phragmites australis* as well as annual crops such as sunflower, maize and rape and irrigation was one of the various agronomical measures tested (Claus *et al.* 2007). Increasing soil humidity by additional irrigation enhanced the Zn uptake and removal by *Phragmites australis*.

Tobacco mutant lines showed outstanding performance in drought resistance, in shoot DW yield, and shoot metal (Cd, Zn and Cu) removals on contaminated soils at the Biogeco site (Kolbas 2012). However, water supply throughout the growing season, notably from April to end of August, was necessary. It was particularly limiting tobacco and sunflower growth in 2011 and 2012, and irrigation (i.e. 8 times 7.5 L m<sup>-2</sup>) was required to optimize shoot Cu removal (Kolbas 2012). At the Bettwiesen site, tobacco was the only crop that survived during the extreme drought of 2003, whereas other crops such as wheat, barley and rape dried up (Herzig, Nehnevajova and Schwitzguébel 2007).

In arid areas, irrigation of phytomanaged sites may both increase the site treatment costs and also cause water competition with food crops. Irrigation with wastewater may be a sustainable practice to meet both the plant water requirements and an alternative treatment of wastewater, especially since phytomanaged sites are already under continuous monitoring. To

our knowledge, such options have not been practiced or tested at a field scale and some relatively cheap technologies for practical site use of wastewater irrigation are available.

#### 4.4 Harvest management

Since the GRO objectives are often to insure high plant productivity, and in the case of phytoextraction to maximize shoot TE removal, the optimum harvesting time for phytoremediation crops may differ from harvesting times that are considered appropriate for conventional agricultural purposes. For example, in a phytoextraction trial on a former sewage sludge deposit site (Leipzig, Germany) harvesting of *Phragmites australis* in October and November instead of December (which is more common practice) resulted in an increase in plant dry biomass and higher metal extraction. On the other hand, multiple harvesting of this common reed over the whole growing season did not result in any beneficial increase in dry biomass production (Claus *et al.* 2007). In phytoextraction methods based on the annual cultivation of tobacco, the topping of plants before flowering, and then shoot harvest can enhance the development of bottom suckers and allow a second harvest, especially in southern Europe and depending on water supply especially from August to October (Kolbas 2012).

Considering the cultivation of grassland (green fodder, hay, and silage) on contaminated soils, harvest management should aim avoiding possible TE transfer into the food chain over common values. Therefore, the Saxon State Office for Environment, Agriculture and Geology (LfULG, Germany) published guidelines for grassland management to help farmers comply with the legal threshold values and to practice sustainable farming on contaminated land (Klose (2013) according to Elsässer *et al.* 2007): (1) obtaining a robust grassland cover and tough sod

density through more frequent mowing or fertilization regimes which promote grasses that take up 50-70% less Cd compared to dicotyledonous herbaceous plants; (2) grading molehills and increased cutting height (mowing up to 5-7 cm); (3) improved setting of hay turning machines and hay rake to avoid soil dispersion; (4) keeping tires clean, in particular when placing and compacting harvested grass or hay; and (5) adjusting pasture stocking with cattle. Concerning cereal production, LfULG is running a program which offers the opportunity of a pre-harvest control routine to the farmers. Through this initiative the farmers can gain insight into the contamination level of the corn several days before the harvest date, allowing them to decide in advance if the harvest can be used as foodstuffs, as forage or if it can only be used for energy production. In the latter case, instead of threshing the farmers will harvest the whole plant (Klose 2013).

## 5. FERTILIZATION MANAGEMENT

Contaminated sites not only present (phyto)toxic concentrations of pollutants, but are also typically characterized by edaphic properties which can severely limit plant growth (nutrient deficiency, poor soil structure, low organic matter (OM), etc.). Fertilization regimes can be designed with the aim of improving plant growth and establishment or increasing plant uptake of TEs. Pioneer studies demonstrated the importance of fertilization during the re-vegetation of TE-contaminated mining areas in the USA (Pancholy, Rice and Turner 1975; Cundell 1977). Table 4 summarizes the effects of different fertilizer regimes on plant growth and yield, TE accumulation and soil TE behavior.

Improvements in the biomass production of TE hyperaccumulators using inorganic fertilizers has been reported (Robinson et al., 1997; Schwartz et al., 2003; Bani et al., 2007) (see Table 4). Herzig, Nehnevajova and Schwitzguébel (2007) assessed various rates/types of fertilizer on the productivity/metal extraction of sunflowers and tobaccos at the Bettwiesen field site. Based on these results sunflower (5<sup>th</sup> to 8<sup>th</sup> generation) mutants and controls were then fertilized with 60 kg N ha<sup>-1</sup>, 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 400 kg K<sub>2</sub>O ha<sup>-1</sup>, and the tobacco clones of M<sub>1</sub> and M<sub>2</sub> generations and controls with 170 kg N ha<sup>-1</sup>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 260 kg K<sub>2</sub>O ha<sup>-1</sup> according to Swiss agricultural standards; N and potassium (K) were added as sulfate form (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, and P as triple superphosphate. One option to avoid N leaching from the root zone is to split the applications of NPK fertilizers. For example, addition rates of 40 kg N, 40 kg P<sub>2</sub>O<sub>5</sub>, 40 kg K<sub>2</sub>O, and 60 kg SO<sub>3</sub> per ha in May and 20 kg N, 20 kg P<sub>2</sub>O<sub>5</sub>, 20 kg K<sub>2</sub>O, and 30 kg SO<sub>3</sub> in July were used for sunflower plantations at the Biogeco site (Kolbas *et al.* 2011).

On the SRC field in Lommel, chemical soil fertilizers (NH<sub>4</sub>NO<sub>3</sub> and Patentkali®) were applied resulting in nutrient additions of 55 kg N ha<sup>-1</sup>, 250 kg K ha<sup>-1</sup> and 90 kg magnesium (Mg) ha<sup>-1</sup>. To obtain and maintain biomass production over several years, fertilizer application is needed to replace exported nutrients with the harvest. In Saxony (Germany, Table 1) a SRC with poplar and willow at a medium yield (10 t DM ha<sup>-1</sup> yr<sup>-1</sup>) led to the following export of nutrients (kg ha<sup>-1</sup> yr<sup>-1</sup>): N 37, P 6, K 26, Mg 8 and calcium (Ca) 54 (Röhricht and Ruscher 2009). In the early years of a poplar SRC, mycorrhized trees were found to take advantage of the inorganic fertilization. At the Biogeco site, the poplars inoculated with ectomycorrhiza in plots adjacent to the NPK fertilized plots managed with the sunflower-tobacco crop rotation were producing more

wood than the other ones, due to the horizontal development of their root system in these fertilized plots.

