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GOLD

Growing energy crops on contaminated
land for biofuels and soil remediation

D1.6

Optimised phytoremediation solutions

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1. Introduction

Under the frame of GOLD project, and within WP1 “Optimization of lignocellulosic energy crops for phytoremediation purposes”, four selected lignocellulosic industrial crops are tested for their phytoremediation potential when cultivated in contaminated soils. These crops are industrial hemp, sorghum, miscanthus x giganteus and switchgrass. Two phytoremediation practices are evaluated: plant-associated microorganisms and biostimulants (fulvic/humic acids and protein hydrolysates). Trials are conducted to identify the best combination of phytoremediation practice with the most effective crop (in terms of biomass yields, quality, and uptake of inorganic /degradation of organic contaminants), across a wide range of soil contaminants and under real field conditions. All partners used the same crop varieties and they applied the same protocols. In Europe, five experimental fields representing different climatic zones and types of soil pollutants have been established (Figure 1). Additionally, two field trials are being carried out in China, while in India the trials are being carried out on pots.

The produced biomass is feeding the conversion routs of WP2 for the production of sustainable, clean biofuels. In addition, all the collected data and information are supporting the activities of WP3 in order to bridge the gap between clean biofuel production and advanced phytoremediation solutions.

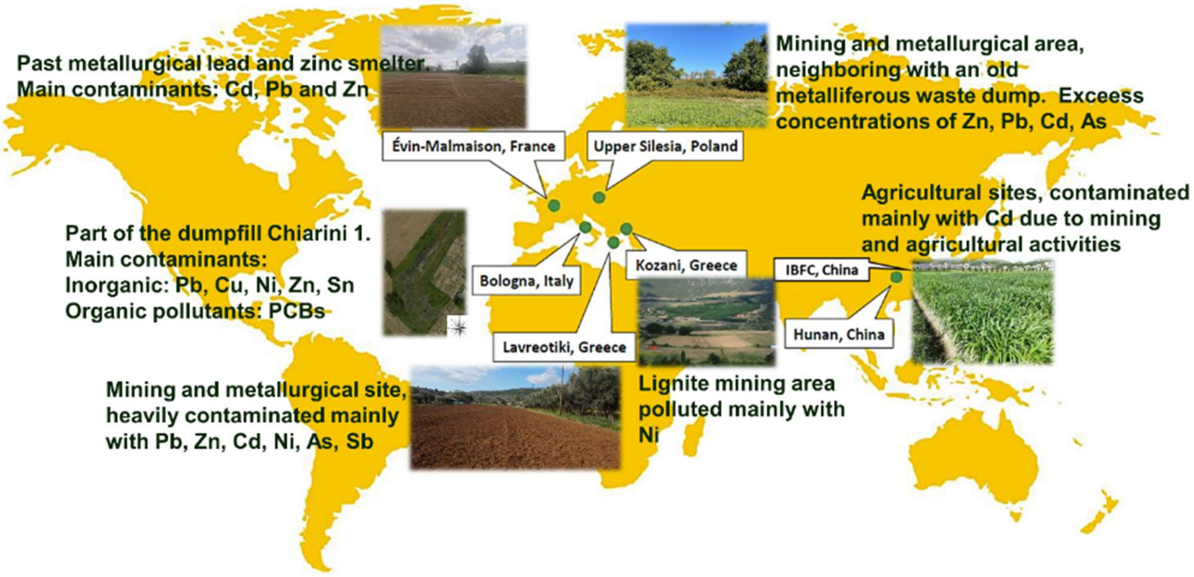


Figure 1. Field trials of GOLD project.

The specific objectives of WP 1 are (Figure 2):

- to compare various phytoremediation practices on soils contaminated by organic and inorganic pollutants, when cultivating the selected high-yielding lignocellulosic energy crops.
- to apply the best performing phytoremediation practices on pilot field trials.
- to develop optimized phytoremediation solutions for the selected crops in the form of lessons learnt.

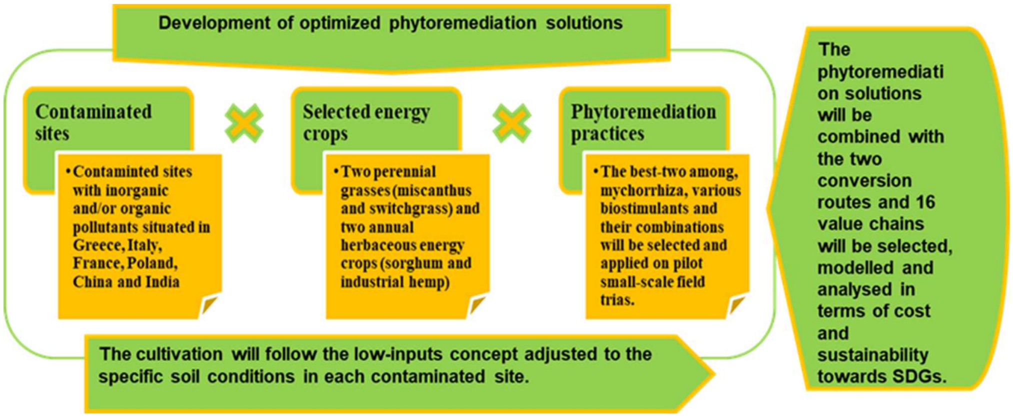


Figure 2. Development of optimized phytoremediation solutions.

In Europe, biofuels (biodiesel and bioethanol) are mainly produced from cereals (e.g. wheat), bio oil (e.g. rapeseed) and sugar crops (e.g. sugar beet) using established technologies (first generation biofuels). In line with the Renewable Energy Directive (RED II), the European Union aims to achieve a renewable energy consumption target of at least 27% by 2030. The directive also stipulates a gradual reduction in the production of biofuels, bioliquids, and biomass fuels derived from food and feed crops, commencing from the end of 2023. In Annex IX of the RED II non-food lignocellulosic materials are specifically listed. Therefore, emphasis is given to biofuel production with low ILUC risks by promoting the cultivation of lignocellulosic non-food energy crops on unused, abandoned and severely degraded land (the latter including contaminated land). Several energy crops have been tested by several EU research projects (i.e. MAGIC, FORTE, BECOOL, GRACE, Greenland, New-C-Land, OPTIMA, OPTIMISC, SEEMLA, FORBIO, Phy2SUDOE) with promising results. Among the lignocellulosic energy crops, the perennial grasses miscanthus and switchgrass and the annual herbaceous crops biomass sorghum and industrial hemp are considered as ideal feedstock for advanced biofuels production with low ILUC risks (Figure 3).

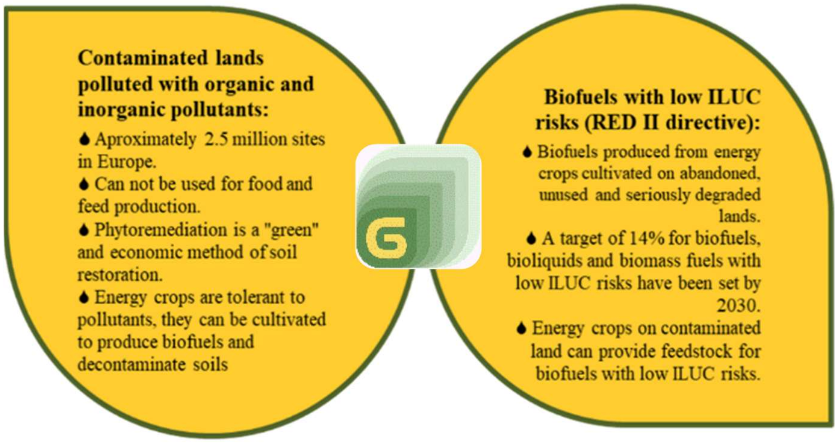


Figure 3. GOLD aims to produce clean low-ILUC biofuels by growing selected high-yielding lignocellulosic crops on contaminated lands, and, in long-term, to return these lands back to the agricultural production.

In Task 1.4 “Optimised phytoremediation solutions” of WP1, the results of the previous tasks and the field trials are critically reviewed, along with results from published scientific papers and from several ongoing and/or completed projects on soil phytoremediation using energy crops (like FORTE, INTENSE, New-C-Land, MAGIC, Phy2Sudoe). The aim of this task is twofold: a) to provide information regarding optimised phytoremediation solutions for the selected energy crops on specific contaminated soils, which will be further analysed in WP3 and b) to outline lessons learnt for optimised phytoremediation solutions in the form of factsheets per case study. All the information and data of this task will be presented in the deliverables D1.6 and D1.7. The present deliverable 1.6 is the initial version of the work accomplished so far under Task 1.4. The final outcomes and results will be incorporated into D1.7 at the end of the project.

The D1.6 includes a concise introduction to soil pollution, to remediation techniques (with emphasis on phytotechnologies) and to the crops suitable to be used for phytoremediation. Additionally, it provides details on the annual energy crops—industrial hemp and sorghum—selected for GOLD's objectives. A comprehensive literature review on publications regarding their phytoremediation efficacy under real field conditions is also included. The corresponding information for the perennials miscanthus and switchgrass will be included in D 1.7. Furthermore, brief summaries of previous and ongoing phytoremediation projects involving the selected energy crops, and in which GOLD partners were involved, are provided in this deliverable. Given that the field experiments of Work Package 1 are ongoing, the final results on the optimal phytoremediation solutions will be presented in Deliverable 1.7 at the project's completion.

2. Soil pollution

Soil is a natural, non-renewable resource, providing the basis for the production of food, feed, and other resources for a circular bio-economy. Soil also supports biodiversity, plays a central role in carbon sequestration and storage, and provides a number of other ecosystem services, such as water regulation and nutrient cycling.

Soil pollution refers to the presence in the soil of a chemical or substance out of place and/or present at a higher than normal concentration that has adverse effects on any non-targeted organism. Because soil formation is extremely slow and soil systems have a great resistance to change, the degradation of soils and its consequences can only be perceived with a considerable temporal delay: It takes 1,000 to 10,000 years to build up fully functional soil layers of 30 cm (Heuser, 2022). Although the majority of pollutants have anthropogenic origins, some contaminants can occur naturally in soils as components of minerals and can be toxic at high concentrations. Soil pollution often cannot be directly assessed or visually perceived, making it a hidden danger. Currently, more than 10 million major sites worldwide are contaminated. In Europe, a survey conducted across 39 countries (including the EU 27, the UK, and the EIONET cooperating countries), has estimated a total of 2.5 million potentially contaminated sites. Of these, approximately 14% (340,000 sites) are expected to be contaminated and in need of remediation measures (Figure 4) (Van Liedekerke et al., 2014; Mench et al., 2018; Paya Perez and Rodriguez, 2018).

