# PROJECT FINAL REPORT

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# 1. Executive summary

Soil contamination by trace elements (TE) is still one of the major environmental problems in Europe. Consequently, soil pollution has been listed by the European Commission as one of the eight major threats to European soils. Soil remediation is therefore an urgent requirement for maintaining one of the most important natural resources for maintaining safe agriculture for producing food and fodder, but also biomass for various other purposes, e.g. production of energy or raw materials such as fibre.

Conventional soil remediation technologies are very often costly, energy intensive and may negatively affect the soil quality and functions. However, gentle soil remediation options (GRO) using plants, associated microbes and soil amendments may serve as an environmentally friendly and cost-efficient alternative. Although the effectiveness of GROs has been demonstrated in the lab and greenhouse scale, information on long-term efficiency in the field was still missing. Furthermore, valorisation options for GRO biomass and methods to assess GRO efficiency were hardly available and GROs were hardly considered in decision support tools and remediation guidelines.

The main objectives of the GREENLAND project were to overcome the above listed reasons hindering the wider application of GROs. A network of 16 case studies on 13 long-term field experiments in Europe has been established to evaluate and compare the long-term effectiveness of GROs, including TE removal by phytoextraction, or TE immobilisation by (aided) phytostabilisation and in situ stabilization/phytoexclusion. The promising results were summarized in success stories and published together with a detailed guideline for GRO implementation. These instructions also include tools for enhancing the GRO efficiency by choosing more efficient plants, associated microorganisms, mineral and/or organic soil amendments, according to the site-specific conditions. Since GROs often mainly affect the labile TE pool in soils, specific soil tests are required for measuring GRO efficiency. The GREENLAND best practice guidance handbook provides specific instructions for the assessment of GRO progress and success.

GREENLAND has demonstrated that GRO biomass can be valorised in several ways, e.g. by combustion, anaerobic digestion and pyrolysis, thereby providing energy, but also raw material for further use, e.g. polymetallic catalysts for chemo-catalytic processes.
In order to enable stakeholders and decision makers deciding for GROs, a decision support tool (DST) has been developed by GREENLAND. This DST contains on the one hand a technical part that allows selecting the most suitable GRO for a specific site, but on the other hand it also provides specific instructions for stakeholder engagement, which is a key requirement for the optimal application of sustainable remediation strategies and in site regeneration more widely.

The DST and the best practice guidance handbook are available on www.greenland-project.eu.

## 2. Description of project context and objectives

Rapid urbanization and industrial development has caused serious environmental problems, leading to **contamination of soils** in many areas across Europe. Soil is a non-renewable resource, performing vital functions in the biosphere and acting as a basis for the majority of human activities (biomass and food production; storage and cycling of nutrients; water regulation; C sequestration and storage; etc.). The European Environment Agency (EEA) estimates that **remediation** is required for approximately 250,000 sites across Europe and that more than 80,000 sites have been cleaned up during the last 30 years in EU countries for which data on remediation are available. Among the most common harmful contaminants are toxic trace elements (also commonly called heavy metals), present in excess at 37% of the contaminated site.

Soil contamination due to trace elements (TE) in excess has been identified by the European Commission (EC) as one of the eight **major threats to European soils**. Soil contamination alters soil quality and functions, and can negatively affect water quality, biodiversity, food security and human health. As part of an initiative to address this problem the EC adopted a Soil Thematic Strategy (COM(2006) 231) and proposed a Soil Framework Directive (COM(2006) 232) with the objective of developing sustainable technologies for soil protection and remediation across the EU. However this proposal has not yet reached full political agreement among the Member States. Mitigation of pollution and remediation of soils will be crucial if European society is to ensure that soil remains one of its key natural resources. **Remediation of soils** has already been applied for soil clean up, but there have been wide variations between Member States. It has been estimated that the turn-over of the soil remediation industry in EU-27 amounted to €5.2 billion, of which 21.6% was spent in Germany, 20.5% in the Netherlands, and 5.9% each in France and the United Kingdom (COM(2012) 46 final). Estimated clean-up costs for contaminated soils across Europe are between €59 and €109 billion (COM(2012) 179 final). Paganos et al. (2013; http://dx.doi.org/10.1155/2013/158764) estimated that the management costs for contaminated sites in the EU are about 6 billion € annually.

Over the years several technologies have been developed for reducing risks posed by polluted soils. The conventional option for remediating TE contaminated soils has been through "hard" engineering approaches such as excavation and removal (often called "dig and dump"). However, many of these techniques are cost and energy intensive and often destroy soil structure and functions. As an alternative, **gentle remediation options (GRO)** have been developed and proposed as a cheap and environmentally friendly alternative. GROs include technologies based on the use of plants, associated microbes, and soil amendments for removing or immobilizing TEs in contaminated soils. Although the GRO efficiency has often been demonstrated in bench scale or greenhouse experiments, results from long-term field experiments were still limited. In order to demonstrate the GRO efficiency in real world conditions and to gain information on associated issues (e.g. biomass use, GRO assessment and improvement), the GREENLAND project has been initiated in January 2011.

GREENLAND has established, integrated and/or extended **16 case studies on 13 long-term field trials** across Europe, covering different environmental conditions (soil, climate, etc.) and different pollution scenarios. Based on this network of case studies, the main objectives of GREENLAND were as follows:

- **GRO option appraisal in the field – comparison of GRO efficiency and progress in large-scale and long-term field experiments under different conditions**
    - Assessing the performance of most promising GRO at large field scale using the network of the GREENLAND case studies
    - Designing, preparing, improving and applying new and or optimised GROs
    - Optimising the biomass production for various valorisation options and related ecological and financial returns

- **Assessment of valorisation options for biomass harvested on GRO sites**
    - Reviewing existing processes and types of biomass used in these processes
    - Compiling information on current and under development processes for biomass cultivated on TE contaminated lands
    - Testing the feasibility of using the various types of plant biomass collected from the case study sites, in existing or under development processes to assess advantages and limitations regarding technical aspects, regulations, acceptance and costs.

- **Development of a tool set for monitoring the progress and success of GRO**
    - Selecting/harmonising methods assessing the bioavailable/bioaccessible TE fractions among the GREENLAND case studies
    - Selecting methods to be used as indicators for GRO success and as sustainability monitoring tools.

- **Improving GRO through selection of plants and associated microbes and modification in soil trace element bioavailability**
    - Selecting plant species, varieties, cultivars or clones for highest TE resistance, TE extraction potential, and/or biomass production.
    - Improving plant performance and/or TE accumulation-exclusion potential using (a) conventional agronomic practices or (b) biotechnological approaches (microbial inoculants).
    - Identifying effective soil amendments and/or amendment-microbial inoculant combinations, for TE immobilisation (phytostabilisation).

- **Development of implementation guidance and decision support**
    - Preparation of a (multi-lingual) best-practice guidance document for the application of GRO at field-scale (including appraisal of the various options available, evaluation of large scale field trials, analysis of valorisation potential, and suggested methods and monitoring)
    - Development of guidelines for stakeholder participation, engagement and empowerment when implementing GRO
    - Developing and evaluating a decision support tool, focussed on GRO, which can be integrated into existing, well-established and utilised (national) DSTs / decision-frameworks

3. description of the main S&T results/foregrounds (not exceeding 25 pages),

## 3.1 Sustainable management adapted to trace element-contaminated sites and deployment of GRO at field scale

The GREENLAND network of field sites is a cross-European network of metal(loid)-contaminated sites where the efficiency of phytomanagement strategies has been investigated on a medium- to long-term, under various contaminant (trace element) types and loadings and soil and climatic conditions, with various plant species and cultivars. The main S&T results / foregrounds delivered by WP1 of the GREENLAND project are summarized in relation to WP deliverables and are more detailed in the appendix of this document, as well as success stories and datasheets of field trials available on the GREENLAND website (www.greenland-project.eu).

### 3.1.1. Remediation option appraisal

The general scheme for the remediation and phytomanagement of trace-element contaminated soils (TECS) comprises four stages: (1) risk assessment, (2) option appraisal, (3) implementation of remediation strategy and (4) phytomanagement (including biomonitoring and maintenance). Nine partners were deploying GRO at field scale. Five main types of historically contaminated sites were investigated, under different climatic and soil conditions, with either diffuse contamination on a large area (generally with agricultural soils) or local contaminations at mining sites, industrial facilities and landfills (with technosols at several sites) (Tab. 3.1.1). Case studies were categorized using a bioassay battery (with WP3). Five exposure patterns were defined based on main contaminants involved in pollutant linkages: Cu (Touro, Biogeco), Cd/Zn (Pb) (Lommel, Bettwiesen, Phytosed, Phytoagglo, Arnoldstein, Piedrafita, Högbytorp, Freiberg), As (Reppel, Jales, Freiberg), Cr/Mo (Rive de Gier), and metal/PAHs (Biogeco, Chaban-Delmas, Phytoagglo, Borifer).

**Tab. 3.1.1 Summary of the Greenland network investigating GRO in long-term field trials**

| Partner | Sites | Country | Sources | GRO | Main contaminants |
|---|---|---|---|---|---|
| ***Landfill*** | | | | | |
| PT-F | Bettwiesen | Switzerland | former hot dip Zn galvanizing plant | 1 | Cd, Zn, Pb |
| IUNG | Piekary | Poland | Cd/Zn/Pb tailings | 3 | Zn, Cd, Pb |
| INRA | Chateauneuf | France | steel mill wastes | 3 | Cr, Mo, Zn, Ni, Cu, Pb |
| ***Atmospheric depositions on a large agricultural area*** | | | | | |
| HAU | Lommel | Belgium | Zn/Pb smelter | 1 | Cd, Zn, Pb |
| AIT | Arnoldstein | Austria | Zn/Pb smelter | 3 | As, Cd, Zn, Pb |
| LfULG | Freiberg-Halsbrücke | Germany | Zn/Pb smelter | 1, 2 | As, Cd, Pb |
| HAU/INRA | Reppel | Belgium/France | As refinery | 1 | As, Zn, Pb, Cd |
| ***Wood preservation facility*** | | | | | |
| INRA | Biogeco | France | wood preservative | 1, 2, 4 | Cu, Cu/PAHs |
| ***Mine, tailings*** | | | | | |
| CSIC | Touro | Spain | Cu mining | 1, 2 | Cu |
| CSIC | Piedrafita, Rubiais | Spain | Pb/Zn mining | 1 | Zn, Pb, Cd |
| INRA | Jales | Portugal/France | Au mining | 2, 3 | As, Zn |
| ***Technosols, other sources*** | | | | | |
| SLU | Högbytorp French trial | Sweden | Irrigation with landfill leachates | 1 | (Cd), Cr, Zn |

| | | | | | |
|---|---|---|---|---|---|
| INERIS | Phytosed ech 1 | France | dredged sediments | 1, 2 | Zn, Cd, Cu, Cr, Pb, Mo |
| INERIS | Phytagglo | France | brownfield | 1, 4 | Zn, Cd, PAHs |
| INRA | Chaban Delmas | France | embankments, harbor facilities | 2, 4 | Zn, Pb, Cu, Cd, Ni, As, Hg, PAHs |
| INRA | Borifer | France | metal surfacing | 2, 4 | Zn, Pb, Cu, Ni, As, Hg, PAHs |

1: (aided) phytoextraction, 2: (aided) phytostabilization, 3: in situ immobilization/phytoexclusion, 4: rhizodegradation

### 3.1.1.1. Setting of conceptual models

Conceptual models (CM) were built for all sites (n=16) summarizing main sources of soil contamination, exposure to TE and soil ecotoxicity, biological receptors such as plant and microbial communities, initial pollutant linkages and risks on site and nearby. At five sites, vertical migration from the topsoil as well as wind erosion were considered. Clusters with different soil ecotoxicity were defined at most sites. Diverse physico-chemical parameters, soil and technosol types were of concern, from acid sandy soils to calcareous soils and alkaline technosol. Mixed contamination (TE and organic contaminants such as PAHs) was taken into account at 4 sites. Climatic conditions varied from cold climate and short summer (Sweden) to Mediterranean climate and dry summer (Spain).

Different end land uses were considered: landscaping, recreation area, production of annual crops for (non-food) plant-based feedstock and biosourced chemistry, production of metal-excluder crops (grasses, cereals), production of wood from short rotation coppice (SRC) and tree planting in line with eco-technologies. .

### 3.1.1.2. Risk assessment – pollutant linkages

Spatial distribution of soil contaminants, soil physico-chemical parameters, labile soil TE and ecotoxicity were characterized (with WP3). Initial and residual risks and pollutant linkages were quantified, in line with the GRO implementation and their (bio)monitoring. The TE concentrations in harvested plant biomasses were determined at each harvest for all sites, and were compared with the legislation, common values, and the needs of local conversion chains. Concentrations of TE in leachates from the topsoil, changes in labile TE pools in the soils and residual risks for plants and microbial communities were generally monitored over four years. Organization of plant communities and interspecific variability of TE concentrations in plant parts were investigated.

### 3.1.1.3. Option appraisal

This stage aims at establishing which remediation option, or combination of options, can alleviate all pollutant linkages that present an unacceptable risk at the site. It includes: identification of feasible remediation options, detailed evaluation of options, and developing the remediation strategy. The Greenland project added another aim: the implemented GRO must improve the ecosystem services, notably provisioning services through biomass production for the bio-economy and other ecosystem services such as carbon sequestration, recycling of organic matters, water filtration, quenching of soil erosion and restoration of plant-microbe communities, without generating wastes and pollutant linkages. The main concerns were to ensure that remediation option criteria selected for the soil are protective for controlled waters, plant, microbe and animal communities, and in compliance with current legislation for labile (extractable) TE fraction in the soil, forages and feedstuffs, foodstuff, groundwater or on upper critical threshold values according to experts. To better determine the benefits and limits of feasible GRO for some or all clusters at one site, according to the selected conceptual scheme and end land use, it is recommended to compare them with the best relevant conventional remediation options, in parallel in pot and field experiments (with similar soil contamination). In case of failures of GRO in the long-term, the other remediation options would be deployed on the site clusters. Soil amendments and plant materials were investigated in coordination with WP4.

Option appraisals resulted in the selection of:

- **phytoextraction** at 13 field trials (5 with SRC, 5 with high yielding crops, and 3 with hyperaccumulators),
- **(aided) phytostabilisation:** 11 field trials (3 with SRC and 8 with perennial herbaceous plants)
- ***in situ* immobilization/phytoexclusion**: 5 field trials.

### 3.1.2. Implementation of remediation strategies

Most GRO were selected based on pot and mesocosm experiments (option appraisal). Main lessons gained on GRO implementation were:

- quantify the spatial variability of parameters driving the choice of feasible GRO according to the current/future land uses (for each cluster) and the related target/trigger values (notably those from the legislation and exposome) and other drivers (land value, time constraints, etc.).
- account for any specific requirements related to the selected feasible GRO and the best conventional option (to be compared).
- compare the best conventional technology(ies) in parallel with the selected GRO emerging from option appraisal.
- don’t upscale directly from ‘pot experiments’ to ‘full-scale’ (*in situ*) deployment on the cluster(s) without the return skill of biomonitoring and maintenance for several years.
- set fences around small clusters, especially at the start of the phytomanagement, to protect the trees and other attractive plant species;
- define reasonable plot size for avoiding the edge effects and permitting a long-term (>5 years) monitoring and maintenance.,
- don’t forget to monitor the foliar exposure
- adopt appropriate agronomic practices:
- the choice of initial plant/microbe partnerships must account for the local conversion chains of biomass (generally the biomass production on one rather small site is not enough to financially support a dedicated local valorization plan; this biomass must be commonly merged with similar biomass from other sites (forest, SRC, agricultural field, green wastes, etc), provided that their composition is suitable with the process or its marketing image. Plants must not only show tolerance to the contaminant(s) present but also resist other abiotic and biotic potential stresses, *e.g.* water stress, soil acidity, frost, soil erosion/compaction, herbivory, pests, nutrient deficiency, salinity, etc.

Try to implement the young trees before to implement the herbaceous crops underneath and in between. It is pivotal to irrigate trees in year 1 (and sometime year 2) during dry periods to increase the survival rate and promote the establishment of their root systems (depending on soil type, climatic conditions, etc.). Pay attention to the slope, potential soil erosion and/or flooding. In case of excluder-based SRC for bioenergy purposes, the selection of genotypes can be based on their characteristics in line to conversion processes, *e.g.* calorific value, bulk density, moisture content, ash and extractive content. Transplantation of mycorrhizal trees was more successful than that of non-mycorrhizal ones and the on site mycorrhization of tree cuttings.

*Salix* and *Populus* clones show high variations in biomass production, TE tolerance and accumulation patterns in roots, leaves, and even in wood between clones. Some species and clones of willow have high bioconcentration factors (BCFs) for Cd (up to 27) and Zn (up to 3). Given the ample variation in metal accumulation, best-performing clones can be selected based on their TE-tolerance, uptake efficiency (accumulating clones for phytoextraction *vs.* excluding clones for phytostabilisation), translocation from roots to shoots, and biomass production. Clones can be selected for their ability to accumulate certain metals (*e.g.* Cd and Zn) while at the same time immobilizing elements such as Cu and Pb. Evidences of tolerance to TE and fungal and insect infection, *e.g.* leaf rust (*Melampsora* sp.) and lace bug (*Monosteira unicostata* Muls. and Rey), cold and drought adaptation were revealed at the Lommel site.

● combine phytomanagement and ecology: establish natural and passive habitats to host and promote reproduction of the biological auxiliaries (notably beneficial insects and birds) and counteract bioagressors. Think about the connection of clusters with the other ones nearby. Use corridors allowing the predators (fox, raptors, etc.) to hunt; these corridors can be combined with the access required for monitoring and sampling as well as the harvest machines. Avoid a full site monocultures to alleviate the selection of pest populations (*e.g.* use diverse clones/genotypes for trees in clusters; use a crop rotation in case of annual plants.

● Phytomanagement can combine some GRO: The phenotype of plant species in response to TE excess is element dependent and a plant assemblage can support various GRO at the same time on mixed-contaminated soils. For example a poplar SRC can simultaneously phytostabilize Cu/Pb in its root system, phytoextract Cd/Zn in its aerial parts and promote the rhizodegradation of xenobiotic organic compounds.

### 3.1.3. GRO implemented and biomass production

#### 3.1.3.1. In situ immobilization/phytoexclusion

This GRO can be implemented as either a long-lasting (phyto)management option or a temporary, reversible one that can be later modified based on the monitoring results from the phytomanaged plots. Decreasing the labile TE pools in TECS by incorporation of soil conditioners and the use of excluder plants are both main approaches. Different **soil conditioners** were investigated on a long-term and at field scale, *i.e.* phosphates, composts and technosols, iron bearing materials (iron grit, gravel sludge), and alkaline materials such as alumino-silicate slags, marl lime, biosolids, and dolomitic limestone (see Appendix)

**End land use: annual crop production**

<u>Staple crops and oilseeds</u>: Selection of efficient excluder cultivars of wheat, barley, rice, potato and maize for cultivation on contaminated and remediated land contributes towards reducing the entrance of non-essential TE, and also avoiding the excess of essential ones, into the food chain. Cd is of highest concern regarding metal uptake into the food chain as well as As, Mo, Se, Tl and Hg. Excluder maize, barley and potatoes cultivars were long-term assessed at Arnoldstein: use of the excluder-phenotype Bodega *vs.* accumulator-phenotype Hellana reduced barley grain Cd by over 40 %. In combination with the incorporation of gravel sludge and red mud into the contaminated soil, a further >30 % Cd uptake could be avoided.

Crop rotation included winter oilseed rape, winter wheat and spring barley at Freiberg-Hilbersdorf in combination with marl lime application. Soil pH was slightly changed by lime and P application and was generally increased in year 4, which resulted in a decrease of mobile Cd (by 50-75%). Based on BCF of Cd, Zn and Pb, the barley cultivar "Salome" was shown to less accumulate these metals in its grains than the Marthe cultivar. Based on EU directive 2002/32/EC (2002) these barley grains were suitable as single fodder. Considering changes in element transfer into plant parts as affected by amendment options, grain Pb differed between the control and P treatment with highest concentration and the limed treatments with low concentration, especially for the combined fertilized treatment. The biomass production of winter oilseed rape for both cultivars was within the common range of yields for this German region (2.4 – 4.4 t/ha). Those of winter wheat were below the range (5 – 8 t/ha), especially for the low accumulating cultivar Türkis, which produced a lower grain yield than to the high accumulating cultivar Tiger. The grain yields of spring barley were below the common range (4.2 – 7.4 t/ha) with slight differences between both cultivars.

<u>Grassland management</u>: Grassland based on TE excluder grassy crops is one relevant GRO to alleviate windblown dust and water runoff on large TE-contaminated areas, notably with low fertility.
At Arnoldstein, shoot DW yield reached 5 t/ha/yr. The most efficient soil conditioner (gravel sludge and red mud, slurry management) was reducing the labile pools of Zn (-90%), Cd (-80%), and Pb (-90%) in the soil. Plant monitoring based on *Plantago lanceolata* indicated reduced shoot concentrations for Cd (-70%) and Zn (-77%). Shoot Cd and Pb concentrations of harvested grass mixture just exceeded the maximum permitted concentrations (MPC) in forages.

At Freiberg-Hilbersdorf, soil pH ($CaCl_2$) varied between 4.3 and 5 in the unamended soil. It reached pH 6 at 2t marl lime/ha and 6.5 at 4t/ha. Extractable soil Cd was reduced from 0.6-0.7 to 0.05-0.1 mg/kg soil DW. Grass shoot DW yield varied from 2 to 5.75 t DW/ha depending on season (3 cuts/yr) and soil amendments. It was enhanced in May by marl lime application at 4t/ha. Shoot As concentrations of grass did not differ between unamended and 2t/ha-amended soils on the 2012-2013 period and was in the 0.25-0.5 mg/kg DW range. It started to decrease on the third year. Shoot As concentration was higher in the 4t/ha-treated soil, reaching 1.5-1.75 mg As/kg DW in 2012-2013, despite high shoot DW yield, and also decreased to 0.6 mg As/kg DW in 2014 after the last marl lime application. Shoot Cd concentration ranged between 1-1.5 mg/kg DW in 2012 and did not differ across the treatments. It decreased in all treatments in 2014 (0.5-0.8 mg/kg DW), but lower values in marl lime-treated soils were similar to the unamended soil. Shoot Pb concentration varied from 0.3 to 4 mg Pb/kg DW in average and reached 8 mg/kg DW in some shoot samples from the 4t/ha-amended plots. It was decreased in year 3 for all plots with a lower value at 2 t/ha (0.3 mg Pb/kg DW) compared to the unamended soil.