Inorganic fertilizer application can affect TE bioavailability, and much research has been carried out towards a better management of fertilizers and reduction in food-chain transfer of TE in agriculture (McLaughlin *et al.* 1996; McLaughlin, Parker and Clarke 1999; McLaughlin and Singh 1999; Singh *et al.* 2011; Chaney 2012). The type and amount of fertilizer used and interactions between TE and major nutrients (N, P and S) and among TE themselves (e.g. ZnóCd and FeóCd) are key-players in TE uptake by crops (Tiller *et al.* 1997; Mench 1998). Plant availability of Cd can be affected directly through the addition of Cd as a contaminant in P fertilizer, or indirectly through ion exchange reactions in the soil solution (McLaughlin and Singh 1999; Wangstrand, Eriksson and Oborn 2007). Furthermore, fertilizers can influence Cd speciation and complexation. Chloride can increase crop Cd uptake more than other soil factors due to the formation of chloride complexes (Smolders *et al.* 1998), and this is particularly pronounced in alkaline soils (Hattori *et al.* 2006). Ammonium fertilizers frequently cause higher Cd concentrations in crops than nitrate fertilizers as a result of the pH decrease caused by nitrification or plant uptake of  $\text{NH}_4^+$  (Grant 1999) (see Table 4).

The chemical form of fertilizers can therefore be selected according to the GRO technique to be implemented: in the case of phytoextraction, ammonium-, sulfate- or chloride-based fertilizers may be managed so as to enhance the phytoavailability and hence uptake of metallic cations, while in the case of GRO where TE stabilization is the aim avoiding certain fertilizer types can minimize plant TE uptake. The effects of plant N nutrition can be exploited to enhance the efficiency of phytoextraction (Salt, Smith and Raskin 1998). Acidification induced

by ammonium-fed plants can promote localized mobilization of some metallic cations in neutral to alkaline polluted soils, thereby improving phytoextraction (Zaccheo, Crippa and Pasta 2006). Furthermore, the amount of metals taken up by roots can be replenished both by the soil buffering capacity and by diffusion processes extending the metal mobilization to a wider volume of soil than the rhizosphere itself (Whiting *et al.* 2001a, b; Kashem and Singh 2002). However, the effects may largely vary depending on the plant physiological requirements.

The application of elemental sulfur (S) may increase the metal solubility for plant uptake (Wenger *et al.* 2002; Wang *et al.* 2008; Iqbal *et al.* 2012). Microbial oxidization of elemental S leads to the production of sulphuric acid and consequent decrease of soil pH (depending on the soil pH buffering capacity). Sulfur oxidation along with microbial and root respiration might also lead to partly anoxic conditions in the rhizosphere, inducing reductive dissolution of manganese oxides, which further enhances the Zn and Cd solubility (Iqbal *et al.* 2012). However, a soil-specific balance between increasing metal solubility and potential negative effects (e.g. Al toxicity) due to decreasing pH needs to be found.

Incorporation of organic residues (in particular composts, manure, sewage sludge or biosolids) improves soil physical properties, water infiltration and water holding capacity. They further contain essential micro- and macronutrients for plant growth, and also decrease bulk density (Vangronsveld *et al.* 2009; Soriano-Disla, Navarro-Pedreño and Gómez 2010). Addition of this type of amendment is a common practice to facilitate re-vegetation of contaminated soils and at the same time provides a viable manner of recycling waste products. Their effects on metal(loid) bioavailability depend on the nature of the organic matter, and on the particular soil type and elements concerned (Clemente, Walker and Bernal 2005; Ruttens *et al.* 2006b; Goecke

*et al.* 2011; Kumpiene *et al.* 2011; Lagomarsino *et al.* 2011). Some debate remains regarding the possible addition of trace metals to the soils, or the potential mobilization of Pb and Cu (or As) by dissolved organic matter (DOM) (Geebelen *et al.* 2003; Mench *et al.* 2003, 2006; Clemente, Escolar and Bernal 2006; Ruttens *et al.* 2006a; Marchand *et al.* 2011; Carcamo *et al.* 2012; Kumpiene, Fitts and Mench 2012). Some forms of unstabilized organic matter such as manure or biosolids may increase metal mobility due to their high DOM content that can complex metals and facilitate their movement through soil (Almås, Singh and Salbu 1999; Kiikkila *et al.* 2002a; Tandy *et al.* 2009). Composting stabilizes organic wastes and can reduce their DOM content and the potential for metal leaching (Kiikkila *et al.* 2002b). Application of sewage sludge to willow SRC generally add TEs (notably Cu, Zn, Pb, and Cd), but several studies of their balances in willow stands suggest that the shoot metal removal compensates for this addition (Dimitriou *et al.* 2012). A sewage sludge treatment (600 t ha<sup>-1</sup>) to SRC implemented in landfill sites increased tree yield by up to 56 % in *Salix*, 81 % in *Populus*, 120 % in *Betula* and 6 % in *Alnus* (French, Dickinson and Putwain 2006).

For Cu- and Pb-contaminated soils phytomanaged with incorporation of organic matter and annual crops with high shoot biomass, the sustainability of metal binding by organic matter and its influence on plant growth is questionable. The need for a second compost dressing (at the 5% w/w application rate) was investigated with potted soils collected in the Biogeco site five years after the first compost dressing and sunflowers were cultivated for five weeks. At both median and high total soil Cu (268 and 820 mg Cu kg<sup>-1</sup>), the second compost dressing enhanced (+27% and +25%) the maximum shoot length and shoot DW yield of sunflower plants. Such



beneficial effects were confirmed in field plots in 2013 and 2014 for both sunflower and tobacco, notably for the highest soil Cu contamination (Mench, unpublished results).

These studies point towards a key role of N and N-related biogeochemical activities in TE-contaminated sites, and the search for low C-footprint N fertilizers for a sustainable management of this type of area. In relation to this, soil organic amendments and fertilization increase soil microbial biomass and enzyme activity, and enhance N transformation rates (i.e. ammonification, nitrification), thus leading to reduced chemical assistance in the long-term (Lagomarsino *et al.* 2011).

## 6. NEGATIVE ASPECTS ASSOCIATED WITH THE INTRODUCTION OF NON-NATIVE PLANT SPECIES AND GRO TO MANAGE INVASIVE SPECIES

When selecting the plant species to be cultivated care must be taken to avoid the unnecessary introduction of non-native and particularly of invasive plant species. For example, the giant reed (*Arundo donax*) may be well adapted to a wide range in climates and suitable for plantation in contaminated soils but can be an invasive crop in certain areas, such as the Mediterranean area (Zegada-Lizarazu *et al.* 2010; Nsanganwimana *et al.* 2013). However, invasive plant species may already be present at TE-contaminated sites. For instance, at the Biogeco site, of a total of 91 species occurring on the site four are considered as invasive (*Cyperus eragrostis*, *Phytolacca americana*, *Senecio inaequidens*, and *Sporobolus indicus*) (Bes *et al.* 2010).

