Phytoremediation monograph

Final Report

Date of report: 24 April 2024
Report number: 2022/11 PHYTO EN

Introduction to IMPEL

The European Union Network for the Implementation and Enforcement of Environmental Law (IMPEL) is an international non-profit association of the environmental authorities of the EU Member States, acceding and candidate countries of the European Union and EEA countries. The association is registered in Belgium and its legal seat is in Brussels, Belgium.

IMPEL was set up in 1992 as an informal Network of European regulators and authorities concerned with the implementation and enforcement of environmental law. The Network's objective is to create the necessary impetus in the European Community to make progress on ensuring a more effective application of environmental legislation. The core of the IMPEL activities concerns awareness raising, capacity building and exchange of information and experiences on implementation, enforcement and international enforcement collaboration as well as promoting and supporting the practicability and enforceability of European environmental legislation.

During the previous years IMPEL has developed into a considerable, widely known organisation, being mentioned in a number of EU legislative and policy documents, e.g. the 7th Environment Action Programme and the Recommendation on Minimum Criteria for Environmental Inspections.

The expertise and experience of the participants within IMPEL make the network uniquely qualified to work on both technical and regulatory aspects of EU environmental legislation.

Information on the IMPEL Network is also available through its website at:www.impel.eu

Suggested citation:
Falconi M. et al. (2025), Phytoremediation Monograph. IMPEL, COMMON FORUM, EIONET, NICOLE report no 2022/11 PHYTO, 199 pages. Brussels, ISBN 978-2-931225-36-3

ISBN 978-2-931225-36-3
9 782931 225363

Title of the report: Phytoremediation monograph	**Number report:** 2022/11 PHYTO EN
Report adopted at IMPEL General Assembly Meeting: Budapest, 27-29 November 2024	**Total number of pages:** 199 **Report:** 82 pages **Annexes:** 117 pages

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Executive Summary

Keywords

Phytoremediation, Phytostabilization, Phytoextraction, Phytodegradation, Phytovolatilization, Phytomining, Remediation, Environmental benefits, Health benefits, Well-being benefits, Green environment, Rhizospheric mechanisms, Site management, Long-term evolution, Feasibility study, Plant selection, Amendment optimization, Operational aspects, Pollutant transport, Field test design, Regulatory aspects, Performance monitoring, Vegetal cover monitoring, Ecosystem health, Phytocapping

Target groups

Competent authorities for remediation technology approval/application/monitoring, industrial operators, environmental protection agencies, nature protection bodies, environmental inspectorates, environmental monitoring, and research institutions, technical universities, environmental associations, NGOs, insurance companies and associations, environmental consultants.

As part of its 2020 Work Programme, the IMPEL Network set up this project Water and Land Remediation (2020/09), concerning the criteria for evaluating the applicability of remediation technologies.

The Water and Land Remediation project takes guidance on definitions and key steps of remediation technology application as a springboard and focuses on the technical procedures connected with the remediation technologies. The ultimate goal of the project is to produce a document proving criteria for the assessment of the proposal of remediation technology application, to understand the applicability, what to do in the field tests, and in the full-scale application. Annex 1 covers a number of case studies, that may help the reader to anticipate any problems they may encounter and see if the provided solution applies to their site, knowing that every contaminated site differs from others and it is ever needed a site-specific approach.

The Water and Land Remediation project for 2022-2024 has the objective was to concentrate on two remediation technologies, for 2023 the technologies are Phytoremediation and In Situ Thermal Desorption.

Finally, Water and Land Remediation project intends to contribute to promoting the application of in situ and on-site remediation technologies for soil and groundwater, and less application of Dig & Dump and Pump & Treat that are techniques widely used in Europe but not sustainable in the middle-long term. Soil and water are natural resources and, when it is technically feasible, should be recovered not wasted.

Acknowledgements

This report has been peer reviewed by a wider IMPEL project team and by the IMPEL Water and Land Expert Group, Common Forum network, NICOLE network, EIONET WG Contamination and a group of external reviewers.

Disclaimer

This publication has been prepared within the IMPEL Water & Land Remediation project with the support of partner networks interested in Contaminated Land Management. Written and reviewed by a team of authors the document on hand intends to serve as primary information source to bridge and broaden knowledge among European countries and regions. In aiming support for a joint understanding the potentials of the specific remediation technology it seeks to facilitate.

The content reported here are based on relevant bibliography, the authors' experience, and case studies collected. The document may not be extensive in all situations in which this technology has been or will be applied. Case studies (see annex) are acknowledged voluntary contributions. The team of authors had no task like evaluating or verifying case study reports.

As well some countries, regions, or local authorities may have launched particular legislation, rules, or guidelines to frame technology application and its applicability.

This document is NOT intended as a guideline or BAT Reference Document for this technology. The pedological, geological and hydrogeological settings of contaminated sites across Europe show a broad variability. Therefore, tailor-made site-specific design and implementation is key for success in remediating contaminated sites. So, the any recommendation reported could be applied, partially applied, or not applied. In any case, the authors, the contributors, the networks involved, cannot be deemed responsible.

The opinions expressed in this document are not necessarily those of the individual members of the undersigned networks. IMPEL and its partner networks strongly recommend that individuals/organisations interested in applying the technology in practice retain the services of experienced environmental professionals.

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Glossary

TERM	DEFINITION	SOURCE	PARAGR.
'compliance point'	location (for example, soil or groundwater) where the assessment criteria shall be measured and shall not be exceeded	ISO EN 11074	3.4.5
'compliance or performance control'	investigation or program of on-going inspection, testing or monitoring to confirm that a remediation strategy has been properly implemented (for example, all contaminated have been removed) and/or when a containment approach has been adopted, that this continues to perform to the specified level	ISO EN 11074	6.1.5
'contaminant'[1]	substance(s) or agent(s) present in the soil as a result of human activity	ISO EN 11074	3.4.6
'contaminated site'[2]	site where contamination is present	ISO EN 11074	2.3.5
'contamination'	substance(s) or agent(s) present in the soil as a result of human activity	ISO EN 11074	2.3.6
'effectiveness'[3]	<remediation method> measure of the ability of a remediation method to achieve a required performance	ISO EN 11074	6.1.6
'emission'	the direct or indirect release of substances, vibrations, heat or noise from individual or diffuse sources in the installation into air, water or land;	IED	Art. 3 (4)
'environmental quality standard'	the set of requirements which must be fulfilled at a given time by a given environment or particular part thereof, as set out in Union law;	IED	Art. 3 (6)
'Henry's coefficient'	partition coefficient between soil air and soil water	ISO EN 11074	3.3.12
'*in-situ* treatment method' [4]	treatment method applied directly to the environmental medium treated (e.g. soil, groundwater) without extraction of the contaminated matrix from the ground	ISO EN 11074	6.2.3
'leaching'	dissolution and movement if dissolved substances by water	ISO EN 11074	3.3.15

[1] There is no assumption in this definition that harms results from the presence of contamination

[2] There is no assumption in this definition that harms results from the presence of contamination.]

[3] In the case of a process-based method, effectiveness can be expressed in terms of the achieved residual contaminant concentrations.

[4] Note: ISO CD 241212 suggests as synonym: 'in-situ (remediation) technique' [Note 1 to entry: Such remediation installation is set on site and the action of treating the contaminant is aimed at being directly applied on the subsurface.] ISO CD 24212 3.1

‘pollutant’	substance(s) or agent(s) present in the soil (or groundwater) which, due to its properties, amount or concentration, causes adverse impacts on soil functions	ISO EN 11074	3.4.18
‘pollution’	the direct or indirect introduction, as a result of human activity, of substances, vibrations, heat or noise into air, water or land which may be harmful to human health or the quality of the environment, result in damage to material property, or impair or interfere with amenities and other legitimate uses of the environment	IED	Art. 3 (2)
‘remediation objective’	generic term for any objective, including those related to technical (e.g. residual contamination concentrations, engineering performance), administrative, and legal requirements	ISO EN 11074	6.1.19
‘remediation strategy’[5]	combination of remediation methods and associated works that will meet specified contamination-related objectives (e.g. residual contaminant concentrations) and other objectives (e.g. engineering-related) and overcome site-specific constraints	ISO EN 11074	6.1.20
‘remediation target value’	indication of the performance to be achieved by remediation, usually defined as contamination-related objective in term of a residual concentration	ISO EN 11074	6.1.21
‘saturated zone’	zone of the ground in which the pore space is filled completely with liquid at the time of consideration	ISO EN 11074	3.2.6
‘soil’	the top layer of the Earth’s crust situated between the bedrock and the surface. Soil is composed of mineral particles, organic matter, water, air and living organisms	IED	Art. 3 (21)
‘soil gas’	gas and vapour in the pore spaces of soils	ISO EN 11074	2.1.13
‘unsaturated zone’	zone of the ground in which the pore space is not filled completely with liquid at the time of consideration	ISO EN 11074	3.2.8

[5] The choice of methods might be constrained by a variety of site-specific factors such as topography, geology, hydrogeology, propensity to flood, and climate

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1 INTRODUCTION

IMPEL, the European Union Network for the Implementation and Enforcement of Environmental Law developed, under the Water and Land Remediation (WLR) project, a series of guidelines focusing on the most common and most used soil and groundwater remediation technologies. These guidelines summarize the latest and most updated information on these remediation technologies that could help the distinct stakeholders such as site owners, surrounding community, project managers, contractors, regulators, and other practitioners to understand all the information emanating from each remediation project. It uses information supplied from the involved contributors, obtained in peer-reviewed scientific sources and official reports.

This guideline compiles the most recent knowledge on phytoremediation.

1.1 Phytoremediation background

Phytoremediation is the general technique that applies the use of plants (herbs, shrubs, trees) to partially or substantially remediate selected pollutants in contaminated soil, sludge, sediment, groundwater, surface water, and wastewater, using a variety of plant biological processes and the physical characteristics of plants (USEPA, 1998) (USEPA, 2000a). Growing and, in some cases, harvesting plants on a contaminated site as a remediation technology is an aesthetically pleasing, solar energy driven, passive technique that can be used to clean up sites with low to moderate levels of contamination. This technique can be used along with or, in some cases, in place of mechanical remediation technologies. Phytoremediation includes a series of processes, with are used to different degrees for different media, pollutants, and physic-chemical conditions. Also, the selection of plants used for phytoremediation depends on the specific purpose (USEPA, 2000b). Phytoremediation works best where pollutant levels are low, because high concentrations may limit plant growth and diversity. Moreover, at high concentrations the remediation process could take long time periods, up to decades or even centuries. Plants also help to prevent wind, rain, and groundwater flow from carrying pollutants away from the site to surrounding areas or deeper underground (Erakhrumen et al., 2007).
Phytoremediation includes several different processes that can lead to pollutant degradation, removal (through accumulation or dissipation), or immobilization (Sharma et al., 2023; Barceló et al., 2003).

1.2 Types of phytoremediation

Degradations lead to destruction of alteration of organic pollutants and occurs through the following processes:

- Rhizodegradation: the enhancement of biodegradation in the below-ground root zone by microorganisms. It consists of the decomposition of pollutants in the soil through microbial and fungal activity. The root exudates stimulate the growth of micro-organisms with the capacity to degrade organic pollutants. Through their metabolic and physiological activities, plants release simple sugars, amino acids, aliphatic and aromatic compounds, nutrients, enzymes and oxygen, which are transported from their upper parts to the root, favouring the growth of fungi and bacteria, which through their metabolic activities cause the mineralisation of pollutants. Rhizodegradation is a much slower process than phytodegradation.
- Phytodegradation: pollutant uptake and metabolism above or below ground, within the root, stem, or leaves. Some plants can break down or transform pollutants into less toxic forms. They produce enzymes that can degrade organic pollutants, such as petroleum hydrocarbons or pesticides. The plants metabolize these substances, converting them into harmless or less harmful by-products

- Phytovolatilization: Some plants can take up pollutants and release them into the atmosphere through a process called volatilization. This technology is commonly used for volatile organic compounds (VOCs), such as gasoline or solvents.

Phytoremediation occurs also through accumulation processes, and concerns both metals and organic pollutants. Two examples of these accumulation processes are:

- Phytoextraction: pollutant uptake and accumulation for removal. Also called phytoaccumulation, it is based on the ability of some plants to accumulate pollutants in their roots, stems or foliage. It is mainly used for metals, but also with certain types of organic pollutants and radioactive elements and isotopes. It is generally implemented using metal-tolerant and -accumulating plants known as metallophytes and/or (hyper)accumulators. It is applied by using one or several plants, allowing them to grow for several weeks or months. Subsequently, the plants can be harvested and valued in various processes to recycle the metals and/or the plant biomass (e.g. composting). If the plants are burnt, the ashes should be analysed before any valuation (i.e. agriculture) to complain with regulation and Standards. The volume of ash will be less than 10% of the volume that would be generated by the soil if it was dug up for treatment. This procedure can be repeated as necessary until acceptable levels in soil/groundwater are reached.
- Rhizofiltration: pollutant adsorption on roots for containment and/or removal. This technique involves using plants to treat contaminated water or wastewater. Plants are grown hydroponically or in constructed wetlands, and their root systems act as filters. The roots absorb and accumulate pollutants, improving the water quality as it passes through the plant system.