At **Piekary** (PL): The grass mixture consisted of local cultivars: *Festuca rubra* L. cv. Atra, *Poa pratensis* L. cv. Alicja, *Festuca arundinacea* Schreb. cv. SZD, and *Festuca ovina* L. cv. Sima. 17 years after biosolid incorporation, water-soluble fractions of major contaminants (Zn, Cd, and Pb) in the soils remained at low levels, in line with soil pH and Ca-carbonate distribution over the field. Soil bacterial communities were highly diversified in amended soils. Dehydrogenases activity increased as water extractable metal (Cd, Zn) fractions in the soils were reduced. Plant cover and biomass production depended on the soil treatment being highest soils amended with biosolid combined with byproduct lime. Most persistent grass species were *P. pratensis*, *Agrostis capillaris* and *F. ovina*. These species covered the largest area of the field. A substantial part of the areas was covered by colonists: *Calmagrostis epigejos*, *Hypochoeris radicata*, *Melandrium* *album*, *Artemisia vulgaris*, *Daucus carota* and *Solidago gigantean*. Untreated tailings outside the reclamation area remained barren.

### 3.1.3.2. (aided) phytostabilisation

#### 3.1.3.2.1. SRC with and without grass cover/herbaceous layer

***SRC parameters*:** Many tree species are 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, and As) in the soil by physically preventing migration, leaching, and soil dispersion; alternatively, they can immobilize TE through uptake and accumulation by the roots into the plant, adsorption on the root, and precipitation in the rhizosphere.

At **Biogeco** (Cu-contaminated soils) fertilized mycorrhizal poplars were harvested two times, whereas minimum values of potential biomass to initiate the harvest of willows and non-mycorrhizal poplars were not reached. For willow SRC, it can be done in year 9 only for ectomycorrhizal trees. Shoot DW yield of poplar SRC varied from 20 to 270 t DW/ha showing the spatial variability of soil exposome, fertility and water supply, 135 t DW/ha was even reached in some untreated plots nearby other fertilized plots managed by phytoextraction. In year 6 after amendment incorporation, compost (OM) increased poplar growth compared to the untreated soil (UNT), whereas addition of dolomitic limestone (DL) resulted in less significant increases.

At **Phytosed,** two willow cultivars (Tordis and Inger) were planted. Their survival rate in year 2 was 89% accounting for all plots, but it dropped to 75% for Tordis in several amended plots and the rate of chlorotic leaves reached 30-50% in these plots. The foliar Cd concentrations were high (10-30 mg Cd/kg), the Tordis willow clone showing higher values than the Inger in both the Thomas basic slag (TBS)-amended plots and in the control plots. Similarly foliar Zn concentrations ranged from ~ 2000 to 3500 mg $kg^{-1}$ DW whereas common values varied from 81 to 296 mg $kg^{-1}$ DW. Wood and bark Zn and Cd concentrations in year 2 (2 mg Cd/kg DW) were lower than those in willow leaves. Bark concentrations (10-15 mg Cd/kg) were higher than wood concentrations and concentrations increased with the height of willow due to the increase of bark proportion. Tordis willows accumulated more Cd than Inger, in accordance with the leaf results. The alkaline amendment did not reduce or at least stabilize the TE concentrations in aerial plant parts of willows.

***Herbaceous layer*:** at **Phytosed** (FR) a commercial alkaline by-product of steel industry used in agriculture was incorporated (rate 9 t $ha^{-1}$, pH 8) into the technosol for reducing the metal mobility and promoting the grassy crop.
After 2 years, the commercial cultivar *Barchampsia cespitosa* is a good candidate for phytostabilisation (*i.e.* success of the plant cover, tolerance to the technosol conditions, shoot TE concentrations close to common values for grasses on uncontaminated soil). This grass competes well against the invasive species (beneficial effect of phytostabilisation; see previous report).

***Responses to bioagressors*:** Both willow cultivars Inger and Tora at **Phytosed** were susceptible to the imported willow leaf beetle (*Plagiodera versicolora*). A similar biotic interaction was occurring at Lommel. The use of native poplar and willow at Biogeco reduced disease incidence, particularly from *Melampsora* rust.

**Touro** (ES): the Cu-mine tailings were amended with three mixtures: composted municipal solid wastes (compost) and two technosol mixtures and planted with metal-tolerant clones of *Salix* (*S. caprea* and *S. viminalis*) and *Populus nigra*, or with a grass cover *Agrostis capillaris* cv. Highland. Mortality was high on technosol-amended plots but low on compost-amended plots. Growth and survival (70-80%) of woody trees was optimal in compost-amended plots. After three years tree height was highest in *S. viminalis* and *P. nigra* (reaching up to 3-4 m).

***Changes in soil exposome / TE mobility in soils***

Touro: In year 3, soil $NaNO_3$-extractable Cu concentrations remained low (<1 mg/kg) in all treated soils without influence of the vegetation cover type. Soil pH was 3.5 before GRO implementation, and in year 3 remained between 6.0 and 7.0 in compost-amended soils. Soil pH was higher in soils under *Salix*, followed by *Agrostis* and finally, unplanted soils.

Phytosed: In year 2 extractable Zn and Cd fractions (roughly 0.4-0.8 mg Zn and 0.001-0.0015 mg Cd/kg soil) did not differ between the amended and unamended technosols.

Biogeco: In year 5, Cu concentration in the soil pore water was higher in the compost-amended soils for both mycorrhizal and non-mycorrhizal trees and the lower in the limed soils with mycorrhizal trees.

***Restoration of soil microbial activity and communities:*** At Touro, soil enzyme activities (involved in C, N and P cycles) were lowest in untreated soils and increased with time in amended soils. A plant-induced effect was observed: activities were higher in plots planted with woody trees, followed by *Agrostis*, and lowest in unplanted plots. Shifts in the structure of the soil bacterial community (total Eubacterial community, a- and *b-proteobacteria*, *Actinobacteria* and *Streptomycetaceae*) were compared over time. At each sampling period (after 1, 2 and 3 years) the bacterial community of soils sampled before GRO implementation differed from that of phytomanaged soils. At all sampling periods, the soils amended with compost, technosol 1 or technosol 2 differed. In general the soil bacterial communities continue to cluster separately according to the plant species, either *Agrostis* or *Salix* cultivation. There was a trend towards an increase in bacterial diversity with time, and also a higher bacterial diversity in planted soils (albeit *Agrostis* or *Salix*) compared to unplanted (but amended) soils.

### 3.1.3.2.2. Grassy crops (only)

***TE excluder perennial herbaceous crops*** such as switchgrass (*Panicum virgatum*), *Miscanthus* spp. and vetiver (*Vetiveria zizanioides*) have wide climatic adaptability, low production costs, suitability to marginal lands, relatively low water requirements, low nutrient and agrochemical needs, and potential environmental benefits. They can provide feedstock for the energy sector or essential oils. *Miscanthus* x *giganteus* and Vetiver were implemented at Biogeco. For **Vetiver** in year 4, shoot DW yield reached 38t/ha in uncontaminated plots, 7-15t/ha in the amended Cu-contaminated plots, and only 2.6 t/ha in the highly Cu-untreated plots, as total Cu in soil pore water increased from 0.2 to 0.9 mg Cu/L. It always demonstrated a Cu-excluder phenotype, shoot Cu concentration being in the 10-13 mg/kg range with no influence of soil Cu contamination. For ***Miscanthus,*** shoot DW yield in year 3 varied from 0.07 (Unt) to 1.8 (compost and dolomitic limestone, OMDL) t DW/ha. Its shoot Cu concentration ranged from 7 (OMDL) to 95 mg Cu/kg DW (Unt). The single incorporation of compost and dolomitic limestone still reduced labile soil Cu and *Miscanthus* exposure in year 7. Shoot Cu removals in year 3 varied from 3 to 17 g Cu/ha depending on shoot Cu concentration and shoot DW yield, with maximum at median soil Cu contamination and minimum for soil Cu/PAH contamination. For **Sorghum** (*Sorghum* spp.) both cultivars for biomass and bioenergy were not successful at field scale, being too sensitive to Cu excess and low water supply in sandy soils.

***Grassland***: At Biogeco compost (OM) and dolomitic limestone (DL), singly and in combination (OMDL) were assessed. In year 7, most plots initially planted with grasses were dominated by an assemblage of Cu-tolerant *Agrostis capillaris* and *A. gigantea* whatever the soil treatments. Other introduced grassy species such as *Sporobolus tenacissimus* and *B. cespitosa* are disappearing. Shoot DW yields were higher in the compost-amended plots compared to the limed ones. Shoot Cu concentration was slightly lower for the grass species harvested in the OMDL plots. Highest shoot DW yields in the compost-amended plots led to maximum shoot Cu removals. Total Cu concentration in the soil pore water was increased in the compost-amended soils as compared to the limed soils.

Chaban-Delmas (FR): A total of 72 plant species were identified in the grassland, 32 species being occasionally present. Main species discriminated three plant subsets, one dominated by *Medicago sativa* and *Lolium perenne* with the lowest plant species richness, one dominated by *Arrhenatherum elatius*, *Bromus sterilis*, *Holcus lanatus* and *Dactylis glomerata*, with a median value for the species richness, and one *Melilotus albus*, *Trifolium arvense*, and *Trifolium pratensis* as dominant with the highest bare soil percentage but the highest species richness.

Touro (ES): *Agrostis capillaris* cv. Highland was successfully established in both compost- and technosol-amended plots. Shoot Cu concentrations were within normal levels for grass species growing in uncontaminated soils, and lower than in grass species colonizing the surrounding untreated tailings. Nutrient concentrations were increased in all amended soils, notably in technosol-amended plots. Shoot DW yield was highest in compost-amended plots.

### 3.1.3.2.3. (Aided) phytoextraction

The aims were to quantify (1) the biomass production, (2) the plant ionome (notably TE concentrations), and (3) the TE phytoextraction. Additional aims were (4) to improve agronomic practices, (5) to enhance ecosystem services such as C sequestration and microbial activities, and (6) to create economic opportunities from the biomass. Plants must be able to accumulate high TE concentrations in their harvested parts and have a reasonably high biomass production. Relevant options were TE-hyperaccumulators and secondary TE accumulators. Three main options were implemented to address three mains situations, Cd/Zn (Pb), Cu (Cu/PAH) and As/metal excess: high-yielding crops (HYC), short rotation coppice (SRC), and herbaceous hyperaccumulators, in monoculture and co-cropping. Influences of soil conditioners such as compost, Linz-Donawitz slags, soil acidifying agents (citric acid, S), and co-cropping were investigated to enhance TE phytoextraction.

### 3.1.3.2.3.1. High-yielding crops (HYC)

High-yielding crops (annuals or perennials) are recognized as viable alternatives for TE phytoextraction (particularly Cd, Se and Zn) if they show relevant shoot TE removals (*i.e.* moderate-high BCF and high shoot yield). *In vitro* breeding and chemical mutagenesis can improve the metal tolerance and phytoextraction capacity of high-yielding crops such as tobacco and sunflower (see WP4). At the Rafz site (Switzerland), shoot metal removals by the sunflower mutants were up to 7.5-, 9.2- and 8.2-fold higher for Cd, Zn and Pb than the inbred line, respectively. As 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, crop rotations such as sunflower/tobacco (with winter fodder pea at Bettwiesen, and white clover at Biogeco as cover crops during winter for green manure and limiting soil erosion) were investigated. Fibre hemp (*Cannabis sativa*) and kenaf (*Hibiscus cannabinus*) were cultivated at Lommel. Tobacco and sunflower mother-clones and variants were cultivated at 5 sites. Datasets are available for shoot DW yields, shoot metal concentrations and shoot metal removals. The influences of soil contamination levels, fertilization, maintenance through compost dressing, plant species and genotypes (mother-line, somaclonal tobacco variants, and sunflower mutants), agronomic practices such as irrigation, co-cropping and flower topping were considered.

**Touro** (ES): Shoot DW yield of **tobacco** primarily depended on the climatic conditions during the growth season: the highest biomass for all genotypes was achieved in the 2014 harvest and reached 3400-4000 kg DW ha$^{-1}$ (levels comparable to those obtained in the agricultural soils of Bettwiesen and Lommel). Differences between the BAG motherline and the 10-8 and 10-4 variants were not pronounced, and biomass tended to be higher for BAG. Shoot Cu removal in 2014 (60-70 g Cu/ha) was lower than that obtained in Biogeco.

**Piedrafita** (ES): Sunflower could only grow in the compost-amended plots, while tobacco could grow in both compost-amended and untreated mine-soils (after fertilization with inorganic NPK). **Tobacco:** Annual shoot DW yield varied widely according to climatic conditions and to competition with weeds; biomass production was highest in 2012. There were no consistent differences between motherlines and other variants. Biomass production and Cd/Zn extraction potential was lower than that observed in Lommel and Bettwiesen. **Sunflower:** its cultivation was more successful than tobacco at this site. Annual shoot DW yields were similar to that obtained at the other Greenland field sites. Cd/Zn extraction potentials were similar to that obtained in Bettwiesen and Lommel in the 2012 and 2013 harvests, but lower in 2014. Mutant 1 reached up to 6772 g Zn and 23 g Cd /ha extraction potential.

**Biogeco** (FR):
***Tobacco*****:** its shoot DW yield depended on total soil Cu, soil amendments, the genotype in some plots, and annual climatic conditions. Flower topping in years 6 and 7 allowed the development of bottom suckers, which increased the shoot biomass. Depending on climatic conditions, early flower topping in Southwest France allowed to harvest tobacco shoots two to three times per year and to avoid loss of dried leaves.

● At moderate soil Cu contamination (258 – 382 mg Cu/kg): in year 6, shoot Cu removal by the OMDL plants reached 84-132 g Cu/ha, without genotype influence. The second compost dressing in year 6 (OM2DL) reduced shoot Cu removal as compared to OMDL, likely due to a decrease in mobile soil Cu. However, in year 7, the OM2DL plants had a higher shoot yield than the OMDL ones, likely due to nutrients released by compost mineralization.

● At high soil Cu contamination (894 – 1020 mg Cu/kg): in year 6, shoot Cu removals by OMDL plants varied from 68 to 193 g Cu/ha, with no consistent influence of genotypes. For the OM2DL plots, the genotype did not influence shoot Cu removal and its values were similar or higher. In year 7, shoot length and DW yield were higher for the OM2DL plants than for the OMDL ones, which promoted shoot Cu removal. This was sometime more marked for the 10-8 variant.

● For the Linz-Donawitz slag (LDS) amended-plots in year 6, shoot Cu removal peaked with the variant 10-8 in one plot, reaching 254 g Cu/ha. For all genotypes, differences were not significant between the P-spiked LDS and Unt plots. The LDS less enhanced shoot DW yield than two compost dressings combined with dolomitic limestone. In year 7, tobacco from untreated and LDS-amended plots had again a lower shoot biomass than the OM2DL plants. The 10-8 variant best developed in some cases, but the genotype influence was mostly not consistent. Soil amendment, especially the second dressing of compost was a key factor to maintain the shoot yield.

***Sunflower***: in year 6 and 7, leaf chlorosis occurred on many sunflower plants growing in OMDL amended plots with high total soil Cu and in both LDS-treated soils. No sunflowers were growing in the UNT soil.

● at moderate soil Cu contamination (258 – 382 mg Cu/kg): in year 6, the M2 mutant performed best in comparison to other genotypes, without effect of the second compost dressing. The shoot DW yield of the M3 mutants was lower than that of motherline plants in the OM2DL plots. In overall, shoot Cu concentrations were similar for all plants. The M2 mutant showed a higher shoot Cu removal in the OM2DL plot. Shoot Cu removal was in the 42 g Cu/ha range. Due to lower shoot Cu concentrations than in previous years, this was lower than the 100 g Cu/ha reported in years 1 and 2. In year 7, shoot DW yield varied from 0.5 to 36 t/ha depending on plots and genotypes.

● at high soil Cu contamination (894 – 1020 mg Cu/kg): plants best developed in years 6 and 7 in all OM2DL plots with a second compost dressing in year 6, showing the need to maintain soil organic matter. A single compost dressing increased the shoot DW yield more than the incorporation of LD slags. The Carmeuse-LDS and P spiked-LDS similarly influenced sunflower growth, and both M3 mutants and motherline plants died in these plots during summer. In year 6 for the compost-amended plots, M2 mutants produced a higher shoot biomass than other genotypes. Both mother-line plants and M3 mutant developed only in the plots with recent compost dressing. The second compost dressing decreased shoot Cu concentrations for the mutants. Low shoot Cu concentrations (close to the upper critical threshold value for Cu in higher plants) for the OM2DL plants matched with their high shoot biomass, suggesting a dilution effect. In overall, shoot biomass is a main driver for shoot Cu removal. According to the plant density, plots and sunflower genotypes, shoot Cu removal varied in the 21-105 g Cu/ha range.

● LDS plots: only M1 and M2 mutants developed on these plots. Their shoot Cu concentrations were higher in LDS amended plots than in the OM2DL ones. Shoot Cu removal peaked for the M1 mutant mostly due to their higher biomass.

In year 7, shoot DW yield was enhanced in the OM2DL and the LDS amended plots. Sunflowers did not grow on the untreated plots. In overall, genotype influence was not consistent. The second compost dressing in year 6 was the key factor to promote both shoot yield and Cu removal. Between years 4 and 6, extractable Cu fraction in the OMDL plots was reduced by 38%. Since the experiment started, shoot Cu removal fit with a quadratic function, likely following reactions of compost with Cu, nutrient release from compost decay and bioavailable Cu stripping.

**Lommel** (BE): Tobacco clones and sunflower mutants were cultivated from years 1 to 4. *Brassica napus* and *Cannabis sativa* (hemp) were also implemented. In 2013, shoot DW yield of tobacco was similar for all genotypes ranging from 1.3 to 1.7 t/ha. For sunflower, shoot DW yield varied from 3.5 to 7.5 t/ha, with the M1 mutant producing lower shoot biomass than the control plants and the other mutants. Hemp developed well and its shoot DW yield reached 17.5 t $ha^{-1}$ $yr^{-1}$. Shoot metal removals for tobacco were in the following ranges (in g/ha/yr): Cd 14 – 20, Pb 8.6-11.9, and Zn 252-331. Genotype had an influence (*e.g.* higher shoot removal for Cd/10-8 variant and Zn/7-19 variant). Compared to tobacco, shoot metal removals of sunflower were higher for Zn (1992-2504) and slightly higher for Cd (17.7-23.6) and Pb (17.7-42.5), with a genotype influence. The phytoextraction by hemp was 7 g Cd, 41 g Pb, and 1355 g Zn/ha. The phytoextraction of Cd, Pb and Zn by all tested sunflowers was higher than that of the tobacco clones and hemp. Tobacco clones had higher shoot Cd and Pb concentrations but the higher shoot DW yield of sunflowers lead to higher shoot metal removals. Hemp production on metal-contaminated soil could be relevant if cutting the pollutant linkage to food along with an economic profit from the plant-based feedstock (e.g. fibre) is the primary goal instead of the other ecosystem services (*e.g.* decreases of labile metal pools, decontamination, and soil remediation).

In 2014, the biomass production, and consequently the shoot metal removal, was higher than in 2013, especially for tobacco (tobacco 3.6-4.9 t/ha; sunflower 5.8-9.5 t/ha). Shoot removals were 59.6-122 g Cd, 38-70 g Pb and 1027-1926 g Zn/ha for tobacco, 32-61 g Cd, 17.5-34.5 g Pb, and 2624-5745 g Zn/ha for sunflower. This highlights the influence of annual climatic conditions on shoot metal removals.

**Bettwiesen** (CH): The crop rotation is based on four sunflower and five tobacco genotypes with higher metal tolerance and accumulation properties for stripping bioavailable Zn and Cd excess in topsoil. After 5 years, the labile Zn pool in soil was lowered by 45-70%. A Mass Balance Analysis confirmed soil Zn decontamination in line with plant Zn uptake. The plants partially take Zn from the non-labile pool of the total. The 'stripping' of bioavailable Zn is feasible within a few years period. To decrease available soil Zn below the Swiss threshold value, the phytomanagement would take 3-12 years at moderate available Zn levels and 5-25 at high levels. Various plant densities and intercropping of sunflower with tobacco in early spring are further explored.

### 3.1.3.2.3.2. (aided) phytoextraction using woody SRC

The capacity of poplar and willow to colonize hostile environments such as mine wastes is recognized. Numerous *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. Most promising poplars and willows (according to climatic conditions and shoot TE concentrations) were assessed at 6 sites: Lommel (HAU), Högbytorp and French trial (SLU), Freiberg (LfULG), Piedrafita (ES) and Phytagglo (FR).

**Freiberg, Halsbrücke Krummenhennersdorf** (DE): this 9-old field trial is a SRC plantation on contaminated agricultural land. Shoot DW yields reached 15 t/ha/yr for poplar SRC and 14-19 t/ha/yr for willow SRC, corresponding to common values (6-16 t DM $ha^{-1}$ $yr^{-1}$). Stem wood and bark ionomes of poplars and willows depended on genotypes, particularly for Cd (3-10 mg/kg in wood; 8-30 mg/kg in leaves), and clonal differences were higher across willows. Values were higher in willows compared to poplars. Willow cultivars Tora, Tordis and Gudrun displayed the highest wood and foliar Cd concentrations among all cultivated clones of poplars and willows. The ratio of the 3-year-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. Bark Cd concentrations account for triple (poplars) to fourfold (willows) of those in stem wood while Pb-concentrations did not differ between the compartments. Foliar As-concentrations were mostly below the detection limit. Wood As concentrations varied from 2 to 4 mg/kg. For the third rotation, willow Tora produced the highest biomass out of all poplars and willows (followed by Tordis) and it displayed relatively high Cd and Pb accumulation capacity. This confirmed Tora as a relevant choice for metal (Zn, Cd) phytoextraction, and Tordis as well (for Cd).

**Piedrafita do Cebreiro** (ES): plots with *Salix smithiana* and *S. atrocinerea*, in monoculture and inter-cropped with *Alnus glutinosa*, and *Salix* cv. Tora were established in autumn 2012. Plots were unamended or amended with 5% (w/w) compost. Plant survival was higher for *S. smithiana* than either *S. atrocinerea* or Tora. Plant growth (height/spread) and leaf/stem Cd/Zn concentrations were recorded in 2013 and 2014. No significant effects of inter-cropping on plant growth have been recorded to date (although there was a tendency to increased tree height in intercropped plots in 2012). Intercropped *S. smithiana* plots showed higher leaf Cd and Zn concentrations (approx.. 12 and 1400 mg/kg). Changes in total or $NH_4Cl$-extractable concentrations of Cd and Zn are not yet consistent.

***Changes in soil exposure***: At Freiberg, generally total soil Cd decreased (e.g. 3.4 to 2.9 mg/kg for Weser 6 poplar; 2.5 to 1.0 for Tora willow) between 2011 (year 6) to 2013 (year 8). The rhizosphere of willow and poplar clones at contaminated and adjacent reference sites showed a lower pH for SRC compared to arable land (with a higher decrease for willow SRC). Since the SRC implementation in 2005, the initial soil pH of 5.7 ($CaCl_2$) dropped in average to 5.2. However, soil pH increased in poplar plots after the harvest in year 8, whereas a further decrease occurred for willow plots especially for Tordis cultivar. $NH_4NO_3$-extractable soil Cd and Pb were roughly 10 fold higher under SRC and grassland compared to annual cropped land. In contrast, mobile As in soil decreased for SRC (3 to 9 fold) compared to annual cropped land (lowest values for willow SRC). Soil pH was higher under winter wheat with the lowest $NH_4NO_3$-extractable soil Cd in year 2. $NH_4NO_3$-extractable soil Cd decreased for Jorr, Sven, Tora willow SRC and Max 3 poplar SRC.