Japanese knotweed (*Fallopia japonica*) is a very tolerant, metal-excluding species found on many heavily contaminated (smelter) sites. Due to its invasive trait and despite its high

productivity, it is neither relevant for phytoextraction nor phytostabilization. Phytostabilization with SRC can be a strategy to avoid its further propagation. The first step is to weaken the plant by cutting it, then to sow a metal-tolerant grass at a high density, and finally to plant unrooted cuttings of willow or poplar to cover the plants. In such conditions, the Japanese knotweed is less competitive and its growth decrease represents a beneficial effect of phytostabilization in terms of ecological services. At the Phytosed Ec 1 site (Table 1), using this strategy a decrease in Japanese knotweed was visible after one year of monitoring and corresponded to a reduction in coverage of 27% of the surface area (Bert, unpublished results).

On the other hand, the invasiveness of plants native to one area (e.g. China) is not necessarily the same in a different climatic area (e.g. Europe). For example, *Elsholtzia splendens* is a Cu-tolerant plant growing on Cu deposits in China that could be grown on potted Cu-contaminated soils from the Biogeco site (Bes 2008). Its invasiveness in Southern France is unlikely since it flowers in November and there is no chance for this plant to complete the cycle and produce seed before the first frosts. The same applies for other plants such as *Pteris vittata* also used in greenhouse experiments at the Biogeco site, which has little chance to survive outside of the greenhouses. DiTomaso *et al.* (2013) concluded that the risks of invasion associated with cultivating switchgrass in riparian or wetland areas in California (USA) could be minimized by employing mitigation practices to limit propagule movement.

## 7. PREVENTIVE MEASURES, WEED CONTROL AND PESTICIDE USAGE

GRO implementation may fail due to a lack of proper weed control. Weeds normally dominate plant communities in the early stages of natural re-vegetation of TE-contaminated sites

as pioneer plants, and therefore weed control should be an integrative part of the phytomanagement, especially in the early stages of the site management. In conventional agriculture, weed control can be carried out via a combination of physical, chemical and biological techniques. However, feasible techniques for TE-contaminated sites have not been specifically designed, and their weed management may require modification through time as changes occur in the main physico-chemical properties of the soils, particularly with the increase in soil fertility. Chemical (e.g. herbicide) and mechanical (e.g. tilling, disking, mulching) weed control can be necessary, particularly in the early stages. For example, applying herbicides before plant harvesting in a phytoextraction field site (Leipzig, Germany) improved the phytoextraction of TE such as Cr, Ni and especially Zn by a factor of two to three in comparison with control plants, mainly due to enhanced transpiration (Claus *et al.* 2007).

Biological pest control by inclusion of plant species antagonistic of crop pests may be an option for reducing the use of agrochemicals in sites that are already highly contaminated so as to increase the speed of plant colonization and rapidly improve the habitat for microbial communities. Amount and timing of fertilizer applications (particularly N) are additional factors that affect the attractiveness of crops to pests. The introduction of spring crops into crop rotations implies the need for soil tillage in the spring which destroys weeds that emerged during winter (Chauvel *et al.* 2001). Rotational crop cultivars can act as natural pesticides. Following a particular crop with a taxonomically different crop (one not susceptible to the same pests) often eliminates pest problems and as mentioned above, can result in growth and yield increases (Stapleton and Bañuelos 2009).

At the Lommel site during the first growing season of SRC, two weed control actions were undertaken, the first in the middle of May and the second at the end of June. Between the twin rows (1.50 m space) weed control was done mechanically by means of a 1.40 m large full automatic lawnmower. Within the twin rows the glyphosate-based herbicide Roundup MAX™ (3% solution; 8L ha<sup>-1</sup>) was applied using a handmade protection device, to avoid damage to the young trees. At the Krummenhennersdorf site, five days after planting of poplars and willows in May 2005, the herbicide Flexidor (with the active agent Isoxaben; 1L ha<sup>-1</sup>) was applied to reduce weed infestation during the first growing season of SRC (Röhricht and Kiesevalter 2008). This selective pre-emergence herbicide effectively inhibits germination of dicotyledonous weeds for three months after planting. In some areas *Rumex obtusifolius* established and were treated with the glyphosate-based herbicide Glyfos® (100 mL ha<sup>-1</sup>). Additionally, Ratron® was used against field mouse (*Microtus arvalis*). Due to the location of the plantation adjacent to smaller forested areas, a fence was necessary to protect young plants against browsing by deer (Röhricht and Kiesevalter 2008). The use of pesticides can be forbidden at some contaminated sites (e.g. ISO14001). In such cases, weed control and plant management can be performed manually or by thermal weeding. In the case of SRC of willows and poplars, the use of horticultural cover or mulching is also recommended.

## FINAL REMARKS

Upscaling of GRO from greenhouse to field conditions clearly requires incorporating agronomical knowledge into the remediation process. The number of field case studies for assessing GRO is increasing in the literature; however, the number of successful cases is still

limited. More information can be found on plant selection and the comparison of various plant species or cultivars (for their growth and TE accumulation) but studies investigating the influence of management options are scarce. This review attempted to summarize the available information on aspects which are rarely considered such as soil management practices, crop rotation, intercropping/row cropping, planting methods and plant densities, harvest management, pest and weed control and irrigation management. Certain aspects such as tillage practices or ploughing methods are impossible to evaluate at a bench-scale. Other aspects, such as intercropping, show promise in terms of promoting plant biomass, TE accumulation and soil quality, but again these have primarily been assessed in pot experiments. It is clear that further field studies are greatly needed in which these practical aspects can be developed and optimized within a GRO framework. Applying suitable agronomic measures will be a crucial factor for enhancing the success of GRO application.

The use of bioaugmentation appears to be an effective means of improving plant growth, biomass production, and tolerance to TE excess. There is a large body of evidence demonstrating that bacteria, which are typically, found in association with TE-tolerating or -accumulating plants, can contribute to improve shoot TE removals and thus the efficiency and rate of phytoextraction (reviewed in Sessitsch *et al.*, 2013). However, these techniques have rarely been tested at a field scale and several issues still limit their upscaling to a field level (e.g. biosafety aspects, performance of inoculants under natural conditions and their persistence and competition capacity).