The third way plants support risk reduction for polluted soil is through immobilization processes:

- Hydraulic Control: control of ground-water flow or infiltration rate or precipitation by plant uptake of water.
- Phytostabilization: pollutant immobilization in the soil. Certain plants can immobilize pollutants in the soil, reducing their mobility and, hence, bioavailability. This approach is useful for stabilizing sites with heavy metal contamination. The plants create a barrier that prevents the pollutants from spreading or leaching into groundwater

A specific type of phytoremediation is phytomining. Through phytomining, metals from low-grade ore bodies or polluted areas are retrieved using plants, aiming to extract valuable metals. For contaminated soils rich in heavy metals phytomining offer eco-friendly alternatives to destructive mining. Hyperaccumulator plants, with their ability to tolerate and accumulate metals, enable this technique by transporting metals from roots to above-ground parts. This method finds utility in low-grade mining and recycling metals from polluted soil in the metal industry.

For practical purposes, the following distinction in types of phytoremediation is made in this report:

- phytoextraction (chapter 2);
- phytostabilization (chapter 3);
- phytodegradation (chapter 4);
- phytovolatilization (chapter 5);
- phytomining (chapter 6).

In chapter 7, an innovative remediation train is described. In chapter 8 conclusions have been formulated.

1.3 Phytoremediation applicability

Phytoremediation has a potential of application for a wide range of pollutants, such as petroleum hydrocarbons, chlorinated solvents, heavy metals, nutrients, radionuclides, polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, and xylene (BTEX), polychlorinated biphenyls (PCB), trichloroethene (TCE) and other chlorinated solvents, pesticide waste (Bartucca M. L. et al., 2023). Phytoremediation is apparently a simple process. However, for application it requires knowledge from different disciplines, i.e. on plant physiology, ecology, pedology, chemistry, and physical sciences. Given the great number of potential candidates, the achievement of a relatively limited number of plants have been investigated. Screening studies are important in selecting the most useful plants. Sometimes, the same cultivar on different soils containing similar kind of pollutants have not the same decontamination efficiency, since many external variables affect phytoremediation efficiency (Chirakkara et al., 2016).

Generally, phytoremediation is a remediation technology, requiring a relatively long time, needing an area (where the plants can grow), and its performance strongly depends on the specific site conditions. Plant uptake of organic pollutants, for example, can also depend on the type of plant, age of the pollutant, and many other physical and chemical characteristics of the soil (Figueroa et al., 2014).

There is potential to use phytoremediation beneficially under a wide variety of site conditions. Type of sites at which phytoremediation has been applied or evaluated includes pipelines; industrial and municipal landfills; agricultural fields; wood treating sites; military bases; fuel storage tank farms; gas stations; army ammunition plants; sewage treatment plants; and mining sites. Phytoremediation is often applied at brownfield sites, mostly in case of the combination of large areas and low pollutant concentrations, with the purpose of redevelopment of the brownfield.

1.4 Health and well-being benefits from a green environment

One drawback of phytoremediation is that it takes a relatively long time to complete restoration of the site (at least several years and often decades). Since the mid 1990s, a significant change in mentality in terms of contaminated site management has taken place in most developed countries (Swartjes, 2011). Before this reference date, it was common to strive towards a complete removal of contaminants (multi-functional approach), within a short time frame. Today, from a sober perspective, it could be stated that 'contaminants that have been in soil for many decades need not necessarily be removed within a timespan of months up to a few years', if unacceptable risks for humans and the environment are excluded.

In highly densely polluted regions, where ground prices are high, application of phytoremediation is often interpreted as soils that are eliminated from beneficial use, for years to decades. From this perspective, 'beneficial use' is usually defined as built-up areas, with houses, commercial buildings and infrastructure. However, it is generally acknowledged that in particular in highly densely populated areas, green areas are just another version of 'beneficial use'. The advantages of green in urban areas are enormous. Access to healthier environments will reduce the prevalence of health conditions that affect our daily quality of life, such as cardiovascular disease, stroke, asthma, hypertension, dementia and stress (Ganzleben and Marnane, 2020). The authors claimed further that high-quality natural environments offer health benefits through physical activity, relaxation and restoration and social cohesion, and by supporting the functioning of the immune system. These pathways deliver improved mental health and cognitive function, reduced cardiovascular morbidity, reduced prevalence of diabetes, improved maternal and foetal outcomes and overall reduced mortality. A green environment also reduces the number of premature deaths. Pareira- Barbosa et al. (2021)

investigated the number of deaths that could be prevented by increasing green space in European cities, with a focus on 978 cities and 49 greater cities, in 31 European countries. The authors showed the highest mortality burdens due to the lack of green space in the European capitals, Athens, Brussels, Budapest, Copenhagen and Riga. They concluded that in average 43 thousand (95% confidence limit 32 – 64 thousand) deaths annually could be prevented, which represents 2·3% of the total natural-cause mortality, if the WHO recommendation for universal access to green space was achieved. When phytoremediation is used as a remediation technology, the expansion of green in urban areas offers a 'win-win' by mitigating environmental pollution, at the same time improving the health and well-being of urban populations.

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2 PHYTOSTABILIZATION

2.1 Description of the technique

2.1.1 General description

Phytostabilization is an in-situ remediation technology based on plant use, which aims to decrease the exposure to pollutants (metals and metalloids) by reducing pollutant transport into the air and other environmental compartments. It is particularly suitable for large surfaces of polluted soils such as waste dumps or sites where the vegetal cover is lacking or is not enough to reach the pollutant transport reduction objectives. Plant species can be aided by mineral or biological amendments which are incorporated in soil to immobilize pollutants.

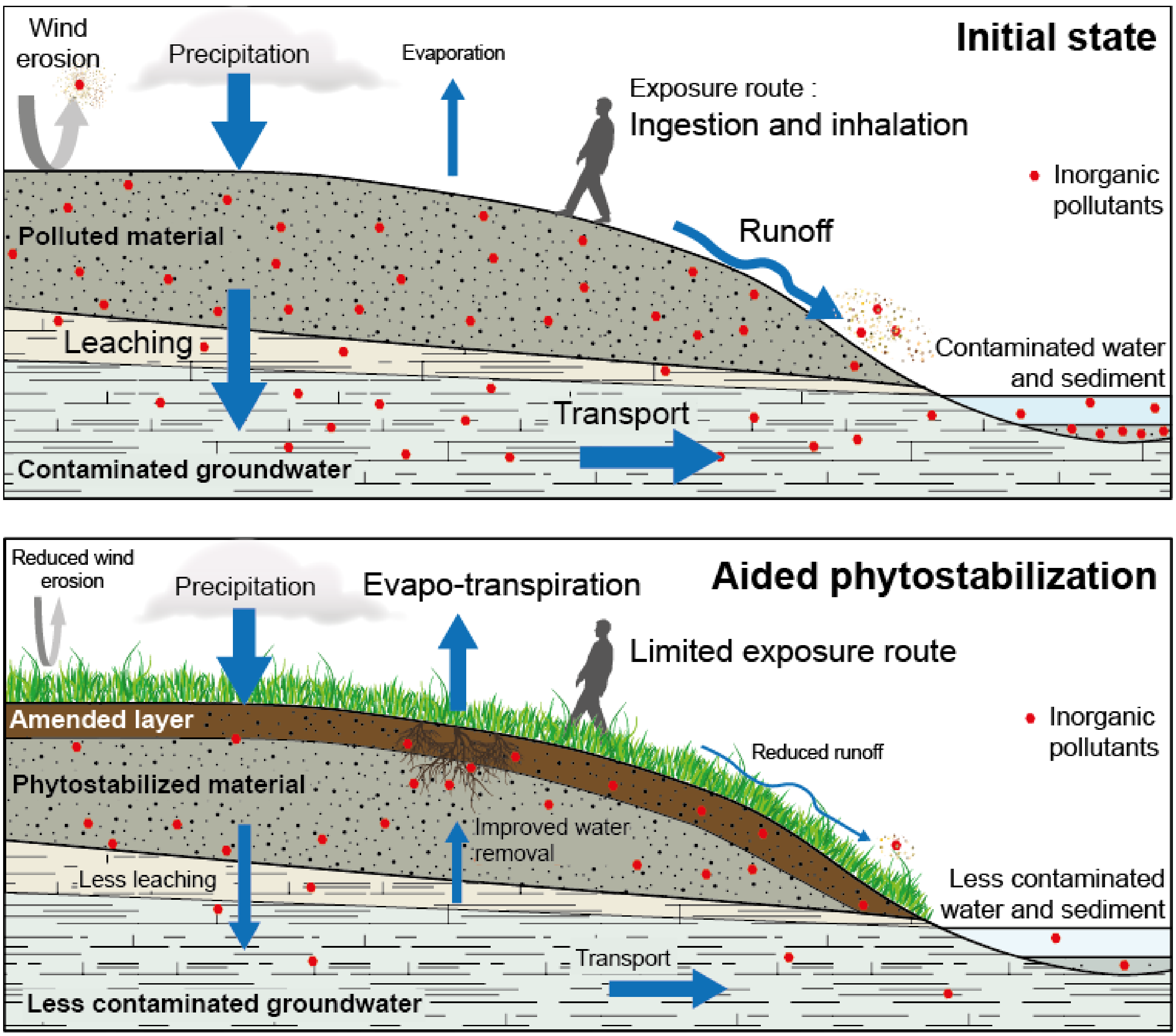


Figure 2.1- Principle of aided phytostabilization

The process consists in the assisted development of a vegetal cover on the soil /waste surface that induces the physical and chemical immobilization of the pollutants at the plant root surface and in the rhizospheric soil. The principle of the technology is shown in Figure 2.1.

The timeframe of the implementation of phytostabilization can be relatively short: the vegetal cover can develop in less than one year, depending on plant life cycle. This will be modulated, notably, according to the climate. However, while the pollutant stabilization in soil can be achieved quickly the impact on ecosystem services like pedogenesis or biodiversity can take several months or years.

Phytostabilization is a polluted soil management technology, which does not aim to decrease the total concentration of metals and metalloids in the soil or waste. When organic pollutants are also present in the soil, degradation may be achieved by microorganisms stimulated by the plant roots activity (see chapter 4 on phytodegradation).

2.1.2 Rhizospheric mechanisms

The rhizospheric mechanisms involved in the beneficial effects of phytostabilization are the following (Figure 2.2):

- physical sequestration of particles in the surface soil/waste by the root network;
- physical sequestration of particles in mineral/organic aggregates;
- chemical sequestration of pollutants through bio-mineralization processes in the rhizosphere.

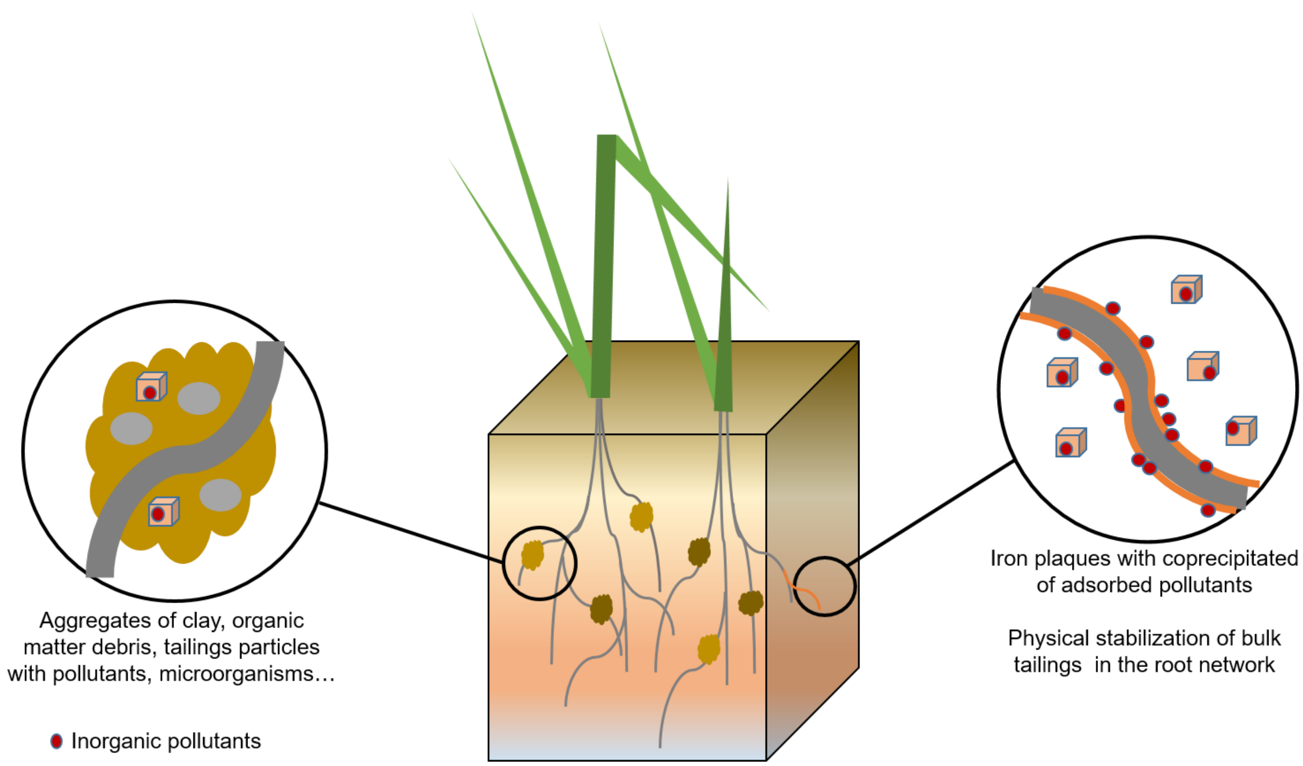


Figure 2.2- Main rhizospheric mechanisms involved in the beneficial effects of phytostabilization

The risks and exposure pathways that are decreased and/or controlled when the site is phytostabilized are the following:

- Air pollution by airborne particles containing pollutants, that could be inhaled or transported by air, thus inducing contamination of land outside the source site.
- Direct contact between humans/animals and polluted particles: ingestion of polluted particles and dermal uptake.
- Pollution of surface water and aquatic sediments by particulate material runoff associated with precipitation (e.g., rain, snowmelt), transporting polluted particles.
- Polluted water flow (infiltration towards groundwater and surface runoff), due to plant evapotranspiration and rain interception by plants.