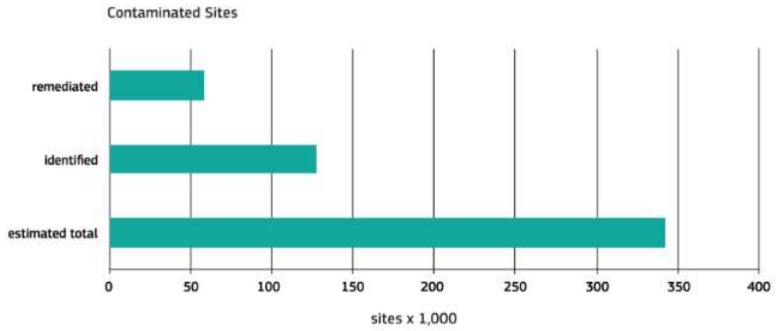


Figure 4. Identified potentially contaminated sites and contaminated sites (Van Liedekerke et al., 2014).

Main sources of soil pollutants (i.e. inorganic or organic substances) are anthropogenic activities, including industrial processes, mining and smelting, domestic effluents, waste treatment, extensive use of agrochemicals, extraction and processing of fossil fuels, and transport emissions (Figure 5).

Anthropogenic metal(loid) contamination of soils has become a global environmental problem due to intensively increasing industrialization and agricultural activities leading to one of the most pressing concerns in the debate about food security and food safety in Europe and globally (Kong, 2014; Ma et al., 2020; Yang et al., 2023; Rashid et al., 2023). A number of urban and industrial activities including mining, metal(loid) smelters and steel industry, road transport, waste incineration and unsafe disposal of industrial waste, the use of fertilizers and agrochemicals, land application of industrial and domestic sludges, biosolids and manure are identified as the main human sources of metal(loid)s in soils and water in the superficial ecosystems (Rodríguez-Eugenio et al., 2018; Haroon et al., 2019; Zine et al., 2021; Adnan et al., 2024). In addition, geogenic emissions from volcanoes, degassing processes in the Earth's crust, forest fires or the chemical composition of the parent material and rock weathering can be also important sources of metal(loid)s in soils (Abraham et al., 2017; Koul and Taak, 2018; Dupla et al., 2023).

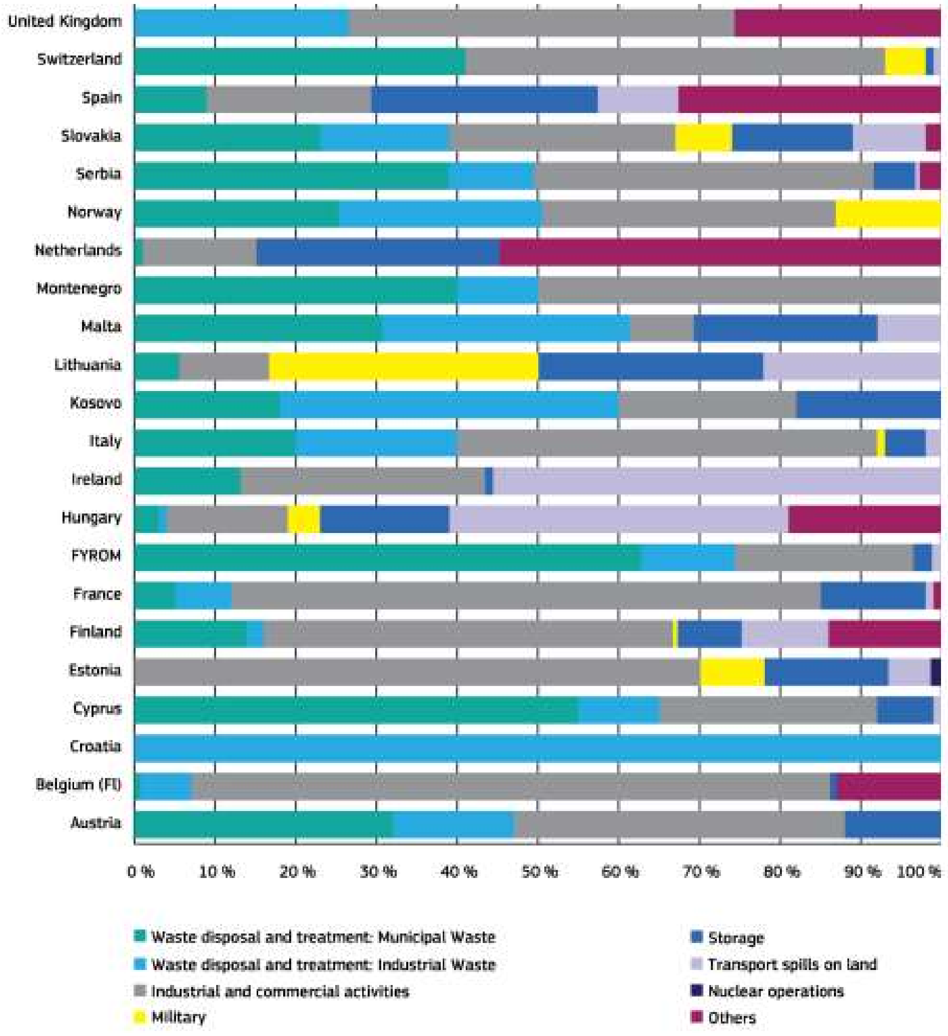


Figure 5. Breakdown of activities costing soil contamination (Van Liedekerke et al., 2014).

Contrasting to organic contaminants, metal(loid)s are somewhat unique by the fact that they are highly resistant to either biologically or chemically induced degradation result in accumulation in the tissues of living organisms (but some organometallic chemical forms, such as methyl-Hg, tetraethyl-Pb, methylated forms of Se, Sb, and Sn, Pd, As, etc. are prone to synthesis and (bio)degradation). Therefore, total metal(loid) contents of soil persist for a long time after being introduced into the soil, while the most mobile of them (e.g. Cd, Zn, As, Ni, Se, and Mo) could induce groundwater pollution as they are leachable (Vangronsveld et al., 1995; Hadia-e-Fatima, 2018; Heuser, 2022). Excess metal(loid) accumulation in soils can be phytotoxic, as well as toxic to humans and other animals through food chain transfer (i.e. the more mobile metal(loid) in the soil solution matter: e.g. Cd, Zn, Ni, As, Se, Sn, and Mo) (Papazoglou, 2011; Cuypers et al., 2013; Ma et al., 2020; Ceramella et al., 2024). Of all the elements, the most important to consider in terms of food-chain contamination are arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se).

Furthermore, soil pollution impacts biodiversity both above and below ground (Feckler et al., 2023; Adewara et al., 2024). This occurs through a reduction in organism numbers due to contaminant toxicity and by altering communities, favoring pollution-tolerant species over sensitive ones. Even low contaminant levels can prompt adaptive responses, such as physiological and behavioral changes in organisms. Shifts in soil organism activity can disrupt biogeochemical cycles. Moreover, polluted soil can contaminate groundwater via leaching and affect freshwater and marine environments through wind and water erosion. These changes may be gradual or dormant until a tipping point, leading to severe degradation and a cascade of ecosystem processes, ultimately resulting in the loss of ecosystem services (Figure 6).

Figure 6. Soil pollution causes a cycle of degradation processes that leads to the reduction and ultimately to the loss of ecosystem services (Source: FAO and UNEP. 2021. Global assessment of soil pollution - Summary for policy makers. Rome, FAO https://doi.org/10.4060/cb4827en).

3. Remediation techniques

The remediation processes used for clean-up organic and inorganic contaminated soils or (progressively) quenching the pollutant linkages from the soils (secondary sources) to the biological receptors may be biological, physical and chemical (Figure 7) (Liu et al., 2018). These techniques are often used in combination with each other for more economical and efficient remediation of a contaminated site. Physicochemical methods of soil remediation are usually expensive when large sites are of concern and often result in a deterioration of the soil ecosystem. Therefore, since the 90's, the development of environmentally-friendly biological technologies to economically remediate these soils has been stimulated (Hernandez-Allica et al., 2006; Cundy et al., 2016; Moreira et al., 2021; Drenning et al., 2022; Vilela et al., 2022). Among biological techniques, phytoremediation is encompassed a relevant set of phytotechnologies, associating plant species, associated microorganisms and mesofauna, for remediating pollutant linkages due to contaminated soils. A new and more recent aspect of phytoremediation is phytomanagement in which remediation strategies are combined with sustainable and profitable site management options, resulting in a net gain in soil functions and ecosystem services, while producing economic revenue (Evangelou et al., 2015; Perlein et al., 2023; Saran et al., 2024).

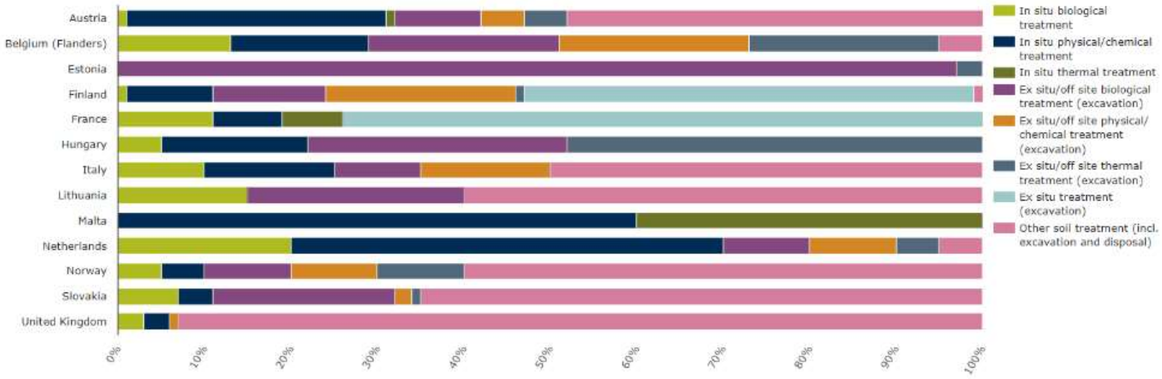


Figure 7. Most frequently applied remediation techniques for contaminated soil (EEA, 2014).