Based on case studies carried out within the EU FP7-funded GREENLAND project, several recommendations can be summarized for increasing the GRO success: (1) the initial

spatial variability in the total and labile TE pools should be well characterized in order to enable efficient installation and monitoring during long-term trials. The same is true for the distribution of the (labile) TE pools through the soil profile (and this is pivotal in the case of SRC). Soil (and plant samples) should be archived to facilitate any retrospective monitoring; (2) it is not recommended to up-scale directly from studies carried out at a bench-level (e.g. pot experiments) to large-scale site applications. At least one step on an intermediate scale should be conducted on site (e.g. several small plots of some 10 to 1000 m<sup>2</sup>) and if possible for more than one growth season. This is recommended so as to detect any potential failures due to long-term changes, such as ageing of soil amendments, inter-annual changes in climatic conditions, pest attacks, litter build-up and release of dissolved organic matter, changes in plant and animal communities, etc.). Additionally, in a best case scenario it would be better to compare in parallel the best GRO and conventional technique for this site (for better demonstrating the pros and cons, and having an immediate alternative in case of GRO failure; (3) soil conditions (e.g. regarding root penetration, water retention, organic matter content, nutrient supply, factors which may lead to plant toxicity) should enable plant growth, otherwise intervention (e.g. sub-soiling, soil amendments) is needed; (4) weed control is essential during the early establishment of plantations due to competition for resources; (5) multi-species/multi-cultivar/multi-clone plantations are recommended due to enhanced plant cover resistance against unknown or unexpected impacts which may otherwise lead to total plant loss and due to associated benefits related to pest control or biodiversity. The biomass production should be considered in line with its use by local conversion chains; (6) water supply vs. water requirements is vital during early plant establishment, and therefore irrigation should be considered; and (7) fencing or some

means of protection are recommended to reduce plant loss due to local wildlife herbivory, several clusters being better than only one large fence around the GRO field trial.

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**Table 1.** Characteristics of the field case sites included in the EU GREENLAND project (FP7-KBBE-266124).

Site	Arnoldstein	Bettwiesen	Biogeco	Freiberg	Krummenhennersdorf	Lommel	Phytosed Ec 1
Country	Austria	Switzerland	France	Germany	Germany	Belgium	France
GRO	<i>In situ</i> stabilization / phytoexclusion	Phytoextraction	(Aided) phytoextraction and phytostabilization	<i>In situ</i> stabilization / phytoexclusion	Phytoextraction	Phytoextraction	(Aided) phytostabilization
Plant species	Maize, barley cultivars	Somaclonal variants and motherlines of tobacco, mutant lines and controls of sunflower, and selected maize cv.s, winter fodder pea EFB33	Tobacco, sunflower, sorghum, willows, poplar, Miscanthus, vetiver, <i>Agrostis capillaris</i> , <i>Agrostis gigantea</i>	Winter oilseed rape (Visby, Lorenz); Winter wheat (Türkis, Tiger); Spring barley (Salome, Marthe)	Willows (Jorr, Sven, Tora, Tordis, Gudrun), poplars (Weser 6, Max 3, Hybride 275)	Willows, poplar, tobacco, sunflower	Willow (Inger, Tordis), <i>Deschampsia cespitosa</i>
pH <sub>(KCl)</sub>	5.0	6.8	6.3	4.6	4.9	5.8	-

## ACCEPTED MANUSCRIPT

OM (%)	2.6	3.6	1.4-3.4	4.6	2.5	32.8	31.0
CEC	5.0	32.8	2.9-4.8	22.9	8.4	6.2	23.5
	(cmol(+) kg <sup>-1</sup> )						
As	36.5±2.7	11.0±0.8	8.5±1.1	837.2±133.2	128±18	7.4±0.8	42.9±3.5
Cd	6.1±0.3	1.2±0.3	0.13±0.06	16.2±2.6	3.4±0.4	10.9±0.9	9.39 ±0.9
Cr	30.9±3.9	168±38	19.7±0.7	49.2±4.1	29.7±2.1	4.8±1.0	99.7±4.2
Cu	58.6±4.8	40.7±2.4	1259±379	92.6±55.8	28.3±4.5	26.7±1.5	110±7.2
Pb	791±70	59.5±10.2	21.0±2.2	1719.5±285.6	302±53	238±20	956±88.6
Zn	712±58	1698±411	53.3±20.2	542.1±112.7	255±38	682±64	6089±825

**Table 2.** Examples from the literature of studies assessing various woody crops for application in GRO (mainly as short rotation coppice).

Woody species	Genotype selection criteria	Study type	Soil characteristics	PTE (mg kg <sup>-1</sup> )	GRO assessed	References
<i>Alnus cordata</i> (Lois), <i>A. glutinosa</i> , <i>Salix atrocinerea</i> , <i>S. caprea</i> x <i>cinerea</i> x <i>viminalis</i> Calodendron, <i>Salix viminalis</i> (Jorum), <i>Populus deltoides</i> x <i>nigra</i> (Ghoy), <i>P. trichocarpa</i> (Trichobel)	Deep rooting systems Fast growth rates Tolerance of nutrient-poor soils	F	Dredged contaminated canal sediment	As, Cr, Cu, Pb, Zn, Ni	Phytostabilization	Hartley <i>et al.</i> 2011
<i>Salix alba</i> var. <i>alba</i> (Belders), <i>S. x rubens</i> var. <i>basfordiana</i> (Belgisch Rood), <i>S. viminalis</i> (Christina), <i>S. triandra</i> x <i>S. viminalis</i> (Inger), <i>S. viminalis</i> (Jorr), <i>S. dasyclados</i> (Loden), <i>S. schwerinii</i> x <i>S. viminalis</i> (Tora), <i>S. triandra</i> (Zwarte Driebast)	Metal accumulators	F	Smelter-contaminated agricultural soil; sandy soil pH 4.8 (limed to pH 6.6)	Cd, Zn (6.5/377)	Phytoextraction; removal rates of 72g Cd and 2.0 kg Zn ha <sup>-1</sup> y <sup>-1</sup> Planting density: 15,000 cuttings per ha	Van Slycken <i>et al.</i> 2013
<i>Salix schwerinii</i> x <i>S.</i>	High biomass	F	Dredged sediment	Cd	Phytoextraction (and	Delplanque <i>et al.</i> 2013

<i>viminalis</i> (Tora)	production		disposal site		biomass production)	
<i>S. viminalis</i> (Jorr)	Disease		pH 7.9; organic C		Planting density: 17,800	
<i>Salix schwerinii</i> x <i>S. viminalis</i> (Björn)	resistance (against rust)		22.8 g kg <sup>-1</sup> & N 1.99 g kg <sup>-1</sup>		cuttings per ha	
<i>Populus alba</i> var. <i>pyramidalis</i> (Bunge)		F	Cd-contaminated calcareous agricultural soils	Cd (29)	Phytoextraction	Hu <i>et al.</i> 2013b
			pH 7.5; OM content 15.3 g kg <sup>-1</sup>			
<i>Populus deltoides</i> × <i>Populus nigra</i> ( <i>P.</i> × <i>euramericana</i> (Dode) Guinier)		G	Cd-spiked commercial peat	Cd (40)	Phytoextraction (and Bioaugmentation)	Cocozza <i>et al.</i> 2014
<i>Populus alba</i>		Semi-F	Two TE-polluted soils affected by a mine spill; sandy loam texture; pH 3.0 and 7.4	As, Cd, Cu, Pb, Zn	Phytostabilization	Ciadamidaro <i>et al.</i> 2013; Ciadamidaro, Madejon and Madejon 2014
<i>S. smithiana</i> Willd. ( <i>Salix caprea</i> × <i>Salix viminalis</i> (BOKU 03 CZ-001))	Metal-accumulator	G	7 TE-contaminated soils	Cd, Pb, Zn	Phytoextraction	Puschenreiter <i>et al.</i> 2013
<i>Salix viminalis</i> x <i>Salix schwerinii</i> (Björn)	Metal-excluder	G (76 d)	Sandy (pH 5.84)	Hg (30)		Wang <i>et al.</i> 2005
<i>Salix viminalis</i>	Metal-accumulator	G	Calcaric regosol (pH 7.8)	Cd, Cu, Zn (1.76/547/666)	Phytoextraction	Hammer, Kayser and Keller 2003
		G	Fluvisol (pH 5.15)	Cd/Cu/Zn (2.49/227/1144)	Phytoextraction	Hammer, Kayser and Keller 2003
<i>Salix dasyclados</i> SW890129 (Loden),	S. d: Cd accumulator;	F	Sewage-sludge amended	Cd/Zn (41.6/2418)	Phytoextraction (SRC)	Maxted <i>et al.</i> 2007