Aided phytostabilization is a technology already applied at field scale (Technology readiness level TRL 9, real system qualified by successful operational missions), for example on mining sites. However, operational maturity is still lower than for conventional remediation technologies (e.g., containment), due to limited operational feedback.

2.1.3 Implementation

The technology involves the following steps (Mendez and Maier, 2008; Bert 2012):

- Choice of the vegetal cover.
  The plants, in general an association of several species, must be adapted to the climate and presenting resistance to the main pollutants present on the site. The association preferably includes species able to fix atmospheric nitrogen in case of nitrogen deficiency. Ideally, the choice of plants should be based on an inventory of species found on the site in order to select adapted plants and increase implementation success. The selected plants should not accumulate pollutants in their above-ground parts, in order to limit the transfer of pollutants in the trophic chain. Covering and herbaceous plants are preferred. Trees alone are not appropriate as they cannot cover the soil.
  In case of infiltration or hydraulic containment, the water extraction rate of the trees, especially,t can contribute to limit groundwater contamination.
- Optimization of the amendment
  Amendments are applied during aided phytostabilization. In most cases, the absence of development of a vegetal cover on the site is mainly linked to poor agronomical characteristics of the soil/waste. The poor characteristics include low nutrient contents, bad texture and low water retention capacity. The soil/waste pH can be extreme (acid or alkaline) for most plant species. In rare cases, a very high toxicity might also contribute to the absence of vegetal cover development for decades. In order to allow an accelerated growth of plants, the soil/waste surface layer colonized by plant roots is supplemented with amendments that will increase the nutrients availability (sources of N, P and K, bioavailable for plants), improve the soil texture and modify the soil/waste pH. Soil amendments must not increase the mobility and bioavailability of pollutants through geochemical or biogeochemical mechanisms; on the contrary, they must decrease pollutant mobility when initial site conditions evidenced pollutant leaching. Amendments may combine highly biodegradable organic materials (for example compost), nearly non-biodegradable biochars, and inorganic substances (for example limestone). The costs and the availability near the site should be considered for the choice of amendments.

- Micro-rhizosphere inoculation

The growth of plants is more rapid and abundant in the presence than in the absence of a rich rhizosphere microbiome, including bacteria and fungi named plant-promoting microbes. Among them, some bacteria are involved in atmospheric nitrogen fixation, providing some bioavailable nitrogen to nitrogen fixing plants through symbiosis in root nodules. Phytostabilization applications increasingly include a step of selection of plant-promoting microorganisms that are cultivated ex-situ and then used to inoculate the plant seeds.

- Erosion prevention in earlier phases of the technology application
  In areas presenting high erosion, such as steep slopes, anti-erosion nets can be deployed together with the seeding step, thus limiting the loss of seeds by runoff. The use of organic biodegradable nets (e.g. coconut) can contribute to the increase of the organic matter content, water retention, and the agronomic quality of the soil/waste.

2.1.4 Long-term site management and evolution

The primary objective of phytostabilization is the reduction of pollutant transport and exposure. However, other benefits may be linked to the implementation of this technology, i.e. landscape quality improvement including health benefits for humans, ecosystem health or site valorisation, i.e. for energy or industrial purposes.

The sown plant species selected to start the process must not be accumulators of pollutants in their above-ground organs. However, they are not necessarily pollutants excluders. Consequently, the above-ground biomass can contain pollutants in a range above common levels. In addition, plants from the soil seed bank may naturally grow, thanks to the amendments and to their ability to be resistant to pollutant toxicity and show excessive levels of pollutants in their above ground biomass. Thus, the management of the phytostabilized site should take the transfer of pollutants in the above-ground plant biomass into account to avoid pollutant transport in environment over time.

Several options of biomass harvest and transformation have been proposed to financially value the phytostabilized site (Perlein et al., 2021a,b,c; 2023; Chai et al., 2022; Khan et al, 2023): production of biogas or biofuels, composting, production of dyes, essential oil or fibres for industrial uses. The adopted strategy for the management of biomass will depend on the quantity and quality of harvested biomass, and on the local availability of processing units. On phytostabilized sites, the harvest of biomass will depend on the management strategy that was adopted. Currently on mining sites, the vegetal cover could be let evolve freely as an ecosystem (Corbett et al., 1996; Juge et al., 2021), but sometimes it would be necessary to clear.

2.2 Feasibility study

A conceptual model of the site (indicating links between sources of pollutions, transport routes and sensitive environmental compartments) is a relevant tool to synthetize results of site characterisation and develop the phytostabilization strategy.

The effectiveness of phytostabilization depends on the amendments and selection of plant species, but also of the physico-chemical parameters of the site material (texture, pH, organic matter content, pollutants content, …) and external factors such as climatic conditions.

2.2.1 Selection and optimization of amendment

The feasibility study should involve orientation and optimization tests for the choice of the best amendment to supply to soil or waste. This amendment should promote / allow the development of plants by providing nutrients (N, P, K) and adjusting the pH at suitable values for the plant growth. The amendment should not induce an increase of mobility and/or bioavailability of the pollutants. A preliminary characterization of the site material (pH, main pollutants, agronomic parameters) will help the choice of amendments according to information from the state of the art.

The orientation tests could include slurry batch leaching tests: the site material is mixed with different amendments applied at different concentrations, alone and in combination, and submitted to short-term (24 h) batch leaching with a solution of rainwater composition. Final measurements of the pH and the concentration of pollutants will indicate a first idea of the best combination of amendment that will minimize the pollutant leaching.

Optimization tests could include microcosm experiments performed in small pots or columns containing amended material, equipped with a drainage system to maintain non-saturated conditions. These microcosms will be regularly watered as simulation of rain. Amendment conditions tested at this step are determined based on results of the batch orientation tests. The percolation water will be collected to perform measurements of pH and dissolved elements. These tests could be performed for 1 – 3 months, in order to evaluate the geochemical / biogeochemical behaviour of the amended material. Results will indicate the influence of amendment on the biogeochemical stability of pollutants in the amended zone of the site (Thouin et al., 2019).

2.2.2 Plant selection

Many criteria must be considered when selecting suitable plants, including root system, transfer of metals to the above-ground parts of plants, resistance to pollutants, adaptation to climate and soil, seed costs, etc. Finding a plant that meets all these criteria is sometimes a real challenge (Sheoran et al., 2013). Native species are considered to have more chance of success (ITRC, 2009), provided they have appropriate properties in terms of transfers reduction (low translocation of pollutants in aerial parts, immobilization of pollutants in the root system) and coverage. For sites with historical high pollution levels (for example mining sites with tailings), native plants (with a short reproductive cycle as many herbaceous) could have adapted to the local conditions over many generations. Thus, plants from seeds collected on site often better perform than plants from commercial origin. However, costs associated with seed collection and delay (ex-situ plant cultivation and crop of seeds) should be considered. Pioneer species tend to perform better, especially in case of difficult environments (Larcheveque et al., 2014). A botanical inventory is often necessary to determine the local flora and identify candidate species.

2.2.3 Operational aspects for phytostabilization pot tests

At locations where many species of herbaceous are valuable candidates, initial screening tests in pots can be carried out over a short duration (e.g., three weeks) in small pots, in order to preselect the relevant species. Then, the experiments can be carried out in larger pots (several litres of capacity) and over a period of several months. The duration of the tests can be determined by the development of vegetation: when the development of above-ground vegetation proceeds, the limited volume available for roots due to the size of the pot may become a limiting factor.

For reasons of representativeness, the tests shall be carried out at least with triplicates for each of the combinations of plants/soil improvers/micro-organisms. In addition, control tests are usually carried out with unpolluted soil and/or a neutral substrate (sand, clay). Thus, when several combinations are tested, the total number of pots typically approaches or exceeds one hundred. The quantity of the substrate to be sampled on site is therefore often several hundred of kilos. This aspect must be taken into account for the organization of field sampling campaigns, especially at locations that are not readily accessible.

The tests are usually carried out in climatic chambers under controlled conditions or in greenhouses. Most often, these tests are carried out without limiting parameters (water, light in particular), so as to be sensitive only to the effects of polluted soil (phytoxoxicity) and of the amendments. These conditions can therefore be significantly different from on-site conditions. It is recommended to make screening tests directly on the site in real conditions in small field plots, when possible.

2.2.4 Impact on vertical pollutant transport: laboratory pilot scale

Phytostabilization can impact the infiltration rate and, hence, vertical pollutant transport. In order to complete the feasibility study, a laboratory pilot test enables a precise evaluation of the effect of combined amendments and plant growth on the vertical fluxes of water and pollutants. This is currently not widespread in the community of users; however it should be recommended, particularly when a risk for groundwater quality on the site was identified by preliminary hydrogeological characterizations. This test (Thouin et al., 2022) can simulate the optimized combination of amendments and plants in controlled laboratory conditions, and the impact of the technology on the transport of pollutants in the different compartments of the polluted site, i.e. the amended surface, the underlying unsaturated zone, and the underlying saturated zone (shallow groundwater). The laboratory pilot tank must be filled with materials sampled on the site, representative of the different compartments. However, given the size of the pilot, the materials must be sieved, keeping the fractions < 5 mm before filling the tank. After determination of the initial baseline of parameters, the surface material is amended and planted, applying the protocol previously optimized in batch microcosms and pot experiments. The pilot conditions can include application of specific temperatures, water flux mimicking rain, light intensity and depth of the water table (Figure 2.3). Porewater sampling at different depths allows measurements of physico-chemical parameters (such as pH, redox potential, dissolved oxygen, major ions, pollutant concentrations). Precise quantification of the outlet water flow will indicate the effect of plants development on infiltration. Core sampling can be performed during different steps of the process in order to analyse the evolution of solid phases (mineral, chemical, biological parameters). The duration of this test should be at least 1 year and up to 2-3 years.