***Soil microbial communities***: At **Freiberg,** arylesterase and arylsulfatase activities did not differ between SRC and control plots. Alkaline phosphatase was higher in SRC plot. At **Piedrafita**, the phytomanagement (willow SRC) induced changes in soil microbial communities showing influence of compost and the vegetation covers, notably between mono- and co-cultivated *S. smithiana*. The diversity of bacterial communities decreased with time, with higher value in phytomanaged soils, but without clear plant-induced effect. Most soil enzyme activities increased in the phytomanaged soils, with a plant-induced effect more pronounced in the compost-amended soils. No clear effect of the co-cropping of *S. smithiana* with *Alnus glutinosa* was evidenced.
At **Phytagglo**, willows (*S. viminalis*) were planted on an alkaline technosol developed on dredged sediments contaminated by Zn, Pb, Cu, and Cd. Citric acid based product, ferrous sulfate, and elemental S were separately incorporated for investigating the effects of soil acidification. In year 1, extractable metal fraction decreased for Pb, and remained steady for Cu, Cd and Zn, and the survival rate of willows was 90%. Their foliar Cd and Zn concentrations ranged between 1.5-3.8 and 233-1176 mg $kg^{-1}$. Foliar Cu concentrations (7-12 mg $kg^{-1}$) were similar to common values of willows.

**Högbytorp** (landfill leachate trial, SE): For 14 commercial willow SRC plantations long-term grown (ca. 15 years) on agricultural soil in Sweden, total topsoil Cd decreased (ca. 13% on average) compared to adjacent fields cultivated with cereals in common crop-

rotations. The biomass productions on these SRC fields were lower than the indicative 10 t DM $ha^{-1}$ $yr^{-1}$ expected nowadays in well-managed fields. Farmers had lack of experience in growing such crops, and beneficial incentives in terms of subsidies caused limited engagement throughout the process. Here, treatments consist of three supply rates of landfill leachate (irrigation started in 2005 and carried out until 2010) and a control, with two willow clones, *i.e.* Tora (*Salix schwerinii* x *viminalis)* and Gudrun (*Salix dasyclados* variety with partly Russian origin, more frost-tolerant than Tora).
For willows, shoot concentrations varied in the 1-4.5 mg Cd and 40-120 mg Zn $kg^{-1}$ DW ranges. Tora showed higher shoot Cd, Co, Mn, Pb and Zn concentrations than Gudrun for leachate irrigated-plots. Tora had higher shoot Cd, Co and Zn concentrations for the plots irrigated at the second supply rate compared to other treatments and the control. Gudrun displayed higher shoot concentrations for all metals in the control plots. The leachate treatments did not influence the shoot Cr and Cu concentrations in both clones, even though these concentrations were low for Tora on the control plots compared to the treatments 1-3. Nickel was the only metal with higher shoot concentrations in Gudrun for all treatments. Total shoot N concentration was roughly similar for both clones, except a higher concentration for Gudrun on the control plots.

**Lommel** (BE): Willow clones 'Belgisch Rood (BR)' (*Salix* x *rubens* var. *basfordiana*) and 'Tora' (*Salix schwerinii* x *Salix viminalis)* were compared. Shoot DW yields after 3 years were 5.4 (BR) and 9.0 (Tora) t/ha. Again shoot Cd concentrations were higher in Tora (30 mg/kg) than in BR (24 mg/kg) and shoot Zn concentration as well (1268 and 918 mg Zn/kg). The Tora willow clone had a Cd and Zn removal capacity (274 g Cd and 11 417g Zn $ha^{-1}$) which is twice as high as that for BR clones. Both, the higher biomass production (ton $ha^{-1}$) and metal uptake capacity (mg $kg^{-1}$ DW) make the TO willow clone the favorable clone to select for phytoextraction applications.

**1.3.3.2. TE hyperaccumulators:** can accumulate high concentrations of metal(loid)s (*e.g.* Cd, Ni, Zn, Se, and As) in their above-ground biomass and possess some economic added value (renewable biomass for bio-economy and bio-ores). Variations in both biomass production and TE accumulation within populations of hyperaccumulators, such as *Noccaea caerulescens* (for Zn/ecocatalysis), *Alyssum murale*, *A. bertolonii* and *A. corsicum*, (for Ni phytomining) allows for the selection and breeding of improved phytoextractor plants. The main bottleneck limiting their practical application is the low biomass production of most species (except some Ni-hyperaccumulators) and the number of cropping cycles required for clean-up. However this number is generally reduced when the option of bioavailable TE stripping is considered.

At **Piedrafita**, the Cd/Zn hyperaccumulator *N. caerulescens* and its inter-croppings with *Lupinus albus* and *Lotus corniculatus* were assessed. Inter-cropping *N. caerulescens* with the legume *L. corniculatus* tended to increase Cd accumulation by the hyperaccumulator. Other *Lotus* species show potential for incorporating into GRO due to their worldwide distribution and high adaptation to a number of abiotic stresses. Candidates with good potential for cultivation in degraded or marginal soils include *L. corniculatus*, *L. uliginosus*, *L. tenuis* and *L. creticus*. The soils planted with *N. caerulescens* and those co-cropped with *L. corniculatus* have similar soil microbial communities. Compost and plants induced changes in soil microbial communities. Mono-cultures and co-cultures tend to separate over time. The diversity of bacterial communities increased with time, with a higher diversity in phytomanaged soils compared to untreated soils, and a higher diversity in planted soils compared to unplanted soils.

At **Phytagglo**, seven plots were set up with potentially acidifying properties, *i.e.* legume plant (*Lupinus albus*), a citric acid based product, peat-like, ferrous sulfate and elemental sulphur, *Arabidopsis halleri* and control.
At **Reppel,** since 2004, *Pteris vittata* L., an As hyperaccumulator was cultivated for bioavailable As stripping. Generally, frond DW yield was doubled in the contaminated soils compared to the uncontaminated control soil. Soil treatments, *i.e.* Beringite (B, 5% w/w), iron grit (Z, 1% w/w) and their combination (BZ), and season did not influence annual frond yield, except differences between B and BZ in November and between November and May for the untreated (Unt) and B soils. On the 2006-2013 period, leachate As concentration remained lower in Z-treated soils than in the Unt and B soils. Mean values of frond As concentrations (in mg As/kg) varied in the 60-171 range for the control soil and in the 970-2870 range for the contaminated soils. Frond As removal varied from 3.89 to 2.28 g As/m$^2$ in the decreasing order: Unt, B > BZ, Z.

## 3.2 Valorization of plant biomass produced on TE-contaminated sites

As a result of plant and culture management, Gentle Remediation Options (GRO) produce plant biomass (herbs or woody biomass). Depending on the GRO set up on the polluted site and the type of plant used, harvested plant parts may contain concentrations of TE that may be higher than those found in similar vegetation grown on uncontaminated soils. This is, in particular, the case of phytoextraction, which leads to metal-enriched plant biomass. These plants may enter valuation pathways if (i) TE do not disturb the functioning and the performance of the process, (ii) if the TE transfer is controlled and (iii) if such plant use complies with current regulation. To our knowledge, by far, plant biomass on contaminated lands was only produced for scientific purpose to be used in demonstration projects such as GREENLAND. As a potential advantage, these plants will not compete with plants grown on agricultural lands as contaminated lands are not suitable for food production. On contaminated lands, plants may serve to provide feedstocks and non-food products for bioenergy and, thus, may contribute to achieve the EU aim by 2020, i.e. to get 20% of its energy from renewable sources.

In GREENLAND, our approach was to select routine pathways for plant biomass as a basis to discuss the possible advantages and potential limitations, regarding technical, social and regulatory aspects, of using plant biomass produced from TE contaminated soil into these pathways. In addition, three emerging processing pathways were selected and discussed based on existing knowledge. Thus, combustion and anaerobic digestion were selected as established pathways whereas solvolysis, flash and slow pyrolysis were selected as emerging technologies. Technical assessment was based on assays. They were performed with plants cultivated for the purpose of phytoextraction leading to metal-enriched biomass. All plants used in assays were provided by GREENLAND partners who owned field sites. Assays were performed with equipments owned by GREENLAND partners. Table 3.2.1 details the processes and plants used in assays.

**Table 3.2.1:** Type of process and plant used in assays.

| **Process** | **Test scale** | **Plant** | **Targeted metal** |
|---|---|---|---|
| Combustion | Pilot (40kW) | Willow 'Tora' | Zn, Cd |
| | | Poplar 'Max3' | Zn, Cd |

| | | | |
|---|---|---|---|
| | | Mix willow, poplar | Zn, Cd |
| Anaerobic digestion | Laboratory (5L reactor) | Sunflower | Zn |
| Solvolysis | Laboratory ($110 cm^3$ reactor) | Tobacco | Zn, Cd Cu |
| Flash pyrolysis | Laboratory (100g reactor) | Willow | Zn, Cd |
| | | Sunflower | Zn |
| | | Tobacco | Zn, Cd Cu |
| Slow pyrolysis | Laboratory (100g reactor) | Tobacco | Zn, Cd |

Acceptance and feasibility assessment were realized for combustion and anaerobic digestion based on interviews of installation operators in several European countries (France, Austria, Germany, Sweden). Regarding regulatory aspects, the assessment consisted in a review of current European regulation and examples of national regulations related to combustion and anaerobic digestion focused on plant biomass utilization. This review was the basis to discuss possibilities to use plant biomass produced on TE contaminated lands in these processes.

## KEY RESULTS

**I Assays** were performed to determine the fate of the TE in the resulting products of each conversion process.

**Combustion**, defined as thermochemical conversion of biomass, occurs in combustion plants or boilers in which fuels are oxidized to use the heat generated. For all assays, Zn occurred mainly in the fly ashes. The bottom ashes represented the second compartment for the occurrence of Zn whereas the gaseous fraction of the flue gases represented a minor compartment for Zn emissions. The distribution was not depending on the initial burnt wood, i.e. virgin wood (control) or Zn enriched wood (phytoextraction). Similar results have been found for Cd. Independently of regulation issues, assays allowed to conclude that the burning of plant biomass naturally enriched with metals in industrial or collective boilers could be possible, as they are normally equipped with efficient systems to reduce dust emissions. Depending on the TE concentration in bottom ashes and national legal framework, bottom ashes could be valued by land spreading. Concerning fly ashes, the results invite to perform further in-depth analysis of current practices regarding separation of ashes and valorisation pathways.

**Anaerobic digestion**, a biological process performed by the combined action of several micro-organisms in the absence of oxygen, ends up in partial degradation of organic matter and leads to formation of biogas and digestate. Medium Zn-enriched sunflower showed similar biogas composition as typical biogas. This result evidenced that the presence of Zn in sunflower did not modify the composition of biogas. Results also showed that Zn did not inhibit biogas production. Due to technical problems, the assay performed on high Zn-enriched sunflower was not conclusive. Nevertheless, during the biogas monitoring which lasted 10 days, we could observe that biogas production was not inhibited. As expected, Zn was measured in digestates. Indeed, at 55°C, the temperature of the anaerobic digestion, no Zn volatilization can occur. Depending on TE concentration in digestates and legal framework, digestates could be valued by land spreading or by composting.

**Solvolysis,** chemical decomposition of biomass with a solvent under pressure, investigated metal behaviour in biomass converted by sub- and supercritical conditions. Cu was mainly found in the liquid phase during the heating step or in the residual solid, depending on the temperature. Zn was mainly found in the liquid phase during the heating step whereas Cd was mainly found in the residual solid. Carbon is almost exclusively found in the residual solid. Some molecules of interest for fine chemistry were found in the liquid phase but in very small amount which not allowed to quantify them. In the solid residues, Cu concentrations were too high to consider the usage of the solid residue as an organic amendment. The idea was then to use the solid phase enriched with metals as raw material to produce polymetallic catalysts which could be used in industrial biotechnologies and chemocatalytic processes. Preliminary assays showed that the metal concentrations were too low to evidence a catalytic activity of these residues. Solvolysis can be used as a pre-treatment leading to a significant reduced biomass and a liquid. From the view-point of the industry, it would be easier to get a metal free liquid phase that could be rejected in nature.

**Flash and slow pyrolysis.** Pyrolysis is the thermochemical decomposition of (biomass) material at moderate temperature and in oxygen deficient conditions resulting in 3 end products: char, oil and gas. Flash pyrolysis typically uses moderate temperatures (450 – 600°C), a very high heating rate and a very short vapor residence time. Flash pyrolysis targets the pyrolysis liquid as end product. Low process temperature and long vapour residence times ("slow" pyrolysis) favour the production of char. In flash pyrolysis, the Cd concentrations in the aqueous fractions were never higher than 12.3% of the %wt of Cd present in the original biomass. The recovery of Zn in the aqueous fraction is much lower and did not exceed 2.8% of the %wt of Zn present in the biomass. Cu content in the aqueous pyrolysis oil after flash pyrolysis of the Cu-rich biomass was relatively low. The tar fractions of tobacco and sunflower contained in all cases more target metals than the corresponding aqueous fractions. To be used as a renewable fuel, the physicochemical properties of the liquid must be investigated as well as the potential impact and constraints associated to the presence of the metals for this usage. Further research efforts are needed to investigate these points. Metal enriched biomass was successfully valorized by slow pyrolysis and subsequent physical activation by steam in products with added value, in particular low cost activated carbons.

From safety point of view, considering previous cited valuation pathways of metal enriched biomass, we were not able to identify any major reason to stop further consideration of any of those routes for safety reasons.

## II Interviews

Operators of anaerobic digestion (AD) platforms and actors of the wood bioenergy sector were interviewed to assess the potential acceptance of using plant biomass produced by GRO on metal contaminated lands in their installations and network. The reasons of acceptance or not were investigated by separating phytostabilisation from phytoextraction.

Selection of AD platforms and boiler operators/owners was based on countries among those represented in GREENLAND which used wood and energy crops as fuel/feedstocks at a significant rate in combustion and AD. As a result, 8 actors of the wood energy sector from France, Germany and Sweden, and 11 AD platforms operators from France, Germany and Austria were interviewed. The questionnaire was asking about installation characteristics, plant characteristics, performed analyses and phytotechnologies.

Results from questionnaires suggested that plant biomass from phytotechnologies could be used in AD and combustion, under conditions. From the view-point of interviewed actors, main limitations related to additional controls in process end-products and installations that might generate additionnal costs. In most cases, price of phytotechnologies biomass was mentionned as a driver to potentialy use plants from metal contaminated soils. It should be similar to market price for feedstocks and fuels, less expensive or free. Plants used in phytostabilisation or phytoexclusion were thought to be less risky and, consequently, benefited from a better theoritical acceptance than those issued from phytoextraction.

## III Regulation

The classification of the plant biomass produced on contaminated land (biomass or waste?) is essential to choose the appropriate valuation pathway, and thus, assess the profitability or the cost due to gentle remediation options. By far, this question is solved neither at the European level nor at the local/national level. To know how European regulators would consider biomass produced on contaminated soils by phytotechnologies, we asked some of them through the GREENLAND advisory board. A first comment from regulators related to the fact that this point was never discussed yet. One reason could be that the amount of plants produced on contaminated sites for remediation purpose or for bioenergy production is by far not significant, as it is only produced for scientific purpose. Except one regulator who had the feeling that this biomass could be classified as agricultural product, other regulators had the tendency to classify the biomass from phytotechnologies as waste. These answers could be related to the way of the questions were formulated that could have orientated the regulator answers. Nevertheless, these answers highlighted the fact that it is necessary to evidence harmlessness of metal-enriched biomass, bring information on TE transfer control to regulators and clarify product vs waste consideration.

A state of the art on European and national regulations was performed related to combustion aspects, as this valuation pathway is the most important energy conversion route for biomass produced on uncontaminated soils, and anaerobic digestion aspects, highlighting metal emission limit values or metal input fuel concentrations. Finally, plant biomass from phytotechnologies are not specifically addressed in regulation (national and European level). Results of the regulation study and the combustion assays performed on plant biomass used in phytoextraction highlighted the need to separate fly ashes from bottom ashes (in countries where it is not already done) to valuate bottom ashes more easily and to manage fly ashes accordingly to their TE content. Results and regulation interpretation with less metal enriched plant biomass, i.e. phytostabilising or phytoexclusing plants, could obviously be different. As Zn and Cd are mostly recovered in fly ashes than bottom ashes, it should be possible to land spread more easily bottom ashes than fly ashes. Nevertheless, a low concentration in plant biomass doesn’t not imply that ashes resulting from this plant can be spread on land. Further research might focus on combustion assays with phytostabilising plants to answer more precisely this point.

## 3.3 Harmonisation of methods to assess the bioavailability of TE and development of tool set to monitor the sustainability of GRO

*Objectives*

For the GRO options to be accepted by decision makers, the methods for evaluation of GRO success should be widely available, provide robust results and should be suitable for monitoring of soil health and sustainability. For this, we need to provide proof of the suitability of GROs for risk reduction of contaminated sites by collecting comparable results using the same methods throughout Europe. Hence, the objectives of this WP were: i) to select methods to be used as indicators for GRO success and as sustainability monitoring tools; and ii) to select/harmonise methods describing the bioavailable/bioaccessible TE fractions among European case studies.

*Methods*

Two sets of tests (so called test batteries) were pre-selected based on available literature and the experience of the consortium; *a chemical* one to quantify TE exposure in untreated soils and GRO-managed soils, and *a biological* one to characterize soil ecotoxicity and functionality. The chemical tests included extractions with *aqua regia*, 0.05 M EDTA, 1 M $NH_4NO_3$, 0.1 M $NaNO_3$ and $H_2O$. The biological test battery included ecotoxicity tests (plantox with dwarf beans, lettuce and turnip; activity of plant stress enzymes, and soil invertebrates, such as earthworms and nematodes); specific soil biochemical functions and a number of tests with soil microorganisms.

The common sampling strategy was developed for collection of soil from the test fields. Soil was sampled following the agreed procedure from the case sites representing the main GROs: phytoextraction in Belgium, Sweden, Germany and Switzerland; aided phytoextraction in France and Spain; and aided phytostabilisation or *in situ* stabilization/phytoexclusion in Poland, France, Spain and Austria.

All partners managing case sites applied the chemical test battery on their own soils two times during the four years: one run during the first year and the second run during the third year of the project. At least one sample from untreated and treated plots per treatment method was analyzed in at least 3 replicates.

For the implementation of the biological test battery, the sub-samples were sent for analysis to HAU, INRA and INERIS (ecotoxicity tests). These tests were implemented twice, the first and the third year. Methods that are more demanding and require an advanced analytical base, samples were sent to selected consortium laboratories that are able of performing those tests (HAU, UF, CSIC). Only one measurement occasion was applied (the first year of the project).

*Results*

The chemical test battery showed that extractable trace element (TE) concentrations generally decreased more significantly in soils managed by *in situ* stabilisation combined with phytoexclusion, phytostabilisation or phytoextraction than in soils only managed with phytoextraction.

The extractant strength towards dissolution of several tested TE (e.g. Cd and Zn) was in increasing order: $H_2O < NaNO_3 < NH_4NO_3 <$ EDTA < *aqua regia,* despite the broad range of the total soil Cd and Zn concentrations. Good result reproducibility was achieved as similar results were obtained by repeating the extractions on the samples collected at the same places of each site two years later. Salt solutions were less effective in extracting oxyanions, such as arsenic (As), than distilled water. Extractions that best describe phyto- and bioavailable As should be further investigated.

Among the chemical extractions, the $NH_4NO_3$ and EDTA-extractions showed the most frequent differences in the extracted TE concentrations between the treated and untreated soils, while the most frequent correlations with the biological responses occurred for $NH_4NO_3$, followed by $NaNO_3$-extractable TE pools. Pseudo-total (*aqua regia* extractable) concentrations showed no significant correlation with the biological responses.

Among the bioindicators (plants, earthworms and nematodes), dwarf beans, especially through root mass, followed by shoot length, and stress enzyme activities, were the most responsive indicators to the soil treatments. Even though the selective chemical extractions did not always show statistically significant changes in TE extractability, dwarf beans and stress enzymes developed a stronger response to the tested GRO options. Generally, the plant growth decreased with higher extractable TE concentrations in soil, while bean stress enzymes reacted in the opposite way, *i.e.* increased with increasing TE extractability.

The soil biochemical properties positively responded to the different phytoremediation options, with arylesterase, urease and protease enzymatic activities, nitrification and ammonification potentials responding in all studied cases. The β-glucosidase activity only responded in sites where organic amendments were used. Similar responses were observed for soil N and P, which significantly changed only in soils amended with organic matter.

The measured microbiological and biochemical endpoints indicated significant improvement of soil functionality in soils with the heaviest contamination and where organic amendments were used such as in the French, German and Swiss sites. In soils with moderate contamination such as those of Belgian and Swedish sites, only some of the microbiological and biochemical endpoints were improved by the adopted GRO strategy.

Among the microbiological endpoints, the most responsive were nitrification and ammonification potentials, followed by soil enzymatic activities, which were similar to soil respiration, and the least responsive was microbial biomass. These microbiological and biochemical endpoints are in a good agreement with soil toxicity data and TE solubility and mobility estimated by chemical extractions, and therefore are robust indicators on which management decisions can be based on. It is expected that soil microbial biomass and functional activity increases during the early stages of phytomanagement TE contaminated sites up to typical levels. This is because soils, like any other ecosystem, have an own maximum carrying capacity, which is site specific and depends on the pedo-climatic conditions, vegetation and site management.

Functional gene diversity of the soil microbial communities that was measured in soils managed by phytoextraction using willow short-rotation coppice (SRC) showed no significant changes. The functional diversity of the soil significantly increased only in soil where amendment with a mixture of organic matter and dolomitic limestone were used in addition to the willows, indicating that microbial communities responded to SRC-based GRO by enriching carbon degradation, nutrient cycling (nitrogen and phosphorous) and metal resistance response gene families.

The analysis of the microbial community structure by the clustering of DGGE profiles showed clear differences between the treated and the untreated soils (at both the total community and phylogenetic group level), while the qPCR technique showed differences in the number of gene copies (nirK, nirS, nosZ, amoA), demonstrating that GRO implementation can lead to a shift in bacterial community and diversity. Shifts in community structure were more pronounced in soils where phytoexclusion or phytostabilisation had been implemented.

*Conclusions*

Differences between the treated and untreated soils were detected mainly for soils that were treated by addition of soil amendments, especially those containing organic matter. The tests that showed differences include chemical extractions, enzyme activities, soil N and P content, microbiological and biochemical endpoints, the functional diversity and structure of the soil microbial communities.

In general, the microbiological and biochemical endpoints were in a good agreement with soil toxicity data, TE solubility and mobility estimated by chemical extractions, and can be robust indicators suitable for making decisions on which management option should be used.

Based on the obtained results with the selected evaluation methods, it is suggested that a minimum risk assessment battery to compare or monitor the sites phytomanaged by GROs might consist of the 1M $NH_4NO_3$ extraction and the dwarf bean Plantox test including the plant stress enzyme activities.