<i>S. viminalis</i> (Sv-78101, Sv-78198 and Sv-78198), <i>S. caprea</i> x <i>cinerea</i> x <i>viminalis</i> Calodendron, <i>S. aurita</i> x <i>cinerea</i> x <i>viminalis</i> Rosewarne White <i>S. spaethii</i> Spaethii	S. v: Cd / Zn accumulators		agricultural soils			
<i>Populus nigra</i> var. <i>italica</i> <i>Paulownia tomentosa</i>	Rapid growth, High biomass, Stress tolerance	F	Industrial soil; TE- and hydrocarbon- contaminated; clay loam soil; pH 8.0- 8.8	Cd/Cu/Ni/Pb/ Zn (1.53- 3.35/137- 180/407- 619/133- 275/493-628)	Phytoextraction	Macci <i>et al.</i> 2013
<i>Salix pseudo-lasiogyne</i> , <i>S. koreensis</i> , <i>S.</i> <i>bracteosa</i> , <i>S. purpurea</i> var. <i>japonica</i> , <i>S. caprea</i> , <i>Populus alba</i> × <i>glandulosa</i>		G	Cd/Zn-spiked soils	Cd/Zn (112/260)	Phytostabilization	Han, Kim and Shin 2013
<i>Populus tremula</i>	Rapid growth, tolerance to various climatic conditions and high adaptability to a wide range of soils and presence of TEs	G	TE-contaminated soil affected by former Smelter; Calcaric Cambisol; pH 7.2; organic C 27.3 g kg <sup>-1</sup> and total N 2.1 g kg <sup>-1</sup>	Cd/Pb/Zn (24.3/3560/19 60)	Phytoextraction	Langer <i>et al.</i> 2012b
<i>Alnus glutinosa</i> , <i>Acer</i> <i>pseudoplatanus</i> , <i>Robinia</i> <i>pseudoacacia</i>		F	Former agricultural soils highly contaminated by Cd,	Cd/Pb/Zn (19.2/1000/11 94)	Aided-phytostabilization	Pourrut <i>et al.</i> 2011

			Pb, Zn; pH 7.3; organic C 23.8 g kg <sup>-1</sup>			
<i>Salix caprea</i> (Boku 04 CZ-024)		G	Moderately TE-contaminated soil; pH 7.5; organic C 38.5 g kg <sup>-1</sup>	Cd/Zn (4.9/608)	Phytoextraction (and bioaugmentation)	De Maria <i>et al.</i> 2011
<i>Populus trichocarpa</i> x <i>P. deltoides</i>		F	Dredged sediment soil; nutrient-rich clay loam soil; pH 7.5	Cd/Cr/Pb/Zn (9.4/191/173/986)	Phytoextraction	Lettens <i>et al.</i> 2011
<i>P. trichocarpa</i> x <i>P. balsamifera</i> (Balsam Spire) <i>P. trichocarpa</i> x <i>P. deltoides</i> (Beaupre, Hazendans, Hoogvorst, Raspalje and Unal) <i>P. trichocarpa</i> (Columbia River, Fritzi Pauley and Trichobel) <i>P. deltoides</i> x <i>P. nigra</i> (Gaver, Gibecq and Primo) <i>P. nigra</i> (Wolterson)	High biomass TE-accumulator	F	Old household waste disposal site; heavy clay-loam; pH 7.3-8.1	Cd/Mn/Zn (0.056 1.6/1096 210/606486)	Phytoremediation Planting density: 10,000 cuttings ha <sup>-1</sup>	Laureysens <i>et al.</i> 2004
<i>Salix fragilis</i> (Belgisch Rood) <i>S. triandra</i> (Noir de Villaines)	Easy to propagate, fast growing, metal tolerant, perennial crops, extensive root system and high	F	Dredged sediments	Cd/Cr/Zn (4.6-9.4/67-138/361-475)	Phytoextraction	Mertens <i>et al.</i> 2006

		evapotranspiration that can stabilize pollutants			
<i>P. deltoides x nigra</i>	F	Dredged sediment disposal site	contained 8.36 10.6 mg Cd/kg, 9876 1279 mg Zn/kg and 90061100 mg Mn/kg		Vandecasteele, Buysse and Tack 2006
<i>Populus tremula</i>	F	Metal-contaminated wastes derived from pyrite ore roasting	As/Co/Cu/Pb/ Zn	Phytostabilization	Vameralli <i>et al.</i> 2009
<i>Populus deltoides x maximowiczii</i> (Eridano) <i>P. x euramericana</i> (I-214)	F	Industrial organic wastes	Cd/Cr/Cu/Zn (4.4/14800/10 2/ 10300)	Phytoextraction/ phytostabilization	Sebastiani, Scebba and Tognetti 2004
<i>Alnus cordata</i> <i>A. glutinosa</i> <i>A. incana</i> <i>Populus deltoides x nigra</i> (Ghoy) <i>P. trichocarpa</i> (Trichobel) <i>Salix atrocinerea</i> <i>S. caprea x cinerea x viminalis</i> (Calodendron) <i>S. fragilis</i> <i>S. viminalis</i> (Jorunn) <i>S. viminalis x burjatica</i> (Ashton Stott)	F	Canal sediment	As, Cd, Cr, Cu, Pb and Zn (420/20/980/7 40/1445/4285)	Phytoremediation Planting density: 26,700 plants ha <sup>-1</sup>	King <i>et al.</i> 2006