3.1. Phytoremediation

Phytoremediation is a cost-effective and environment friendly set of phytotechnologies, which are used to remove contaminants from the environment or to render them harmless (Chaney et al., 1997; Eevers et al., 2017; Salifu et al., 2024). The main purpose is to quench the pollutant linkages by actions on either the sources, the exposure pathways, or the biological receptors (or even all) and to enhance the ecosystem services. Unlike physical and chemical treatments that irreversibly alter soil properties, phytoremediation generally improves the physical, chemical, and biological quality of contaminated soils. For the various types of phytoremediation now practiced, demonstration and pilot projects have been translated into commercial-scale operations, so establishing the credibility of the technology. Phytoremediation is more effective and economically viable when: (i) it is applied in large areas with low to medium concentrations of pollutants so that phytotoxicity on plant remains low and plants can grow effectively, (ii) the crops used produce high added-value biomass providing a revenue, (iii) the site is in unused/abandoned arable land and agricultural practices and mechanization can be applied.

Phytomanagement is to pair phytoremediation with sustainable and profitable site management options, using plant species that will produce marketable biomass and thus the landowners /land managers / stakeholders will have an economic revenue. The harvested biomass can be used as feedstock for bioenergy purposes, and concurrently, plants are decontaminating the soil. In this way, marginal or degraded soils that cannot be given over for food production will be exploited and upgraded, the energy targets of RED II will be supported, new jobs will be created, local farmers will have the possibility to maintain and/or increase their income, and the development of rural areas will be reinforced.

There are various types of phytotechnologies used for soil and water remediation, including phytoextraction, phytostabilization, phytodegradation, phytovolatilization, phytostimulation and rhizofiltration (Figure 8) (Eevers et al., 2017; Burges et al., 2018; Ashraf et al., 2019; Mai et al., 2025). Phytodegradation is the breakdown of organic and organometallic compounds either by metabolic processes of plants or enzymes produced by plants, independent of microbial communities. Phytodegradation is only beneficial for decomposition of organic and organometallic compounds.

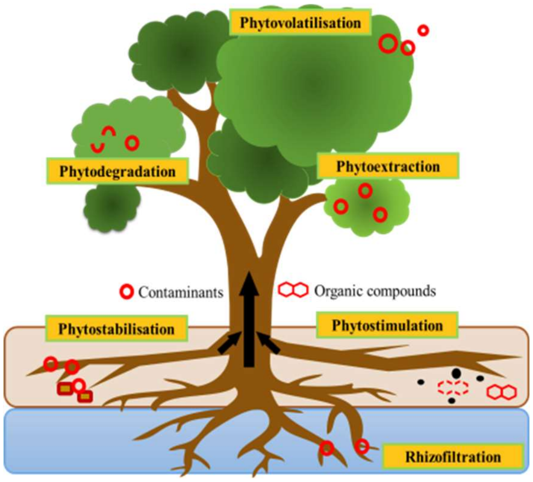


Figure 8. Phytoremediation for polluted soils with inorganic and organic pollutants

The term phytovolatilization refers to the release of volatile compounds into the atmosphere through plant transpiration. In this technique plants uptake contaminants from soil, transform them to volatile compounds and subsequently release these compounds into the atmosphere. However, phytovolatilization is applicable only to organic compounds, as well as to Hg (in transformed plants) and Se. Phytostimulation, also referred as rhizodegradation, is the disintegration of organic pollutants in rhizosphere through enhanced microbial activity. Rhizofiltration is the use of plant roots for reclamation of surface and groundwater and wastewater with low level of contaminants.

3.2 Phytoextraction – Phytostabilization

The two main strategies for metal(loid) phytoremediation are phytoextraction, the use of plants and associated microbes to extract metal(loid)s from the soil and phytostabilization, the use of plants and associated microbes to reduce metal(loid) bioavailability (Cundy et al., 2016; Neaman et al., 2020; Moreira et al., 2021).

Phytoextraction involves generally fast-growing plants to remove metal(loid)s from the environment, both soil and water (Yanai et al., 2006; Van Nevel et al., 2007; Pajevic et al., 2016). There are two primary approaches of phytoextraction: continuous or natural and chemically induced phytoextraction (Ghosh and Singh, 2005). In continuous phytoextraction, a network of roots removes metal(loid)s from the soil, directing them to the upper plant tissues above ground (Jadia and Fulekar, 2008). This approach is best to progressively reduce the bioavailable fraction of excess metal(loid)s from the soil by plant roots and shoots without affecting soil properties. Metals can be restored from harvestable plant parts (Garbisu & Alkorta, 2001). Employing a continuous cropping and harvesting system can significantly reduce the bioavailable fractions of contaminants in the soil (Vandenhove et al., 2001). In contrast to prevailing remediation techniques, this green technology is estimated to be ten times more cost-effective (Wan et al., 2016).

Phytostabilization is plant-based inactivation approach to deal with metal contaminated soil (Singh, 2012; Ramanjaneyulu et al., 2017; Khalid et al., 2017; Ashraf et al., 2019). The objective of this technique is to diminish the mobility and bioavailability of metal(loid)s, thereby restricting their leaching into groundwater and entry into the food chain though root uptake. In aided phytostabilization plants play secondary role as compared to soil amendments. This technique involves physical and chemical immobilization of metal(loid) excess in the soil solution by their sorption onto roots, reaction with various soil amendments and changes in soil properties (Wuana and Okieimen, 2011). Organic matter, biosolids, clay minerals, Fe/Mn oxy-hydroxides and phosphate fertilizers are most efficient soil amendments for metal(loid) immobilization. While phytostabilization serves as a management strategy, it is not a permanent solution as metal(loid) contaminants persist in the soil over time (Vangronsveld et al., 2009).

4. Energy crops used for phytoremediation

There has been a growing global interest in energy crops because of their potential to serve as a carbon-neutral, environmentally friendly source of renewable energy, which could help to fulfill global energy needs to some extent. Among the various types of renewable energy, biomass based energy is one of the most important renewable energy due to resource abundance. The increased requirement of energy across domestic, industrial, transportation and agricultural sectors had required the rapid deployment of bioenergy options. Globally, biomass based renewable energy is expected to expand to 80–160 EJ yr−1 in 2050 from 50 EJ yr−1 today (Johansson et al., 2012). Energy crops supply about 1.5% of electrical power, 3% of heat generation, and close to 3% of liquid transport fuels globally (Panday et al., 2016). Unfortunately, current bioenergy production levels are insufficient to meet the growing energy demands of the global population. Additionally, contaminated lands are often unsuitable for traditional agricultural production. Therefore, there is an urgent need to cultivate energy crops on contaminated lands for bioenergy production, with the aim of achieving economic, social, and environmental sustainability.

Hyperaccumulators are plants capable of growing in metalliferous soils, and accumulate extremely high levels of metals in their aerial tissues, far in excess of the levels found in the majority of species, without suffering phytotoxic effects. Approximately 800 plant species have been identified to be hyperaccumulators, out of which 110 belongs to the Brassicaceae family and approx. 340 are Ni hyperaccumulators (Van den Ert et al., 2013; Reeves et al., 2021; Fadzil et al., 2024). Hyperaccumulators could be used for phytoextraction purposes. However, the majority of these species are small, giving low biomass and have poor agronomic traits.

Several recent studies and funded projects have been focused on fast growing and high biomass-yielding non-food crops, which are tolerant to soil contaminant and are able to be cultivated in metalliferous soils. Plants species used for phytoremediation, except from metal tolerance, should have rapid growth, high biomass production and extended root network, and –if used for phytoextraction- should bear and store elevated levels of metal(loid)s (Römkens et al., 2002; Sytar et al., 2016). Moreover, more suitable crops are non-food industrial crops characterized by low production costs. These crops should possess the ability to be cultivated in contaminated agricultural land, yielding satisfactory harvests and resulting in a profitable net gain. High biomass crops usually accumulate much lower concentrations of metal(loid)s in their aboveground tissues, but their use for phytoextraction sometimes results in an over-all metal(loid) removal comparable or even higher to that of hyperaccumulators due to their higher biomass production (Kayser et al., 2000). In any case, crops with such traits can be used for phytostabilization.

Several high biomass yielding non-food industrial crops have attracted much interest for their phytoremediation potential, such as miscanthus, industrial hemp, castor bean, cardoon, giant reed, kenaf, switchgrass, sorghum, poplar, willow, etc. These crops can provide feedstock for the production of bioenergy and for several industrial applications.

In GOLD project, four high-yielding lignocellulosic energy crops have been selected, i.e. two perennial grasses: miscanthus and switchgrass and two herbaceous annuals: sorghum and industrial hemp (Figure 9). The key criteria used for their selection were:

- ✓ They provide feedstock for advanced biofuels with low ILUC risks since they can be grown on abandoned, idle and/or seriously degraded/contaminated lands (result of other projects e.g. MAGIC, GRACE, OPTIMA, BECOOL, FORTE).
- ✓ They are rapidly growing and tolerant to contaminants and other stresses (pest, low soil fertility, drought, etc.).
- ✓ They are non-food crops with high biomass yields and relatively low input requirements (fertilizers, pesticides and irrigation).
- ✓ Even though their phytoextraction potential is low, it is expected that, with the application of plant associated microorganisms and biostimulants in GOLD field trials, their final uptake will be increased [Uptake=biomass produced X % of metal(loid)s in the biomass].

Figure 9. Selected energy crops for GOLD

5. Phytoremediation potential of the annual energy crops selected for GOLD project

5.1. Industrial hemp (*Cannabis sativa* L.)

5.1.1. Crop description, requirements, agronomic management and uses

Industrial hemp (*Cannabis sativa* L.) is an annual C3 dicotyledonous angiosperm belonging to the Cannabaceae family (Figure 10). It is a naturally dioecious, flowering plant, but monoecious cultivars also exist (Placido and Lee 2022). Hemp grows best at temperatures ranging from 16 to 27 °C, and it is a short-day plant, requiring less than 12 to 14 h of sunlight to flower. Its planting season in the Northern hemisphere is in spring, from the second half of April to mid-May. The plants are tall (maximum shoot length can reach 6 m) and have deep roots (45–90 cm). The flowers take 10 to 16 weeks to mature, and fiber production is at its highest at the full-flowering stage. At full flowering, fiber maturation at higher internodes is more advanced and fiber is more homogenous. Hemp is adapted to different pedo-climatic conditions and it can produce up to 20 t DW ha^{-1} (Blandinières and Amaducci, 2022; Visković et al., 2023; Mariz et al., 2024).