It can be also recommended to include several biochemical analyses into test batteries, but not rely on a single endpoint, especially in soils where organic amendments and pH conditioners are used. This is because soil microbiological and biochemical endpoints respond to several environmental co-variables such as soil organic matter, nutrient content and availability, pH value, water holding capacity, plant and litter cover.

Although the microbial community structure showed clear differences between the treated and the untreated soils, these methods are costly and labour intensive and at this day could not be applied on a commercial basis.

## 3.4 Improving GRO through plant selection and modification in soil trace element bioavailability

The objectives were to evaluate and identify methods or tools for improving the performance of different gentle remediation options. The work carried out within this working package was distributed amongst 5 main tasks, targeting the following strategies: (1) selection of appropriate plant species, cultivars, varieties or clones for application in distinct GRO; (2) application of microbial inoculants for improving plant performance and/or their phytoremediation potential; (3) addition of amendments for reducing trace element (TE) bioavailability (*in situ* stabilisation and phytoexclusion, and (aided) phytostabilisation); (4) incorporation of appropriate agronomic techniques to improve plant performance, TE removal or immobilisation; and finally, (5) the use of effective soil amendments and/or amendment-microbial inoculant combinations for TE immobilisation (phytostabilisation).

**Selection and screening of plant species, cultivars, varieties or clones for application in GRO**

For phytoextraction plants must be able to accumulate high concentrations of TEs in their harvested parts 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 in their above-ground biomass and at the same time possess some economic added value (renewable biomass for bio-economy and/or bio-ores). High-biomass crops (annuals or perennials) and woody plants (short rotation coppice (SRC)) 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). Phytostabilisation can be combined with excluder-based SRC for bioenergy purposes (selection of genotypes is based on TE exclusion and also based on their characteristics in relation to conversion processes, e.g. calorific value, bulk density, moisture content, ash and extractive content). Selection of pollutant-excluding agricultural cultivars for cultivation on contaminated and/or remediated land contributes towards reducing the entrance of harmful trace elements into the human food chain.

Various plant groups were assessed throughout the duration of Greenland on both a field scale and at a bench level (greenhouse pot experiments). The range of plant types/species assessed by the different groups is presented in Table 1. Plant species, cultivars, varieties or clones were assessed for distinct GRO: phytostabilisation (TE tolerance and exclusion capacity), *in situ* stabilisation and phytoexclusion (TE-excluding phenotypes) and phytoextraction (TE accumulation capacity). Field/pot experiments were established and carried out to evaluate plant species for their TE resistance, TE extraction potential and/or biomass production.

In particular, well assessed mutant-lines of sunflower and tobacco with enhanced yield and stress tolerance were provided by PT-F to partners for further assessment and development at the different Greenland sites. From the existing seed bank of 230 sunflower inbred-lines (M3-M6), the most promising sunflower mutant-lines were selected and tobacco in vitro-selections were performed. Using high yielding M3-6 sunflower mutant inbred-lines with large genetic variability enables more efficient mutant selection for enhanced TE tolerance, extraction and high yield characteristics, and speeds up fast track-breeding. Four successive crops of sunflower and tobacco variants were carried out in five Greenland sites over the period 2011-2014. The plant biomass production and metal extraction potentials for the different variants were assessed and compared between sites and years. The progress and results of this comparative screening were presented in five deliverables (see Table 3.4.1).

**Table 3.4.1. Plant groups assessed by different partners at a field- and bench-scale throughout Greenland.**

| **Plant group tested** | **Partners involved** | **Related deliverables** |
|---|---|---|
| ***Woody plant species:*** | | |
| *Salix, Populus* clones | CSIC;<br>INRA; UH;<br>LfULG | D4.17: List of Cu-tolerant grasses/trees and TE-excluding crop cultivars<br>D4.21: First screening of Cu-tolerant grasses/trees |
| ***Grassy species:*** | | |
| *Agrostis* sp.,<br>*Festuca* sp.,<br>*Vetiveria* | CSIC;<br>INRA;<br>IUNG; | D4.17: List of Cu-tolerant grasses/trees and TE-excluding crop cultivars<br>D4.21: First screening of Cu-tolerant grasses/trees |

| | | |
|---|---|---|
| *zizanioides, Agropyron elongatum* | LfULG | |
| ***High biomass annual crops:*** | | |
| Tobacco, sunflower variants | PT-F; UH; INRA; CSIC | D4.16: Provision of a selection of improved sunflower and tobacco genotypes;<br>D4.19: First mutant screening on pilot sites;<br>D4.23: Second mutant screening and candidate selection; D4.25: First comparative mutant screening and assessment; D4.29: Second comparative mutant screening and assessment of top ten sunflower and tobacco traits. |
| Brassica spp. | UH | - |
| ***Agricultural crops (metal-excluding phenotype):*** | | |
| Barley, wheat, maize cultivars | AIT; LfULG | D4.17: List of Cu-tolerant grasses/trees and TE-excluding crop cultivars<br>D4.20: First screening of TE-excluding agricultural crops |
| ***Hyperaccumulating plant species:*** | | |
| *Noccaea caerulescens* | CSIC; BOKU | - |

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

Upscaling of GRO from greenhouse to field conditions clearly requires incorporating agronomical knowledge into the remediation process. The influence of management options, such as planting densities and harvest systems, or the need for crop rotations or intercropping, irrigation, or weed and pest control, are not taken into account under bench scale evaluations. Based on the experience and lessons learnt from the long-term Greenland field case studies, the partners involved in this task produced a review article summarising agronomic practices against their demonstrated or potential positive effect on GRO performance. Potentially negative effects of GRO, such as the introduction of potentially invasive species, were also discussed. A series of recommendations were made for increasing the GRO success and aiding stakeholders in related decision-making, and these can be summarised as follows:

(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 $m^2$) 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;

(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.

The main findings related to this task formed part of one deliverable (D4.22: *Knowledge of agronomic practices for improving plant establishment*) and were published in the joint publication:

Petra Kidd, Michel Mench, Vanessa Álvarez-López, Valérie Bert, Ioannis Dimitriou, Wolfgang Friesl-Hanl, Rolf Herzig, Jolien Olga Janssen, Aliaksandr Kolbas, Ingo Müller, Silke Neu, Giancarlo Renella, Ann Ruttens, Jaco Vangronsveld, Markus Puschenreiter (2015) Agronomic practices for improving gentle remediation of trace element-contaminated soils. *International Journal of Phytoremediation* (DOI:10.1080/15226514.2014.1003788, in press).

### Use of microbial inoculants for improving plant performance and/or phytoremediation potential

Exploiting the plant-microbial partnerships in phytoremediation is generally based on the capacity of the bacteria to, on one hand, improve establishment, growth and plant survival (plant-growth promotion); and, on the other hand, to act directly on the contaminant. Contaminated sites are not only a source of interesting plant species for application in phytoremediation but also of microorganisms. During GREENLAND numerous collections of plant-associated bacteria were obtained from a series of trace element-contaminated or TE-rich soils and from a variety of plant host types (from hyperaccumulating plant species to high-biomass crops and woody trees) (Table 3.4.2). Cultivable strains were isolated and characterised for their PGP traits, capacity to modify soil TE bioavailability and their potential application in phytoremediation of TE-contaminated soils.

**Table 3.4.2. Bacterial collections obtained by different partners during the duration of Greenland.** Based on D4.18: List of microbial strains or consortia with PGP and/or TE solubilising/immobilizing capacities.

| Partner | Host plant species | Bacterial type | Characterised traits | References |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| CSIC | Grass species, woody trees and shrubs | Rhizobacteria | 16S rDNA sequence identification, Cd/Zn MTC, $PO_4$, Surf, Sid, IAA | Becerra-Castro et al. 2012 |
| CSIC | Hyperaccumulating plant species | Rhizobacteria | 16S rDNA sequence identification, Ni MTC, $PO_4$, Surf, Sid, IAA | Álvarez-López et al. 2015 |
| INRA | *Agrostis capillaris* | Root endophytes | 16S rDNA sequence identification, $PO_4$, Surf, Sid, IAA | Kolbas et al. 2015 |
| UHASSELT | Willow clones | RhizobacteriaRoot endophytes | 16S rDNA sequence identification, Cd MTC, $PO_4$, Surf, Sid, IAA, OA | Janssen et al. 2015 |
| UHASSELT | *Brassica napus* | Rhizobacteria<br>Endophytes | 16S rDNA sequence identification, Cd MTC, $PO_4$, Surf, Sid, IAA, OA | Croes et al. 2015 |
| UHASSELT | *Agrostis capillaris* | Seed endophytes | 16S rDNA sequence identification, Cd MTC, $PO_4$, Surf, Sid, IAA, OA | Truyens et al. 2014 |
| UHASSELT | *Lupinus* sp. | Rhizobacteria<br>Endophytes | 16S rDNA sequence identification, Cd MTC, $PO_4$, Surf, Sid, IAA, OA | Weyens et al. 2014 |
| AIT | *Zea mays* cvs. | Rhizobacteria<br>Endophytes | 16S rDNA sequence identification, Cd/Zn MTC, $PO_4$, Surf, Sid, IAA, ACC | Touceda-González et al. 2015 |

*Abbreviations:* maximum tolerable concentration, MTC; $PO_4$, inorganic $PO_4$-solubiliser; Ac, acid producer; Surf, biosurfactant producer; Sid, siderophore producer; IAA, indoleacetic acid producer; OA, organic acid-producer

*References:*

C. Becerra-Castro, C. Monterroso, A. Prieto-Fernández, L. Rodríguez-Lamas, M. Loureiro-Viñas, M.J. Acea, P.S. Kidd (2012) Pseudometallophytes colonising Pb/Zn mine tailings: A description of the plant–microorganism–rhizosphere soil system and isolation of metal-tolerant bacteria. J. Hazard. Mat. 217– 218: 350– 359.

Álvarez-López, V., Prieto-Fernández, A., Becerra-Castro, C., Monterroso, C., Kidd, P.S. (2015) Rhizobacteria associated with the flora of three serpentine outcrops of the Iberian Peninsula. Plant Soil (submitted).

Kolbas, A., Kidd, P., Guinberteau, J., Jaunatre, R., Herzig, R., Mench, M. (2015) Endophytic bacteria take the challenge to improve Cu phytoextraction by sunflower. Environ Sci Pollut Res (in press), DOI 10.1007/s11356-014-4006-1.

Janssen, J., Weyens, N., Croes, S., Beckers, B., Meiresonne, L., Van Peteghem, P., Carleer, R., Vangronsveld, J. (2015) Phytoremediation of metal contaminated soil using willow: exploiting plant-associated bacteria to improve biomass production and metal uptake. International Journal of Phytoremediation (submitted).

Truyens, S., Jambon, I., Croes, S., Janssen, J., Weyens, N., Mench, M., Carleer, R., Cuypers, A., Vangronsveld, J. (2014) The effect of long-term Cd and Ni exposure on seed endophytes of *Agrostis capillaris* and their potential application in phytoremediation of metal-contaminated soils. International Journal of Phytoremediation, 16, 643–659. DOI: 10.1080/15226514.2013.837027

Weyens, N., Gielen, M., Beckers, B., Boulet, J., van der Lelie, D., Taghavi, S., Carleer, R., Vangronsveld, J. (2014) Bacteria associated with yellow lupine grown on a metal-contaminated soil: *in vitro* screening and *in vivo* evaluation for their potential to enhance Cd phytoextraction. Plant Biology, 16: 988-996. doi:10.1111/plb.12141

Croes S., Weyens N., Colpaert J., Vangronsveld J. (2015) Characterization of the cultivable bacterial populations associated with field grown *Brassica napus* L.: an evaluation of sampling and isolation protocols. Environmental Microbiology, doi:10.1111/1462-2920.12701.

Touceda-González, M., Brader, G., Antonielli, L., Balakrishnan Ravindran, V., Waldner, G., Friesl-Hanl, W. Sessitsch, A. (2015) Bioavailability of heavy metals, plant growth and microbiome characteristics due to the amendment of immobilizers and the plant growth-promoting strain *Burkholderia phytofirmans* PsJN. Soil Biology and Biochemistry (submitted).

A large part of the Greenland project was dedicated to carrying out bench-scale bioaugmentation trials using different combinations of potential PGP/metal-(im)mobilising bacterial strains and plant species. Promising plant-bacterial partnerships were presented in the deliverable D4.24: *Testing of candidate plant-microbial partnerships*. In general these studies showed that the inoculation of selected plant species/cultivars/clones with beneficial plant-associated bacteria can significantly enhance biomass production and/or plant metal uptake, but that the results are highly plant-, bacterial strain- and soil-specific. Some advances were also made in small-scale field trials, these studies underlined the need for further research and also showed that results obtained in *in vitro* or using pot-scale experiments do not always correspond with the effects obtained in the field where additional complicating factors can influence the overall outcome and performance of the inoculant. The main results obtained at a field-scale were presented in deliverable D4.26: *Identification of microbial strains or consortia with PGP and/or TE solubilising/immobilising capacities at field-scale*.

In addition to the publications of the individual partners (listed in previous reports and D6.40) some joint publications resulted from this task:

Angela Sessitsch, Melanie Kuffner, Petra Kidd, Jaco Vangronsveld, Walter W. Wenzel, Katharina Fallmann, Markus Puschenreiter (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology & Biochemistry* 60: 182-194.

Álvarez-López, V., Prieto-Fernández, A., Janssen, J., Herzig, R., Vangronsveld, J., Kidd, P.S. (2015) Bacterial inoculation methods influence the phytoextraction capacity of *Nicotiana tabacum*. *International Journal of Phytoremediation* (in preparation).

### Use of amendments for reducing TE bioavailability (*in situ* stabilisation and phytostabilisation)

Soil amendments including liming agents (calcite, burnt lime, slaked lime, dolomitic limestone), phosphates and apatites, Fe, Al and Mn oxyhydroxides, biochars (carbon-rich end product of the pyrolysis of biomass), organic amendments, and industrial waste products have been widely used in phytostabilisation and some (aided) phytoextraction experiments. The formation of insoluble TE chemical species reduces leaching through the soil profile and the labile metal pool in the soil. Amendment trials were carried out throughout the full duration of the Greenland project by the different partners on both a field- and bench-scale (see Table 3.4.3). In addition, several collaborative initiatives were carried out between WP4 members, and one collaborative initiative between Greenland and the FP7 HOMBRE project (Nº. 265097). The main results of these screenings were presented in the deliverable D4.27: *Database of efficient amendments and/or combinations of amendments for use in phytostabilisation approaches and selection of treatments for particular site conditions*. The effectivity of soil amendments is generally assessed on the basis of physico-chemical and selective chemical extractions demonstrating a reduction in soil metal mobility and availability. However, biological evaluations are also vital when assessing the potential use of a soil amendment in a given remediation procedure. In these studies biochemical properties (soil enzyme activities) as well as an ecotoxoxicity assay (using earthworms) was carried out in order to obtain a more reliable estimation of the efficiency of the metal immobilisation obtained. Effective amendments were identified for the *in situ* stabilisation and phytostabilisation of Cd-, Pb- and Zn-contaminated soils. Results indicated that amendment combinations were the most successful in reducing toxicity and promoting plant growth and soil enzyme activities, and reducing metal mobility and bioaccumulation in earthworms. The best-perfomring combinations for this type of contaminated soil were the $CaHPO_4$+drinking water residues (DWR)+compost, iron grit(IG)+Linz-Donawitz slag+compost, and IG+cyclonic ashes+compost.

**Table 3.4.3 Soil amendments used for *in situ* stabilisation and (aided) phytostabilisation and assessed during the Greenland project**

| **Inorganic amendments** | **Organic amendments** |
|---|---|
| Rock phosphate (a major source of P fertilizers) | Manures |
| Thomas basic slag (a by-product of the iron industries) | Biosolids (sewage sludge), Composted biosolids |
| Wood ashes | Green waste composts |
| Cyclonic ashes | |
| Zerovalent iron grit | **Others** |
| Linz-Donawitz slag | Biochar |
| Siderite | |
| Gravel sludge | |
| Red mud | |
| Drinking water residues | |

Bench-scale evaluations were carried out to assess the potential of different biochars and green waste compost to immobilise Cu in a contaminated soil. Biochar and compost were shown to reduce leachable copper in contaminated soil, the combined application of the two amendments was shown to be effective for immobilisation and plant growth, biochars and composts were shown to increasingly immobilise copper with increased application rate, and the amendments do not effect a reduction in copper bioavailability alone, but rather initiate multiple concomitant changes to soil which contribute to reduced phytotoxicity. These results indicate that these amendment types could be successfully used in combination with phytoremediation to further decrease pollution risks and potentially provide a saleable energy crop. However, more research is required to further establish the detailed operating windows of these amendments and to more clearly define the influence of different feedstock materials on biochar and recycled organic matter properties. Additionally, the amendments successfully trialled will require field trials to determine their efficacy on a larger scale and confirm their potential for deployment on a full-scale remediation site. Nonetheless, both recycled organic matter and biochar have the potential to simultaneously stabilise soil contaminants, improve soil quality and offer carbon sequestration benefits. Biochar has been shown to have significant longevity and therefore may be economically attractive, as it may provide a long-term effect without repeat applications.

Finally, GREENLAND assessed the combined use of TE-immobilising soil amendments and beneficial plant-associated bacteria as a means of simultaneously improving plant growth/establishment and reducing metal mobility and bioavailability. Again this was assessed on a bench- and field-scale, and presented in the deliverable D4.28: *Report on possibility to enhance assisted phytostabilisation through microbial inoculation.*

## 3.5 Appraisal of current GRO practice, and development of implementation guidance and decision support

This document summarises the main S&T results / foregrounds delivered by WP5 of the GREENLAND project (Appraisal of current GRO practice, and development of implementation guidance and decision support). Results are presented in relation to WP deliverables.

### D5.1: (Multi-lingual) Best-practice guidance document for the application of GRO at field-scale (including appraisal of the various options available, evaluation of large-scale field trials, analysis of valorisation potential, and suggested methods and monitoring)

Outputs from WP1-4 were collated, evaluated and reviewed to produce a best-practice guidance document for the application of GRO at field-scale. Following detailed discussions at the regular Greenland project meetings (including with the project Advisory Board members), and critical review of existing guidance documents from (a) project partners AIT and BOKU, and (b) ADEME (Fr), a first draft of the guidance document was tabled at the October 2013 periodic meeting (Brighton). A finalised version of the Best Practice Guidance document was completed in December 2014, following tabling (and input from Greenland partners and Advisory Board members) at the Frankfurt end of project meeting. Versions of the document have been produced in English, French and German, with the Best Practice Guidance now available through the Greenland project website (www.greenland-project.eu) and via the EUGRIS web portal (www.eugris.info). The Best Practice Guidance document, which cross-links to the project decision support tool (DST, deliverable 5.3), is structured as follows:

1. Definitions and context – what is GRO and how does it work?
2. Overview of current state of development and risk management capability
3. Case / success stories
4. Potential economic, environmental and social benefits
5. Operating windows for GRO
6. Further information sources

*Appendices:*
*Appendix 1: Design and implementation (WP1)*
*Appendix 2: Cultivars and amendments (WP4)*
*Appendix 3: Safe biomass usage (WP2)*
*Appendix 4: Indicators of success and methods (WP3)*
*Appendix 5: DST and cost-calculator (WP5)*
*Appendix 6: Stakeholder engagement guidelines (WP5)*
*Appendix 7: Further examples and case studies (WP1)*

Within the document, section 3 presents three case studies from WP1, representing cases where application of phytoextraction, aided phytostabilisation, and *in situ* stabilisation / phytoexclusion phytomanagement strategies have led to demonstrable source removal, pathway management or receptor protection. Further examples are given in Appendix 7. In section 4, the guidance cross-refers to three assessment tools which allow assessment of wider benefits from GRO application: the European Union FP7 HOMBRE project (grant 265097, www.zerobrownfields.eu) Brownfield Opportunity Matrix (BOM); the SURF indicator sets on sustainability; and an outline Cost-Calculator (developed within the Greenland project). These tools were felt to provide a more thorough and defensible assessment of wider benefits from GRO application than a previous tabular indicator set approach used in earlier draft versions of the Best

Practice Guidance, and are discussed further under deliverable 5.3. Under section 5 (Operating Windows) of the guidance we present quick reference tables on GRO applicability (*Are GRO applicable to your site?*) and treatable contaminants (*Which metal(loid) contaminants can GRO treat?*), and a link to outline Operating Window Matrices in the project DST (see Deliverable 5.3).

## D5.2: Guidelines for stakeholder participation, engagement and empowerment when implementing GRO

Following collation and review of existing methods of stakeholder engagement (MS34, submitted at end of project year 1), and stakeholder engagement discussions held at the periodic Greenland meeting in Pulawy and at the 9th International Conference on Phytotechnologies at Hasselt University in September 2012 (via a special round-table session), a review publication with recommendations and stakeholder engagement guidelines for GRO was published in the Journal of Environmental Management (JEM, Impact Factor 3.245) in 2013. This review article (co-authored by representatives of 6 Greenland partner organisations) incorporates case study material from the Greenland test sites Biogeco platform (SW France), and Krummenhennersdorf (Saxony, Germany), and reviews the current state of the art in stakeholder engagement strategies. It identifies key principles for stakeholder engagement when implementing GRO. Following publication of this article, work under this deliverable focused on:

1. Integrating stakeholder engagement strategies and guidance into the project decision support tool (DST) and the Best Practice Guidance;
2. Developing criteria for the identification of different stakeholders profiles/categories - their expectations, influence, characteristics, preferred approaches to engagement and levels of engagement.

Under these points, a stakeholder engagement module has been included in phase 2 of the project DST which gives a context and rationale for stakeholder engagement, shows (a) methods and strategies for effective stakeholder engagement and (b) stakeholder classification criteria, and which presents the key guidance for stakeholder engagement published in the Journal of Environmental Management paper. Under point 2 specifically a table and representative listing for identification and classification of stakeholders, based on published literature and current practice at the Greenland and other sites, has been presented and finalised at the final project meeting in Frankfurt, and included in the stakeholder engagement module of the DST. Key information from the stakeholder engagement module, including stakeholder engagement guidelines for GRO, is also presented in Appendix 5 of the Best Practice guidance.

## D5.3: Practical GRO-focused decision support tool (DST), for integration into existing national decision support frameworks

A working GRO-focused DST was developed in the early project phases (deliverable MS35 – Production of Outline (Generic) DST), following the “tiered” (or layered) model proposed in the earlier ERA-NET SUMATECS project and in Onwubuya et al. (2009), initially within the framework of the UK Model Procedures for the Management of Land Contamination (CLR11). Research underpinning this DST development was submitted as a PhD thesis by K. Onwubuya (University of Brighton) in early 2013, which also includes validation and testing of this outline model using 3 case studies (sites in east

London, Lommel, and the Biogeco platform), the latter two drawn from the Greenland test sites. This thesis was successfully defended in April 2013. Following this testing and evaluation of the outline model produced (milestone MS36) the project DST was further developed and populated during year 4 of the project, drawing on outputs from the other Greenland WPs (1-4), and following tabling (and feedback) at:

(a) the 11th International Conference of the International Phytotechnology Society (Sept 30th – Oct. 3rd 2014, Heraklion, Crete), to a dominantly European and US academic and industry audience; and

(b) the "CABERNET 2014: Tailored & Sustainable Redevelopment - towards Zero Brownfields" conference (14-16th October 2014 in Frankfurt am Main, Germany), to a dominantly EU-based research, industry and consultancy audience,

and following feedback from project Advisory Board members at the final Greenland project meeting (also Frankfurt).