<i>S. viminalis x caprea</i> (Sericans)						
<i>S. viminalis x schwerinni</i> (Tora)						
<i>Salix viminalis</i> (Orm)		F	Dredged sediment disposal site	Cd/Cu/Pb/Zn (mineral oil, PAHs)	Phytoremediation	Vervaeke <i>et al.</i> 2003; Meers <i>et al.</i> 2005
<i>Alnus incana</i> <i>Betula pendula</i> <i>Fraxinus excelsior</i> <i>Salix viminalis</i> <i>Sorbus mougeotii</i>	<i>S.v &amp; B. p:</i> Cd and Zn phytoextraction ability	F	Former landfill; organic C 0.06 g kg <sup>-1</sup> , pH 7.4	Cd, Cu, Zn (1.8/557/620)	Phytoremediation	Rosselli, Keller and Boschi 2003
<i>Larix eurolepis</i> <i>Betula pendula</i> <i>Alnus incana</i> <i>Populus deltoides x nigra</i> (Ghoy) <i>Populus trichocarpa</i> (Trichobel) <i>Salix caprea x cinerea x viminalis</i> (Calodendron) <i>Salix viminalis</i> (Orm) <i>Salix caprea x viminalis</i> (Coles) <i>Salix burjatica</i> (Germany) <i>Salix viminalis x schwerinni</i> (Tora)	Based on UK Forestry Commission guidance	F	Five brownfields (landfill, industrial waste, sewage sludge); clay loams or silt loams with 5.6-13% OM, 0.14-0.3% total N, pH 5.4-7.4	As/Cd/Ni/Pb	SRC and biomass production	French, Dickinson and Putwain 2006
<i>Betula pendula</i> <i>Alnus cordata</i> <i>Alnus incana</i> <i>Alnus glutinosa</i> <i>Crataegus monogyna</i>		F	Cu refinery site; pH 6.4-8.4	Cd/Cr/Cu/Ni/Pb/Zn	Phytoremediation	Dickinson 2000

<i>Salix caprea</i>						
<i>S. purpurea</i>						
<i>S. caprea</i>						
<i>S. alba</i>						
<i>S. rubens</i>						
<i>S. viminalis</i>						
<i>S. fragilis</i>						
<i>S. calodendron</i>						
<i>Populus alba</i>	Cd-, Zn- accumulator	F	Area affected by mine spill; neutral or slightly alkaline soils; loamy sand to silty clay	As/Cd/Cu/Pb/ Tl/Zn	Phytoremediation	Dominguez <i>et al.</i> 2008
<i>Salix viminalis</i> (78183)		F	Sewage sludge- amended agricultural soils	Cd	Phytoextraction (SRC)	Klang-Westin and Eriksson 2003
<i>Acer campestre</i>		F	Sites affected by Zn smelter	Cd/Pb/Zn	Phytoremediation	Migeon <i>et al.</i> 2009
<i>Acer pseudoplatanus</i>						
<i>Alnus glutinosa</i>						
<i>Betula pendula</i>						
<i>Fraxinus excelsior</i>						
<i>Prunus avium</i>						
<i>Quercus robur</i>						
<i>Robinia pseudoacacia</i>						
<i>Salix alba</i>						
<i>Salix caprea</i>						
<i>Salix purpurea</i>						
<i>Populus deltoides</i> ×						
<i>Populus nigra</i> P. <i>tremula</i>						
× P. <i>alba</i>						
P. <i>tremula</i> × P.						
<i>tremuloides</i>						
P. <i>trichocarpa</i> × P.						

<i>deltooides</i>						
<i>Salix caprea</i> <i>S. purpurea</i> <i>S. fragilis</i> <i>Populus tremula</i> <i>P. nigra</i> <i>Betula pendula</i>		F	Cd/Zn-contaminated soils across Central Europe	Cd/Zn	Phytoextraction	Unterbrunner <i>et al.</i> 2007
<i>Salix caprea</i> (BOKU 01 AT-004) <i>S. fragilis</i> (BOKU 01 CZ-001) <i>S. × smithiana</i> ( <i>S. caprea</i> × <i>S. viminalis</i> BOKU 03 CZ-001) <i>S. × dasyclados</i> ( <i>S. caprea</i> × <i>S. cinerea</i> × <i>S. viminalis</i> BOKU 03 CZ-002)	Metal-accumulators	G	Agricultural soils affected by former Zn/Pb smelter; loamy sand; pH 6.5-7.5	Cd/Zn (4.0-13.4/490-955)	Phytoextraction	Wieshammer <i>et al.</i> 2007
<i>S. viminalis</i> , <i>S. caprea</i> , <i>Populus nigra</i> , <i>Amorpha fruticosa</i>	Cu-excluder	F	Wood preservation site	Cu (674)	Aided phytostabilisation	Bes <i>et al.</i> 2007

Study type: F, field experiment; G, greenhouse based;

**Table 3.** List of Cd-excluding crop cultivars\*

Crop	Cultivar	Tolerance to TE	Reference
Maize	cv. Fuxxol cv. Morisat cv. Acces cv. Die Samanta cv. Antonio cv. Atletico cv. Fransisco cv. LaFortuna	Cd	Friesl-Hanl <i>et al.</i> 2011
Spring barley	cv. Streif cv. Sebastian cv. Sunshine cv. Auriga cv. Bodega cv. Ursa cv. Pasadena cv. Xanadu cv. Hanka cv. Felicitas cv. Messina	Cd	Friesl-Hanl <i>et al.</i> 2009; Klose 2013; BfUL/LfL 2002-2013; Spiegel <i>et al.</i> 2009
Spring durum	cv. Astradur cv. Rosadur cv. Floradur cv. Helidur	Cd	Wenzel <i>et al.</i> 1996; Spiegel <i>et al.</i> 2009
Winter durum	cv. Inerdur cv. Prowidur cv. Aradur cv. Superdur	Cd	Spiegel <i>et al.</i> 2009
Winter rey	cv. Agronom cv. Ero cv. Kier cv. Picasso cv. Nikita		Spiegel <i>et al.</i> 2009
Winter wheat	cv. Batis cv. Skagen cv. Türkis cv. Orkas	Cd	Klose 2011, 2013; BfUL/LfL 2002-2013 Wenzel <i>et al.</i> 1996; Spiegel <i>et al.</i> 2009

	cv. Esket	
	cv. Julius	
	cv. Xenos	
	cv. Josef	
	cv. Fridolin	
	cv. Tommi	
Potato	cv. Ditta	Spiegel <i>et al.</i> 2009
	cv. Nicola	

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\*, the commercially available range of cultivar seed changes yearly, e.g. some cultivars disappear and others enter the market.

Table 4. Fertilizer management trials: effects on plant growth and yield, TE accumulation and soil TE behaviour.