Figure 10. Industrial hemp (Cannabis sativa L.), Cannabinaceae family

Soil preparation requires a fine, homogeneous seedbed. Desired plant density is 90 – 150 plants m^{-2}, so 35 – 45 kg ha^{-1} are sown using conventional seed drills. No weeding is generally required due to fast hemp growth and covering of the soil. This crop needs little fertilizers and, thus, has low environmental impact. Hemp requires 250–700 mm of water (approximately 2500–7000 m3 ha^{-1}) over the entire growing season for optimum yield, with 250–350 mm (2500–3500 m3 ha^{-1}) needed during the vegetative stage (Gill et al., 2023). Without optimum water, hemp shoot yield can decrease by up to 60%, with reports of early maturity, reduced stem mass, and stunted plants, notably when water deficit occurs prior to successful establishment. Seasonal water use between 220 and 450 mm is the most common range for fibre hemp. The hemp nitrogen-use efficiency is high and 60 kg N ha-1 are sufficient to reach maximum biomass yield (up to 20 Mg ha-1) (GRACE 2023).

In the EU, hemp production has recently seen a considerable rise in area, growing by 75% from 19,970 ha in 2015 to 34,960 ha in 2019. Hemp production is up 62.4% during that time, from 94,120 tons to 152,820 tons. More than 70% of the EU's production is coming from France, the Netherlands (10% of production), and Austria (4%), and the rest from other EU-member countries. However, the area under hemp in the EU remained the same in 2020, and then decreased slightly to 32,000 ha in 2021 (European Commission, 2023; Viskovic et al. 2023).

Being a multi-purpose crop, hemp biomass can be processed for many applications: i.e. rope and twine, bio-composites and building materials made of fibres, insulation, animal bedding, textiles, papermaking, cosmetics, biofuels, bio-herbicides from the seeds and medical cannabinol (CBD) from the threshing residues, and food industries (Irakli et al., 2019 ; Gill et al., 2023).

5.1.2. Phytoremediation potential of industrial hemp

Hemp has good prospects as a phytoremediator since it can grow on soils contaminated by metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons, and toxins and produce marketable products used for bioenergy and bioproducts (Bidar et al., 2019; De Vos et al., 2023).

Hemp has been assessed for its potential to phytomanage contaminated soils in several countries. However, despite its ability to accumulate heavy metals to a certain extent, it is primarily considered a metal(loid) excluder (Pietrini et al., 2019; Wielgusz et al., 2022; Flajšman et al., 2023). Phytoextraction strategies involving hemp can be enhanced by soil amendments to improve biomass yield and metal removal, thereby increasing metal availability and mobility (Rheay et al., 2021; Moreira et al., 2021). Hemp can export 7.1 g Cd ha-1, 142.0 g Pb ha-1 and 479.4 g Zn ha-1 in one growing season (Ofori-Agyemang et al., 2024). A literature survey on the phytoremediation potential of hemp under real field conditions resulted in the data presented in Table 1. Since shoot metal concentrations vary widely between pot experiments and field trials, only results from field experiments are presented in Table 1.

Table 1. Results from field experiments regarding the heavy metal concentrations (mg kg^{-1}) in industrial hemp aboveground and underground parts.

Metal	**Concentration (ppm)**		**Reference**
	Underground tissues	**Aboveground tissues**	
Cd	0.35 – 1.03	0.33 – 0.98/ 0.19 – 0.55/ 0.41 – 1.22/0.150 – 0.40/0.34 – 1.00 (stems/leaves/flowers/fiber/seeds)	Angelova et al., 2004
Cd	-	0.8 – 3.5 (hurds/fibres/leaves/seeds)	Linger et al., 2002
Cd	-	2.96 – 151/1.03 – 4.0 (leaves/seeds)	Ahmad et. al., 2016
Cd	1.69 – 2.59	0.14 – 0.30 (leaves/stem)	Di Candilo et al., 2004
Cd	-	1.3 – 4 (shoots)	Mihoc et al., 2012
Cd	-	0.113 & 0.254 & 0.547 (biomass)	Tlustoš et al., 2006
Cd	-	0.12 (aerial biomass)	Kotoula et al., 2023
Cd	-	0.018 – 0.204/0.014 – 0.145/0.019 – 0.164 (leaves/stems/aerial parts)	Luyckx et al., 2022
Cd	0.94	1.80/1.0/0.85 (leaves/stems/seeds)	Canu et al., 2022
Zn	15.5 – 66.8	12.7 – 54.5/ 9.3 – 40.0/ 18.3 – 78.6/1.3 – 18.9/17.8 – 73.5 (stem/leaves/flowers/fiber/seeds)	Angelova et al., 2004
Zn	-	42 – 94 (seeds)	Mihoc et al., 2012
Zn	-	20 & 23.5 & 24.0 (biomass)	Tlustoš et al., 2006
Zn	-	137.96	Kotoula et al., 2023
Zn	-	25.27 – 65.67/8.25 – 16.333/18.508 – 31.196 (leaves/stems/aerial parts)	Luyckx et al., 2022
Zn	43.66	278.75/45.53/143.71 (leaves/stems/seeds)	Canu et al., 2022
Ni	-	6.9 – 63.6 (hurds/fibres/leaves/seeds)	Linger et al., 2002
Ni	35.8 – 321.8	7.1 – 123/1.6 – 6.1 (leaves/seeds)	Ahmad et. al., 2016
Ni	-	51.85 (aerial biomass)	Kotoula et al., 2023
Pb	3.8 – 38.2	2.4 – 23.5/ 1.9 – 16.5/ 4.5 – 44.8/2.1 – 6.3/1.0 – 7.6 (stems/leaves/flowers/fiber/seeds)	Angelova et al., 2004
Pb	-	1.8 – 22.4 (hurds/fibres/leaves/seeds)	Linger et al., 2002
Pb	1.30 – 1.88	0.21 – 1.12 (leaves/stem)	Di Candilo et al., 2004
Pb	-	2.55 – 8.70	Tlustoš et al., 2006
Pb	-	21.19	Kotoula et al., 2023
Pb	-	1.099 – 3.721/0.819 – 1.379/0.974 – 1.893 (leaves/stems/aerial parts)	Luyckx et al., 2022
Pb	5.87	9.05/3.65/2.09 (leaves/stems/seeds)	Canu et al., 2022

Metal accumulation in hemp stems might limit fibers use as a raw material in clothing and the food chain. In most of their uses, products derived from hemp biomass do not generally expose consumers to contaminants. Eventually, several processes can remove excess Cd, Pb, and Ni from leaves and stems, e.g. thermochemical pretreatment prior to enzymatic hydrolysis for conversing hemp biomass into succinic acid, a precursor for biodegradable polymers, food, fine chemicals, green solvents, and pharmaceuticals. Hemp harvested from remediation sites can be safely distilled into ethanol for use as a biofuel. Coupling phytomanagement and bioenergy production from hemp crop is an increasingly well-documented solution to overcoming the economic constraints of soil remediation projects. Hemp biomass grown on metal(loid) contaminated soils can be processed by pyrolysis and gasification to produce biofuels (Das et al., 2017; Sieracka et al., 2023). However, the high moisture content of the shoots at harvest (54%) may necessitate drying prior to their processing, depending on factors such as facility availability, storage, and operational profitability throughout the year. Finally, a comparative cost analysis indicated that hemp is a profitable commodity crop for producing both biofuels and value-added products (Placido and Lee, 2022).

5.2. Sorghum (*Sorghum bicolor* L. Moench).

5.2.1. Crop description, requirements, agronomic management and uses

Sorghum (*Sorghum bicolor* L. Moench) is a C4 annual herbaceous spring crop belonging to the Poaceae family (Figure 11). It exhibits a short growth cycle (120 to 150 days), high photosynthetic rates, tolerance to water deficit, adaptation to a wide range of soil conditions, including salinity and excessive exposure to metal(loid)s. It is also characterized by low production cost, high yields and its ability to grow under suboptimal conditions where other crops would struggle (Steduto et al., 1997; Zegada-Lizarazu and Monti, 2012; Martinez-Uribe et al., 2020).

Figure 11. Sorghum (Sorghum bicolor L. Moench), Poaceae family

This crop grows at altitudes ranging from sea level up to 1000 m, and at latitudes between 40°N and 40° S. It is primarily a plant for worm climates and it performs best on semi-arid tropical environments with an annual rainfall of 400-600 mm. Its resilience to drought can be attributed to its extensive root system, which extends down to 1.5 – 2.5 m into the soil, depending on soil texture and structure. This deep root system enables access to water and nutrients in deeper soil horizons (Anami et al., 2015). Additionally, the extraradical mycelium developed around the roots may enhance the uptake of nutrients and water (Lounès-Hadj Sahraoui et al., 2022; Ofori-Agyemang et al. 2024).

Sorghum is a fully mechanized crop established by seeds, with well-known management practices. The shoot yield of biomass sorghum depends on temperature, precipitation, and crop rotation (Assefa et al., 2010; Bekele et al., 2014; Paesal and Suarni, 2021). Due to its small seed size, it requires adequate preparation of the seedbed. Plant density depends on the variety, environmental conditions, and desired earliness, ranging from 110,000 to 400,000 plants/ha. Optimal sowing distances are typically 45 to 70 cm between rows and 10-20 cm within rows. In fields where soil fertility ranges from low to moderate, fertilisation needs are approximately 100-150 kg N, 60-100 kg P2O5 and 60-100 kg K2O per hectare. Nitrogen application is best split into two times: before sowing and 20-30 days after emergence. Yields of sorghum (both fiber and sweet varieties) can reach up to 140 t/ha (on a fresh basis) and 20 to 30 t/ha (dry weight).

Sorghum is the fifth most important cereal in the world and an important staple food in the semi-arid tropical areas of Africa and Asia. Being a multipurpose crop and it can be cultivated, apart from grain, for: sugar juice from its stalk for making syrup or ethanol, bagasse and green foliage which can be used as an excellent fodder for animals, for gasification, for second generation bioethanol production, as organic fertiliser, for paper manufacturing or for co-generation (Regassa and Wortmann, 2014; Aruna and Visarada, 2019). For developing countries sorghum provides opportunities for the simultaneous production of food and bioenergy, thereby contributing to improved food security as well as increased access to affordable and renewable energy sources. For Europe sorghum is seen as a promising crop for the production of raw material for 2nd generation bioethanol.