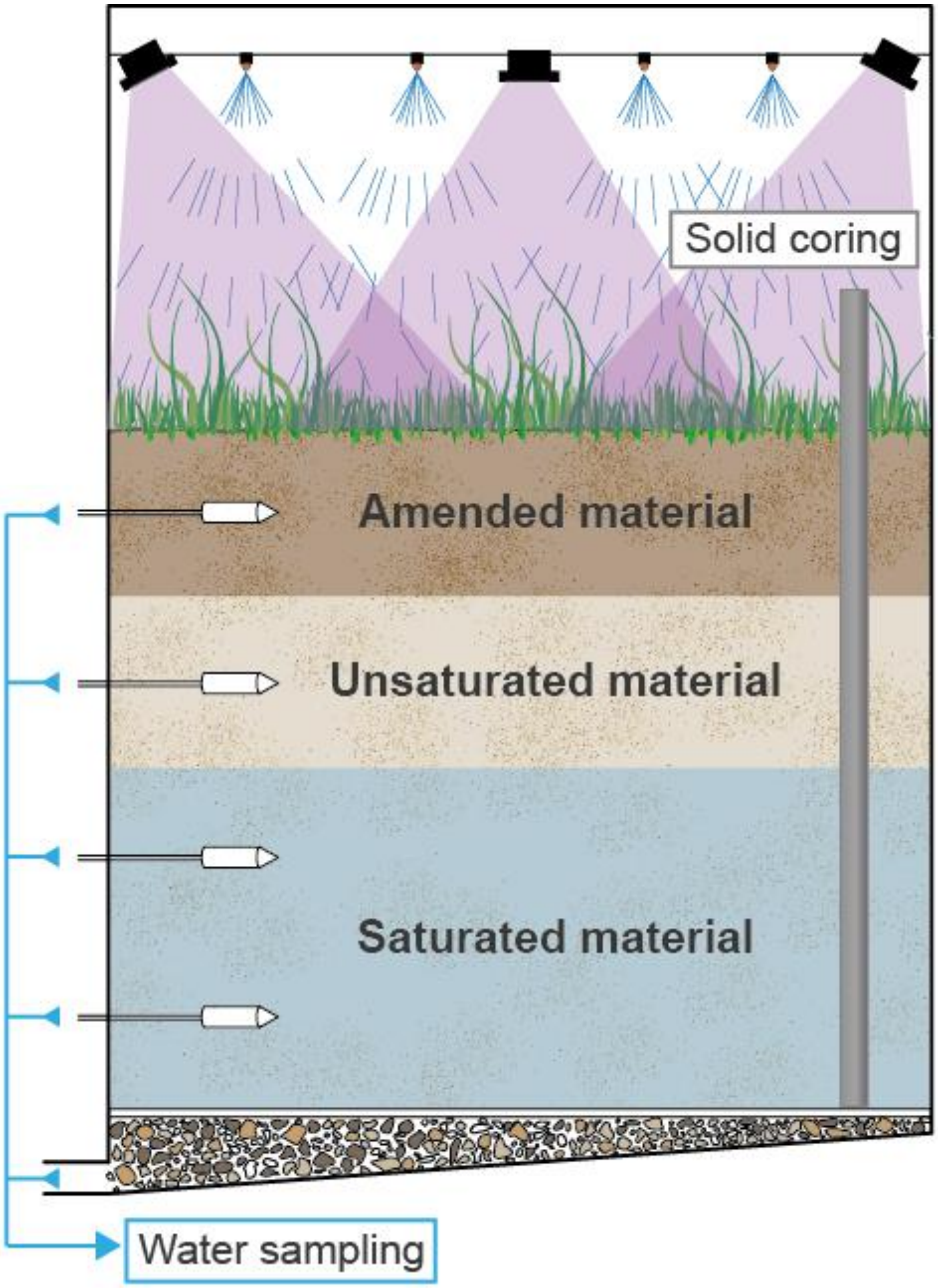


Figure 2.3- Example of experimental pilot test to evaluate the impact of phytostabilization on vertical pollutant transport (Thouin et al., 2022).

2.3 Field test

Field tests are tests performed in-situ on small plots to validate the phytostabilization technique, before full scale deployment. They are usually put in place after laboratory small scale pot tests. Laboratory pilot tests can be implemented in parallel to field tests

2.3.1 Objectives of in field tests

The objectives of field test are to:

- assess the feasibility of the phytostabilization technology with real conditions;
- verify that the objectives could be reached;
- identify the operational constraints specific to the site;
- gather data to optimize costs and design for full scale phytostabilization.

Depending on the country, field tests could be performed following specific guidelines (e.g,. in France field tests are framed by the norm NF X31-620 -3[6]) and could be a part of a remediation plan to manage a polluted site.

2.3.2 Preliminary studies before field tests

A comprehensive site assessment is crucial for the design and installation of a phytoremediation system (ITRC 2009). Even if phytostabilization is based on natural solutions, phytostabilization projects need a sound understanding of site functioning to optimize phytostabilization solutions and budget allocation (de Lary de Latour et al., 2022).

Preliminary studies for site characterization could include:

- Evaluation of the site history (e.g., land use, polluting activities, chemicals used).
- Evaluation of the environmental context (e.g., geology, hydrogeology, climate).
- Identification of the agronomical properties of the soil (e.g., texture and structure of soil, organic matter content, pH, exchange capacity, minerals).
- Identification of native vegetation: botanical inventory.
- Evaluation of the pollution: source of the pollutants, spatial distribution of pollutants.
- Evaluation of transport routes (through erosion, leaching, wind dispersion).
- Identification of environmental compartments that could be affected by pollution (water resources, protected areas).

2.3.3 Design and scale of in field tests

If laboratory tests have been performed previously, the best plants/amendments/microorganisms combinations could be selected as a basis for field tests.

The location of the test plots is an important criterion for the representativeness of the tests, especially if the site has high variability of conditions (nature of substrate, exposure to sun, slope, soil water content, etc). As much as possible, plot locations should reflect the variability of the environmental conditions. For the Abbaretz case study (see annex 1) biomass of herbaceous plant was approximatively 3 times higher in the rather wet zone than in the rather dry zone. Thus, several plots with the same plants/amendments combination are often necessary. In another field study performed on 3 areas with increasing metal pollution and roughly similar agronomic parameters, biomass yield of 10 crops and trees generally were highest in the least contaminated area but for some species the pollution level alone did not explain the yield difference (Perlein et al., 2023). Competition between planted species and colonists is one of the explaining factors (Perlein et al., 2021a).

[6] NF X31-620-3, December 2021, Soil quality - Services related to contaminated sites and soils - Part 3 : requirements in the field of restoration work engineering services

Cation exchange capacity and organic matter content of the soil are other explaining factors (Perlein et al., 2021b).
Minimal area of plot tests depends on the size of the plants that will be planted. It will be larger for woody plants than for herbaceous plants. Areas of the order of several tens to several hundreds of square meters are often used, e.g. as in Larcheveque et al., 2014. In the dimensioning, it is necessary to take the fact into account that the outline of the plot is often not representative, which implies over-dimensioning it.
The duration of the tests depends in particular on the growth rate of the plants. For herbaceous plants, a minimum period of six months (life cycle for annual plants) to one year (seed germination cycle) is necessary to be able to draw the first conclusions on the feasibility. For woody species, several years may be necessary.

2.3.4 Regulatory aspects

If methodological documents or standards exist at national level to specify the conditions of the on-site tests, it is recommended that the tests be carried out in accordance with the requirements of these documents or standards.
The implementation of pilot trials may require regulatory procedures in protected areas for the conservation of habitats or species (e.g., Natura 2000), in particular in cases where it is necessary to clear brush or create access.
Whatever the situation, all precautions must be taken to avoid the introduction and spread of invasive species.

2.3.5 Creation of in field tests

Preliminary work may include site earthwork, such as clearing land and the creation of access. It is necessary to decide to what extent the existing vegetation can be left in place. Existing vegetation can play an important role in protecting soils against erosion, while waiting for selected plant to grow. Nevertheless, the conservation of this vegetation requires tailoring and adapting to site conditions.

Depending on the situation, the amendment can be incorporated in layers, so as to reproduce a fertile horizon on top of the soil, or incorporated homogeneously over one to several tens of centimeters of soil. The incorporation of the amendment in a homogeneous way on the whole surface of the plots can be a delicate task. Mixing with a mechanical shovel or concrete mixer may be necessary in case where it is particularly important to have a homogeneous distribution of the amendment. However, associated costs could be significant for a full-scale project. Solutions must be developed for homogeneous incorporation of amendments at low costs at hectare scale. Land preparation should be done carefully, using existing farming practice and agricultural materials to avoid soil compaction and reinforce water retention and reduce costs (Boisson et al., 2011; Bert, 2012).

The choice of the planting period is a crucial element for the development of plants. This choice must be adapted to the plants put in place as well as to the climate and soil conditions. For a lowland climate with potentially hot and dry summers, sowing in early autumn can be a suitable solution: this allows the plants to develop their root system sufficiently before the arrival of the next summer.

Depending on the context, other related works could be necessary to guarantee the sustainability of the plots:

- Rainwater management: creation of ditches to evacuate rainwater and avoid water stagnation or erosion, installation of infiltration pond.
- Erosion control: soil cover (coco geotextile or any other mulching practice) can be used to stabilize topsoil and reduce water evaporation, brushwood fascines, silt fences.

- Irrigation: may be necessary in dry areas. Nevertheless, phytostabilization should be designed to minimize irrigation and be able to abandon it once the plant cover is well established.
- Installation of fences around the plots to avoid the intrusion of animals and avoid damages on selected plantation.
- Maintenance operations during tests: weeding of undesired or invasive species, regulation of pests, seedling of zones with poor growth, reparation of zones degraded by erosion.

2.3.6 What to do in case of failure of plant growth?

Management of variability is a key aspect for phytomanagement: as plants are biological organisms, their responses to site conditions and stress are inherently variable. In case of total or relative failure of the vegetation cover, analyses of the soil (in the zones with low plant growth) and/or environmental conditions can help understanding the failure and adapt the protocol to be applied. However, it is not possible to control external elements (weather). Thus, when using phytostabilization methods, it is not uncommon to have to reseed one or even several times due to unfavourable weather conditions after seeding. Using diversified grain mixtures and several species of trees often maximizes the chances of establishing a resilient vegetation cover.

2.3.7 Long term evolution

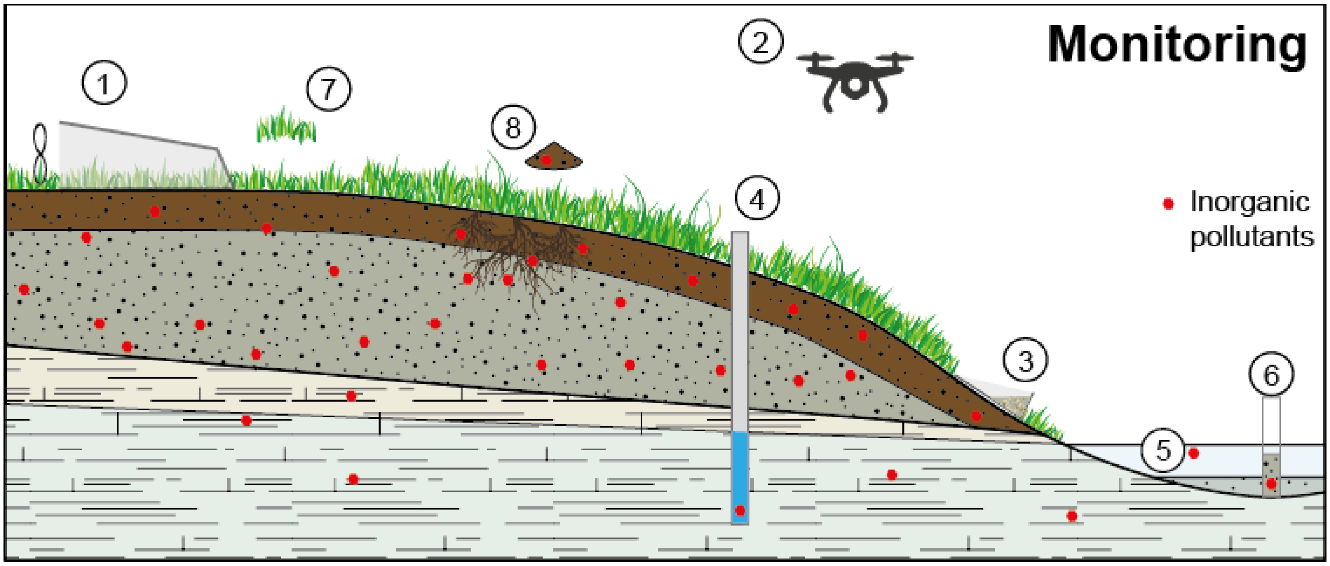
In the absence of special treatment (mowing, sowing, planting) the system will tend to evolve towards a state of equilibrium in accordance with its potential and climate. If the system is sufficiently productive, for a lowland temperate climate several types of ecological succession could be expected, resulting in a gradual closure of the environment: from herbaceous plants, to shrubs, pioneer trees, high trees and at the end afforestation.
Over time, the species initially sown may be gradually replaced by native species, because they tend to be more resilient in the long term. In this case, the species sown are still useful, because they have triggered the process of phytostabilization. Nevertheless, it should be ensured that native species have self-established properties consistent with the desired objective (stabilization of pollutants, limitation of transfers to the biosphere).

2.4 Performance monitoring

2.4.1 Performance for decreasing pollutant transport

The main objectives of the phytostabilization technology are the decrease and stabilisation of pollutant exposure and fluxes at a low/acceptable residual level and the development and sustainability of the vegetal cover.

The evaluation of the performance implies the quantification of the initial reference values of pollutant transport fluxes. According to the conceptual model of the site, these include quantification of erosion linked to runoff, airborne particles, pollutants quantification in porewater and quantification of the water infiltration from the soil surface to the groundwater (Figure 2.4). The evolution of results obtained using the corresponding monitoring devices are compared before and after phytostabilization in order to evaluate the efficiency of the technology application.



1: Wind tunnel for wind erosion monitoring
2. Drone pictures monitoring of vegetal cover intensity and erosion figures
3. Particles sink for water runoff erosion monitoring
4. Piezometer for groundwater monitoring
5. Surface water sampling for monitoring
6. Coring for sediment monitoring
7. Pant organs sampling for monitoring of inorganic pollutants transfer to vegetal biomass and stress monitoring
8. Soil sampling for inorganic pollutants bioavailability monitoring and soil bio-indicators

Figure 2.4- Site monitoring devices for phytostabilization

The airborne particles can be quantified using wind tunnels equipped with dust samplers or dust sensors (Park et al., 2019; Jiang et al., 2022).