Crop type	Soil type	Fertilizer form	Effect on TE behaviour in soil	Effect on plant biomass, TE accumulation and removal	References
<b>INORGANIC FERTILIZATION</b>					
<i>Annual plants</i>					
Winter wheat cv.s	AG	Nitrate of lime (mostly Ca(NO <sub>3</sub> ) <sub>2</sub> ; rates of 75-175 kg N ha <sup>-1</sup> )	Possible increase in Ca <sup>2+</sup> which caused increased [Cd <sup>2+</sup> ] in soil solution	Grain [Cd] increased with increasing N rate. Grain [N] and [Cd] correlated.	Wangstrand, Eriksson and Oborn 2007
Sceptre durum wheat	AG	Urea (0-800 mg N kg <sup>-1</sup> )	Solution [Cd] and DTPA-extractable soil [Cd] increased with increasing N rate	Plant [Cd] increased with increasing N rate (up to 800 mg kg <sup>-1</sup> ); yield increased with N rate (only up to 200 mg kg <sup>-1</sup> )	Mitchell, Grant and Racz 2000
Durum wheat	AG	(NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub> (20-80 mg P kg <sup>-1</sup> )	Soil solution [Cd] unaffected	Plant yield increased with P rates; grain [Cd] unaffected	Gao <i>et al.</i> 2011
Durum wheat	AG	NH <sub>3</sub> ; urea; NH <sub>4</sub> NO <sub>3</sub>	Possible increase in soil solution [Cd]	Grain [Cd] lower in clay loam soil compared to sandy loam soil; N fertilizers increased grain [Cd] and decreased grain [Zn]	Gao <i>et al.</i> 2010
Malting barley cv.s	AG	NH <sub>4</sub> NO <sub>3</sub> ; (NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub> ; KCl	Possibly related to increase in soil solution [Cd]	NH <sub>4</sub> NO <sub>3</sub> increased grain [Cd] and yield when soil nitrate levels were low; (NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub> and KCl tended to increase grain [Cd]	Grant, Bailey and Therrien 1996
Sunflower	AG	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , K <sub>2</sub> SO <sub>4</sub> ,	NH <sub>4</sub> NO <sub>3</sub> enhanced labile	NH <sub>4</sub> NO <sub>3</sub> increased shoot	Nehnevajova

			and and $\text{NH}_4\text{NO}_3$ , KCl, as $60\text{kg N ha}^{-1}$ , $50\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$ , $400$ $\text{kg K}_2\text{O ha}^{-1}$	Zn & Pb, $(\text{NH}_4)_2\text{SO}_4$ enhanced labile Cd in top soil	Cd uptake, $(\text{NH}_4)_2\text{SO}_4$ increased shoot Zn & Pb uptake	<i>et al.</i> 2005
Sunflower	AG		$\text{Ca}(\text{NO}_3)_2$ , $\text{CO}(\text{NH}_2)_2$ (urea), $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_4)_2\text{S}_2\text{O}_3$	$\text{NH}_4^+$ -based treatments decreased soil pH	Shoot [metal] higher in $\text{NH}_4^+$ -fed plants	Zaccheo, Crippa and Pasta 2006
Radish	AG		$\text{NH}_4\text{NO}_3$ ; $\text{KNO}_3$	$\text{NH}_4^+$ -based treatments increased soil solution [Cd, Zn]; decreased soil solution pH	Increased shoot [Cd]	Lorenz <i>et al.</i> 1994
Water spinach, radish, oat	AG		$\text{NH}_4\text{NO}_3$ ; $\text{KNO}_3$	Increase in soil solution [Cd, Ni, Zn] but plant species-specific (soil pH unaffected)	Increased [Cd, Ni, Zn] in water spinach and radish	Kashem and Singh 2002
Potato	AG		$(\text{NH}_4)_2\text{H}_2\text{PO}_4$ ; $\text{K}_2\text{SO}_4$ ; KCl	-	Tuber [Cd]: $\text{K}_2\text{SO}_4 <$ KCl (by 20-30%)	Sparrow, Salardini and Johnstone 1994
Sorghum, sunflower	AG		Urea; $\text{CaHPO}_4$ , $\text{K}_2\text{O}$	-	Increased crop yield; TE accumulation unaffected; TE removal increased	Marchiol <i>et al.</i> 2007
Sunflower, tobacco	AG		$(\text{NH}_4)_2\text{SO}_4$ and $\text{NH}_4\text{NO}_3$ ,	$\text{NH}_4\text{NO}_3$ stimulated labile Zn, Cd, Pb soil concentration and pH more efficient than $(\text{NH}_4)_2\text{SO}_4$	$\text{NH}_4\text{NO}_3$ enhanced tobacco Cd, Zn, Pb shoot uptake more effi- cient than $(\text{NH}_4)_2\text{SO}_4$	Herzig <i>et al.</i> 2005
Sunflower, tobacco	AG		Elemental S	S additions caused calcite dissolution and soil acidification	Increased Cd/Zn uptake	Fässler <i>et al.</i> 2010; Fässler <i>et al.</i> 2012
Spring barley	M		NPK fertilizers (single or double	-	Increased yields, particularly double doses	Gilewska 1991

		doses)		(for 10 yr period)	
<b>Hyperaccumulators</b>					
<i>Alyssum murale</i>	S	NH <sub>4</sub> NO <sub>3</sub> ,	-	N fertilization increased plant biomass; shoot [Ni] unaffected but [Ni] removal increased	Li <i>et al.</i> 2003
<i>Alyssum corsicum</i>		Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> , KCl, K <sub>2</sub> SO <sub>4</sub>			
<i>Alyssum bertolonii</i>	S	NPK fertilizer	-		
<i>Alyssum murale</i>	S	NH <sub>4</sub> NO <sub>3</sub> , K <sub>2</sub> SO <sub>4</sub> , Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	-	Increased plant biomass and coverage; Ni removal increased (reaching 25 kg Ni ha <sup>-1</sup> on fertilized plots cf. to 3 kg Ni ha <sup>-1</sup> on unfertilized plots)	Bani <i>et al.</i> 2007; Bani <i>et al.</i> 2014
<b>Grass species</b>					
<i>Phragmites australis</i>		NPK fertilizer	-	Two-fold increase in plant biomass; 2- to 3-fold increase in Ni/Zn removal	Claus <i>et al.</i> 2007
<i>Agropyron spp.</i> , <i>Atriplex canescens</i>	M	NPK fertilizers	-	Aided plant establishment and biomass production of coal mine wastes	Miller 1979
Mixed perennial grasses (+legumes, +sunflower)	M	NPK fertilizers	-	NPK-fertilized plots showed enhanced plant yields as compared to unfertilized plots	Sorenau 1983
<i>Bouteloua gracilis</i> , <i>Atriplex canescens</i>	M	Urea, P <sub>2</sub> O <sub>5</sub> , (+ alfalfa residues)	-	Increased yields and soil biochemical activity of re-vegetated spoils cf. to	Fresquez and Lindemann 1982