The DST is a 3 phase MS Excel-based model (figure 1), designed to build in complexity and time effort (and technical detail) through its 3 phases, and which embeds the best practice guidance produced in D5.1 and the stakeholder engagement principles and identification criteria produced in D5.2. It is designed to link to existing national decision support frameworks at the Options Appraisal stage. A full user's guide for the tool can be accessed by selecting a "User Guide" tab on the front/entry page (figure 1). **Phase 1** (***Initial Concepts/Feasibility***) includes definitions, high level operating windows for GRO (in terms of their scope and risk management capability), "success stories" of GRO (i.e. where GRO strategies have led to demonstrable source removal, pathway management or receptor protection), and an outline contaminant matrix for the applicability of various GRO options at trace element contaminated sites (TECS), based on data from the GREENLAND site network. **Phase 2** (***Exploratory stages/confirmation***) includes modules on stakeholder engagement (including stakeholder engagement principles and stakeholder identification criteria, discussed under D5.2 above), and sustainability/ wider benefits assessment, where links to three matrices/modules are provided:

(a) The European Union FP7 HOMBRE project (grant 265097, www.zerobrownfields.eu) **Brownfield Opportunity Matrix** (BOM). This is an Excel-based qualitative screening tool to help decision makers identify which services they can obtain from "soft reuse" interventions (including GRO) at a site, and how these services interact. Greenland partners have collaborated with HOMBRE to populate the operating and opportunity windows for GRO within the BOM. The matrix can be used to map the prospective range of opportunities that might be realised by a remediation or redevelopment/regeneration project for "soft" reuses[1], and the project's consequent sources of value.

(b) The SURF **indicator sets on sustainability**, which outline the various headline indicator categories that should be considered during sustainability assessment in land remediation projects. These indicator categories provide a checklist for agreeing a scope for a sustainability assessment. A tiered approach to sustainability assessment is suggested, in keeping with guidance from NICOLE (www.nicole.org) and SuRF-UK. Many decisions may be resolvable on the basis of a qualitative

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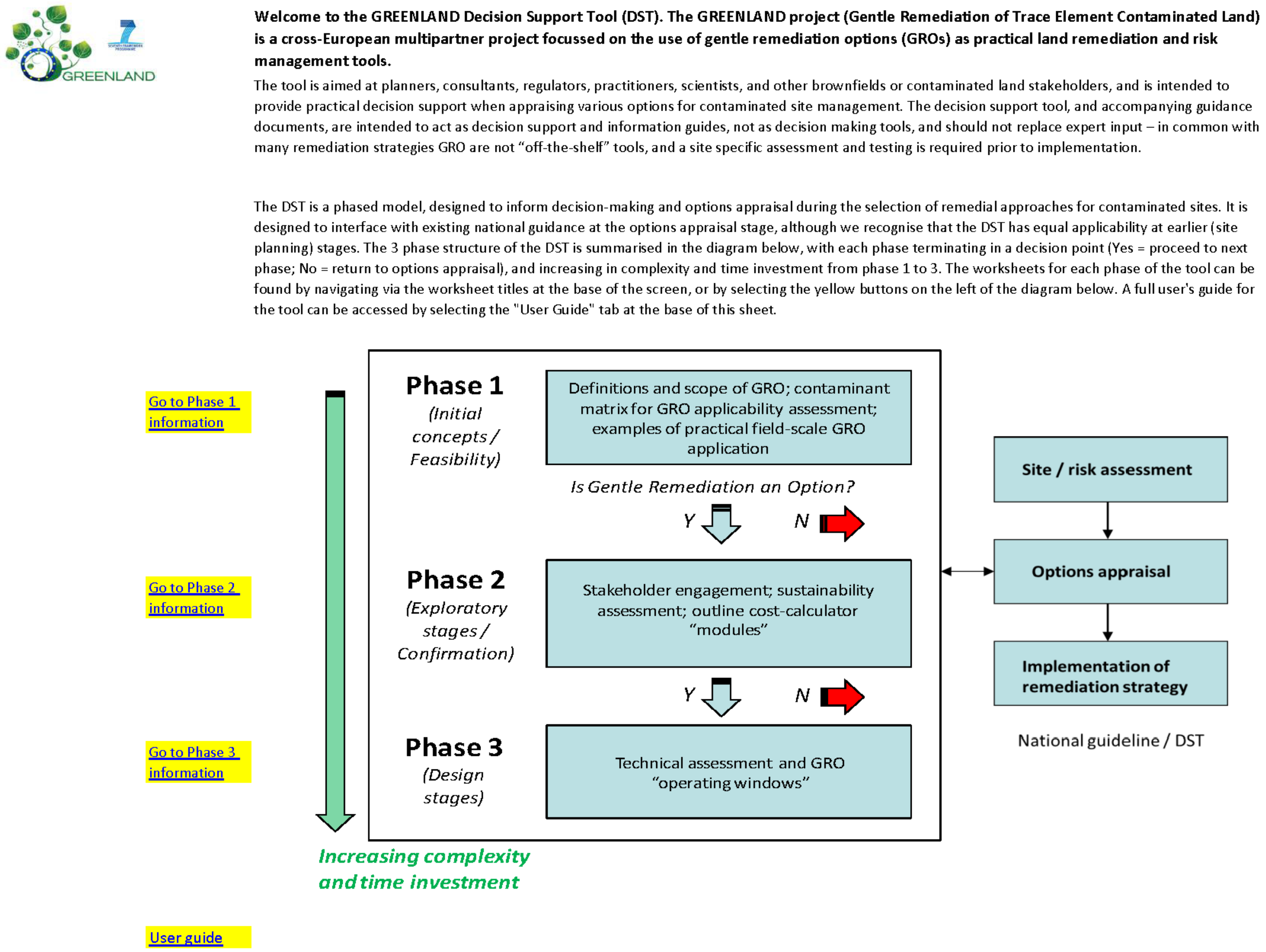
[1] I.e. where the soil surface is not sealed by a building or infrastructure

approach. Where issues are more complex a semiquantitative or even quantitative approach may be used such as Life Cycle Assessment (LCA) and Cost Benefit or Multi-Criteria Analysis (CBA/MCA). However, in most caes quantitative approaches only allow a partial consideration of the full range of possible sustainability issues.

(c) An outline **Cost-Calculator**, which has been developed within the Greenland project and incorporates user-entered cost data (including site preparation costs; plant and planting costs; site costs; biomass costs and revenues; and monitoring costs) to estimate the economic value proposition of GRO at a particular site. This module has been "calibrated" using data from the Greenland site network, which are used to test the cost calculator and give input examples.

**Phase 3** (***Design Stages***) of the DST provides a technical assessment and implementation guidance for GRO, with detail from WP1 – 4, and also outline operating windows for GRO. For the latter, we provide three MS Excel-based operating window matrices which allow the user to check the outline applicability of GRO (grouped as phytoextraction, phytostabilisation, and immobilisation/phytoexclusion) to a specific site, in terms of local soil pH, site plant toxicity, climate, soil type, and depth of contamination. The tool then refers the user to national contact points from the Greenland consortium, and further references.



**Welcome to the GREENLAND Decision Support Tool (DST). The GREENLAND project (Gentle Remediation of Trace Element Contaminated Land) is a cross-European multipartner project focussed on the use of gentle remediation options (GROs) as practical land remediation and risk management tools.**

The tool is aimed at planners, consultants, regulators, practitioners, scientists, and other brownfields or contaminated land stakeholders, and is intended to provide practical decision support when appraising various options for contaminated site management. The decision support tool, and accompanying guidance documents, are intended to act as decision support and information guides, not as decision making tools, and should not replace expert input – in common with many remediation strategies GRO are not "off-the-shelf" tools, and a site specific assessment and testing is required prior to implementation.

The DST is a phased model, designed to inform decision-making and options appraisal during the selection of remedial approaches for contaminated sites. It is designed to interface with existing national guidance at the options appraisal stage, although we recognise that the DST has equal applicability at earlier (site planning) stages. The 3 phase structure of the DST is summarised in the diagram below, with each phase terminating in a decision point (Yes = proceed to next phase; No = return to options appraisal), and increasing in complexity and time investment from phase 1 to 3. The worksheets for each phase of the tool can be found by navigating via the worksheet titles at the base of the screen, or by selecting the yellow buttons on the left of the diagram below. A full user's guide for the tool can be accessed by selecting the "User Guide" tab at the base of this sheet.

**Figure 1 : Front/entry page of the Greenland project decision support tool (DST), showing overall DST structure and introductory text for the user.**

The project DST has been finalised and uploaded to the Greenland project website (www.greenland-project.eu), and forms the focus of a technical paper which has been submitted to the journal "Remediation", targeting this journal's main audience of contaminated land regulators, consultants and practitioners.

# 4. The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

Soil is a non-renewable natural resource. It provides the basis for all terrestrial human activities, including agriculture and forestry, and is therefore an irreplaceable basis for the survival and prosperity of the European society. Due to anthropogenic activities (e.g. industrialisation, intensive agriculture, urbanisation etc.) soil is getting lost every day. Only by soil sealing, 275 hectares of soil were lost per day in the 1990s, with a slight reduction to 252 hectares per day in recent years. In addition to the loss of soil, further soil resources are degraded by organic matter decline, salinization or pollution. The Thematic Strategy for soil protection lists soil contamination as one of the eight major threats to soil. Local soil pollution has caused localised hot spots of contamination, whereas diffuse soil pollution has lead to widespread contamination of soils on a very large scale.

Conventional remediation technologies can (at very high costs) decontaminate small scale contaminations, whereas moderately polluted soils on a much larger scale ($km^2$) remained mostly untreated because of the impossibility to treat millions of $m^3$ of contaminated soils with excavation or washing treatments. GRO may serve as low cost, environmental and socio-economic friendly solutions to remediate polluted soils on a very large scale and thus help to overcome previous limitations. In the GREENLAND project the remaining problems of GRO development, up-scaling and biotechnological improvement have been successfully addressed and a set of well-proven methods allowing the remediation of TE-contaminated soil at low cost and with the potential to deliver additional financial benefits for land owners were delivered. In this way, GREENLAND delivered GROs as a basis for a new generation of green soil remediation technologies and thereby significantly contributed to overcoming one of the most problematic threats to European soils. More widely, promoting sustainable soil remediation methods, such as GRO, contributes to a number of the priorities listed in the Lisbon and Gothenburg agendas, such as climate change, public health and resource management as well as enhancing research and technological development and promoting entrepreneurship and skills.

Contamination of soils with TE is still a major problem in Europe. According to the European Environmental Agency, up to 3 million sites in Europe are contaminated, approximately 37 % of them with TE. This European problem can only be solved on a European scale. The best practice guidance document produced by GREENLAND (http://www.greenland-project.eu/downloads/Greenland_Handout.pdf) provides all the necessary information for applying GRO successfully and the decision support tool (DST; http://www.greenland-project.eu/downloads/DST%20download.xlsx) allows selecting the most suitable GRO for the specific site conditions and offers further information on stakeholder engagement and empowerment for further enhancing the success of the GRO application. The best practice guidance is also available in German (http://www.greenland-project.eu/downloads/Greenland_best-practice_guide_German.pdf) and French (http://www.greenland-project.eu/downloads/Greenland_best-practice_guide_French.pdf). Considering the huge number of contaminated sites, GRO implementation offers also a huge economic

potential for new entrepreneurship in bio-economy. New companies can be founded, focusing on GRO as a new and innovative environmental technology, applied on contaminated arable land (e.g. in cooperation with local farmers and farmer's associations), mine tailings, but also on former industrial sites, i.e. so-called brownfields. In the latter case, GRO application might be integrated into a general brownfield re-development plan (further information is provided by the Brownfields Management and Avoidance brochure, developed and published by the HOMBRE FP7 project: http://www.zerobrownfields.eu/quicklinks/HOMBRE_Broschure_2014_FINAL.pdf).
GRO application includes in most cases the use of specific plants (and associated microbes). In this way, biomass is produced, offering the opportunity of using it in various ways. However, in some cases the biomass is enriched with trace elements, thus GREENLAND focused on biomass valorisation options that are suitable for biomass harvested on GRO sites. By applying the tested technologies (e.g. combustion, pyrolysis, etc.) energy and raw materials for further use are produced. Due to the increased demand for biomass in Europe, e.g. because of the substitution of fossil fuels or the need for raw materials produced from plant biomass, more and more sites are needed for non–food biomass production. In order to avoid a competition for land between production of non-food biomass and food/fodder, additional sites are needed. Marginal lands, e.g. TE-contaminated sites, could offer a great opportunity for providing sites for biomass production, while due to the application of GRO the sites are remediated.

The output of GREENLAND has been presented to different target groups in many ways. The general public has been addressed in several newspaper articles as well as radio and TV broadcasts. The GRO performance in the field has been demonstrated in several field days, where the local community (e.g. farmers, local decision makers, local media) had the opportunity to see GRO sites and to talk with site managers. On one site (Arnoldstein, Austria) also school classes (Fig. 4.1) were invited to experience how GRO works in practice. The role of soils as a natural resource, loss of soil and remediation of soils were discussed at the site, but also in associated workshops in their schools. Newspaper articles as well as radio and TV reports provided information on GRO for the local population. On the GREENLAND website (www.greenland-project.eu) general information and project results (e.g. case studies, success stories, best practice guidance document, DST) were offered for scientists, stakeholders, practitioners and the general public.

Figure 4.1: Left: School children visiting the site Arnoldstein; Austria (source: http://www.nms-noetsch.ksn.at/). Right: Field day at the site Lommel, Belgium, at the harvesting (source: www.niewsblad.be, 25.2.2014).

One major target group of GREENLAND included stakeholders and decision makers on a national level. They were addressed in two major ways: on the one hand, six representatives of national environmental ministries or environmental agencies were invited to join the advisory board (AB) of GREENLAND. This board consisted of representatives of the following organisations:

- AT: Austrian Environmental Agency (Umweltbundesamt); Gernot Döberl
- DE: German Enviromental Agency (Umweltbundesamt): Jörg Frauenstein
- FR: Agence de l'Environnement et de la Maîtrise de l'Énergie (ADEME): Frédérique Cadiere
- SE: 2011-2012: NATURVÅRDSVERKET: Inger Johansson; 2013-2014: Länsstyrelsernas tillsynssamordnare förorenade områden: Klas Köhler
- PL: Polish Ministry of Environment, Department of Environmental Instruments: Joanna Kwapisz

They took part in four project meetings, where the project output was presented and discussed. The AB provided important feedback regarding the project progress and output, especially in the context of the different national circumstances. Furthermore, the AB offered substantial support for the development of the DST and the practical guidance handbook. In particular the handbook, showing not only technical instructions, but also success stories for GRO application, was considered by the AB as a crucial tool for bringing GRO into practical application. In addition, they also acted as "information hubs", transmitting the Greenland information to other stakeholders and decision makers (e.g. the Swedish EPA).

On the other hand, national and regional stakeholders as well as consultancies and environmental enterprises were also addressed in several local workshops and meetings, e.g., R&D days on management of polluted sites and soils Paris 2012 and Paris 2014; INRA Ecotoxicology Network – Biarritz 2014; several meetings with a representative (Dr. Harald Kasamas) of the Austrian Ministry for Environment and the Austrian Society for Management of Landfills and Contaminated Sites as well as the Austrian Soil Science Society; and many more.

The results of Greenland were also presented at several scientific conferences, with a total number of 250 oral and poster presentations (see list of dissemination). In this context, one major event was the joint final conference of the FP7 projects GREENLAND, HOMBRE, TIMBRE and GLOCOM in Frankfurt am Main (Germany) on October 14-17 2014 (http://www.zerobrownfields.eu/Displaynews.aspx?ID=566). In this forum, the main output of GREENLAND was presented to a diverse audience of scientists, students and stakeholders. Together with the results of the other projects, all wider aspects of soil remediation, brownfield regeneration and associated topics such as biomass use and valorisation were presented and discussed. In addition, the results of GREENLAND were prominently presented in several relevant international conferences, such as the International Phytotechnology Conferences, the SETAC (Society of Environmental Toxicology and Chemistry) conferences, the International Conferences of the Biogeochemistry of Trace Elements (ICOBTE), the Sustainable Remediation Conference, and many more. Within these conference series, GREENLAND has become a well-known “trademark” for research work on GRO.

Finally, the output of GREENLAND was published in several scientific journals. In total, 114 papers were published (see dissemination list). The most important publications were focussing on the assessment of GRO efficiency, on agronomic measures to improve GRO efficiency and on the decision support tool and stakeholder engagement (see Table on scientific dissemination).
Three selected key papers are:
Cundy AB, Bardos RP, Church A, Puschenreiter M, Friesl-Hanl W, Müller I, Neu S, Mench M, Witters N, Vangronsveld J (2013) Developing principles of sustainability and stakeholder engagement for "gentle" remediation approaches: The European context. J Environ Manage 129, 283-291
Kumpiene J, Bert V, Dimitriou I, Eriksson J, Friesl-Hanl W, Galazka R, Herzig R, Janssen J, Kidd P, Mench M, Müller I, Neu S, Oustriere N, Puschenreiter M, Renella G, Roumier P-H, Siebielec G, Vangronsveld J, Manier N (2014) Selecting chemical and ecotoxicological test batteries for risk assessment of trace element-contaminated soils (phyto)managed by gentle remediation options (GRO). Sci Tot Environ 496, 510-522.
Kidd P, Mench M, Álvarez-López V, Bert V, Dimitriou I, Friesl-Hanl W, Herzig R, Janssen JO, Kolbas A, Müller I, Neu S, Renella G, Ruttens A, Vangronsveld J, Puschenreiter M 2014. Agronomic practices for improving gentle remediation of trace-element-contaminated soils. Int J Phytorem (DOI:10.1080/15226514.2014.1003788, in press)

# The address of the project public website, if applicable as well as relevant contact details

www.greenland-project.eu

# GREENLAND FINAL REPORT

# Appendix to: Description of the main S&T results/foregrounds

The GREENLAND network of field sites is a cross-European network of metal(loid)-contaminated sites where the efficiency of phytomanagement strategies has been investigated on a medium- to long-term, under various contaminant (trace element) types and loadings and soil and climatic conditions, with various plant species and cultivars.

## Task 1.1. Remediation option appraisal

The general scheme for the remediation and phytomanagement of trace-element contaminated soils (TECS) comprises four stages: (1) risk assessment, (2) option appraisal, (3) implementation of remediation strategy and (4) phytomanagement (including biomonitoring and maintenance).

Nine partners (INRA, CSIC, AIT, HAU, SLU, LfULG, Ineris, and IUNG) were deploying Gentle Remediation Options (GRO) at field scale for research and demonstration purposes. Five main types of historically contaminated sites were investigated, under different climatic and soil conditions, with either diffuse contamination on a large area (generally with agricultural soils) or local contaminations at mining sites, industrial facilities and landfills (with technosols at several sites) (Tab. A1). Case studies were categorized based on soil contamination levels (Kumpiene et al 2014) and exposome types which encompasses life-course environmental exposures for a biological receptor (Wild 2012[1]). Based on the WP3 plant tests, the initial phytotoxicity of untreated soils followed the increasing order for dwarf bean: Bettwiesen, Freiberg, Piekary I & II < Lommel, Biogeco, Arnoldstein < Högbytorp, and for lettuce: Biogeco, Freiberg, Bettwiesen < Lommel, Piekary I & II, Arnoldstein < Högbytorp. Accordingly, the phytotoxicity of Bettwiesen, Freiberg and Piekary topsoils did not reflect their high total soil TE.

Five exposome patterns were addressed based on main contaminants involved in pollutant linkages: Cu (Touro, Biogeco), Cd/Zn (Pb) (Lommel, Bettwiesen, Phytosed, Phytoagglo, Arnoldstein, Piedrafita, Högbytorp, Freiberg), As (Reppel, Jales, Freiberg), Cr/Mo (Rive de Gier), and metal/PAHs (Biogeco, Chaban-Delmas, Phytoagglo, Borifer).

**Tab. A1 Summary of the Greenland network investigating GRO in long-term field trials**

| Partner | Sites | Country | Sources | GRO | Main contaminants |
|---|---|---|---|---|---|
| ***Landfill*** | | | | | |
| PT-F | Bettwiesen | Switzerland | former hot dip Zn galvanizing plant | 1 | Cd, Zn, Cr, Cu, Pb |
| IUNG | Piekary | Poland | Cd/Zn/Pb tailings | 3 | Zn, Cd, Pb |
| INRA | Chateauneuf | France | steel mill wastes | 3 | Cr, Mo, Zn, Ni, Cu, Pb |
| ***Atmospheric depositions on a large agricultural area*** | | | | | |
| HAU | Lommel | Belgium | Zn/Pb smelter | 1 | Cd, Zn, Pb |
| AIT | Arnoldstein | Austria | Zn/Pb smelter | 3 | As, Cd, Zn, Pb |
| LfULG | Freiberg-Halsbrücke | Germany | Zn/Pb smelter | 1, 2 | As, Cd, Pb |
| HAU/INRA | Reppel | Belgium/France | As refinery | 1 | As, Zn, Pb, Cd |
| ***Wood preservation facility*** | | | | | |
| INRA | Biogeco | France | wood preservative | 1, 2, 4 | Cu, Cu/PAHs |
| ***Mine, tailings*** | | | | | |
| CSIC | Touro | Spain | Cu mining | 1, 2 | Cu |
| CSIC | Piedrafita, Rubiais | Spain | Pb/Zn mining | 1 | Zn, Pb, Cd |
| INRA | Jales | Portugal/France | Au mining | 2, 3 | As, Zn |
| ***Technosols, other sources*** | | | | | |
| SLU | Högbytorp French trial | Sweden | Irrigation with landfill leachates | 1 | (Cd), Cr, Zn |
| INERIS | Phytosed ech 1 | France | dredged sediments | 1, 2 | Zn, Cd, Cu, Cr, Pb, Mo |
| INERIS | Phytagglo | France | brownfield | 1, 4 | Zn, Cd, PAHs |
| INRA | Chaban Delmas | France | embankments, harbor facilities | 2, 4 | Zn, Pb, Cu, Cd, Ni, As, Hg, PAHs |

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[1] Wild CP 2012. The exposome: from concept to utility. International Journal of Epidemiology 41: 24–32

| | | | | | |
|---|---|---|---|---|---|
| INRA | Borifer | France | metal surfacing | 2, 4 | Zn, Pb, Cu, Ni, As, Hg, PAHs |

1: (aided) phytoextraction, 2: (aided) phytostabilization, 3: in situ immobilization/phytoexclusion, 4: rhizodegradation

## 1. Setting of conceptual models

Conceptual models (CM) were built for all sites (n=16), with information gained on main sources of soil contamination, soil exposome and ecotoxicity, biological receptors such as plant and microbial communities, initial pollutant linkages and risks on site and nearby. The procedure was derived from those reported by the Environment agency (UK). Exposures, organization/biodiversity and functioning of plant, animal, mesofauna and microbial communities were mostly taken into account, in line with exposures of animal and human populations (Fig. A1). At some sites (*e.g.* Biogeco, Jales, Reppel, Freiberg, and Piekary), vertical migration from the topsoil as well as wind erosion were considered. Clusters with different soil exposome and ecotoxicity were defined at many sites (*e.g.* Biogeco, Arnoldstein, Bettwiesen, Chaban-Delmas, Phytosed, Touro, and Piedrafita). Diverse physico-chemical parameters, soil and technosol types were of concern, from acid sandy soils to calcareous soils and alkaline technosol. Mixed contamination (trace elements and organic contaminants such as PAHs) was taken into account at 4 sites (Biogeco, Chaban-Delmas, Borifer, and Phytoagglo). Climatic conditions varied from cold climate and short summer (Sweden) to Mediterranean climate and dry summer (Spain).