For the quantification of particles runoff fluxes, surface runoff water and sediments sampling devices can be placed in different locations downstream the site slopes (Sun et al., 2014; Thompson et al., 2016; Tosini et al., 2020; Difpolmine project, 2006). If quantification is not possible for budget or technical reasons, visual observation of plots, and in particular of possible erosion phenomena, can enable a qualitative estimate of the magnitude of residual erosion.

The quality of surface water bodies impacted by the contaminated site can also reflect the performance of phytostabilization (like any other remediation technique). River water that is close enough to the site to be impacted, must be monitored upstream and downstream the site, analysing sediments, suspended particle concentration, total and dissolved pollutants, in different seasons, during low water and high water conditions. Sediment cores can be sampled in lakes and dam water reservoirs, when they are present downstream the site. The evolution of metals and metalloids geochemical signatures in sediments reflect the fluxes of pollutants transported by surface water.

The total and dissolved pollutants in surface water compartments must be analysed. If transport to groundwater is potentially significant, it must be monitored in piezometers. It should include the monitoring of both shallow and deep groundwater bodies. Interstitial water from the unsaturated zone can be monitored at different deepness, using porous samplers. Considering the complexity of the site geohydrology, the organization of the monitoring plan will have to consider a statistical approach, with the need to multiply the sampling points, at least for the measurement of the infiltration towards the groundwater table. Regarding runoff, the measurement at the outlets of the watershed of the stabilized zone will be preferred, because the latter changes very little and the quality of the water collected will be easily comparable to the status chosen as a reference. The number of samples per point should make it possible to establish a statistic basis consistent with that of the reference state; it must therefore be designed in relation to local climatic conditions and be based as much as possible on an annual sampling schedule.

2.4.2 Monitoring of vegetal cover

The monitoring of the vegetal cover can be performed using aerial photos taken by drones, before the start of the application, then regularly in the summer and winter seasons. This monitoring can be completed by direct observations and an inventory of vegetal diversity. Cover (as priority) and species composition (as complementary information) can be estimated using transect and quadrat sampling (Elzinga et al., 1998; Gil-Loaiza et al., 2016). Observations can be made within a defined surface (for example 1 m^2) frame placed at regular increments along a diagonal transects across defined plots. The number of plots thus studied must correspond to approximately 10% of the total area of the site. The inventory of vegetal diversity is useful for the monitoring of ecosystem health, and for the detection of the possible development of known pollutant accumulator species. Taking aerial photos, for example by drones, can greatly help to estimate large scale parameters, such as the rate of vegetation cover (Guérin et al., 2019). Quantification of biomass can complete this monitoring, biomass sampling being performed together with vegetal cover/biodiversity evaluation campaigns (Perlein et al., 2023). For trees, the survival rate as well as the vigor of each tree can be established.

The interpretation of the results must take the variability of the responses of the vegetation into account, according to the conditions of each plot as well as the weather before the observation period.

2.4.3 Long-term monitoring of ecosystem health

In addition to performance in terms of stabilizing pollutants, long term monitoring should assess to what extent the system created is in balance and therefore will be sustainable over time.

Evolution of pollutants bioavailability, indicators of soil-plant transfer and soil water transport

The bioavailability of pollutants in soils can be monitored applying selective chemical extraction procedures (see ISO 17402:2008). The bioavailability for plants (phytoavailability) can be evaluated by applying extraction from soil with ammonium citrate (Chojnacka et al., 2005) or other extractants such as NH_4NO_3 (see ISO 19730/2008). Nevertheless, the best way to assess the phytoavailability is to directLy measure the metal concentration in the plant organs. Plant bioindicators such as the Omega3-index (stress indicator) can also be useful tools to monitor plant health status (Le Guedard et al., 2008).

Monitoring of ecosystem health indicators

The ecosystem quality can be considered as a perspective in terms of environmental benefits. For non-vegetated sites the development of a vegetal cover implies an increase of the ecosystem complexity and diversity, from a low diversity environment (mainly composed of microorganisms) to a complex ecosystem composed of micro-organisms, plants and animals. The objectives in terms of of ecosystem evolution must be designed according to the site characteristics, and a selection of microorganisms, plants and animals (invertebrates and vertebrates) should be selected together with the associated parameters (enumeration, diversity, stress indicators, contamination by pollutants) to monitor. New developments in the field of molecular biology (environmental DNA) can provide tools to monitor the evolution of global biodiversity while avoiding sampling of living animals (Ruppert et al., 2019). Bioindicators of soil health, microbial diversity and functions linked with the nutrient status of the soils (cycles of C, N, P) can be included in the long-term monitoring of the site. Among them, nucleic acid-based determinations and enzymatic activities can be considered (Michel et al., 2014; Bhaduri et al., 2022).

Monitoring the agronomic quality of soils (including pH, organic matter rate, soil particle distribution, minerals, exchange capacity, water retention/infiltration capacity, pollutants) will show to what extent the soil is suitable to support a dense and perennial vegetation cover over time. Indeed, the sustainability of an ecosystem is partly linked to the organic matter content of the soil (Sheoran et al., 2013).

2.5 Phytocapping

2.5.1 Scope

Among the interventions of site securing, in addition to phytostabilization which, as reported in the previous sections, is a technology that aims to reduce the risk associated with contaminated soil by reducing the bioavailability of contaminated materials, we also find phytocapping.

Phytocapping is based on the use of higher plants (trees, shrubs and herbaceous) to be inserted directly on the cover layer of a contaminated site, reducing or eliminating the use of waterproofing materials such as clays, geosynthetics or sheets of high density plastic polymers. In phytocapping, plants grow in the clean soil of the cover layer and minimize (or avoid in best conditions) the percolation of rainwater through maximizing evapotranspiration. Consequently, it can strongly reduce the leaching of contaminants present in the underlying layers of contaminated soil. The layer of clean soil on which the plants grow is part of the project, because the goal is to create an “ET - Cover” (EvapoTranspiratio-Cover system) without direct interaction between contaminants and plants. The technique is optimal for areas that are not excessively large and where the pollutants are located in the deep layers. The technique is also used to reduce leaching from old waste landfills.

The blocking of the transport of pollutants occurs through the interception of rainwater mediated by the foliage of the plants and the subsequent water regulation at ground level. This regulation is achieved and depends on the evapotranspiration activity of the plants and on their influence on the physical-chemical characteristics of the surface layers of the soil. Part of the rainwater intercepted by the foliage does not reach the ground and evaporates directly; a fraction of the water that infiltrates into the soil is evapotranspirated by the plants after being absorbed by the roots; the remaining part primarily remains in the cover soil.

The efficiency of phytocapping therefore depends both on the evapotranspiration capacity of the plants and on the water retention capacity of the soil (therefore on its texture and organic matter content) and, ultimately, on the climate that characterizes the area where the contaminated site or landfill is located.

2.5.2 Advantages

Among the advantages of phytocapping, is that it encourages the development of an aerobic microbial community capable of degrading methane gas produced by the landfill, limiting its release into the atmosphere (Lamb et al., 2014). Furthermore, water regulation, by hindering the percolation of water to the contaminated layers at the contaminated site or landfill, also limits their decomposition and the consecutive generation of methane, carbon monoxide and gases whose production is linked to the humidity of the substrate. The containing effect of the roots also favors the stability of the capping against both water and wind erosion. A further advantage of phytocapping is landscape improvement, which contributes to human health and well-being, absorption and storage of atmospheric CO_2, as well as the contribution to the conservation of biodiversity and habitats for fauna and insects.

2.5.3 Disadvantages

Phytocapping has some disadvantages, because plants have physiological limits that cannot be easily overcome. For example, in some cases the plant system may not be sufficient to regulate the water supplies originating from precipitation, and therefore to prevent percolation of water into the contaminated layers. However, it is possible to overcome this limitation by creating an appropriate drainage system to collect excess water which can then be used for surface irrigation in dry periods. In particular and well-confined cases, it is also possible to use the leachate from the landfill for irrigation, reducing or eliminating the management of the drainage water.

Based on the previous considerations, the choice of plant or tree species must take into account the following aspects:

- capacity for horizontal and vertical development of the root system;
- tolerance to water stagnation and any dry periods;
- climate and other environmental conditions relevant to the development and stability of the coverage;
- timeframe for growth and development of the foliage;
- embedding into the landscape and ecological value;
- adequate transpiration rates in optimal conditions and in all seasons;
- possible use of the biomass produced (where relevant).

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3 PHYTOEXTRACTION

3.1 Description of the techniques

Phytoextraction is an in-situ remediation technology using plantsto remove pollutants from soil, through uptake into roots, followed by translocation to stems and leaves. It allows the plants to accumulate metals in their organs (Peng et al., 2009). Subsequently, aerial plant parts are collected to ensure pollutant removal from the site and not only movement of the pollutants from soil to aerial parts. This remediation technique is primarily suited for soils polluted by metals. In a few cases it could be also applicable for radionuclides. When there is a mixture of pollutants, metals and petroleum hydrocarbons for instance, phytoextraction and phytorhizodegradation can act together to extract and degrade pollutants. Soil metal removal is not total, because plants have only access to a fraction of the total metal content in the soil, i.e. the phytoavailable fraction. To implement a phytoextraction program it is necessary to involve the planting of one or more species that are (hyper)accumulators of the pollutants, at the same time or subsequently. Phytoextraction of metals has garnered much attention in the past several decades, since the initiation of its field trials, mainly conducted on metals such as Cd (cadmium) and Zn (zinc) (USEPA, 2000), with potentially important health and environmental benefits. In analogy with other phytoremediation technologies, phytoextraction requires preliminary field testing to ensure successful plant growth and control pollutant exposure pathways.

3.2 Feasibility

A plant suitable for phytoextraction ((hyper)accumulator plants) should have the following characteristics:

- rapid growth rate.
- high biomass production.
- ability to accumulate and tolerate high concentrations of metals in harvestable tissue.

After high level of metal accumulation in the plant parts, the plants are harvested which generates concentrated pollutant containing material. This highly concentrated mass material may contain even higher concentrations of pollutant than the soil, which is what makes this technology successful.

Hyperaccumulator plants for metals found in soils have the capability of accumulating large amounts of metals, without experiencing any obvious physical effects or symptoms (Goolsby, et al., 2015). They are characterized by a high accumulation of metals in shoots compared with the root system (Weber et al., 2004). Hyperaccumulator plants should have a high rate of growth and high production of above- and below-ground organs such as stems, leaves, and roots, so that efficient translocation of metals to all parts can be accomplished in a relatively short timeframe. They should also be tolerant to high concentrations of metals and adaptable to biotic and abiotic stresses so they can be easily cultivated and harvested (Memon, et al., 2009). The capacity to hyperaccumulate metals is a rare phenomenon in the plant kingdom, occurring in about 500 species of vascular plants total (Van der Ent et al., 2013). Also, trees such as willows and poplars can be used for phytoextraction, because of their extensive root systems, high aerial part biomass and minimal cultivation needs. They are accumulators and not hyperaccumulators, but these traits, notably, their high biomass, compensate for their limited metal concentration compared to hyperaccumulators.

Phytoextraction can be induced using chelator agents, such as EDDS, acidification or microorganisms able to enhance uptake of metals from soil through the absorption of the less soluble fractions of metals in soil. Chelators can also assist plants to gain biomass, depending on their composition (Zulkerlain et al., 2023). This biochemical mechanism is usually called 'induced phytoextraction'. Chelators facilitate accumulation of metals in roots, stems and leaves of some plants such as yellow mustard.

An important role in plant uptake, and hence phytoextraction, is that of root exudates. These are chemical compounds likely to occur in the rhizosphere, which are clearly associated with an increase of metal uptake from soil and their translocation to shoots (Wenzel et al., 2003).

Phytoextraction has been presented in many papers as a low-cost method for remediating contaminated soil. However, phytoextraction has one limitation in feasibility, i.e., the long timeframe in combination with a larger area needed to decontaminate soils, i.e., years and sometimes decades (Santa-Cruz et al., 2023). Phytoextraction is a function of the metal extraction rate, which is the biomass of the harvestable organs of the plant multiplied by the metal concentration in the biomass. Its efficiency depends on the clean-up time, i.e. the time needed by the plant to reduce the pollution to an acceptable value. The extent of extraction of metals from soil depends on multiple factors, such as soil properties (like pH, organic matter content, soil type), root interaction with metals, plant capacity for metal adsorption and accumulation in harvestable parts of plants. When the fraction of phytoavailable metal in soil decreases, the extraction rate of metals from soil decreases too. The capacity of metal extraction from soil and accumulation in harvestable parts varies widely among plants species and even among cultivars of the same plants. The plants which have the highest potency in extracting metals are called hyperaccumulators. These plants can accumulate one or more than one metal in their aerial parts, but the affinity for extracting different types of metals differs. *Sauropus androgynus* (L.) Merr, for example, efficiently Zn (zinc), but it is not able to absorb Pb (lead) (Beicheng Xia et al., 2013).