	Ryegrass (+clover)	M	Urea, Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> , KCl	-	unfertilized spoils Increased yields after 5 yrs	Ross <i>et al.</i> 1992b
<b>SRC</b>	<i>Salix</i>	AG	N fertilizers	-	Two-fold increase in biomass production	Aronsson, 2011
<b>ORGANIC FERTILIZATION</b>						
<b>Annual plants</b>						
	Sunflower Sorghum	AG	Cow manure	-	Increased crop yield; TE accumulation unaffected; TE removal increased	Marchiol <i>et al.</i> 2007
	Maize, sainfoin, oat	M	Farmyard manure, Anaerobically digested sludge	-	9 years of fertilization increased crop production. Maize yields after sludge applications (2-3 yrs) were comparable to those obtained with equivalent NPK fertilization.	Bлага <i>et al.</i> 1991
	Alfalfa	M	Topsoil	-	Increased yields (to levels obtained in unpolluted soils)	Shcherbakov, Uskov and Kosolapova 1991
<b>Hyperaccumulators</b>						
	<i>Thlaspi caerulescens</i>	M	Composted MSW	Decrease Cd/Zn availability of mine spoils	Increased biomass; Total Cd/Zn removal increased	Álvarez-López <i>et al.</i> , unpublished data
<b>Grass species</b>						
	<i>Agrostis capillaris</i> cv. Highland	M	Composted MSW	Decreased Cu availability of mine spoils	Aided plant establishment and	Álvarez-López <i>et al.</i> ,

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<i>Bouteloua gracilis</i> , M <i>Atriplex canescens</i>	Alfalfa residues, sewage sludge, topsoil	-	increased biomass production Increased yields and soil biochemical activity but less than with inorganic fertilizers	unpublished data Fresquez and Lindemann 1982
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AG, agricultural soil; S, serpentine soil; M, mine-soils

**FIGURE LEGENDS**

Figure 1. (a) ó (b) Winter wheat cultivars planted at the Krummenhennersdorf (Halsbrücke) site (Germany) where *in situ* stabilization combined with phytoexclusion was implemented (two cultivars each in a crop rotation of rapeseed, winter wheat and summer barley). In the background the chimney stacks of the metallurgic industry can be seen. On the left: *Triticum aestivum* cv. Tiger (relatively high accumulation potential for Cd). On the right: *Triticum aestivum* cv. Türkis (low accumulation potential for Cd). (c ) ó (e) Short rotation coppice established with poplar clones öMax 3ö (*Populus nigra* x *P. trichocarpa*) ,öHybride 275ö (*P. maximowiczii* x *P. trichocarpa*) and öWeser 6ö (*P. trichocarpa*) were planted in 2005 and (f) Willow clone öJorrö (right) and clone öSvenö (left) planted in 2005.

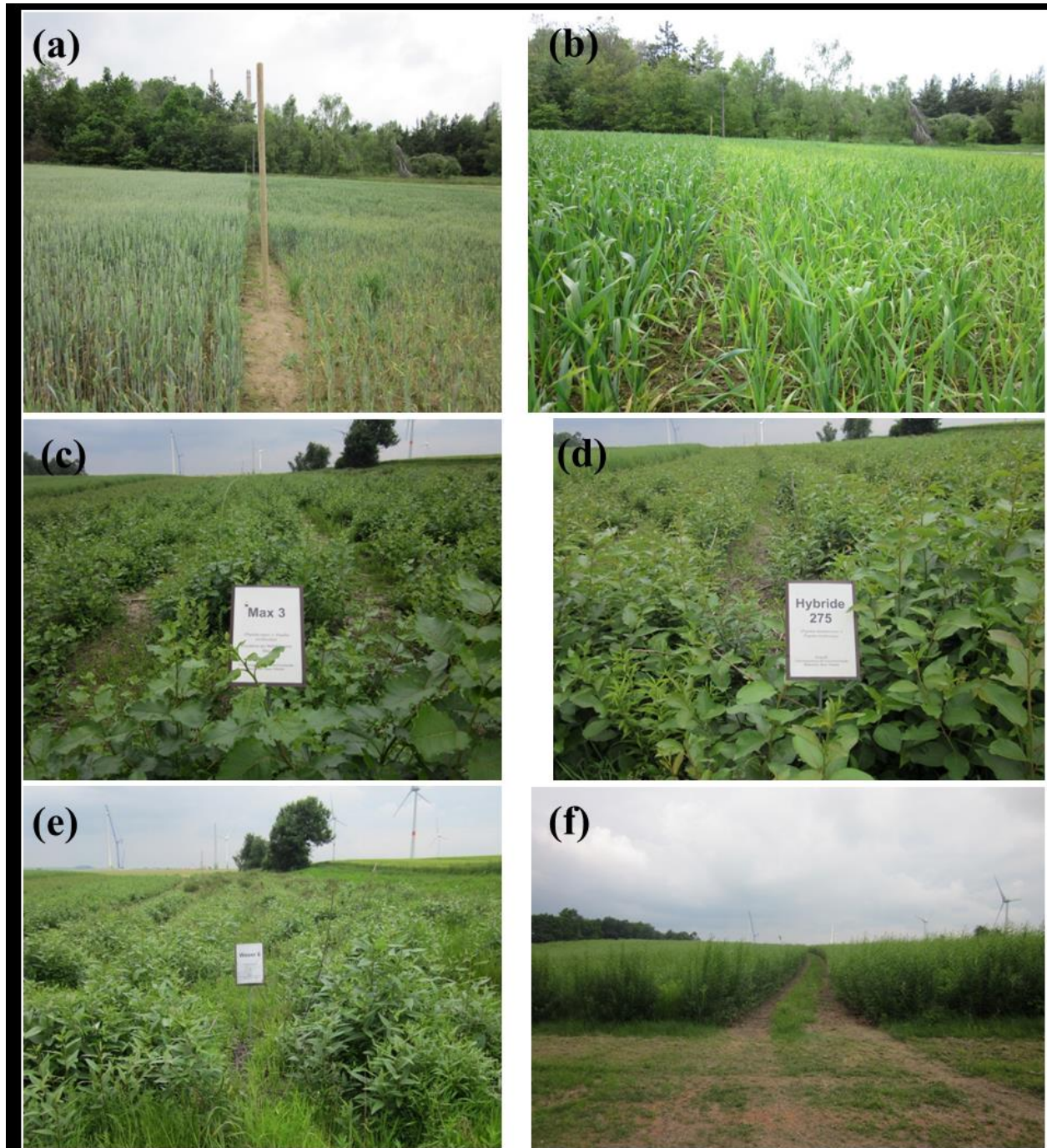


Figure 2. (Aided) phytostabilization and phytoextraction options were implemented at the Biogeco site (France). On the left (a) unmycorrhizal poplar and willows in June 2013 (7 yr), and on the right (b) ectomycorrhizal poplar and willows in June 2013 (7 yr). Photos (c-f) show sunflower (in 2009), tobacco (in 2010), sunflower, tobacco and *Miscanthus* (in 2013) and vetiver (in 2012) also growing at the Biogeco site.