5.2.2. Phytoremediation potential of sorghum

Sorghum is gaining attention as a lignocellulosic crop for phytoremediation due to its low agricultural input requirements, and tolerance to low-medium soil metal(loid) concentrations and harsh environmental conditions, such as drought (Al Chami et al., 2015; Gorelova et al., 2023; Ofori-Agyemang et al., 2024). Furthermore, it produces high biomass on contaminated soils that can be processed for the bioenergy sector (Moreira et al., 2021), including bioethanol, renewable gasoline, diesel, sustainable aircraft fuel, etc., while also phytoextracting some amounts of metal(loids) (Yuan et al., 2019; Perlein et al., 2021; Ofori-Agyemang et al., 2024). This offers economic value for landowners, making phytoextraction an attractive management strategy for metal-contaminated soils (Suman et al., 2018). The theoretical yield of bioethanol production for six sorghum cultivars ranged from 5510 to 7510 L ha-1 (Xiao et al., 2021).

High shoot DW yields (t DW ha^{-1}) were evidenced on contaminated soils: 22.1 (Marchiol et al., 2007) and 20 (Papazoglou et al. 2022a,b) in Italy, 25.8 (Zhuang et al., 2009) and 12.4 – 32.7 (Wang et al., 2023) in China, 11.5 - 15.9 (Ofori-Agyemang et al. 2024) and 9.85 – 21.75 (Perlein et al. 2021) in France, 12.5 – 26.6 in Greece, and 20 in Poland (Papazoglou et al. 2022a, b).

Integrating leguminous cover crops (such as alfalfa, white clover, common vetch, or fava bean) into a crop rotation system with sorghum, being an annual crop, could enhance nitrogen and carbon sequestration while improving soil moisture retention and fertility (Garcia-Gonzalez et al., 2018; Moreira et al., 2021). This approach not only aids in managing soil contamination but also has the potential to enhance soil functionality and ecosystem health, thereby promoting ecosystem services.

Sorghum can accumulate elevated concentrations of heavy metals and metalloids in the shoots, as shown in Table 2. Due to significant variations in shoot metal concentrations between pot experiments and field trials, only results from field experiments are presented in Table 2.

Table 2. Metal(loid) concentrations (mg kg^{-1}) in sorghum aboveground and underground parts in field experiments.

Metal	Concentration (mg kg^{-1})		Reference
	Underground tissues	Aboveground tissues	
Cd	-	0 – 13.6	Yuan et al., 2019
Cd	-	111 / 128 / 50.8 (unwashed shoots /stem / leaves)	Murillo et al., 1999
Cd	4.15	1.16 / 2.31 (stem / leaves)	Zhuang et al., 2009
Cd	1.1 – 1.3	0.14 – 0.19 / 0.19 – 0.35/ 0.04 – 0.05 (stem / leaves /grains)	Angelova et al., 2011
Cd	-	2.0 – 3.8 (shoot)	Phieler et al., 2015
Cd	8.06	4.05/ 0.44/ 0.27 (stem / leaves / panicles)	Xiao et al., 2023
Cd	1.35 – 1.75	0.20 – 0.26 (shoots)	Marchiol et al., 2007
Cd	4.04 – 8.08	3.24 – 12.14 (shoots)	Nejatzadeh-Barandozi & Gholami-Borujeni, 2014
Cd	21.8	7.4	Mayerová et al., 2017
Cd		0.38/0.85 (grain in unamended/amended soil)	Jamali et al., 2008

Cd	-	0.22 – 1.94	Ahmad et al., 2024
Cd		1.25 – 3.25 (shoots)	Perlein et al., 2021
Cd		1.86 – 2.43 (shoots)	Wang et al., 2023
Cd		6.3 – 10.6 (shoots)	Ofori-Agyemang et al., 2024
Cu		1.3 – 1.9 in stem, 3.1 – 4.6 in leaves	Angelova et al., 2011
Zn	-	32.5 – 114.6	Yuan et al., 2019
Zn	-	80.9 /46.3 / 32.7 (unwashed shoots/stem/leaves)	Murillo et al.,1999
Zn	-	67.1 – 80.9 (leaves)	Zhuang et al., 2009
Zn	-	0.42 – 0.43	Galavi et al., 2010
Zn	-	47.0/68.0 (grain in unamended/amended soil)	Jamali et al., 2008
Zn	48.9 – 93.00	11.1 – 15.70/ 31.7 – 45.10/ 25.5 – 25.80 (stems/leaves/grains)	Angelova et al., 2011
Zn	-	13.3 – 30.10 (shoots)	Phieler et al., 2015
Zn	265 – 466	55.5 – 115 (shoots)	Marchiol et al., 2007
Zn	474.9	127.7	Mayerová et al., 2017
Zn		140 - 200	Perlein et al. 2021
Zn	-	18.97 – 28.26	Ahmad et al., 2024
Zn		88.45 – 124.3	Ofori-Agyemang et al. 2024
Ni	-	1.5/2.45 (grains in unamended/amended soil)	Jamali et al., 2008
Ni	-	12.3 – 29.00 (shoot)	Phieler et al., 2015
Ni	-	0.1 – 1.21	Ahmad et al., 2024
Pb	-	2.1 – 31.8	Yuan et al., 2019
Pb	49,6 – 96,2	37.3 – 48.2/4 (leaves/stem)	Zhuang et al., 2009
Pb	-	0.96 – 1.00	Galavi et al., 2010
Pb	-	2.00/3.78 (grain in unamended/amended soil)	Jamali et al., 2008
Pb	6.5 – 8.2	0.01/0.90/0.07 – 0.14 (stem/leaves/grains)	Angelova et al., 2011
Pb	22.7 – 60.10	2.73 – 6.08 (shoots)	Marchiol et al., 2007
Pb	8.99 – 15.18	7.45 – 24.01 (shoots)	Nejatzadeh-Barandozi & Gholami-Borujeni, 2014
Pb	25.5	6.7	Mayerová et al., 2017
Pb		0.5 – 0.8 (shoots)	Perlein et al., 2021
Pb	-	0.05 – 0.12	Ahmad et al., 2024
Pb		6.33 – 12.58 (shoots)	Ofori-Agyemang et al. 2024
Co	-	0.8 – 3.2 (shoot)	Phieler et al., 2015
Co	5.39 – 9.42	0.47 – 1.77 (shoots)	Marchiol et al., 2007
As	-	4161/617/1915 (unwashed shoots/stem/leaves)	Murillo et al.,1999
As	-	0.2/0.14 (grain in unamended/amended soil)	Jamali et al., 2008
As	32.3 – 67.5	5.23 – 13.1 (shoots)	Marchiol et al., 2007
Sb	-	241/72/69 (unwashed shoots/stem/leaves)	Murillo et al.,1999

Perlein et al. 2021, demonstrated that the bioconcentration factors (BCFtot: shoot metal concentration vs. total soil metal) for Cd, Pb and Zn were below 1, which agreed that sorghum exhibited an excluder behavior. In Ofori-Agyemang et al. (2024) the highest BCFtot value occurred for Cd (from 0.70 to 0.94 on average, and up to 0.96), showing sorghum displayed higher shoot Cd concentrations than other lignocellulosic crops such as miscanthus and hemp.

Sorghum biomass grown on metal(loid) contaminated soils can be processed by pyrolysis, liquefaction, fermentation and gasification to produce biofuels (Vintila et al., 2016; Stamenković et al., 2020; Liu et al., 2020; Xiao et al., 2021; Perlein et al., 2021). The high moisture content of the shoots at harvest (e.g. 67%) may necessitate drying prior to their processing, depending on factors such as facility availability, storage, and operational profitability throughout the year.

6. Previous and ongoing phytoremediation projects on the selected energy crops

▶ **The FORTE PROJECT entitled "Supply and application of fiber crops for sustainable soil remediation and bio-based raw material production for industrial uses", 2019-2023, www.forte-project.gr**

FORTE is a 3-year project granted under the frame of bilateral and multilateral joint R&T collaboration between Greece and China. The aim of the project is the cultivation of industrial hemp, flax and kenaf on contaminated mining and agricultural lands with dual scope: the soil remediation and the industrial uses of the produced biomass. The project is implemented in a mining site of Greece (Lavreotiki peninsula, Attika) and in a contaminated agricultural land of China (Hunan Province). Lavreotiki is a long-term multi-metal contaminated site due to ancient (3000-200 B.C.) and more recent (1864-1982 A.D.) mining and metallurgical activities. Hunan ranks first among China's provinces in rice production. However, the soil concentrations of cadmium and arsenic in many areas exceed the threshold limits due to: i) industrial and mining activities that dispose their wastes in the Xiangjiang river. The water of the river is used for irrigation purposes, ii) the intensive cultivation and the extend use of agrochemicals (fertilizers and pesticides). Therefore, gaining knowledge for the remediation of a mining site (in Greece) and an agricultural area (in China) is quite important for both countries and the FORTE results will be used for cross-fertilization purposes.

FORTE is consisted from four main work packages dealing with: A) polluted site characterization and plant biodiversity, B) best crop cultivation strategies and biomass production and characterization, C) use of produced biomass per crop as feedstock for particleboard and insulation panel production, and D) sustainability assessment (economic, environmental and social) throughout the supply chain.

The main expected outcomes were to:

- Remediate and exploit contaminated land with the cultivation of fiber crops, with low ILUC effects, releasing agricultural land from the cultivation of non-food cash crops
- Broaden the range of suitable non-food feedstock candidates for phytoremediation, with optimally-lowered resource inputs and sustainable cultivation strategies for each crop
- Enhance the industrial research in the field of bio-composites, and develop economically viable, eco-friendly and bio-based products
- Strengthen the supply and value chains of raw materials from hemp, flax and kenaf and create further opportunities for extroversion and competitiveness
- Farmers will gain insights on how they can create/improve their business models under supervision of inventors and researchers and by participating in the FORTE dissemination and knowledge sharing events.
- The potential of biosynthetic chipboard production into a marketable product is expected to lead to less use of forest-based materials, which in a larger scale would eventually decrease illegal logging. The production of other possible biomaterials will also substitute their fossil-based alternative.
- Mitigate the exposure route for the intake of contaminants by humans and contribute to the wellbeing of local population.