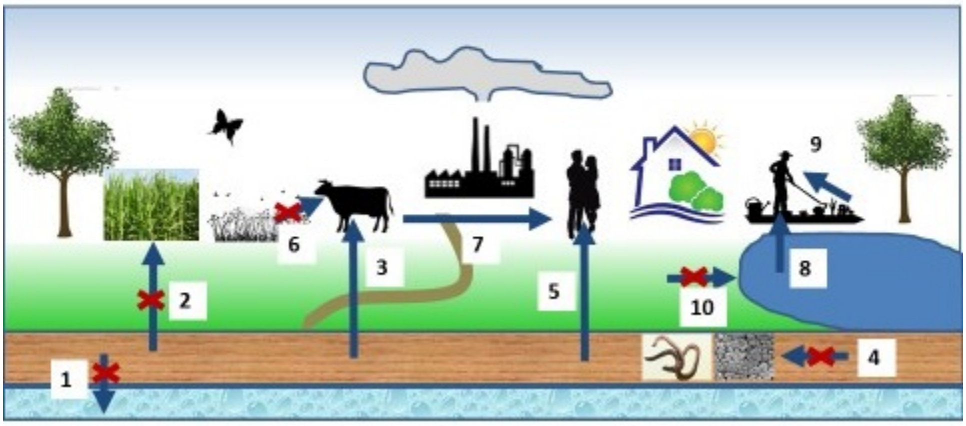

Fig. A1 Conceptual model and pollutant linkages considered on site and nearby.

Different end land uses were considered: landscaping, recreation area, production of annual crops for (non-food) plant-based feedstock and biosourced chemistry, production of metal-excluder crops (grasses, cereals), production of wood from short rotation coppice (SRC) and tree planting in line with eco-technologies. Conceptual models were summarized in the datasheets for all case studies.

## 2. Risk assessment – pollutant linkages

Spatial distribution of soil contaminants, physico-chemical parameters, exposome (notably labile TE pools quantified by either single extractions or DGT) and phytotoxicity were characterized (in coordination with WP3) at most sites. The test battery for assessing soil exposome included generally standard methods (*aqua regia*, 0.1M $NaNO_3$, 1M $NH_4NO_3$, and 0.05 M EDTA BCR), soil pore water (Rhizon MOM) and DGT. Initial and residual risks and pollutant linkages were quantified, in line with the GRO implementation and their (bio)monitoring. Impacts on soil microorganisms and plants were evaluated *ex situ* at most sites in coordination with WP3. Modelling of TE exposure *vs*. plant responses was done for metals (*e.g.* Cu, Zn, and Cd) and As based on total soil content and extractable fractions, soil pore water and the DGT method. The TE concentrations in plant products were determined at each harvest for all sites, and the potential uses of such phytomanagement-borne biomass were compared with the legislation, common values, and the biomass needs of local conversion chains.

Concentrations of TE in leachates from the topsoil in either outdoor lysimeters or column, changes in labile TE pools in the soils and residual risks for plants and microbial communities were generally monitored over four years.

To improve the detailed risk assessment several additional data were determined on site such as organization of plant communities (*e.g.* species richness, Shannon index) and interspecific variability of TE concentrations in plant parts.

## 3. Option appraisal

The option appraisal stage aims at establishing which remediation option, or combination of options, can alleviate all pollutant linkages that present an unacceptable risk at the site (Environment Agency 2005, SUMATECS 2009). It includes: identification of feasible remediation options, detailed evaluation of options, and developing the remediation strategy. The Greenland project added another aim: the implemented GRO should improve the ecosystem services, notably provisioning services through biomass production for the bio-economy and other ecosystem services such as carbon sequestration, recycling of organic matters, water filtration, quenching of soil erosion and restoration of plant-microbe communities, without generating wastes and pollutant linkages.

For the Greenland sites, the main concerns were to ensure that:

- remediation option criteria selected for the soil are protective for controlled waters, plant, microbe and animal communities.

(*here remediation criteria were not based on total soil TE, but on either current legislation for labile (extractable) TE fraction in the soil, such as in Switzerland and Germany, for forages and feedstuffs (e.g. JORF 2003 in France), for foodstuff (EU Directive on Cd and Pb), for groundwater or on upper critical threshold values according to experts for the soil exposome in order to alleviate pollutant linkages*).

- relevant GRO were mainly selected for improving the biomass production and ecosystem services and reducing most pollutant linkages in line with the pathway soil - soil solution – plant, microbe, and animal communities; at some sites the TE bioaccessibility (through soil ingestion) was considered.
- the Remediation Strategy addresses all (or most) relevant pollutant linkages
- in general, requirements for waste management licences, environmental permits, discharge consents etc. were taken into account but not necessary for GRO selected in the option appraisal.

To better determine the benefits and limits of feasible GRO for some or all clusters at one site, according to the selected conceptual scheme and end land use, it is recommended to compare them with the best relevant conventional remediation options, in parallel in pot and field experiments (with similar soil contamination). In case of failures of GRO in the long-term, the other remediation options would be deployed on the site clusters.

Site-specific factors determining the appropriate GRO, *i.e.* nature of the conceptual scheme and risk management, location of treatable contaminants, overall strategy and implementation, and general criteria related to site and contaminants were addressed. Criteria related to technical basis, legal, and financial factors affecting the decision-making process such as engaging with stakeholders, were taken into account in the WP5.

Depending on the sites, data for option appraisal were either previously published or produced by the project. Additional soil amendments and plant materials were investigated in WP4 in coordination with WP1 (*e.g.* biochars derived from either straw, pine bark chips, poplar twigs from Greenland phytomanaged sites or poultry manure, separately and in combination with zerovalent iron grit, compost; and red muds). Some of them (*e.g.* Linz-Donawitz slags) were further tested in field plots. For Cu-contaminated soils, biochar derived from poultry manure had a negative effect on plant growth whereas the C-Cure biochar combined with compost had positive effects. Several plant species were assessed to develop GRO at the Greenland sites (*e.g. Miscanthus sinensis*, *Noccaea caerulescens, Arundo donax, Cana x generalis*) (in coordination with WP4). Three main GRO were considered in the remediation strategies developed at field scale: *in situ* immobilisation/phytoexclusion, (aided) phytostabilisation, and (aided) phytoextraction (Tab A2).

Option appraisals resulted in the selection of:

- **phytoextraction** at 13 field trials (5 with SRC, 5 with high annual biomass crops HBC, and 3 with hyperaccumulators),
- **(aided) phytostabilisation:** 11 field trials (3 with SRC and 8 with perennial herbaceous plants)
- ***in situ* immobilization/phytoexclusion**: 5 field trials.

### Tab A2: Option appraisals for several sites

| **Option appraisals for several sites** | | |
|---|---|---|
| **Remediation option** | **Sites** | **Feasibility** |
| Biochar derived from pine bark chips | Chaban-Delmas, Borifer | + |
| Biochar derived from poultry manure | Chaban-Delmas | - |
| Biochar derived from pine bark chips and compost | Biogeco | + |
| Biochar derived from phytomanaged poplar wood | Biogeco | + |
| C-Cure Biochar and compost | Biogeco | + |
| Biochar derived from pine bark chips and iron grit | Arnoldstein | |

| | | |
|---|---|---|
| Biochar derived from poultry manure and iron grit | Arnoldstein | - |
| Biochar derived from poplar wood and compost | Biogeco | + |
| $CaCO_3$ – reagent grade | Piekary | - |
| Compost (GWDA municipal green waste and sludge) | Piekary | - |
| Drinking water residue (DWR) +GWDA | Piekary | ++ |
| Ca-phosphate – $CaHPO_4$ +DWR + GWDA | Piekary | ++ |
| Thomas basic slag (TBS) +GWDA | Piekary | + |
| Linz-Donawitz slag (LDS) +Z + GWDA | Piekary | + |
| Gravel sludge (GS) | Piekary | - |
| Siderite (SID) – iron carbonate | Piekary | - |
| Cyclonic ashes (CA) +Z + GWDA | Piekary | + |
| Zerovalent Iron grit (Z) | Piekary | - |

## Task 1.2. Implementation of remediation strategies

The Greenland network is currently the frontrunner for the Northern hemisphere and an efficient EU tool, based on the 12thICOBTE and Conferences of the International Phytotechnologies Society (Syracuse 2013 and Heraklion 2014). Datasheets and success stories summarize the key information for each site.

Implementation of the remediation strategy and demonstrating that it is and will continue to be effective. All GRO were implemented after the Tier 1 (Risk assessment) and most GRO were selected based on pot and/or mesocosm experiments (Tier 2, option appraisal). The Tier 2 was completed by the DST outcome from the WP5. In rare cases, GRO were implemented based on Tier 1, literature and information obtained at other sites (e.g. aided phytostabilisation at Phytosed, Touro). Licences were generally not needed as partners were working under the umbrella of local and/or national authorities, without the elimination or production of wastes and by-products.
Main lessons gained on GRO implementation were:

- determine the areas of concern: field experiments were implemented on clusters defined by the initial risk assessment, identified pollutant linkages, and current/future land use. It was crucial to quantify the **spatial variability of parameters** driving the choice of feasible GRO according to the current/future land uses (for each cluster) and the related target/trigger values (notably those from the legislation and exposome) and other drivers (land value, time constraints, etc.). These parameters were (non-exhaustive list): total and labile pool for contaminants (*when possible, including their chemical speciation*) in the soil and soil pore water (if possible in the soil profile), capacity to buffer/resupply the soil solution, leachability, basic physico-chemical properties, texture/composition (define the soil type), and ecotoxicity of the (solid/liquid) matrices, climatic conditions including water supply and its annual distribution, etc. A key point is to survey the water supply and requirements by different plants.
- account for any specific requirements related to the selected feasible GRO and the best conventional option (to be compared).

***Spatial variability of pollutant linkages is a pivotal parameter.*** Before implementing field experiments for testing selected GRO, attention must be paid to the plant communities already colonizing the site/clusters (if any). Presence and habitats of animals (including insects, soil mesofauna, etc.), the slope and the terrain relief in general must be recorded. Information was gathered for most Greenland sites on the spatial variability of pollutants and their linkages, plant candidates for GRO, and eventually (native) plant populations and associated microbes, which can be used directly or selected to obtain efficient partnerships. Sub-site(s) were defined at several sites (*e.g.* Touro, Biogeco, Phytosed, Arnoldstein, etc.) allowing to statistically exploit the field plots. The spatial variability of soil ecotoxicity was assessed for each cluster (at least a plant test with a sensitive plant species and an indicator of pollutant linkage or exposure such as the $NH_4NO_3$-extractable soil fraction).

- compare the best conventional technology(ies) in parallel with the selected GRO emerging from option appraisal. In case of GRO failure, the conventional technology will be an immediate alternative. To better assess the benefits/limits of the GRO's, it is better to compare with the best conventional technology to provide relevant and convincing information to the landowner and the stakeholder core.
- don't upscale directly from 'pot experiments' to 'full-scale' (*in situ*) deployment on the cluster(s) without the return skill of biomonitoring and maintenance for several years. Field plots must be tested on a long-term, especially to address and optimize some aspects that are difficult to investigate with potted soils: *e.g.* variability of climatic conditions, colonization by animal communities, pests, ageing of soil amendments, extension of the root systems, etc. For tree management, enough space must be allowed between the plots, as root systems can extend horizontally more than 10 m for poplars as well as the shading effect. As far as vertical migration to the subsoil and groundwater is of concern, try to establish an *in situ* lysimeter system (even a basic one with containers) or an in situ leachate sampling system underneath the plots to long-term assess the quality and the ecotoxicity of the leachates. Horizontal migration of the contaminants through wind erosion and other natural agents (water runoff), in particular to inland water and allotments, must be considered too.
- fencing: A single fence around the whole site may be necessary (notably to restrict the entrance) but it is generally not sufficient to prevent potential damages caused by the mammal herbivores (*i.e.* rabbits, field rats, deers, etc.). It should be

complemented by fences around small clusters (especially at the start of the phytomanagement, to protect the trees and other attractive plant species; individual fences around trees are less time-consuming but their efficiency is lower.

● plot size: define reasonable plot size for avoiding the edge effects and permitting a long-term (>5 years) monitoring and maintenance, notably for soil and plant samplings. Set up field plots according to the spatial variability of parameters listed above; pay attention to allow sufficient space between the various options; always remember that tree roots, and its associated hyphosphere, can sense better conditions over more than 10-15 m; pay attention to the shading effect which may occur with the canopy development. In case of slopes, the common technique of terraces can be used as well as fiber nets to counteract the soil run-off till the establishment of the vegetation cover, as for to vegetate ski tracks. Starting from seeds, some light mulch (with straw, fern fronds, bark chips, coconut nets, etc.) to trap the seeds can be necessary (and avoid migration with natural agents or bird predation).

● don't forget to monitor the foliar exposure: at some sites, local emissions, atmospheric fallout and windblown dust may occur, contributing to foliar exposure. Place some pots with uncontaminated soil to grow some plants, *e.g.* grassy crops and young trees, for quantifying such foliar exposure. For comparison, potted contaminated soils under remediation must be placed at an uncontaminated site without relevant emissions or under controlled 'clean' conditions.

● adopt appropriate agronomic practices: GROs are essentially based on 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 (Kidd et al 2015). 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. Agricultural practices can be incorporated into GRO as a means of optimizing metal(loid) extraction, immobilization or the prevention of their excessive transfer into the food chain (without inducing TE and (macro)nutrient deficiencies). In addition, these practices can improve plant biomass production, nutritive status or pest management. Sometime during winter, the flooding of soils may prevent the harvest machines to enter in the field. This can be a bottleneck for harvest of SRC, *Miscanthus*, etc.

● Implementation of plant species: for phytomanagement, the choice of initial plant/microbe partnerships must account for the local conversion chains of biomass (generally the biomass production on one rather small site is not enough to financially support a dedicated local valorization plan; this biomass must be commonly merged with similar biomass from other sites (forest, SRC, agricultural field, green wastes, etc), provided that their composition is suitable with the process or its marketing image.

Phytotoxicity and other stress factors can limit the performance of the plant species used in the GRO. The careful selection of plant species and optimization of growth are key elements in successful phytomanagement of TECS under different pedo-climatic conditions. Plants must not only show tolerance to the contaminant(s) present but also resist other abiotic and biotic potential stresses, *e.g.* water stress, soil acidity, frost, soil erosion/compaction, herbivory, pests, nutrient deficiency, salinity, etc. A source of TE-tolerant plant genotypes is the pioneer vegetation colonizing contaminated sites or present nearby. Screening and selection of TE-tolerant plant species and genotypes (e.g. *Agrostis* sp., poplars and willows, tobacco, etc.) were made in WP4 for application under real field conditions. Piekary site provided information on long-term performance of various grass species on tailings reclaimed with biosolids. Within the same plant species different ecotypes, cultivars, varieties and clones can differ in their response to contaminant excess. While tolerance to contaminants in question will always be vital, at other times the selected plant will depend on the GRO to be used *e.g.* TE-accumulating plants (phytoextraction), TE-excluding plants or crop species (phytostabilisation/phytoexclusion). Some herbaceous and woody *Fabaceae* can be included in crop rotation or in mix stand to promote the nitrogen supply by fixation of atmospheric nitrogen.

- for woody crops: competition between young trees and the herbaceous plant communities (notably grassy crops) can be adverse for tree development. Try to implement the young trees before to implement the herbaceous crops underneath and in between, if there is a need to increase the vegetation cover and reduce the contaminant migration through natural agents. It is pivotal to irrigate trees in year 1 (and sometime year 2) during dry periods to increase the survival rate and promote the establishment of their root systems (depending on soil type, climatic conditions, etc.). Pay attention to the slope, potential soil erosion and/or flooding. In case of excluder-based SRC for bioenergy purposes, the selection of genotypes can be based on their characteristics in line to conversion processes, *e.g.* calorific value, bulk density, moisture content, ash and extractive content. Transplantation of mycorrhizal trees was more successful than that of non-mycorrhizal ones and the on site mycorrhization of tree cuttings. Inoculation of native TE-tolerant ectomycorhizes can initiate a synergetic fungi succession.

*Salix* and *Populus* clones show high variations in biomass production, TE tolerance and accumulation patterns in roots, leaves, and even in wood between clones. Some species and clones of willow have high bioconcentration factors (BCFs) for Cd (up to 27) and Zn (up to 3). Given the ample variation in metal accumulation, best-performing clones can be selected based on their TE-tolerance, uptake efficiency (accumulating clones for phytoextraction *vs.* excluding clones for phytostabilisation), translocation from roots to shoots, and biomass production. Clones can be selected for their ability to accumulate certain metals (*e.g.* Cd and Zn) while at the same time immobilizing elements such as Cu or Pb. Evidences of tolerance to TE and fungal and insect infection, *e.g.* leaf rust (*Melampsora* sp.) and lace bug (*Monosteira unicostata* Muls. and Rey), cold and drought adaptation were revealed at the Lommel site.

- combine phytomanagement and ecology: establish natural and passive habitats to host and promote reproduction of the biological auxiliaries (notably beneficial insects and birds) and counteract bioagressors. Think about the connection of clusters with the other ones nearby. Use corridors allowing the predators (fox, raptors, etc.) to hunt; these corridors can be combined with the access required for monitoring and sampling as well as the harvest machines. Avoid a full site monocultures to alleviate the selection of pest populations (*e.g.* use diverse clones/genotypes for trees in clusters; use a crop rotation in case of annual plants.

- Phytomanagement can combine some GRO: The phenotype of plant species in response to TE excess is element dependent and a plant assemblage can support various GRO at the same time on mixed-contaminated soils. For example a poplar SRC can simultaneously phytostabilize Cu/Pb in its root system, phytoextract Cd/Zn in its aerial parts and promote the rhizodegradation of xenobiotic organic compounds.

## Task 1.3. GRO implemented and biomass production

### 1.3.1. In situ immobilization/phytoexclusion

This GRO can be implemented as either a long-lasting (phyto)management option or a temporary, reversible one that can be later modified based on the monitoring results from the phytomanaged plots. Decreasing the labile TE pools in TECS by incorporation of soil conditioners and the use of excluder plants are both main approaches. Different **soil conditioners** were investigated on a long-term and at field scale, *i.e.* phosphates, composts and technosols, iron bearing materials (iron grit, gravel sludge), and alkaline materials such as alumino-silicate slags, marl lime, biosolids, and dolomitic limestone.

- Organic matter: composts are frequent in the amendment combination for promoting crop production and soil quality. Their quality (C/N ratio, seed bank, labile P pool, etc.) is pivotal. They were used, singly and in combination (Biogeco) and compared to technosol mixtures (Touro), especially in Cu-contaminated soils. Compost was more efficient to promote the vegetation cover at Touro. Biosolids can be successful and economically viable option when large volumes of amendment are needed (smelter tailings). Caution must be paid when using rather “fresh” organic material in case of a labile pool of Cu, Pb, As, Mo, Cr, Sb, and Sn. Dissolved organic matter (DOM) may transiently increase the soluble complexed (for metals) or free anion (for metalloids) fraction in the root-zone with consequences on TE leaching when plants were not able to fully use the leaching water and take up soluble TE.

- alkaline materials, through changes in soil pH influenced physico-chemical and biological reactions into the TECS, with consequences on the chemical speciation, sorption by bearing phases and mobility of TE (Biogeco, Phytosed, Freiberg, Piekary). Over-liming may however induce nutrient deficiency and mobilize TE in oxyanion forms. Marl lime (Freiberg), dolomitic limestone (Biogeco), biosolid and by-product lime (Piekary), and alumino-silicate slags such as Linz-Donawitz slags (LDS), with and without P spikes (Biogeco, Phytosed) were assessed.
- other soil conditioners: Fe/Mn bearing materials such as zerovalent iron grit (Z) and water treatment sludge (WTS), gravel sludge, red mud and siderite bearing material were tested singly and in combination notably with compost. All were tested in field plots. Incorporation of Z and LDS into TECS (Biogeco) was split to avoid the pepite formation and better homogenize the amended soil. Various incorporation ways (injection, tillage, slurry) were assessed at Arnoldstein.

**End land use: annual crop production**
Staple crops and oilseeds: Cultivars within species from major staple crops such as wheat, barley, rice, potato and maize differ widely in their ability to accumulate metal(loid)s. Selection of efficient excluder cultivars for cultivation on contaminated and remediated land contributes towards reducing the entrance of non-essential TE, and also avoiding the excess of essential ones, into the food chain. Cd is of highest concern regarding metal uptake into the food chain as well as As, Mo, Se, Tl and Hg. Selection of the most appropriate cultivars for use on TECS can ensure that food and forage production is in compliance with the respective regulations on threshold TE contents. For example, The Operating Company for the Environment and Agriculture of the Saxon State (Germany), the Austrian Agency for Health and Safety (Austria) and Arvalis (France) are testing TE uptake behaviour of currently available cultivars in both field and parallel batch experiments. 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. Moreover, since commercial 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. Excluder maize, barley and potatoes cultivars were long-term assessed at Arnoldstein. Use of the excluder-phenotype Bodega *vs.* accumulator-phenotype Hellana reduced barley grain Cd by over 40 %. In combination with the incorporation of gravel sludge and red mud into the contaminated soil, a further >30 % Cd uptake could be avoided. After five years,

soil amendments at Arnoldstein were still effective immobilizing agents illustrating the efficiency and cost-effectiveness of *in situ* stabilization and phytoexclusion.

Management practices recommended by the authorities to the owners of contaminated land in the Freiberg area were to increase soil pH to values of 5.8 to 6.5, moderate phosphorus (P)-fertilization at the beginning of the growing season, increase redox potential, and use harvesting methods which minimize contamination of grass forage by soil particles. Consequently crop rotation including winter oilseed rape, winter wheat and spring barley at Freiberg-Hilbersdorf in combination with marl lime application. Soil pH at Freiberg-Hilbersdorf was slightly changed by lime and P application and was generally increased in year 4, which resulted in a decrease of mobile Cd (by 50-75%). Based on BCF of Cd, Zn and Pb, the barley cultivar “Salome” was shown to less accumulate these metals in its grains than the Marthe cultivar. Based on EU directive 2002/32/EC (2002) these barley grains were suitable as single fodder. Considering changes in element transfer into plant parts as affected by amendment options, grain Pb differed between the control and P treatment with highest concentration and the limed treatments with low concentration, especially for the combined fertilized treatment. The biomass production of winter oilseed rape for both cultivars was within the common range of yields for this German region (2.4 – 4.4 t/ha). Those of winter wheat were below the range (5 – 8 t/ha), especially for the low accumulating cultivar Türkis, which produced a lower grain yield than to the high accumulating cultivar Tiger. The grain yields of spring barley were below the common range (4.2 – 7.4 t/ha) with slight differences between both cultivars.