It is important to distinguish between total metal concentration in soils and the phytoavailable fraction. Therefore, feasibility of phytoextraction also depends on chemical and physical characteristics of the soil. The most relevant soil parameters controlling bioavailability for plant uptake include pH, organic matter content, clay content and concentration of (hydr)oxides of manganese and aluminium. Alkaline soils generally have lower metal solubility and so plants growing on that type of soil might extract less metal. However, Grignet et al. (2020) showed that the Arabidopsis halleri Zn foliar concentration exceeded the Zn hyperaccumulation threshold (> 10,000 mg/kg DW) in the presence of NPK fertilizer, although the soil was alkaline (pH > 8.2). Bioavailability of arsenic (As) and molybdenum (Mo), on the contrary, may increase at higher pH. These elements are nevertheless rarely translocated in high concentrations in most plants.A way to increase extraction capacity of plants for most metals is therefore enhancing the solubility of metals in soil. This is possible by adding chelating compounds in soil bulk solution to reduce phytoextraction duration. This procedure has some drawbacks, such as excessive costs in using biodegradable chelators. Another option is to harvest aerial parts of the plants several times during its growth life cycle, with the purpose to increase its phytoextraction capacity. Feasibility of phytoextraction depends on the time required to remove pollutants from soil and to translocate them into the roots, stems, and leaves. Predicting the extent of phytoextraction requires determination of the dynamic rate of metal removal from soil.

An experiment of phytoextraction can be defined feasible if plants have the capacity of reducing the soluble fraction of the metal in the soil to reduce the metal phytoavailable fraction (Santa Cruz et al., 2023). A parameter commonly used to evaluate the feasibility and efficiency of phytoextraction is the BCF (BioConcentration Factor), sometimes also called BAF (BioAccumulation Factor). The BCF is defined as the metal concentration presents in the harvestable plant tissue divided by the total metal concentration in soil (Yoon et al., 2006; see Figure 3.1).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration of metal in plant tissue } \left(\text{mgkg}^{-1}\right)}{\text{Concentration of metal in soil } \left(\text{mgkg}^{-1}\right)}$$

Figure 3.1- BioConcentration Factor calculation (Concentrations are expressed in dry weight).

It is an important parameter in phytoextraction determining the magnitude of metal uptake, its mobilization into the plant tissues, and storage in the shoot parts. Metal BCF values >1 indicate a metal accumulating behaviour and that the species is a potential metal hyperaccumulator (Hanan Almahasheer, 2009).

Another factor useful to monitor phytoextraction performance is the translocation factor (TF), also called shoot-root quotient, that represents the ability of a plant to translocate the metal from roots to shoots (leaves or stems) (see Figure 3.2).

$$\text{Translocation factor (TF)} = \frac{\text{Concentration of metal in leaves or stems } \left(\text{mgkg}^{-1}\right)}{\text{Concentration of metal in roots } \left(\text{mgkg}^{-1}\right)}$$

Figure 3.2- Translocation factor calculation (Concentrations are expressed in dry weight)

Generally, plants with BCF and TF >1 are considered relevant candidates for phytoextraction.

3.3 Field test

Although phytoextraction has been known for decades, demonstration in real cases, and its application as a remediation solution for contaminated sites, is still rare. As an example, phytoextraction was set up in an urban area in France (Creil conurbation, Oise, France). The concentration in soil was Cd 1.66 ± 0.2; Zn 616.5 ± 248 mg/kgDW. The objective of the project was twofold; the first was to avoid “dig and dump” by managing in situ the metal pollution of the soil (1) and the second was to green the polluted landscape to form a corridor with other unpolluted green lands (Grignet et al., 2020; 2021). This case study was included in a redevelopment project of a neighborhood.

To make a plant cover, two well-known plants to (hyper)accumulate Zn and Cd were chosen and cultivated together on the polluted site (Figure 3.3). One was the herbaceous hyperaccumulator Arabidopsis halleri (Brassicaceae) and the other was the ligneous species Salix viminalis (Salicaceae). In addition to these traits, both plants were selected due to their suitability for the pedoclimatic conditions of the site. The plantation of unrooted willow stems and the seedlings of A. halleri, both at high density, was performed in the spring of 2013 and 2015, respectively.

Figure 3.3- Impression of the study site, including overview with information panels in front of the plant barrier; phytoextraction panel explaining its principles and used plant species; unrooted willow stems before and after plantation; *A. halleri* seedlings production in greenhouse and after plantation; *A. halleri* flowering stage in willow inter-rows.

In this project, the authors showed that both species coped with the alkaline soil condition without altering their capacity to extract Zn and Cd in their aerial part, evidencing the possibility to use both species for a wide range of soil pH. Fertilization with commercial organic product (NPK) and harvest of A. halleri boosted its Zn phytoextraction performance. When both species are harvested, the clean-up time to reduce the Zn and Cd levels in the soil by 50% was estimated to be 24 and 36 years for Zn and Cd, respectively. Besides this result, no decrease of the phytoavailability of Zn and Cd in soil was visible, likely due to the reloading of this fraction from the less soluble metal pools in the soil. These results are obtained in optimal conditions, i.e., A. halleri harvested at the rosette stage in presence of NPK fertilizer, A. halleri biomass yield extrapolation from 1 m2 field plot to 1 ha, collection of S. viminalis leaves in autumn to get the maximum of metal extraction and constancy of biomass yield over years.

From the beginning of the project to the end (i.e., almost 10 years), the greening of the polluted soil was successful, with these plant species showing their adaptation to the overall agro-physico-chemical conditions, including metal pollution. Nothing in the landscape allows these plants that grow on polluted soil from other plants that grow on non-polluted soils. This greening promotes aesthetics providing cultural services to the citizens, while at the same time detoxifying the soil in the long-term.

As phytoextraction only addresses partial remediation of a soil on a long-term basis, exposure of humans and ecosystems needs to be limited or, even better, reduced. Consequently, a plant barrier was built all around the study site to discourage people, and particularly children, from going inside and being exposed to polluted soil by direct contact through soil ingestion (plant barrier shown in Figure 3.3). In addition, potential exposure of snails, as the first link of the food chain, was determined by feeding these animals with leaves of willows and *A. halleri* containing various metal concentrations. Snails fed on willow leaves, enriched with metals. However, since they did not or hardly consume them, it was concluded that the dispersion of metals via the food chain was low but not negligible. Moreover, metal transfer was evidenced with the most metal concentrated in A. halleri leaves, suggesting bioconcentration in snails in case of consumption and biomagnification in the food chain if snails are consumed in turn. This result might be a concern when phytoextraction is applied at a site to avoid pollutant dispersion in the environment.

Other issues concern the valuation of the plant biomass and/or the metals contained in the plants. In this example, the Zn in A. halleri was used to produce Zn ecocatalysts with similar efficiency as usual ones (Cybulska et al., 2022). Willow leaves collected in autumn could also be used to produce ecocatalyst in green chemistry, whereas wood could be used in biomass boiler (Grignet, 2021). Cd might be separated from Zn in the plant biomass to produce Cd free ecocatalyst.

Finally, this example showed that long clean-up times could be compensated for by several benefits, such as greening of the area, which has a positive impact on health and well-being of people, soil resource economy and land valuation.

3.4 Performance monitoring

Phytoextraction implements plant species with soil metal chelators to increase metal mobility and fertilizers to improve plant growth, if necessary. The performance of phytoextraction will therefore be measured through the effectiveness of plant species and soil amendments. Measurements are made after each harvest and on a control-soil not treated by phytoextraction, for comparison.

The “best” phytoextracting plant is a domesticated herbaceous or ligneous plant which has:

- a dense and depth root system to maximize the polluted soil volume in contact with plant roots.
- a bioconcentration factor (BCF = ratio between the concentration of a metal in the harvestable parts of the plant to the total concentration of the same metal in the soil where the plant grows) > 1;
- a biomass production like the one on non-polluted soil.

Thus, performance monitoring of plant species is based on the following measurements and calculation:

- number of metals annually extracted by the plant per unit area (kg metal ha-1 year-1) calculated from: Biomass production or yield (t ha-1).
- metal concentrations targeted by phytoextraction in harvestable plant parts (mg metal kg plant-1 dry weight). BCF for each targeted metal.

In case of use of soil amendments, measurement of metal bioavailability in soil is necessary to verify their effectiveness. Metals are then measured in soil solution by artificial roots (rhizon) or after chemical extraction. The EN ISO 17402:2011 standard presents requirements and guidance for the selection and application of methods for the assessment of bioavailability of pollutants (metals, including metalloids, and organic contaminants, including organometal compounds) in soil and soil materials.

Soil amendments can sometimes lead to nutrient deficiencies (Ca, Mg, etc.), whereas these essential metals are necessary for the proper development of plant species. Symptoms may be visible on plants. Measuring these elements in the aerial parts of plants helps to verify this aspect. In the case of the use of low biodegradable chelates, it will be necessary to verify that these products and the complexes of these products with the metals are not transported in significant amounts to the groundwater or surface water.

As for any technique for managing polluted soils, once phytoextraction is in place, it is necessary to follow residual risks and perform monitoring. The frequency of this monitoring will mainly depend on the use of the site during the phytoextraction process, frequency of harvesting and level of soil pollution.

The plant biomass produced is enriched with pollutants (often metals), because the plants are selected for their ability to transfer and store large amounts of metals in their harvestable parts, most often aerial parts (stems and leaves). To achieve the partial remediation of the soil, subsequent harvest should be performed regularly, which leads to large amounts of pollutant (metal) enriched plant biomass. In order phytoextraction does not lead to migration of pollutants from one place to another, i.e. from soil to above ground plant parts, but could be considered as a circular economy strategy, the valuation of the harvested plant biomass and the metal inside the plants is necessary. To date, many studies have been conducted showing feasible options for such biomass, such as the production of essential oil or ecocatalyst production (e.g. Cybulska et al., 2022; Perlein et al., 2023. Perlein et al., 2021a, b,c).

In addition to improving the agro-physico-chemical parameters of a soil, phytoextraction should positively impact soil biological parameters or even help restore soil functions when they have been disturbed or inhibited by pollution. To assess the positive effects of phytoextraction, general and specific biological indicators and biomarkers can be used and compared to references (i.e. non-polluted soil, non-vegetated soil). The TRIAD approach, a procedure for site-specific ecological risk assessment (EN ISO 19204:2022), can also be used to evaluate the potential benefits of a phytoextraction management strategy on the ecological status of a specific soil. So far, only a little feedback from real cases is available to quantify these aspects.

An issue that might require attention is the potential pollutant transfer through the plant consumption by herbivores when they are rich in metals. Indeed, due to seed bank and surrounding sites, indigenous species are likely to settle on the soil treated by phytoextraction. Measurements of metal concentrations (particularly

Cd and Zn) in the aerial parts of the most abundant plants may be appropriate, especially in those known to be palatable to herbivorous animals. The ISO 24032:2021 standard on pollutant snail bioaccumulation assessment can help to evaluate these risks to herbivores.

Another issue concerns the sustainability of the plant cover to continuously phytoextract metals over time. As (hyper)accumulating plants are selected for their phytoextraction performance, they might not be from the seed bank of the contaminated soil or the near surrounding zones and consequently not be competitive against indigenous plants. To maintain the selected plants, it might be necessary to plan regular weeding.

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4 PHYTODEGRADATION

4.1 Description of the technology

4.1.1 Scope

Phytodegradation stands as a promising and ecologically sustainable remediation approach. It seeks to mitigate soil pollution by capitalizing on the natural processes orchestrated by plants and their associated microorganisms. Additionally, the presence of plant-associated microorganisms can enhance the bioavailability and mobilization of pollutants, making them more accessible for degradation (Salt et al., 1998).

Phytodegradation refers to the use of plants and associated microorganisms to degrade organic pollutants (Lone et al., 2008). It is a key process in phytoremediation, which aims to clean up polluted areas using plants (Gajić et al., 2018). Phytodegradation involves the uptake, metabolism, and degradation of pollutants within the plant (Sharma & Juwarkar, 2015). Through these processes, pollutants are converted into less harmful forms or mineralized to CO_2 and water. This process occurs through the absorption of pollutants and subsequent metabolic processes within the plant (Ratnawati & Faizah, 2020). Phytodegradation particularly excels in remediating sites polluted with nutrients and organic compounds, such as chlorinated hydrocarbons, petroleum hydrocarbons, BTEX, MTBE, polycyclic aromatic hydrocarbons (PAHs), pesticides, persistent organic pollutants, explosives, and other industrial chemicals. Phytodegradation is also possible with inorganic substances such as cyanides.

Phytodegradation in the rootzone is often referred to as rhizodegradation. Rhizodegradation plays an important role in phytoremediation of mineral oil and polycyclic aromatic hydrocarbons (PAHs).

This chapter delves into the mechanisms of phytodegradation and its pros and cons, guided by the principles outlined in the Code of Good Practice for Phytoremediation (OVAM, 2019).

- **Mechanism of Phytodegradation:** Phytodegradation is a complex, yet elegant, process that unfolds in several interconnected steps. Pollutants present in the soil or groundwater are taken up through the roots of plants. Inside the plant, these pollutants encounter various metabolic and biochemical processes. Some pollutants may undergo direct metabolism by the plant. Others undergo transformations catalyzed by enzymes and microbial activity within the plant's root zone, known as the rhizosphere. This combination of plant-assisted degradation and microbial interactions in the root zone contributes to the degradation of pollutants into less toxic or non-toxic substances.