▶ **The projects PHYTOSUDOE & Phy2SUDOE**
The PHYTOSUDOE entitled "Demonstration of the improvement in soil biodiversity, functionality and ecosystem services through phytomanagement in contaminated and degraded soils, 2016-18", www.phytosudoe.eu/en, https://interreg-sudoe.eu/en/proyectos/phytomanagement-of-contaminated-soils-improves-soil-biodiversity-functionality-and-ecosystem-services/

The Phy2Sudoe entitled "Advancing in the application of innovative phytomanagement strategies in contaminated areas of the SUDOE space", 2020-23, https://www.phytosudoe.eu/en/ https://5.interreg-sudoe.eu/gbr/projects/the-approved-projects/239-advancing-in-the-application-of-innovative-phyto-management-strategies-in-contaminated-areas-of-the-sudoe-space

Soil biodiversity and functionality were improved at French, Portuguese, and Spanish contaminated sites, with energy crops.

The Phy2SUDOE project was extending the network of phytomanaged sites in the EU SUDOE region (PhytoSUDOE), proving the efficiency and the limits of phytotechnologies to remediate contaminated soils. The Phy2SUDOE network has integrated 8 new sites with new case studies (i.e. other soil uses, organic pollutants, and mixed contamination) with various edaphic conditions and future land use, to reach a total of 15 sites (whereas 4 other sites were closed) (https://www.phytosudoe.eu/en/the-project/sites/). These were mining areas and urban and industrial areas to broaden the range of future land uses (i.e. peri-urban green belts, parks, industrial crop production, remediated grassland, etc.). These sites depend on partners and/or associated administrations and companies, facilitating the transfer of results. New phytomanagement solutions were applied at these sites according to the PhytoSUDOE and Phy2SUDOE methodologies and gained knowledge, notably in compliance with the Phy2SUDOE WP1 for the site characterization and tools and with the WP3 as the components of the biodiversity were characterized and preserved.
The objectives were:

(1) Evaluate pollution linkages: sources, exposure routes and risks
(2) Feasibility of solutions based on phytotechnologies
(3) Remediation / Phytomanagement strategies: operation plan, evidence of effectiveness, benefits / limitations of crops, soil functions and ecosystem services

Among others, the following options were evaluated:
- phytostabilization with metal-excluding plants, including perennial grasses such as miscanthus, vetiver and Agrostis sp.;
- phytoextraction with metal-accumulating plants;
- phytomining with high-value metal-accumulating plants;
- rhizo/ biodegradation through the stimulating effect of root systems on soil microbial communities; - bioaugmentation with microbial consortia with the ability to promote plant growth;
- biostimulation with organic and/or mineral amendments

Short rotation coppices and mixed tree stands, mainly based on poplars and willows, were implemented at most sites in France, Spain and Portugal, with a good development of these trees species. *Pinus sylvestris* and Eucalyptus displayed also a very good growth. Data were produced over long-term periods (from 3 to 18 years depending on sites). In addition perennial lignocellulosic crops, i.e. Miscanthus and vetiver, were cultivated, notably at the St-Médard d'Eyrans site (soil contamination by Cu and PAHs) and the Durandeau site (soil contamination by Ni, Cd, Zn, Pb, As, PAHs, PCBs and trichloroethylene) in France.
Maximum shoot length (MSL) of Miscanthus annually peaked up 203 – 223 cm at Durandeau only after 19 months. Its shoot DW yield varied between 26 - 55 t DW ha^{-1} depending on soil treatments and residual contaminant exposure. At St-Médard d'Eyrans, MSL was in the 270 - 324 cm range and shoot DW yield exponentially decreased from18.5 to 7.1 t DW ha^{-1} as the available soil Cu increased.

It was evidenced that Miscanthus (Figure 12) and vetiver are metal(loid) and organic xenobiotic - tolerant plant species, metal(loid)-excluders (no accumulation in shoots, shoot Cu concentration in the 8-12mg kg^{-1} range), relatively adapted to summer drought (in line with climate change) and able to deliver a high shoot biomass for various biomass processing chains. No visible symptoms were recorded across the 8-year-field trials. Both Miscanthus and Vetiver increased the C sequestration whereas available soil Cu was decreased

by 75% in the dolomite and compost-amended soils. Soil amendments, in particular compost made of pine bark and poultry manure paired with dolomite were beneficial with a long-lasting effect.
Giant reed (*Arundo donax*) was also implemented (at the St-Médard d'Eyrans site), notably in plots amended with biochar alone and paired with compost, but the shoot yield was limited by the dryness of the gravel coarse sandy soil during summer.

A lot of results are available at https://www.phytosudoe.eu/en/publications/

Figure 12: View of miscanthus trials in Phytosudoe projects (at Durandeau site in the left and at the St-Médard d'Eyrans site in the right)

- **The MAGIC project entitled "Marginal lands for Growing Industrial Crops: Turning a burden into an opportunity", 2017-21, www.magic-h2020.eu, HORIZON 2020**

MAGIC was a comprehensive project aimed at supporting farmers in deciding which industrial crops could be cultivated in marginal or contaminated areas. It began with mapping marginal lands, selecting suitable crops, investigating the breeding conditions of these crops, and developing cultivation and harvesting methods and machinery tailored to these specific requirements. Additionally, information on conversion technologies for various biomass crops was collected and integrated into the B2Match tool. Furthermore, sustainability assessments were conducted to provide policymakers with recommendations for growing industrial crops on marginal/contaminated lands, as specific political framework conditions are necessary for this new form of agricultural production.

One of the aims of MAGIC project was to screen the most promising industrial crops for cultivation on contaminated and saline soils. The effects of the heavy metals Cd, Pb, Ni and Zn on fifteen industrial crops were examined, namely on: biomass sorghum, camelina, cardoon, castor bean, crambe, Ethiopian mustard, giant reed, hemp, lupin, miscanthus x giganteus, pennycress, safflower, switchgrass, tall wheatgrass, and wild sugarcane (African fodder cane). The experiments were conducted following the same methodology and protocols by all partners involved. The levels of soil contaminations were: for Cd: 0, 4, 8 mg/kg, for Pb: 0, 450, 900 mg/kg, for Ni: 0, 110, 220 mg/kg and for Zn: 0, 450, 900 mg/kg. In addition, UNICT tested giant reed, miscanthus x giganteus and wild sugarcane under increased soil salinity levels. The 'crop X marginality factor' treatment was tested for two subsequent years, applying the completely randomized design in three replications. Growth and yield measurements, biomass characterization, as well as heavy metal determinations were analyzed in order to identify the most promising industrial crops to be cultivated in contaminated and saline soils. The results showed that zinc was the most toxic heavy metal, followed by nickel, cadmium and lead. None of the crops showed to be a hyperaccumulator of the tested heavy metals; however, some crops could accumulate in their aerial biomass metals in concentrations higher than the normal values, namely: (i) Cadmium concentrations were increased in sorghum > tall wheatgrass > castor ≥ safflower > giant reed > Wild sugar cane > switchgrass > pennycress > Ethiopian mustard > miscanthus x giganteus, (ii) Increased lead contents were measured in pennycress > castor > hemp > crambe > safflower > lupin > Ethiopian mustard > switchgrass ≥ sorghum > Tall wheatgrass > giant reed, (iii) increased nickel concentrations were observed in all crops, following the order: miscanthus x giganteus > castor > safflower >hemp > tall wheatgrass > pennycress > Wild sugar cane > giant reed > switchgrass > Ethiopian mustard > lupin > sorghum > crambe, (iv) zinc concentrations were within the normal limits for all crops, apart from hemp. Concerning camelina, the results differed between the four countries and could be grouped in terms of similarity into Greece & Italy and Portugal & Poland.

- **The MISCHAR project entitled "Refunctionalization of multicontaminated soils using a miscanthus biochar: ecological viability and socio-economic benefit of management methods in urban and agricultural environments", 2016-2020, https://mischar-43.webself.net/**

To study the use of the biochar from miscanthus as soil enriching agent to (1) restore urban and agricultural soils affected by industrial activities and (2) reduce mobility and bioavailability of metal(loid) and organic pollutants.

One method of managing soils contaminated by human activities is the use of amendments. Among them, biochar is cited as being able to improve soil fertility and reduce the availability of inorganic pollutants.

This work aims to assess the effects of biochar made from Miscanthus x giganteus grown on contaminated soils affected by the activities of the former smelter Metaleurop Nord (Noyelles-Godault). For this purpose, field experimentations were carried out in two parts: one on carbonated deposits multi-contaminated from coal chemistry (Mazingarbe), and in the other on agricultural soils in the megasite Metaleurop. Although the main objective of the project was to limit environmental and health hazards, expectations were different between the two sites. The aim for the Mazingarbe site was to restore a degraded area, whereas for the mega site Metaleurop, it was to enable the continuation of crop production by diversifying outlets for miscanthus. The interest of using miscanthus biochar was assessed regarding wheat and industrial hemp (cv. Futura 75). To relieve the potential nutrient deficiency of contaminated soils due to high adsorption ability, this amendment (application rate of 2 % w/w) was combined with green waste compost (2.4 % w/w).

Concerning the Mazingarbe field experimentation, the amendment (biochar combined with green waste) weakly affected the soil physicochemical parameters and the environmental availability of Cd, Cu, Pb and Zn. The concentrations of these pollutants in the aerial parts of spontaneous plant species sampled on amended soils were quite similar to those grown in corresponding unamended soils. Soil biological functions and soil fauna were influenced by the amendment.

On the Metaleurop site, biochar and compost applied alone or in combination did not have significant effects on the fertility of soils studied. Nevertheless, a negative effect of biochar was observed on soil biological activities (reduction of functional diversity of nematodes, slow-down of organic matter degradation). The effects of amendments on Cd, Pb and Zn accumulation in wheat grains and straw varied according to plots, elements and the amendment. However, wheat grains are still non-compliant for their marketing. Industrial hemp is tolerant to metallic soil contamination. The biochar – compost mix reduced Cd and Zn concentrations in grains and leaves. Biochar induced a significant increase of Pb concentrations in leaves. Soil contamination affected stem composition and increased the tensile strength resistance of fibers. Although the general context is favorable to biomass valorization, the local situation, notably the building of a biogas plant that will use uncompliant crop production, might compromise the establishment of a new sector like that of hemp.

- **The New-C-land project entitled "Developing marginal land by producing plant biomass used for energy and material", 2018-2021, www.newcland.eu/en/**

To offer advice and implement projects in order to use plant biomass produced on marginal land (contaminated are included). This project was inviting the municipality to declare marginal land able to be phytomanaged and to produce biomass for the bioeconomy. The purpose was to enhanced biodiversity and ecosystem services (including soil and water functions) and to face climate change (C sequestration) as well.