Grassland management: Grassland based on TE excluder grassy crops is one relevant GRO to alleviate windblown dust and water runoff on large TE-contaminated areas, notably with low fertility.
At Arnoldstein, shoot DW yield reached 5 t/ha/yr. The most efficient soil conditioner (gravel sludge and red mud, slurry management) was reducing the labile pools of Zn (-90%), Cd (-80%), and Pb (-90%) in the soil. Plant monitoring based on *Plantago lanceolata* indicated reduced shoot concentrations for Cd (-70%) and Zn (-77%). Shoot Cd and Pb concentrations of harvested grass mixture just exceeded the maximum permitted concentrations (MPC) in forages.

At Freiberg-Hilbersdorf, last marl lime application was made in autumn 2013 and last soil and plant samples were taken in May 2014 from the grassland trial. Soil pH ($CaCl_2$) varied between 4.3 and 5 in the unamended soil. It reached pH 6 at 2t marl lime/ha and 6.5 at 4t/ha. Consequently extractable Cd in the soil was reduced from 0.6-0.7 to 0.05-0.1 mg/kg soil DW. Grass shoot DW yield varied from 2 to 5.75 t DW/ha depending on season (3 cuts/yr) and soil amendments, which high values in May and July and low ones in September. It was enhanced in May by marl lime application at 4t/ha. Shoot As concentrations of grass did not differ between unamended and 2t/ha-amended soils on the 2012-2013 period and was in the 0.25-0.5 mg/kg DW range. It started to decrease on the third year. Shoot As concentration was higher in the 4t/ha-treated soil, reaching 1.5-1.75 mg As/kg DW in 2012-2013, despite high shoot DW yield, and also decreased to 0.6 mg As/kg DW in 2014 after the last marl lime application. Shoot Cd concentration ranged between 1-1.5 mg/kg DW in 2012 and did not differ across the treatments. It decreased in all treatments in 2014 (0.5-0.8 mg/kg DW), but lower values in marl lime-treated soils were statistically similar to the unamended soil. Shoot Pb concentration varied from 0.3 to 4 mg Pb/kg DW in average and reached 8 mg/kg DW in some shoot samples from the 4t/ha-amended plots. It was decreased in year 3 for all plots with a lower value at 2 t/ha (0.3 mg Pb/kg DW) compared to the unamended soil.

At **Piekary** (PL): The grass mixture consisted of local cultivars: *Festuca rubra* L. cv. Atra, *Poa pratensis* L. cv. Alicja, *Festuca arundinacea* Schreb. cv. SZD, and *Festuca ovina* L. cv. Sima. 17 years after biosolid incorporation, water-soluble fractions of major contaminants (Zn, Cd, and Pb) in the soils remained at low levels, in line with soil pH and Ca-carbonate distribution over the field. Soil bacterial communities were highly diversified in amended soils. Dehydrogenases activity increased as water extractable metal (Cd, Zn) fractions in the soils were reduced. Plant cover and biomass production depended on the soil treatment being highest soils amended with biosolid combined with by-product lime. Untreated tailings outside the reclamation area remained barren. The plant cover is not managed and plant community organizes itself. At the field where grass species were tested, the most persistent grass species were *Poa pratensis*, *Agrostis capillaris* and *Festuca ovina*. These species covered the largest area of the field 17 years after remediation among all grasses. A substantial part of the areas was covered by colonists - *Calmagrostis epigejos*, *Hypochoeris radicata*, *Melandrium album*, *Artemisia vulgaris*, *Daucus carota* and *Solidago gigantean*.

### 1.3.2. (aided) phytostabilisation

Two related GRO were assessed:

● phytostabilisation with various plant covers, *i.e.* mycorrhizal and non-mycorrhizal trees, and perennial grasses, matrices (soils, tailings), climatic conditions, strategies and socio-economic opportunities.

● aided phytostabilisation combining TE in-situ stabilization, mainly through changes in sorption processes and pH in the soils, and phytostabilisation, *i.e.* (non-)mycorrhizal trees, perennial grasses.

The purposes were (1) to cultivate trees and/or perennial grasses on TECS, (2) to manage trees as Short Rotation Coppice (SRC) or fast growth plantation, and (3) to decrease labile TE pool in the root zone.

#### 1.3.2.1. SRC with and without grass cover/herbaceous layer

***SRC parameters*:** Many tree species are 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, and As) in the soil by physically preventing migration, leaching, and soil dispersion; alternatively, they can immobilize TE through uptake and accumulation by the roots into the plant, adsorption on the root, and precipitation in the rhizosphere.

At **Biogeco** (Cu-contaminated soils) two cuts were made for fertilized mycorrhizal poplars (in years 4 and 7), whereas minimum values of potential SRC biomass to initiate the harvest of willows and non-mycorrhizal poplars were not reached. For willow SRC, it can be done in year 9 only for ectomycorrhizal trees. Shoot DW yield of poplar SRC varied from 20 to 270 t DW/ha showing the spatial variability of soil exposome, fertility and water supply, 135 t DW/ha was even reached in some untreated plots nearby other fertilized plots managed by phytoextraction.
In year 6 after amendment incorporation into the Cu-contaminated soil, compost (OM) increased poplar growth compared to the untreated soil (UNT), whereas addition of dolomitic limestone (DL) resulted in less significant increases. Both OM and OMDL promoted the growth of *Amopha fruticosa*. Effect of soil amendment was not significant in the long-term for both willow species.

At **Phytosed**, after six months, grass lines were mechanically removed and replaced by a tarpaulin at the expected willow place. Two willow cultivars (Tordis and Inger) were planted (12,000 willows ha$^{-1}$) in SRC for the biomass production. *B. cespitosa* and the natural colonizers were mowed to maintain as low as possible the competition for water and nutrients with the willows. The survival rate of willows in year 2 was 89% accounting for all plots, but it dropped to 75% for Tordis in several amended plots and the rate of chlorotic leaves reached 30-50% in these plots. Consequences of over-liming and Cr/Mo excess were hypothesized. The grass may also compete with willows. The foliar Cd concentrations of willows were high (10-30 mg Cd/kg), the Tordis willow clone showing higher values than the Inger one. This difference in foliar Cd concentration between the willow clones was observed both in the Thomas basic slag (TBS)-amended plots and in the control plots. Values in year 1 were far higher than frequent concentrations in willow leaves from uncontaminated soils (<2 mg kg$^{-1}$ DW). Similarly foliar Zn concentrations ranged from ~ 2000 to 3500 mg kg$^{-1}$ DW whereas common values varied from 81 to 296 mg kg$^{-1}$ DW. The alkaline amendment did not decrease foliar TE concentrations of willows. Wood and bark Zn and Cd concentrations in year 2 (2 mg Cd/kg DW) were lower than those in willow leaves. Bark concentrations (10-15 mg Cd/kg) were higher than wood concentrations and concentrations increased with the height of willow due to the increase of bark proportion. Tordis willows accumulated more Cd than Inger, in accordance with the leaf results. Compared to initial Cd and Zn concentrations in both willow cultivars before plantation (~ 2 and 150 mg kg$^{-1}$ DW in wood with bark, respectively), the Cd/Zn concentrations increased after 2 years. The alkaline amendment did not reduce or at least stabilize the TE concentrations in aerial plant parts of willows.

***Herbaceous layer*:** The sediment landfill site at **Phytosed** (FR) is contaminated by TE, mainly Zn and Cd. A commercial alkaline by-product of steel industry used in agriculture (Optiscor) was incorporated in September 2011 (rate 9 t ha$^{-1}$ to optimize both metal immobilization and willow growth, pH 8) into the technosol for reducing the metal mobility and promoting the grassy crop. *Barchampsia cespitosa* was used as a plant cover to reduce vertical and horizontal TE transfers, and was expected to alleviate the propagation of *Fallopia japonica*, an invasive species colonizing the technosol. After 2 years, the vegetation cover roughly reached 100% and foliar Cd concentrations in *B. cespitosa* were lower than 0.5 mg kg$^{-1}$ DW and approximated common values (0.05-0.2 mg kg$^{-1}$) for grasses grown on uncontaminated soils. Despite the high total soil Cd, this grassy biomass was suitable for composting. Cd foliar concentration was even reduced in year 2. Averaged foliar Zn concentrations ranged from 180 to 270 mg kg$^{-1}$ which overlapped both common (27-150 mg $kg^{-1}$) and upper critical threshold (100-400 mg $kg^{-1}$) values in grass shoots from uncontaminated soils. No phytotoxicity symptoms were observed on aerial plant parts. Concerning the mineral amendment, its efficiency was not demonstrated neither on foliar TE concentrations nor extractable TE concentrations in the technosol. Indeed, no reduction in concentrations was observed.

The commercial cultivar, *B. cespitosa*, is a good candidate for phytostabilisation (*i.e.* success of the plant cover, tolerance to the technosol conditions, shoot TE concentrations close to common values for grasses on uncontaminated soil). This grass competes well against the invasive species (beneficial effect of phytostabilisation; see previous report). Until now, the selected soil amendment did not succeed. Future work will address the expected mechanisms (speciation, OM, $CaCO_3$ stock, etc.). In this case, the combination of aided phytostabilisation using a grass cover with the plantation of willows to produce biomass for bioenergy is not successful (*i.e.* grass and willow competition for water and nutrients, sensitivity of the selected willow clones to the labile pool of contaminants and other factors such as willow leaf beetle, herbivores..., generation of costs rather than economic benefits [see WP5 for economic data]). One alternative would be to put the grass several years after the willow plantation to avoid the grass competition. This option poses the following questions: is it technically feasible? What about the risks in this case? Replace grass by mulch? Is it economically viable? Is it possible to find other fast growing trees (than willows and poplars) or cultivars with very low TE accumulation? In this case study, benefits of biomass production do not compensate costs linked to set up and monitoring of both aided phytostabilisation and willow plantation. This result questions the possibility to decrease these costs. This could be achieved by recalculating cost and benefits with other protocols.

***Responses to bioagressors***: Both willow cultivars Inger and Tora at **Phytosed** were susceptible to the imported willow leaf beetle (*Plagiodera versicolora*). A severe attack occurred in early spring 2014. Consequently an organic insecticide (pyrethrin) was applied after the leaves have flattened out; this efficient treatment was leading to the leaf re-growth. A similar biotic interaction was occurring at Lommel. The use of native poplar and willow at Biogeco reduced disease incidence, particularly from *Melampsora* rust.

### *Assemblages of plants and microbes*

*Effect of ectomycorrhizae and fertilization:* inoculation with mycorrhizal fungi and plant-associated bacteria (rhizobacteria and endophytes) may improve plant growth and modify soil metal mobility and their uptake/translocation by woody crops, notably in TE-contaminated soils and mine sites. At Biogeco, the maximum stem height of mycorrhizal trees was higher than that of non-mycorrhizal ones. However, poplars in plots nearby fertilized plots phytomanaged by phytoextraction have extended their root system and took advantage of these plots for their growth. Their root system was able to detect lower labile Cu pool and NPK supply in plots nearby. This underlines the influence of plot size and the interspaces for long-term assessment of SRC.

Ectomycorrhizal poplars inoculated with Cu-tolerant endophytic bacteria were also obtained in greenhouse and then transplanted at Biogeco.

**Touro** (ES): The mine tailings of the non-active Cu mine cover an area of approximately 550 ha. The implemented GRO involved establishing a short rotation coppicing system or a grass cover with the principal objective of reducing Cu mobility. The geological substrate is amphibolite, with significant quantities of metal sulphides (pyrite, pyrrhotite, and chalcopyrite). The mine-soils (Spolic Technosols (Episkeletic)) are characterized by their extreme acidity (pH 2.8-3.5), low C, N and P, and high concentrations of Cu (319-774 mg/kg). Cu contamination shows considerable heterogeneity across the site. Tailings were amended with three mixtures: composted municipal solid wastes (compost) and two technosol mixtures. Technosols were based on organic (anaerobic and aerobic sewage sludge) and inorganic wastes (aluminium oxides, iron oxides, fly ash from wood bark combustion, and foundry sand). Plots were planted with different metal-tolerant clones of *Salix* (*S. caprea* and *S. viminalis*) and *Populus nigra*, or with a grass cover *Agrostis capillaris* cv. Highland. Mortality was high on technosol-amended plots but low on compost-amended plots. Growth and survival (70-80%) of woody trees was optimal in compost-amended plots. After three years tree height was highest in *S. viminalis* and *P. nigra* (reaching up to 3-4 m).

### *Changes in soil exposome / TE mobility in soils*

Touro: In year 3, soil $NaNO_3$-extractable Cu concentrations remained low (<1 mg/kg) in all treated soils without influence of the vegetation cover type. Soil pH was 3.5 before GRO implementation, and in year 3 remained between 6.0 and 7.0 in compost-amended soils. Soil pH was higher in soils under *Salix*, followed by *Agrostis* and finally, unplanted soils.

Phytosed: In year 2 extractable Zn and Cd fractions (roughly 0.4-0.8 mg Zn and 0.001-0.0015 mg Cd/kg soil) did not differ between the amended and unamended technosols.
Biogeco: In year 5, Cu concentration in the soil pore water was higher in the compost-amended soils for both mycorrhizal and non-mycorrhizal trees and the lower in the limed soils with mycorrhizal trees.

***Restoration of soil microbial activity and communities:*** At Touro, soil enzyme activities (involved in C, N and P cycles) were monitored over time. In parallel, shifts in the structure of the soil bacterial community (total Eubacterial community, $\alpha$- and *$\beta$-proteobacteria*, *Actinobacteria* and *Streptomycetaceae*) were compared over time using the Denaturing Gradient Gel Electrophoresis (DGGE) technique. Soil enzyme activities were lowest in untreated soils, and increased with time in amended soils. A plant-induced effect was also observed: activities were higher in plots planted with woody trees, followed by *Agrostis*, and lowest in unplanted plots. Similarities in DGGE fingerprints based on 16S rDNA amplified fragments were analysed. At each sampling period (after 1, 2 and 3 years) the similarity dendrograms showed a separation (similarity of <20%) between the bacterial community of soils sampled before GRO implementation (i.e. time=0) and that of phytomanaged soils. At all sampling periods (1-3 years) the soils amended with compost, technosol 1 or technosol 2 formed three distinct clusters of DGGE profiles. In year 1, within each of these three clusters there were clear separations corresponding with soils sampled from unplanted plots, or cultivated with *Agrostis* or *Salix* (similarities of <60%). With time the three amendments continue to cluster separately but there is some intermixing of DGGE profiles from either *Agrostis* or *Salix* cultivated plots. In general the soil bacterial communities continue to cluster separately according to the plant species. Similar patterns were also observed in the DGGE profiles of α- and β-*Proteobacteria*, *Actinobacteria* and *Streptomycetaceae* populations, albeit sometimes to a lesser extent than in the DGGE profiles of Eubacteria. Bacterial diversity increased in all phytomanaged soils compared to the untreated soils. There was a trend towards an increase in bacterial diversity with time, and also a higher bacterial diversity in planted soils (albeit *Agrostis* or *Salix*) compared to unplanted (but amended) soils.

#### 1.3.2.2. Grassy crops (only)

***TE excluder perennial herbaceous crops*** such as switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* spp.), giant reed (*Arundo donax*), and vetiver (*Vetiveria zizanioides*) have wide climatic adaptability, low production costs, suitability to marginal lands, relatively low water requirements, low nutrient and agrochemical needs, and potential environmental benefits (e.g. carbon storage through their deep and well-developed root system). They can provide feedstock for the energy sector or essential oils in Europe and North America. The invasiveness of some of these species (e.g. giant reed) is a controversial topic. They were assessed with potted Cu-contaminated soils. Thereafter, *Miscanthus* x *giganteus* and Vetiver were implemented at Biogeco. After four years, *Miscanthus* as Vetiver did not colonize other plots and nearby area. Co-cropping of leguminous species (*Ornithopus compressus*, *Medicago arabica* and *Trifolium pratense*) with *Miscanthus* did not promote its shoot DW yield.

For **Vetiver** in year 4, shoot DW yield potentially reached 38t/ha in uncontaminated plots, 7-15t/ha in the amended Cu-contaminated plots, and only 2.6 t/ha in the highly Cu-untreated plots, as total Cu in soil pore water increased from 0.2 to 0.9 mg Cu/L. It always demonstrated a Cu-excluder phenotype, shoot Cu concentration being in the 10-13 mg/kg range with no influence of soil Cu contamination, which alleviate potential herbivory exposure.

For ***Miscanthus***, shoot DW yield in year 3 varied from 0.07 (Unt) to 1.8 (OMDL) t DW/ha. Its shoot Cu concentration ranged from 7 (OMDL) to 95 mg Cu/kg DW (Unt) (values in year 1 were higher due to a lower shoot biomass: 16.6-507 mg Cu/kg), slightly over the Vetiver values. The single incorporation of compost and dolomitic limestone still reduced labile soil Cu and *Miscanthus* exposure in year 7. Change in labile soil Cu and in shoot DW yield (dilution effect) explained differences in shoot Cu concentration. Shoot Cu removals in year 3 varied from 3 to 17 g Cu/ha as shoot DW yield increased, depending on shoot Cu concentration and shoot DW yield, with maximum at median soil Cu contamination and minimum for soil Cu/PAH contamination (in line with foliar symptoms of N and water deficiencies).

Biomass **sorghum** (*Sorghum* spp.) was assessed in potted soil and field plots (Biogeco). Both cultivars for biomass and bioenergy were not successful at field scale, being too sensitive to Cu excess and low water supply in sandy soils.

***Grassland***: At Biogeco compost (OM) and dolomitic limestone (DL), singly and in combination (OMDL) were assessed in comparison with the untreated soil (Unt). In year 7, most plots initially planted with grasses were dominated by an assemblage of Cu-tolerant *Agrostis capillaris* and *A. gigantea* whatever the soil treatments. Other introduced grassy species such as *Sporobolus tenacissimus* and *B. cespitosa* are disappearing. *Cytisus scoparius* are colonizing the plots and their shoots are annually harvested to avoid the development of a bush canopy. Grass cover is declining in one block, may be due to competition with roots of poplars located nearby. Shoot DW yield was influenced by soil treatments: in year 7, values were higher in the compost-amended plots compared to the limed ones. Shoot Cu concentration was slightly lower for the grass species harvested in the OMDL plots. Highest shoot DW yields in the OM and OMDL plots led to maximum shoot Cu removals. Shoot Mg, K, Na, B and P concentrations were higher whereas shoot Zn and Al concentrations were lower for the OMDL plants compared to the Unt plants. In year 5, total Cu concentration in the soil pore water was increased in the compost-amended soils as compared to the limed soils.

Chaban-Delmas (FR): A total of 72 plant species were identified in the grassland. 32 species were occasionally present, *i.e.* they always represented less than 1% of the vegetation cover on all subplots. For the other 38 species, three plant subsets were determined. The first one (Subset I) included 40 subplots located in the area center and was dominated by *Medicago sativa* and *Lolium perenne*, followed by *Vulpia myuros*, *Holcus lanatus* and *Eleusine tristachya*. This subset had the lowest plant species richness. The second subset (Subset II) including 15 subplots, located at the south part of the platform, was dominated by *Arrhenatherum elatius*, *Bromus sterilis*, *Holcus lanatus* and *Dactylis glomerata*. It displayed a median value for the species richness. *Melilotus albus*, *Trifolium arvense*, and *Trifolium pratensis* were dominant in the third subset (Subset III), located at the north of the platform and with the highest bare soil percentage. This one had the highest species richness.

**Touro** (ES): *Agrostis capillaris* cv. Highland was successfully established in both compost- and technosol-amended plots. Shoot Cu concentrations were within normal levels for grass species growing in uncontaminated soils, and significantly lower than in grass species colonizing the surrounding untreated tailings. Nutrient concentrations were increased in all amended soils but particularly in technosol-amended plots. Grass biomass was harvested each year (2012-2014) and shoot DW yield was highest in compost-amended plots.

## 1.3.3. (Aided) phytoextraction

The aims were to (1) quantify the biomass production, (2) the plant ionome (notably TE concentrations) and (3) the TE phytoextraction. Additional aims were (4) to improve agricultural practices, (5) to enhance ecosystem services such as C sequestration and microbial activities, and (6) to create economic opportunities from the biomass. Plants must be able to accumulate high TE concentrations in their harvested parts and have a reasonably high biomass production. Relevant options were TE-hyperaccumulators and secondary TE accumulators. Three main options were implemented to address three mains situations, Cd/Zn (Pb), Cu (Cu/PAH) and As/metal excess: high-yielding crops (HYC), short rotation coppice (SRC), and herbaceous hyperaccumulators, in monoculture and co-cropping.

Influences of soil conditioners such as compost, Linz-Donawitz slags, soil acidifying agents (citric acid, S), co-cropping were investigated to enhance TE phytoextraction.

### 1.3.3.1. High-yielding crops (HYC)

High-yielding crops (annuals or perennials) are recognized as viable alternatives for TE phytoextraction (particularly Cd, Se and Zn) if they show relevant shoot TE removals (*i.e.* moderate-high BCF and high shoot yield). *In vitro* breeding (cell and callus tissue culturing on metal spiked media) and chemical mutagenesis can improve the metal tolerance and phytoextraction capacity of high-yielding crops such as tobacco and sunflower. These non-genetically modified plants can be tested under real field conditions without legal restrictions. Commercial sunflower cultivars accumulate only moderate metal concentrations, but their high biomass production makes them attractive for Cd/Zn phytoextraction. Some oleic cultivars can provide both relevant oilseed yield and shoot Cu removal. Chemical mutagenesis (EMS) was used to improve shoot metal concentrations and biomass production of a sunflower inbred line IBL04. At the Rafz site (Switzerland), shoot metal removals by the sunflower mutant were up to 7.5-, 9.2- and 8.2-fold higher for Cd, Zn and Pb than the inbred line, respectively. As 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, crop rotations such as sunflower/tobacco (with winter fodder pea at Bettwiesen, and white clover at Biogeco as cover crops during winter for green manure and limiting soil erosion) were investigated. Fibre hemp (*Cannabis sativa*) and kenaf (*Hibiscus cannabinus*) were cultivated at Lommel.

Tobacco and sunflower mother-clones and variants (from PT-F) were cultivated at 5 sites managed by INRA, CSIC, HAU, and PT-F. Datasets are available for shoot DW yields, shoot metal concentrations and shoot metal removals. The influences of soil contamination levels, fertilization, maintenance through compost dressing, plant species and genotypes (mother-line, somaclonal tobacco variants, and sunflower mutants), agricultural practices such irrigation, co-cropping and flower topping were considered.