Plants are as photoautotrophic organisms not evolutionarily equipped with enzymes to metabolize organic substances and pollutants as compared to heterotrophic organisms such as animals and humans. Plants will therefore not degrade substances, but rather transform them into more water-soluble and less harmful forms according to the so-called green liver model (Figure 4.1). Pollutants without a reactive group first enter phase 1 and are activated by redox reactions (e.g., a functional group is put on the molecule such as hydroxyl, amino or sulfhydryl). In phase 2, these substances are conjugated to sugars by e.g. glutathione and UDP-glycosyl transferases. Ultimately, they are sequestered, usually in the vacuole or cell wall and finally stored in less photosynthetically active tissues including old leaves, in the roots, or in the woody material of the plant (OVAM, 2019)

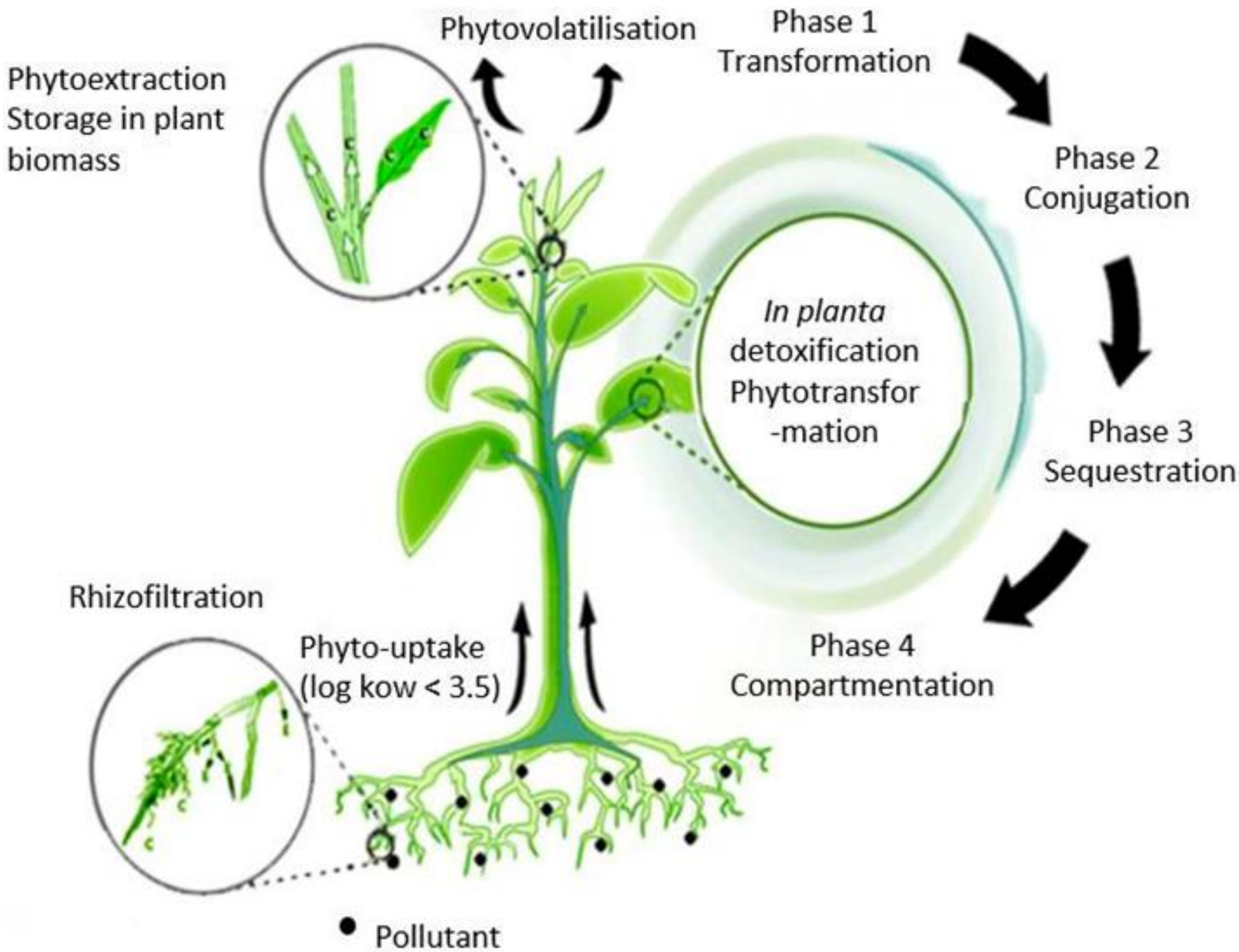

Figure 4.1- Plant uptake, transformation and degradation of pollutants in the plant (green liver model) Adapted from Van Aken et al. (2009) i

In addition to transformation by the plant itself, there are the plant associated microorganisms, the microbiome, which can collectively catalyse complete degradation of organic substances into CO_2 and waterdue to their wide variety of metabolic enzymes (Figure 4.2; OVAM, 2019)

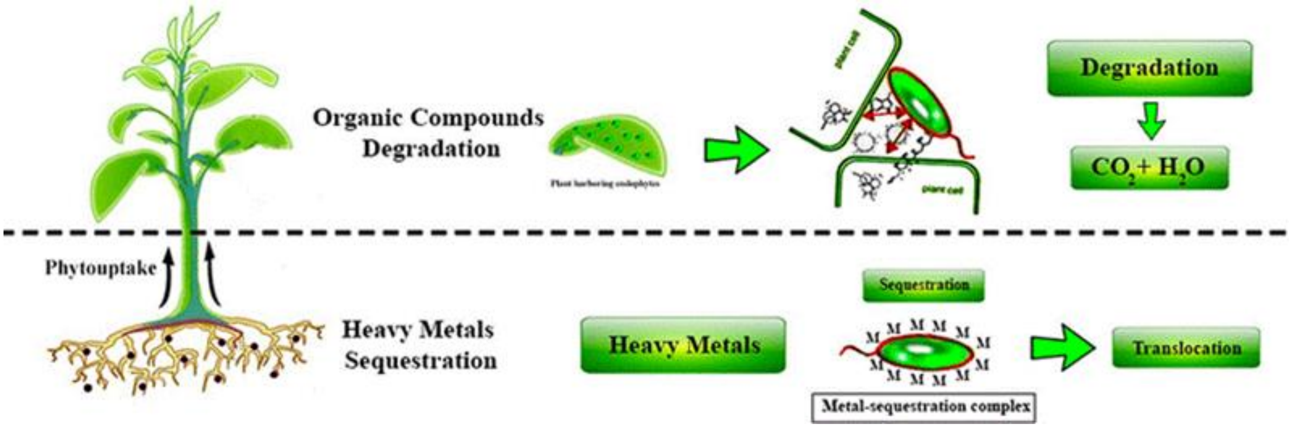

Figure 4.2- Endophytes in action against organic and inorganic pollutants (Weyens et al., 2009) in Code of good practice on phytoremediation, OVAM (2019)

Cyanide is an important source of nitrogen for microorganisms, fungi and plants. Many organisms are capable of degrading cyanide. The most important degradation pathways are hydrolysis, oxidation, reduction, and substitution/transfer (Ebbs, 2004).

Metal cyanide complexes are more resistant to biodegradation than simple cyanides. In the dark they dissociate slowly resulting in low toxicity. Under the influence of UV light, photolysis occurs.

However, several studies have shown that plants can also degrade iron cyanides.

Studies on the transport and metabolism of free cyanide and iron cyanides by willow (Salix sp.). (Ebbs et al., 2003) show that cyanide is taken up by willow and then degraded with the nitrogen being used for amino acid production and other processes in the plant. There is little accumulation of cyanide in the leaves.

Vascular plants possess the enzymes beta-cyanoalanine synthase and beta-cyanoalanine hydrolase that break down free cyanides and convert them to the amino acid asparagine (Larsen et al., 2002). The risk of volatilization of cyanide through the leaves can be neglected because the trees would die even before significant concentrations would be reached.

- **Plant Selection:** The success of phytodegradation hinges significantly on the judicious selection of plant species or cultivars tailored to the specific pollutant profile of the site. Some plant species exhibit remarkable tolerance and accumulation capacities for particular pollutants. The process of plant selection considers factors such as the type and concentration of pollutants, soil characteristics, climatic conditions, and the broader ecosystem. Notably, the use of native plant species often prevails due to their adaptability to local conditions, and associations they form with the native microbiome. For example, Bell et al. (2013) explored the linkage between bacterial and fungal communities in hydrocarbon-polluted soils, specifically focusing on the relationship with plant phylogeny. The researchers found that certain genera of Dothideomycetes, such as Phoma and Preussia, were dominant in hydrocarbon (HC) plots. These genera have been shown to harbor endohyphal bacteria from groups capable of hydrocarbon biodegradation, such as Xanthomonadales, Pseudomonadales, Burkholderiales, and Sphingomonadales (Bell et al., 2013) of which the abundance was related to plant phylogeny, indicating that the type of plant present in the soil may influence the composition of the bacterial community (Bell et al., 2013).

- **Biostimulation/bioaugmentation:** To optimize the phytodegradation process, a range of stimulative techniques can be listed. These include the introduction of beneficial microbes into the rhizosphere (bio-augmentation), fostering pollutant degradation. Soil amendments, such as compost and organic matter (biostimulants), serve to enhance soil structure and bolster microbial activity. Adjusting environmental conditions, such as soil pH and moisture levels, may also be necessary to promote microbial activity and plant health.

4.1.2 Advantages

- **Sustainability:** Phytodegradation is a Nature-based solution that aligns with nature, minimizing disruption to ecosystems and reducing the need for energy-intensive interventions. **Nature-based solutions** are defined as

"actions to protect, conserve, restore, sustainably use and manage natural or modified terrestrial, freshwater, coastal and marine ecosystems, which address social, economic and environmental challenges effectively and adaptively, while simultaneously providing human well-being, ecosystem services and resilience and biodiversity benefits" (UNEA/EA.5/Res.5) (www.biodiversa.eu)

- **Cost-Effectiveness:** Phytoremediation is a cost-effective and environmentally friendly technique that utilizes plants to clean up polluted soils or waters (Abioye et al., 2011).

Once established, phytodegradation systems demand minimal maintenance, translating into lower long-term costs compared to conventional remediation methods.
The cost estimate for remediation with phytoremediation considers four main categories: (1) Design, (2) Organization (3) Maintenance and (4) Monitoring the efficiency and result of remediation (= sampling and analysis).

The costs associated with these four categories are relatively small compared to traditional "engineering-based" remediation technologies. This is especially the case in the operation and maintenance phase, where the primary factor for cost reduction is the energy source for the control systems. Traditional systems use electric power, at considerable costs, to pump water, for example, while phytoremediation systems use free solar energy. Individual sites vary in cost regardless of the technology used. In general, phytoremediation is a cheap alternative to traditional methods (OVAM, 2019)

- **Aesthetic and Ecological Benefits:** Phytodegradation systems often enhance the visual and ecological aspects of polluted sites by introducing vegetation and supporting habitat restoration.
  Phytodegradation systems not only help in the remediation of polluted sites, but also bring about aesthetic and ecological benefits (Thijs et al., 2016).
- **Long-Term Solution:** Phytodegradation offers a sustained, long-term solution as plants continue to grow, adapt, and remediate over time.

4.1.3 Limitations

- **Time-Consuming:** Phytodegradation is generally slower than some conventional methods, as it relies on the growth and metabolic processes of plants. However, depending on the nature and concentrations of pollutants, phytodegradation is often feasible in a timeframe of 5 to 10 years.

- **Site-Specific:** Successful implementation relies on careful plant species selection and site-specific considerations, making universal applicability **possible preceded with a feasibility study for each site.**
- **Bioavailability of pollutants:** Phytodegradation is best suited for sites with low to moderate contamination levels and when pollutants are bioavailable for plant uptake; heavily polluted sites might require supplementary remediation technologies to speed up the process and make plant growth possible (no phytotoxicity).
- **Uncertainty:** The success of phytodegradation varies depending on factors such as plant health, microbial interactions, seasonality,environmental conditions, and time, introducing a degree of uncertainty.

4.2 Practical application

Implementing phytodegradation effectively necessitates a comprehensive approach rooted in site-specific assessments, judicious plant selection, rigorous monitoring, and community engagement.
This chapter serves as a practical guide, drawing inspiration from the principles described in the Code of Good Practice for Phytoremediation, to lead the path towards successful phytodegradation projects.

- **Site Assessment**: The cornerstone of any phytodegradation project is a comprehensive site assessment. This process involves a careful examination of the extent and nature of contamination, alongside an evaluation of soil and hydrogeological conditions. This wealth of information forms the bedrock upon which critical decisions regarding plant selection and remediation strategies are constructed.