Methanization, biofuels and solid fuels such as wood chips were considered as well as ecomaterials, green corridor, market garden vegetables and horticulture (mulching), biorefineries, and textiles

- **The INTENSE project entitled "Intensify production, transform biomass to energy and novel goods and protect soils in Europe", 2016-19, funded by Era-Net Facce Surplus,** www.nibio.no/en/projects/intense

INTENSE project was assessing the efficiency of soil amendments, notably compost and alkaline materials, and inorganic biostimulants based on Si or Se composition, to improve energy crops cultivated at contaminated sites. Field experiments were the project core and were performed by each partner. Fertility and productivity of soils were enhanced by various amendments (manure, biochar and compost pellets). Yield and growth characteristics were recorded by remote and proximal sensing. Data were used in the final analysis to implement models. Soil organisms were analyzed based on the functionality of microbial groups, to unravel key processes responsible for soil fertility and resilience. Mature compost made of pine bark and poultry manure paired with dolomite was the most efficient to increase at long-term the shoot DW yield of miscanthus, vetiver, and annual crops such as winter barley. Foliar fertilization with Si- and Se-biostimulants was not promoting the shoot DW yield of plants, but foliar Se fertilization decreased the shoot Cd and Zn concentrations. Si based-biostimulant did not affect the shoot ionome, but increased the C sequestration in phytolithes. Si-based amendment incorporated into the soil increased the C sequestration. Models are developed to show the economic impact of ecosystem services from farming by soils, with examples from Spain, Germany, Poland and Norway. Guidelines for the treatment of contaminated soils were developed and published. The beneficial influence of soil amendments for reducing contaminat exposure and/or promoting crop yield and plant performance was revealed. Increasing yield with 20 % is possible.

- **The projects MISCOMAR & MISCOMAR+**
  MISCOMAR project entitled "Miscanthus biomass options for contaminated and marginal land: quality, quantity and soil interactions", 2016-19, Financed by FACCE-JPI Programme, https://www.miscomar.eu/, https://project-wheel.faccejpi.net/miscomar/
  MISCOMAR+ project entitled "Miscanthus for Contaminated and Marginal Lands PLUS", 2020-24, funded by FACCE-JPI Programme, https://www.miscomar.eu/, https://project-wheel.faccejpi.net/miscomar/

MISCOMAR/MISCOMAR+ were extending the evidence-base for Miscanthus as a leading perennial bioenergy crop for Marginal, Contaminated, and industrially damaged Land (MaCL) using interdisciplinary academic and industrial expertise, with novel Miscanthus hybrids bred for climate change resilience. MISCOMAR+ upgraded the knowledge of Miscanthus optimization on MaCL and on its subsequent conversion options. The practical results were helping commercial partners to expand their business opportunities moving from TRL 3-4 to TRL 5-8

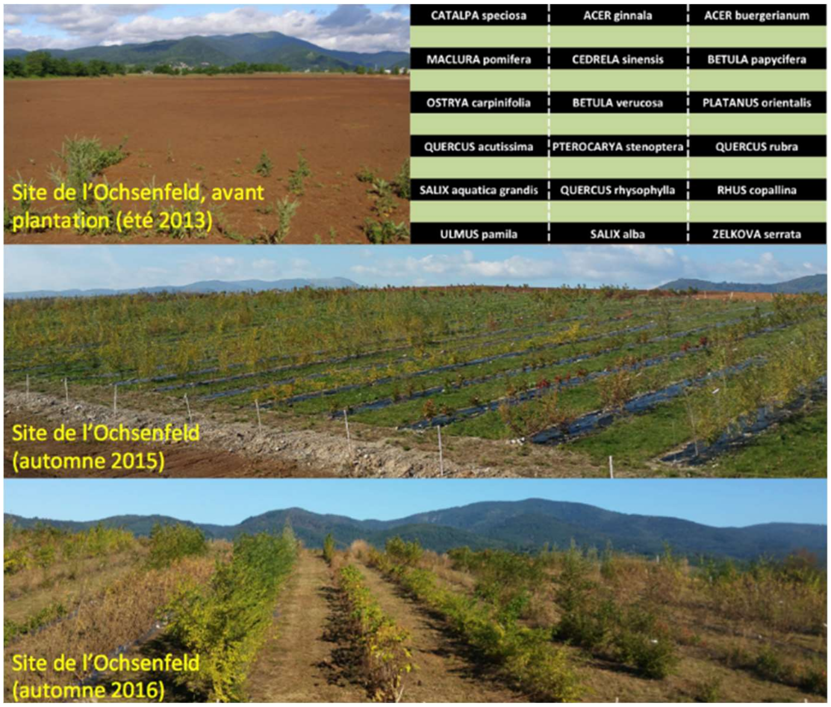
- **The PHYTOCHEM project entitled "Developing eco-innovative chemical processes to valorize phytoremediation-borne biomasses" 2013-18, funded by ANR (French National Research Agency), grant ANR-13-CDII-0005-01, https://chrono-environnement.univfcomte.fr/spip.php?page=projet&projet=30, https://anr.fr/en/funded-projects-and-impact/funded-projects/project/funded/project/b2d9d3668f92a3b9fbbf7866072501ef-1f811ab2c5/?tx_anrprojects_funded%5Bcontroller%5D=Funded&cHash=10299504d452801e76d3758fdecced5b**

The PHYTOCHEM consortium aimed at developing chemical conversion processes for phytotechnology-borne plant biomass. It was in line with both the potential use of metal(loid)-rich biomass as ecocatalysts (biosourced fine chemistry; ecocatalysis), and the production of biomasses for the biorefinery sector. The consortium was assessing a wider panel of plant species on polluted sites to identify those potentially usable by these chemical processes. The researches also aimed at improving plant productivity on sites that are particularly constraining for plant growth. Our researches in this project have led to an eco-innovative valorization of polluted sites and soils, by increasing the available biomass amounts, likely to meet the emerging needs of industrialists and managers of polluted sites, all by integrating into the energy transition law for green growth.

Development of eco-innovative chemical processes related to ecocatalysis and biorefineries to valorize biomasses from phytotechnologies. The chemical processes were assessed for some plant species from experimental sites in line to recover the accumulated metals. Prehydrolysis and "organosolv" pretreatment were tested on wood and crop biomass with high metal concentrations from experimental plots for biomass production and its use in the biorefinery sector. The consortium also tested a wider panel of plant species on polluted sites to identify those potentially usable by these chemical processes. The production of such biomasses was tested through the implementation of demonstrators.

Major results

- The set-up of two field plots, with nearly 30 woody species planted (Figure 13), has delivered relevant results (plant growth and metal(loid)-accumulation) for the selected woody species.
- The originality and effectiveness of polymetallic ecocatalysts were illustrated through some demonstrative examples, from willow biomass.
- The use of phytoremediation-borne biomass in biorefinery is possible with a better knowledge of the distribution of metals in the various co-products to facilitate their proper downstream management.



CATALPA speciosa	ACER ginnala	ACER buergerianum
MACLURA pomifera	CEDRELA sinensis	BETULA papycifera
OSTRYA carpinifolia	BETULA verucosa	PLATANUS orientalis
QUERCUS acutissima	PTEROCARYA stenoptera	QUERCUS rubra
SALIX aquatica grandis	QUERCUS rhysophylla	RHUS copallina
ULMUS pamila	SALIX alba	ZELKOVA serrata

Figure 13: Field trial to assess the various tree species

▶ The PHYTEXPPO project entitled "Phytodisponibilité des ETM pour les Plantes Potagères et extrapolations dans la quantification de l'exposition des consommateurs » 2016-18, financed by ADEME (French agency for the environment and energy management)

Trace element contaminants in kitchen garden soils can contribute to human exposure through the consumption of homegrown vegetables. In urban areas, these soils can be contaminated to various degrees by trace element (TE). They are characterized by a great variability in their physicochemical parameters due to the high anthropization level, the wide variety and combination of disturbance sources, as well as the diversity of cultivation practices and the large range of contamination levels. Pollutants can be taken up by vegetables cultivated in these soils and be concentrated in their edible parts. In this review, the behavior of vegetables cultivated in contaminated kitchen gardens is assessed through six examples of the most widely cultivated vegetables (lettuce, tomato, bean, carrot, radish, potato). The role of soil parameters that could influence the uptake of As, Cd, Cr,Ni, Pb, and Zn by these vegetables is also discussed

- **The POTAGER project funded by ADEME (French agency for the environment and energy management), 2019-2021**

As part of the POTAGERS project, the aim is to study the benefits of organic and mineral soil amendments for managing urban vegetable garden soils presenting the geogenic and/or anthropogenic contamination classically found. More specifically, the aim is to assess the potential of these amendments to sustainably reduce the phyto-availability of trace elements (TEs), as well as the exposure of gardeners and their families through the ingestion of soil particles or self-produced vegetables, and the associated risks. Among the soil amendments studied, compost received particular attention. For several years now, there has been a growing interest in composting and, more generally, in the recycling of biowaste. In the case of domestic composting, however, the quantities produced, and the quality of the compost are heterogeneous and unregulated. The project therefore involved analyzing cultivation and composting practices in urban collective gardens, and characterizing composts collected nationwide. The sociological study revealed that composting practices are highly diverse, flexible and non-standardized, that flow management is complex and that there is a lack of awareness of all the risks associated with composting. On the technical side, 150 compost samples were collected across France, for which numerous physical, chemical and biological parameters were analyzed. The results revealed considerable variability in terms of compost quality. With the aim of investigating the value of soil amendments for managing contaminated urban vegetable garden soils with arsenic, cadmium, lead and zinc, ex situ experiments were carried out on 3 soils presenting different geo-pedo-climatic contexts (a soil from a plot of the Les Eglantiers collective garden in Nantes, a soil from a private garden in Evin-Malmaison and a soil from a plot of a former Bazinghien collective garden in Lille). To determine more concretely the overall benefit of using certain amendments in the presence of a plant system, and their potential under in situ conditions, the impact of the amendments was assessed in terms of environmental, agronomic and human exposure aspects. In view of the results obtained, certain amendments appeared to be effective in reducing the (phyto-)availability of TEs, such as compost and zeolite alone or mixed, potting soil, hydrated lime or bone meal. These results, obtained under controlled conditions, were used as a tool to help select the amendments to be tested in situ on these three gardens, where 5 vegetables were grown. This section was completed by a study of the health risks associated with gardening and the consumption of self-produced vegetable gardens, carried out using the MODUL'ERS software. The study of these three gardens showed that each situation is unique and that a specific study, from pot experiments to on-site soil testing, is necessary in order to select an amendment suited to the soil and geo-climatic context of the garden. This approach was fairly conclusive with the addition of compost for Les Eglantiers, a site with mainly geogenous As and Pb contamination, but remains limited because it does not offer any real benefit in terms of reducing exposure and risks for gardeners. The approach of applying one or more amendments to a site is not operational at present, and when it is implemented, it must be done on a case-by-case basis and according to a monitoring program over several years. The presence of pollutants, particularly TEs, in the soil of vegetable gardens in general, whether community or private, is a current problem. But let's not forget the benefits of these spaces in terms of social links, educational facilities, biodiversity, savings and, quite simply, places that promote moral and physical health. These spaces and soils must be preserved because they provide numerous ecosystem services. It is important to continue developing ways of passing on the knowledge acquired to associations and amateur gardeners, as well as participatory science tools.