**Touro** (ES): **Tobacco:** Shoot DW yield was primarily dependent on the climatic conditions during the growth season and varied accordingly: the highest biomass for all genotypes was achieved in the 2014 harvest and reached 3400-4000 kg DW ha$^{-1}$ (levels comparable to those obtained in the agricultural soils of Bettwiesen and Lommel). Differences between the BAG motherline and the 10-8 and 10-4 variants were not pronounced, and biomass tended to be higher for BAG. Shoot Cu removal in 2014 (60-70 g Cu/ha) was lower than that obtained in Biogeco.

**Piedrafita** (ES): At this site sunflower could only grow in the compost-amended plots, while tobacco could grow in both compost-amended and untreated mine-soils (after fertilization with inorganic NPK). **Tobacco:** Annual shoot DW yield again varied widely according to climatic conditions and also to competition with weeds: biomass production was highest in 2012. There were no consistent differences between motherlines and other variants. Biomass production and Cd/Zn extraction potential was significantly lower than that observed in Lommel and Bettwiesen. **Sunflower:** Sunflower cultivation was more successful than tobacco at this site. Annual shoot DW yields were similar to that obtained at the other Greenland field sites. Cd/Zn extraction potential were similar to that obtained in Bettwiesen and Lommel in the 2012 and 2013 harvests, but lower in 2014. Mutant 1 reached up to 6772 and 23 g/ha Zn and Cd extraction potential, respectively.

**Biogeco** (FR):

***Tobacco*:** its shoot DW yield depended on total soil Cu, soil amendments, and the genotype in some plots. Flower topping in years 6 and 7 allowed the development of bottom suckers, which increased the shoot biomass. Depending on climatic conditions, early flower topping in Southwest France allowed to harvest tobacco shoots two to three times per year and to avoid loss of dried leaves.

- At moderate soil Cu contamination (258 – 382 mg Cu/kg): in year 6, shoot Cu removal by the OMDL plants reached 84-132 g Cu/ha, without significant genotype influence. The second compost dressing in year 6 (OM2DL) reduced shoot Cu removal as compared to OMDL, likely due to a decrease in mobile soil Cu. In year 7, the OM2DL plants had a higher shoot yield than the OMDL ones.
- At high soil Cu contamination (894 – 1020 mg Cu/kg): in year 6, the 10-8 variant best performed in only one OMDL plot thanks to its higher biomass. Shoot Cu removals varied from 68 to 193 g Cu/ha. For the OM2DL plots, the tobacco genotype did not significantly influence shoot Cu removal and its values were similar or higher. In year 7, shoot length and DW yield were higher for the OM2DL plants than for the OMDL ones, which promoted shoot Cu removal. This was sometime more marked for the 10-8 variant.
- For the Linz-Donawitz slag (LDS) amended-plots in year 6, shoot Cu removal peaked with the variant 10-8 in one plot, reaching 254 g Cu/ha. For all genotypes, differences were not significant between the P-LDS and Unt plots. The LDS plants produced less shoot DW yield than the OM2DL ones. In year 7, tobacco from untreated and LDS-amended plots had again a lower stem length than the OM2DL plants. Differences between genotypes were significant only in 3 plots, 10-8 variant best developing in two cases, but no general trend was found. The influence of soil amendment, especially the long-term effect of the compost second dressing was key factor to explain the shoot yield.

***Sunflower*:** At Biogeco, in year 6 and 7, leaf chlorosis occurred on many sunflower plants growing in OMDL amended plots with high total soil Cu and in both LDS-treated soils. No sunflowers were growing in the UNT soil.

- at moderate soil Cu contamination (258 – 382 mg Cu/kg): in year 6, the M2 mutant performed best in comparison to other genotypes, without effect of the second compost dressing. The shoot DW yield of the M3 mutants was lower than that of motherline (IBL04) plants in the OM2DL plots. Overall, shoot Cu concentrations were similar for all plants. The M2 mutant showed a higher shoot Cu removal than the other genotypes in the OM2DL plot. Shoot Cu removal was in the 42 g Cu/ha range. Due to lower shoot Cu concentrations than previous years, this was lower than the 100 g Cu/ha reported in years 1 and 2. In year 7, shoot DW yield varied from 0.5 to 36 t/ha depending on plots and genotypes.
- at high soil Cu contamination (894 – 1020 mg Cu/kg): Sunflower plants best developed in years 6 and 7 on all OM2DL plots that got a second compost dressing in year 6, which illustrated the necessity to maintain soil fertility through organic matter. For the genotype influence, the M2 mutant displayed higher stem length in some plots. A single compost dressing increased the shoot DW yield more than the incorporation of LD slags. The Carmeuse-LDS

and P-LDS had a similar influence on sunflower growth, and both M3 and IBL04 plants died in these plots during summer. In year 6 for the OM2DL plots, M2 mutants produced a higher shoot biomass than other genotypes. The same trend was noticed in the OM amended-plot but the M2 variant only differed from other variants. Other genotypes had similar shoot DW yield in the OM2DL plots. Both mother-line and M3 mutant developed only in the plots with recent compost dressing (OM2DL and OM).

Shoot Cu concentration was the highest in the OMDL plots for the M3 variants followed by the M2 ones. For the M3 plants, this corresponded with their low shoot DW yield. All mutants showed the lowest shoot Cu concentrations in the OM2DL plots compared to the OMDL plots demonstrating the influence of the second compost dressing. The M1 mutant contained a higher shoot Cu concentration when grown on the OM plot compared to other plants. The low shoot Cu concentrations (close to the upper critical threshold value for Cu in higher plants) for the OM2DL plants corresponded with their high shoot biomass, suggesting a dilution effect. Shoot Cu removal was the highest for the M2 mutant in the OM2DL plots, thanks to their high shoot biomass. The trend was similar for M2 mutants from the OM plots, notably compared with the IBL04 plants. In overall, the high shoot biomass would be the main driver for the high shoot Cu removal. According to the plant density, plots and sunflower genotypes, shoot Cu removal varied in the 21-105 g Cu/ha range.

- <u>LDS plots</u>: only M1 and M2 mutants developed on these plots with a better growth of M1 plants. Shoot Cu concentrations of M1 and M2 mutants were higher in both LDS amended plots than in the OM2DL plots. High values for shoot Cu removal were obtained for the M1 mutant in both LDS plots, mostly due to their higher biomass.

In year 7 the shoot DW yield was enhanced in the OM2DL plots, and the LDS amended plots. Sunflowers were unable to grow on the untreated plots. The influence of the genotype was insignificant, but the second compost dressing in year 6 was the key factor to promote the shoot yield and Cu removal. Between years 4 and 6, extractable Cu fraction in the OMDL plots was reduced by 38%. Since the experiment started, shoot Cu removal fit with a quadratic function, likely following reactions of compost with Cu, nutrient release from compost decay and bioavailable Cu stripping.

**Lommel** (BE): phytoextraction of Cd/Zn was assessed. Tobacco clones and sunflower mutants were cultivated from years 1 to 4. *Brassica napus* and *Cannabis sativa* (hemp) *were also* implemented. In 2013, shoot DW yield of tobacco was similar for all genotypes ranging from 1.3 to 1.7 t/ha. For sunflower, shoot DW yield varied from 3.5 to 7.5 t/ha, with the M1 mutant producing lower shoot biomass than the control plants and the other mutants. Hemp developed well and its shoot DW yield reached 17.5 t $ha^{-1}$ $yr^{-1}$.

Shoot metal removals for tobacco were in the following ranges (in g/ha/yr): Cd 14 – 20, Pb 8.6-11.9, Zn 252-331. Genotype had an influence (*e.g.* higher shoot removal for Cd/10-8 variant and Zn/7-19 variant). Compared to tobacco, shoot metal removals of sunflower were higher for Zn (1992-2504) and slightly higher for Cd (17.7-23.6) and Pb (17.7-42.5), with a genotype influence. The phytoextraction by hemp was 7 g Cd, 41 g Pb, and 1355 g Zn/ha.

The phytoextraction of Cd, Pb and Zn by all tested sunflowers was higher than that of the tobacco clones and hemp. Tobacco clones had higher shoot Cd and Pb concentrations but the higher shoot DW yield of sunflowers lead to higher shoot metal removals. Hemp production on metal-contaminated soil could be relevant if cutting the pollutant linkage to food along with an economic profit from the plant-based feedstock (e.g. fibre) is the primary goal instead of the other ecosystem services (*e.g.* decreases of labile metal pools, decontamination, and soil remediation).

In 2014, the biomass production, and consequently the shoot metal removal, was higher than in 2013, especially for tobacco (tobacco 3.6-4.9 t/ha; sunflower 5.8-9.5 t/ha). Shoot removals were 59.6-122 g Cd, 38-70 g Pb and 1027-1926 g Zn/ha for tobacco, 32-61 g Cd, 17.5-34.5 g Pb, and 2624-5745 g Zn/ha. This highlights the influence of annual climatic conditions on shoot metal removals.

**Bettwiesen** (CH): The crop rotation is based on four sunflower and five tobacco genotypes with higher metal tolerance and accumulation properties for stripping bioavailable Zn and Cd excess in topsoil. After 5 years, the labile Zn pool in soil was lowered by 45-70%, and up to 67% for Cd and 62% for Pb. A Mass Balance Analysis confirmed soil Zn decontamination in line with plant Zn uptake. The plants partially take Zn from the non-labile pool of the total. Moreover the results confirm a strong immobilization effect of the plant rhizosphere (by increasing soil pH up to one unit) due to phytoextraction treatment. The 'stripping' of bioavailable Zn is feasible within a few years period. To decrease available soil Zn below the Swiss threshold value, the phytomanagement would take 3-12 years at moderate available Zn levels and 5-25 at high levels. Various plant densities and intercropping of sunflower and tobacco with fodder pea during winter until in early spring, and wild type *Galingsoga parviflora* are further explored.

Due to long lasting cold and rainy weather condition in 2014, the biomass production, and consequently the shoot metal removal on the B3 and B4 plots was lower than in 2011-13, especially for tobacco (tobacco 1.9-4.1 t/ha; sunflower 3.5-11.7 t/ha). Shoot tobacco removals were 381-1148 g Zn/ha, 3-11 g Cd/ha, 25-80 g Cu/ha, and for the sunflower 815-3528 g Zn/ha, 1-14 g Cd/ha, 83-193 g Cu/ha. This highlights the influence of annual climatic conditions on shoot metal removals. The metal extraction efficiency of the tobacco and sunflower mutants and *in vitro bred* cultivars and its controls (motherline inside the brackets) followed the following hierarchy for the experimental years 2011-14:
tobaccos: NBCu10-8 > NBCu10-4 > (BAG)
sunflowers: mutant 3 ≅ mutant 1 > mutant 2 > (IBL04). Mutant 4 additionally tested in 2014 was best for shoot yield, Cd and Cu extraction. In spite of good shoot yield of the tobacco and sunflower controls, their shoot metal extraction efficiency was constantly lower, compared to the sunflower mutants and *in vitro* optimized variants of tobacco.
Based on representative and randomly taken soil samples on the B3 and B4 plots, the phytoextraction efficiency along the four years period (2011-14), was 73–94% for labile Zinc and 73-95% for labile Cd topsoil concentrations.

### 1.3.3.2. (aided) phytoextraction using woody SRC

The capacity of poplar and willow to colonize hostile environments such as mine wastes is recognized. Numerous *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. Most promising poplars and willows (according to climatic conditions and shoot TE concentrations) were assessed at 6 sites: Lommel (HAU), Högbytorp and French trial (SLU), Freiberg (LfULG), Piedrafita (ES) and Phytagglo (FR).

**Freiberg, Halsbrücke Krummenhennersdorf** (DE): this 9-old field trial is a SRC plantation on contaminated agricultural land. Shoot DW yields reached 15 t/ha/yr for poplar SRC and 14-19 t/ha/yr for willow SRC, corresponding to common values (6-16 t DM $ha^{-1}$ $yr^{-1}$). Stem wood and bark ionomes of poplars and willows depended on genotypes, particularly for Cd (3-10 mg/kg in wood; 8-30 mg/kg in leaves), and clonal differences were higher across willows. Values were higher in willows compared to poplars. Willow cultivars Tora, Tordis and Gudrun displayed the highest wood and foliar Cd concentrations among all cultivated clones of poplars and willows. The ratio of the 3-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. Bark Cd concentrations account for triple (poplars) to fourfold (willows) of those in stem wood while Pb-concentrations did not differ between the compartments. Foliar As-concentrations were mostly below the detection limit. Wood As concentrations varied from 2 to 4 mg/kg. For the third rotation, willow Tora produced the highest biomass out of all poplars and willows (followed by Tordis) and it displayed relatively high Cd and Pb accumulation capacity. This confirmed Tora as a relevant choice for metal (Zn, Cd) phytoextraction, and Tordis as well (for Cd).

**Piedrafita do Cebreiro** (ES): plots with *Salix smithiana* and *Salix atrocinerea*, in monoculture and inter-cropped with *Alnus glutinosa*, and *Salix* cv. Tora were established in autumn 2012. Plots were unamended or amended with 5% (w/w) compost. Plant survival was significantly higher for *S. smithiana* than either *S. atrocinerea* or Tora. Plant growth (height/spread) and leaf/stem Cd/Zn concentrations were recorded in 2013 and 2014. No significant effects of inter-cropping on plant growth have been recorded to date (although there was a tendency to increased tree height in intercropped plots in 2012). Intercropped *S. smithiana* plots showed significantly higher leaf Cd and Zn concentrations (approx.. 12 and 1400 mg/kg). Significant changes in total or $NH_4Cl$-extractable concentrations of Cd and Zn have not yet been observed.

***Changes in soil exposome***: At Freiberg, generally total soil Cd decreased (e.g. 3.4 to 2.9 mg/kg for Weser 6 poplar; 2.5 to 1.0 for Tora willow) between 2011 (year 6) to 2013 (year 8).
The rhizosphere of willow and poplar clones at contaminated and adjacent reference sites showed a lower pH for SRC compared to arable land (with a higher decrease for willow SRC). Since the SRC implementation in 2005, the initial soil pH of 5.7 ($CaCl_2$) dropped in average to 5.2. However, soil pH increased in poplar plots after the harvest in year 8, whereas a further decrease occurred for willow plots especially for Tordis cultivar. $NH_4NO_3$-extractable soil Cd and Pb were roughly 10 fold higher under SRC and grassland compared to annual cropped land. In contrast, mobile As in soil decreased for SRC (3 to 9 fold) compared to annual cropped land (lowest values for willow SRC).
Soil pH was higher under winter wheat with the lowest $NH_4NO_3$-extractable soil Cd in year 2. $NH_4NO_3$-extractable soil Cd decreased for Jorr, Sven, Tora willow SRC and Max 3 poplar SRC.

***Soil microbial communities***:
At **Freiberg,** arylesterase and arylsulfatase activities did not differ between SRC and control plots. Alkaline phosphatase was higher in SRC plot.

At **Piedrafita do Cebreiro**, shifts in the structure of the bacterial communities, *i.e.* total Eubacterial community, *Alpha*- and *Beta-proteobacteria*, *Actinobacteria* and Streptomycetaceae, were assessed over two years in the compost-amended plots (aided-phytoextraction) and unamended plots (phytoextraction), the vegetation cover being SRC (with *Salix* sp.). The DGGE profiles of soil samples collected before GRO implementation (time=0) are always separated from phytomanaged soils (similarity <20%). In general, compost-amended plots were separated from untreated soils, although this separation was more pronounced in year 2. In both years 1 and 2, there are some clear separations between soil groups under the different vegetation covers, and between mono- and co-cultivated *Salix smithiana*. The Shannon diversity index indicated a decrease in the diversity of these bacterial communities with time (from 1 to 2 years). A higher diversity always occurred in phytomanaged soils compared to untreated soils, without clear plant-induced effect. After 3 years of SRC, most soil enzyme activities increased in the phytomanaged soils, with a plant-induced effect, and this was more pronounced in the compost-amended soils than the unamended soils. However, a clear effect of the co-cropping of *Salix smithiana* with *Alnus glutinosa* was less evident.

**At Phytagglo** (FR), 350 willows (*Salix viminalis*) were planted on 9 raws in April 2013. Inter-rows were covered with beech mulch. This alkaline technosol (pH 8.1) developed on dredged sediments is contaminated by Zn (1117), Pb (262), Cu (100) and Cd (2.9), with a high spatial variability for Cd and Zn and a relatively high OM content (2.3%). In some plots, citric acid based product, ferrous sulfate, and elemental S were separately incorporated in order to investigate the effects of soil acidification. $NH_4N0_3$-extractable metal fractions were low (*e.g.* 0.014% for Cd, 0.2% for Zn). In year 1, extractable metal fraction decreased for Pb, and remained steady for Cu, Cd and Zn. The survival rate of willows was 90% in year 1. Their foliar Cd and Zn concentrations ranged between 1.5-3.8 and 233-1176 mg kg$^{-1}$, which is quite high when considering the low Cd and Zn mobility and alkaline soil pH. Foliar Cu concentrations (7-12 mg kg$^{-1}$) were similar to common values of willows on uncontaminated soils. In year 1, maximum stem height increased by 4 fold and trunk diameter by 2 fold. After one month, soil pH did not decrease in the amended soils and further inputs of potentially acidifying compounds are planned.

**Högbytorp** (landfill leachate trial, SE): For 14 commercial willow SRC plantations long-term grown (ca. 15 years) on agricultural soil in Sweden, total topsoil Cd decreased (ca. 13% on average) compared to adjacent fields cultivated with cereals in common crop-rotations. The biomass productions on these SRC fields were lower than the indicative 10 t DM ha$^{-1}$ yr$^{-1}$ expected nowadays in well-managed fields. Farmers had lack of experience in growing such crops, and beneficial incentives in terms of subsidies caused limited engagement throughout the process. Here, treatments consist of three supply rates of landfill leachate (irrigation started in 2005 and carried out until 2010) and a control, with two willow clones, *i.e.* Tora (*Salix schwerinii* x *viminalis)* and Gudrun (*Salix dasyclados* variety with partly Russian origin, more frost-tolerant than Tora).

For willows, shoot concentrations varied in the 1-4.5 mg Cd and 40-120 mg Zn kg$^{-1}$ DW ranges. Tora showed higher shoot Cd, Co, Mn, Pb and Zn concentrations than Gudrun for leachate irrigated-plots. Tora had higher shoot Cd, Co and Zn concentrations for the plots irrigated at the second supply rate compared to other treatments and the control. Gudrun displayed higher shoot concentrations for all metals in the control plots. The leachate treatments did not influence the shoot Cr and Cu concentrations in both clones, even though these concentrations were low for Tora on the control plots compared to the treatments 1-3. Nickel was the only metal with higher shoot concentrations in Gudrun for all treatments. Total shoot N concentration was roughly similar for both clones, except a higher concentration for Gudrun on the control plots.

**Lommel** (BE): Willow clones 'Belgisch Rood (BR)' (*Salix* x *rubens* var. *basfordiana*) and 'Tora' (*Salix schwerinii* x *Salix viminalis)* were compared. Shoot DY yields after 3 years were 5.4 (BR) and 9.0 (Tora) t/ha. Again shoot Cd concentrations were higher in Tora (30 mg/kg) than in BR (24 mg/kg) and shoot Zn concentration as well (1268 and 918 mg Zn/kg). The Tora willow clone had a Cd and Zn removal capacity (274 g Cd and 11 417g Zn ha$^{-1}$) which is twice as high as that for BR clones. Both, the higher biomass production (ton ha$^{-1}$) and metal uptake capacity (mg kg$^{-1}$ DW) make the TO willow clone the favorable clone to select for phytoextraction applications.

**1.3.3.2. TE hyperaccumulators:** can accumulate high concentrations of metal(loid)s (*e.g.* Cd, Ni, Zn, Se, and As) in their above-ground biomass and possess some economic added value (renewable biomass for bio-economy and bio-ores). Variations in both biomass production and TE accumulation within populations of hyperaccumulators, such as *Noccaea caerulescens* (for Zn/ecocatalysis), *Alyssum murale*, *A. bertolonii* and *A. corsicum*, (for Ni phytomining) allows for the selection and breeding of improved phytoextractor plants. The main bottleneck limiting their practical application is the low biomass production of most species (except some Ni-hyperaccumulators) and the number of cropping cycles required for clean-up. However this number is generally reduced when the option of bioavailable TE stripping is considered. Additional limiting factors include the absence of commercially available seeds/seedlings, their sensitivity to the presence of contaminants other than the hyperaccumulated TE, a general lack of knowledge related to their cultivation, climate needs and competition with other TE-tolerant plants.

**Piedrafita** (ES): sub-plots were established in May 2012 with the Cd/Zn hyperaccumulator *Noccaea caerulescens* and inter-croppings of *N. caerulescens* with *Lupinus albus* and *Lotus corniculata*. Unamended plots were fertilized with inorganic commercial fertilizers. Nitrogen fixation can decrease soil pH due to nitric acid accumulation in the rhizosphere, 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 *N. caerulescens* with the legume *Lotus corniculatus* tended to increase Cd accumulation by the hyperaccumulator. Other *Lotus* species show potential for incorporating into GRO due to their worldwide distribution and high adaptation to a number of abiotic stresses. Candidates with good potential for cultivation in degraded or marginal soils include *Lotus corniculatus*, *L. uliginosus*, *L. tenuis* and *L. creticus*.

***Restoration of soil microbial activities and community***: The DGGE profiles showed similar clusters for the soils planted with *N. caerulescens* and those co-cropped with *L. corniculatus*. The separation between unamended and compost-amended soils was clearer in year 2. Unplanted soils formed clusters, and were well separated from planted soils. Mono-cultures and co-cultures tended to cluster separately. The diversity of bacterial communities increased with time, with a higher diversity in phytomanaged soils compared to untreated soils, and a higher diversity in planted soils compared to unplanted soils.

**Phytagglo** (FR): Seven plots were set up with potentially acidifying properties, *i.e.* legume plant (*Lupinus albus*), a citric acid based product, peat-like, ferrous sulfate and elemental sulphur, *Arabidopsis halleri* and control.

**Reppel** (BE/FR): Since 2004, *Pteris vittata* L., an As hyperaccumulator was cultivated for bioavailable As stripping in this Belgian soil polluted by atmospheric fallout. Generally, frond DW yield was doubled in the contaminated soils compared to the uncontaminated control soil. Soil treatments, *i.e.* Beringite (B, 5% w/w), iron grit (Z, 1% w/w) and their combination (BZ), and season did not influence annual frond yield, except differences between B and BZ in November and between November and May for the untreated (Unt) and B soils. On the 2006-2013 period, leachate As concentration remained lower in Z-treated soils than in the Unt and B soils. Mean values of frond As concentrations (in mg As/kg) varied in the 60-171 range for the control soil and in the 970-2870 range for the contaminated soils. Frond As removal varied from 3.89 to 2.28 g As/m² in the decreasing order: Unt, B > BZ, Z. For vegetables cultivated after several fern crops, root DW yield of lettuce was higher in BZ-treated soils and lower in the B-treated ones, whereas shoot DW yield did not differ across soil treatments. Shoot As concentration (in µg/g DW) varied from 1.28±0.25 to 2.5±0.5 and was lower in BZ-, B-, and Z-lettuces.