The COST Action FA 1103 entitled "Endophytes in Biotechnology and Agriculture", 2011-15, funded by European Cooperation in Science and Technology, http://www.endophytes.eu

Plants are associated with different microorganisms: Endophytic bacteria and fungi, which live inter- and intracellularly in plants without inducing pathogenic symptoms, interact with the host biochemically and genetically. Endophytic microorganisms may function as plant growth and defense promoters by synthesizing phytohormones, producing biosurfactants, enzymes or precursors for secondary plant metabolites, fixing atmospheric nitrogen and CO2 or control plant diseases as well as providing a source for new bioactive natural products with utility in pharmaceutical, agrochemical and other LifeScience applications. The use of these endophytic microorganisms to control plant-pathogenic bacteria and fungi is receiving increasing attention as a sustainable alternative to synthetic pesticides and antibiotics. Furthermore, these endophytic microorganisms are likely to be adapted to the presence and metabolism of complex organic molecules and therefore show useful biodegradation activities. In order to reduce the input of pesticides and fertilizers and to bring European added value to an eco-friendly agriculture, it will be important to develop inocula of biofertilizers, stress protection and biocontrol agents. The aim of the Action was to identify bottlenecks limiting the use of endophytes in biotechnology and agriculture and to provide solutions for the economically and ecologically compatible exploitation of endophytes.

The GREENLAND project entitled "Gentle remediation of trace element contaminated land", funded by FP7, 2011-14, https://cordis.europa.eu/project/id/266124

Following the SUMATEC Era-Net project (2008-2009), the EU GREENLAND project was setting the concept and rational of phytomanagement of metal(loid)-contaminated soils.

Gentle remediation options (GRO) include various (mostly plant based) approaches to remediate contaminated soils at low cost and without significant negative effects for the environment. Although GRO comprise very innovative and efficient technologies, they are still not widely used as practical site solution due to several reasons of hindrance for applying GRO as practical solution.

Greenland was pairing gentle remediation options (e.g. phytoremediation, in situ stabilization) with the production of useable biomass for the bioeconomy. It was bringing GRO into practical application to solve the final problems comprising still major reasons of hindrance.

The major objectives were:
- test the remediation efficiency and success in pilot field case studies
- develop a toolkit to quantify the remediation progress and targets (not total soil metal(loid)s, but the bioavailable metal(loid) fractions)
- assess the potential biostimulation of plant traits via inoculation of selected endophyte bacteria
- test different technologies for biomass processing (incineration, gasification, biodiesel production, etc.)
- develop a decision support tool
- publish a best practice guide

Greenland was including two groups of end users:
1) companies that offer GRO commercially (including the treatment of metal(loid)-rich biomass) - these group is part of the project consortium
2) stakeholders (including environmental agencies) that decide for GRO

Efficiency of phytomanagement was proved at 11 field trials in 6 EU countries. Poplar and willow SRC, *Amorpha fruticosa*, annual crops (sunflower, tobacco), perennial grasses (miscanthus, vetiver, Agrostis sp.) were assessed in long-term field trials.
The results were collated and published together with detailed best practice guidelines for implementing GROs. The outcomes also include tools for increasing efficiency by selecting the most effective plants, associated microorganisms, and mineral and organic soil amendments in accordance with site-specific

conditions. A decision-support tool, accessible through the website, can be used to select the most suitable GRO for a site's particular conditions.

- **The PHYTENER project entitled "Phytostabilisation on soils contaminated by metals for energy purposes", funded by ADEME, 2009-13, www.ademe.fr/phytenerdeveloppement-phytostabilisation-sols-contamines-metaux-a-finsenergetiques**

At the heart of the former mining basin of Nord - Pas de Calais, the lead foundry Metaleurop Nord (1894 - 2003) severely polluted the surrounding soils (agricultural, urban, and forested) with atmospheric emissions containing cadmium, lead, and zinc. The metallic contamination has left its mark on the landscape, fragmented and bearing witness to past industrial activities. This situation is exacerbated by a high population density and precarious socio-economic conditions. Beyond defined contamination thresholds, plant productions do not meet the food standards for humans and animals. Conventional methods for cleaning contaminated soils are inadequate, but phytotechnologies offer a solution. Two management approaches, based on assisted or unassisted phytostabilization, were evaluated within the Phytener program, focusing on the production of non-food biomass, particularly wood and herbaceous plants.

The management approach focused on the wood sector involved adding mineral amendments to the soil and planting trees to reduce the mobility and bioavailability of metals. After ten years, a significant transformation of the soils was observed, transitioning from an agro-system to a young forest soil. The amendments improved soil characteristics, with a notable increase in certain element concentrations. Analyses showed a reduction in the mobility and availability of cadmium, lead, and zinc, as well as low plant contamination compared to those growing on uncontaminated soils. The amendments also had an impact on soil microbial populations and earthworm biomass, although some organisms such as mites and ground beetles were not affected.

The Miscanthus sector involves the cultivation of Miscanthus x giganteus on contaminated plots. Studies revealed physico-chemical differences between the soils of Miscanthus plots, as well as increased respiratory activity in the most contaminated soils. Miscanthus cultivation favors fungi over bacteria, but no significant influence of genotype was observed. Results showed that Miscanthus cultivation reduces metal mobility in soils, although this varies depending on the years of cultivation and environmental conditions. Metal accumulation in different parts of the plant varies depending on the degree of soil contamination and their physico-chemical characteristics.

Studies on human exposure to metals showed variations in the oral bioaccessibility of cadmium, lead, and zinc depending on soil management practices. Avoidance and Microtox® tests indicated that soils contaminated with Miscanthus had no toxic effects, but precautionary measures are necessary during harvesting to avoid the suspension of metal-containing dust. Combustion of Miscanthus showed that metals primarily concentrate in ashes and soot, with low presence in combustion gases.

In conclusion, the Phytener program demonstrated the effectiveness of both phytomanagement approaches for soil decontamination, while highlighting the economic and technical challenges associated with Miscanthus cultivation.

- **The PHYTAC project entitled "Development of systems to improve phytoremediation of metal contaminated soils through improved phytoaccumulation" funded by 5th framework program, 2002-2005, https://cordis.europa.eu/project/id/QLK3-CT-2001-00429**

The widespread moderate levels of metal pollution, clearly above European standards, have led to limitations in land use, and soil remediation is needed. Phytoremediation, the use of plants for cleaning metal-polluted soils, is considered as environmentally sound and equally protective of human health and the environment, and should be considered a good alternative to very expensive civil-engineering based techniques. However, natural plants have serious limitations. The state of knowledge on the mechanisms of metal tolerance, uptake and accumulation at the time of the project was limited and did not allow construction of designer plants for remediation. Metal-responsive genes were characterized (functional genomic) from metal-hyperaccumulating Noccaea and expressed in yeast, Arabidopsis and higher biomass producing tobacco. Endophytes and rhizosphere microorganisms were isolated, modified and tested, in combination with the plant, for improved metal accumulation. GMO and non-GMO approaches were compared in laboratory and greenhouse studies. Risk assessment of the approach will form a central core of the studies. The goal is to develop systems for improved phytoaccumulation of metals.

Also, a big field experiment was initiated for testing poplar, willow, rapeseed, maize, hemp and tobacco as candidates for sustainable use of polluted soils (phytomanagement) in function of both biomass production and remediation efficiency.

- **The ENDEGRADE project entitled "Endophytic degrader bacteria for improving phytoremediation of organic xenobiotics", funded by 5th Framework program, 2001-2003, https://cordis.europa.eu/project/id/QLK3-CT-2000-00164**

In this project, the first proof of concept was provided for a crucial role of engineered endophytic bacteria in improving phytoremediation of volatile organic contaminants. The general idea behind this use of engineered endophytes is to complement the metabolic properties of their host plant. Proof of this concept was provided by inoculating yellow lupine plants and poplar cuttings with endophytic bacteria able to degrade toluene, which resulted in decreased toluene phytotoxicity and significantly lowered toluene evapotranspiration.

7. Conclusions and further steps

From the surveys accomplished in this D1.6, it has been highlighted how phytoremediation has attract the interest worldwide in the last decades. This set of phytotechnologies can contribute to the exploitation and remediation of polluted sites, releasing at the same time valuable agricultural land for food and feed production, and supporting the targets of the Renewable Energy Directive for 2023 (consumption of at least 27% of renewable energy).

The implementation of WP1 so far showed that the four energy crops of GOLD were successfully selected since they were well established in all the field trials, despite the type and level of contamination and the pedo-climatic conditions of each site. In addition, the biostimulant used in most cases improved the phytoremediation capacity of the crops. Details on these results are given in Deliverable 1.4.

The further steps are to:

- present the data and information gathered from the literature and other projects for the perennials miscanthus and switchgrass
- finalize the activities of Task 1.3 (field trials) and to gather the final results for the optimised phytoremediation solutions concerning the selected energy crops
- outline lessons learnt for optimized phytoremediation solutions in the form of factsheets per case study
- evaluate and present the conclusions on how the contamination site X energy crops X management practices affects: (i) the growth, yield and quality of biomass and yields of the cultivated crops, and (ii) the land decontamination either via pollutants uptake for the inorganics or via degradation for the organics.

For preparing this report, the following deliverable/s have been taken into consideration the GOLD deliverables **D1.1, D1.2, D1.4**.

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