Successful implementation of any phytodegradation project requires careful consideration of site-specific factors such as soil conditions, sunlight exposure, and climate patterns. Moreover, this step calls for monitoring and evaluation of the drivers of degradation of ecosystems or ecological stressors on or nearby the project site (Varshney et al., 2022). Furthermore, the understanding and assessment of factors such as root development, groundwater velocity, type of contamination and existing vegetation may facilitate effective management strategies for climate resilience (Arora & Kaur, 2023), as well as improve phytoremediation project efficacy, potentially leading to the regeneration of the degraded ecosystem (Varshney et al., 2022) (Salt et al., 1998).

1. **Plant Selection and Design**: Guided by the insights gleaned from the site assessment, the careful selection of appropriate plant species assumes paramount importance. The chosen species should exhibit a high degree of tolerance to the specific pollutants present, possess favorable growth characteristics, and demonstrate a propensity to facilitate microbial interactions. The design of the planting layout, including considerations of spacing and density, must be executed with precision to maximize the contact between plant roots and pollutants.
   In general, willow trees used for phytoremediation are planted close together in rows to maximize their pollutant uptake and create a dense network of roots. A spacing of 1-2 meters (3-6 feet) between trees within the rows and 3-4 meters (10-13 feet) between rows is commonly recommended. This spacing allows the trees to form a dense canopy and root system, enhancing their ability to absorb toluene and other pollutants from the soil.
   Additionally, willow short rotation coppice (SRC) crops have been used in buffer strips to mitigate water eutrophication and reduce heavy metal mobility in phytoremediation interventions (Liu et al., 2022). Willows are also suitable for phytoremediation in urban areas due to their high ornamental value and potential for bioenergy production (Capuana, 2020).
2. **Soil Amendments and Nutrient Management**: Soil amendments, such as compost and organic matter, are introduced to improve soil structure and enhance microbial activity. Nutrient management is a critical aspect, ensuring that plants have access to essential elements that underpin robust growth and

metabolic functioning. These strategies are firmly anchored in the principles of sustainable remediation.

3. **Microbial Inoculation**: Beneficial microbes can be introduced to the rhizosphere to enhance pollutant degradation. These microbes support the plants' metabolic processes and contribute to the breakdown of pollutants. This can be achieved by stimulating existing microorganisms or introducing new ones that can accelerate the biodegradation of pollutants, thus enhancing the plant's overall capacity for remediation (Furini et al., 2015; Boorboori & Zhang, 2022).

   In microorganism-assisted phytoremediation, the most beneficial microorganisms for the active phytoremediation mechanism are selected and enriched via inoculation. In many cases, the inoculation will also have to be repeated several times to ensure the presence of the inoculated microorganisms. In some cases, consortia (groups of microorganisms) can also be used that can better maintain and establish themselves in the soil under controlled conditions (OVAM, 2019).

   An important strategy that can be applied to increase the success of colonization is to use **endophytes**, bacteria that live in the plant in the intracellular spaces or in the plant's xylem and phloem without negative effects for the plant. The "environment" _in the plant is less stressful for microbes, there is a lower biodiversity and therefore less competition between microorganisms, which can increase the success of establishing specific bacteria (OVAM, 2019).

4. **Monitoring and Maintenance**: Vigilant monitoring forms the backbone of a successful phytodegradation project. A battery of parameters, including pollutant concentrations, plant health indicators, and assessments of microbial activity, are investigated at regular intervals. These data-driven insights empower project managers to make informed decisions regarding adjustments to the remediation strategy, thus ensuring the efficacy of the endeavor.

5. **Community Engagement**: Community involvement and stakeholder engagement are pivotal components of any phytodegradation project. Engaging local communities in the decision-making process fosters understanding and support for the project's objectives. Public awareness campaigns and educational initiatives underscore the many benefits of this sustainable remediation technology. Studies have demonstrated this in various fields, such as public health initiatives (Huang et al., 2022), regenerative architectural design, genomic research, urban development projects, and environmental sanitation infrastructure planning.

6. **Long-Term Management**: Phytodegradation represents a protracted journey, demanding meticulous long-term management. Factors such as ongoing plant growth, potential biomass harvesting, and continuous monitoring are pivotal to sustaining the effectiveness of the remediation endeavor. Implementing a robust long-term management plan is essential to ensure the enduring success of the phytodegradation project.

   The Code of Good Practice for Phytoremediation employs a table (Table 4.1) known as the Phytotechnology matrix. This matrix outlines the phytotechnology mechanisms associated with each contaminant category, presents implemented and proven successful applications, specifies the applied scale, and provides a concise overview of the main findings along with references.

Table 4.1- The Code of Good Practice for Phytoremediation (the Phytotechnology matrix)

Pollutant	Phytotechnology mechanism						Applications					Scale					Main results	Reference
	Phytostabilisation	Rhizodegradation	Phytohydraulics	Phytoextraction	Phytodegradation	Phytovolatilisation	Biofilters, reed beds	Capping, vegetation cover	Recovery of fens	Hydraulic barrier	Buffer zones, green spaces	Greenhouse	Laboratory	Field	Pilot study	Large-scale		
BTEX		✓	✓		✓		✓			✓	✓	✓	✓	✓	✓	✓	Poplar could efficiently remediate a BTEX groundwater plume	(Barac *et al.*, 2009)
Chlorinated solvents		✓	✓		✓	✓	✓			✓	✓	✓	✓	✓	✓		Oak, ash and associated microorganisms remediate TCE groundwater contamination	(Weyens *et al.*, 2009)
PCBs	✓	✓	✓										✓		✓		Often difficult to solve PCB contamination with phytotechnology, rather for residues	(Sylvestre *et al.*, 2009), (Slater *et al.*, 2011)
Explosives	✓	✓	✓		✓					✓	✓	✓	✓				Grasses and trees present on military sites can stabilise or rhizodegrade explosives contamination (TNT, DNT).	(Thijs *et al.*, 2014a), (Thijs *et al.*, 2014b), (Rylott *et al.*, 2011)
PAHs		✓	✓		✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		Quite difficult to break down, yet poplar, willow and their microbial communities do have potential	(Bell *et al.*, 2014)
Pesticides	✓	✓			✓		✓		✓	✓	✓	✓	✓	✓	✓		Courts can be used to take up and take down DDE.	(Wang *et al.*, 2004), (White *et al.*, 2003), (White *et al.*, 2006)
Mineral oil, petroleum		✓	✓		✓			✓		✓	✓	✓	✓	✓	✓		Alkanes and low molecular weight PAHs can be remediated by willows, poplars, grasses and leguminous plants.	(Gkorezis *et al.*, 2016), (Page *et al.*, 2015)
Arsenic	✓		✓	✓			✓	✓	✓	✓		✓	✓				Poplars have already been used to cut down landfills.	(Ma *et al.*, 2011) (Mesa *et al.*, 2017)
Cadmium	✓			✓						✓	✓	✓	✓	✓	✓	✓	Experimental willow clones with high biomass yield improve cadmium and zinc extraction from soil in the stem	(Janssen *et al.*, 2015), (Bell *et al.*, 2015), (Croes *et al.*, 2013)
Chrome	✓			✓			✓					✓	✓	✓			Willow and birch absorb chromium but it stays in the roots.	(Pulford *et al.*, 2001), (Gardea-Torresdey *et al.*, 2005)
Copper	✓			✓			✓					✓	✓	✓			Soil additives can improve copper uptake in Indian mustard, but more field studies are needed	(Mleczek *et al.*, 2013), (Fang *et al.*, 2012)
Nickel	✓			✓			✓					✓	✓	✓			Plants of the mustard family can accumulate nickel	(Chaney *et al.*, 2007)
Selenium	✓			✓			✓					✓					Duckweed and water hyacinth have already been used to absorb selenium from water basins and reed beds	(Pal & Rai, 2010)
Radionuclides	✓			✓		✓	✓					✓	✓		✓		Sunflowers can remove uranium, caesium and strontium from hydrocultures. Soil additives can improve uptake.	(Lee & Yang, 2010), (Fuhrmann *et al.*, 2002), (Entry *et al.*, 2001)
Cyanides	✓	✓	✓		✓		✓	✓		✓	✓	✓	✓	✓	✓		Vascular plants are able to break down free cyanides. Absorption of Berlin blue can occur either in the form of colloidal Berlin blue, hexacyanoferrates, hydrogen cyanide or free cyanide ions. There is no accumulation of cyanide in the leaves and little or no volatilisation occurs.	(Dimitrova *et al.*, 2015), (Ebbs, 2004), (Ebbs *et al.*, 2003, (Larsen *et al.*, 2002), (Trapp *et al.*, 2003)
Nutrients	✓	✓	✓				✓	✓		✓	✓	✓	✓	✓	✓		Soil with high biodiversity increased maize yield by 20% and significantly reduced leaching of nitrate and phosphate to water.	(Garnier *et al.*, 2016) (Bender & van der Heijden, 2015)

In summary, phytodegradation emerges as a beacon of hope in the realm of environmental remediation. By harnessing the natural abilities of plants and their symbiotic microorganisms, this technique adeptly transforms pollutants into less harmful forms while conferring ecological benefits. While its universal applicability may be constrained, phytodegradation excels in a spectrum of scenarios where its merits clearly outweigh its limitations. Its effective implementation necessitates meticulous planning, adaptive management, and the harmonious collaboration of experts and stakeholders, culminating in efficacious and enduring results. This chapter stands as a guide, navigating the path towards sustainable remediation through the application of phytodegradation.

4.3 References

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5 PHYTOVOLATILIZATION

Phytovolatilization is one of the types of phytoremediation processes, which is a plant-based remediation technique that eliminates pollutants from soil and water via transpiration, by absorbing and metabolizing pollutants. Pollutants that have been taken up by plants are discharged into the atmosphere in less harmful and volatile forms (Muthusaravanan et al., 2020). Air compartment pollutants can diffuse through plant parts before reaching leaves and shoots, converting them into less harmful chemicals. However, this also increases the risk of resettlement of (transformed forms of) the pollutants on the soil and other environmental compartments. This method offers possibilities for the management of sites polluted with organic pollutants like tetrachloroethane, trichloromethane, and tetrachloromethane, as well with high-volatility metals like Se and Hg (Wang et al., 2012; San Miguel et al., 2013; Van Oosten and Maggio, 2015; Zhang and Dong, 2006). Mercury ions can be converted into less harmful forms and released into the atmosphere, but this increases the possibility of additional emissions from precipitation on oceans and lakes and the production of pollutants like methylmercury (Sharma and Pandey, 2014). Mercury emission from leaf tissue is influenced by environmental conditions such as light intensity and air temperature (Muthusaravanan et al.,2020). Wang et al. (2012) investigated mercury exchange fluxes among soil-air and plant aerial parts and found *Caulanthus sp.* has a higher emissions rate into the air during the day compared to other plant species such as *Eucalyptus globulus*, *Artemisia douglasiana*, *Lepidium latifolium*, and *Fragaria vesca.* A distinct diurnal pattern can be seen in the mercury emissions from soils, which peak at 11 A.M. in the spring and summer and fall to 2 P.M. and 8 P.M. in the fall and winter. Ozone and soil temperature both have an impact on the autumn Hg flux, while soil temperature controls the winter and spring Hg flux. Awa and Hadibarata (2020) discovered that the plant transpiration rate influences phytovolatilization effectiveness. However, the transpiration mechanism for removing volatile organic pollutants and metals is not discussed in detail by the authors. The authors discussed the phytovolatilization of metals and volatile organic compounds, focusing on its description and laboratory reports. It also discussed recent challenges and perspectives for future research.

5.1 Description of the technique

Phytovolatilization is a technology in which plants absorb pollutants, transform them into more volatile forms (in some cases), and release them into the atmosphere through volatilization. This technology is effective for organic pollutants, with some pollutants volatilizing directly from stems and leaves while others are lost from the soil, without plant uptake, due to root-soil interactions (indirect volatilization) (Muthusaravanan et al., 2020).

5.1.1 Direct volatilization

Direct phytovolatilization occurs when plants release volatile chemicals from polluted soil or water via transpiration through their stems, trunks, and leaves, as shown in Figure 5.1. This process also causes physical changes in the subsurface, which may enhance the reduction of pollutants in the soil and water (Limmer and Burken, 2016). Direct volatilization rates are influenced by the physical phenomena groundwater table changes, transpiration rates, and preferential routes generated by tree roots are all that influence. These activities can potentially increase the pace at which pollutants are directly volatilized via the soil, which has substantial consequences for clean-up. Plant-produced and emitted volatile organic compounds (VOCs) and transformation products such as selenite's phytotransformation to dimethylselenide are examples of molecules that are not directly phytovolatilized, emphasizing the varied character of these substances (Limmer and Burken, 2016). An increased groundwater flow rate to plant roots may provide additional opportunities for mass transfer, allowing a greater mass of pollutants to volatilize out of the water and into the gas-phase pore space. The following equation calculates the rate of direct phytovolatilization (Limmer and Burken, 2016):

$$\text{Direct phytovolatilization (\%)} = \frac{\text{mass phytovolatilized}}{\text{mass phytovolatilized} + \text{mass in harvestable part of plant}}$$

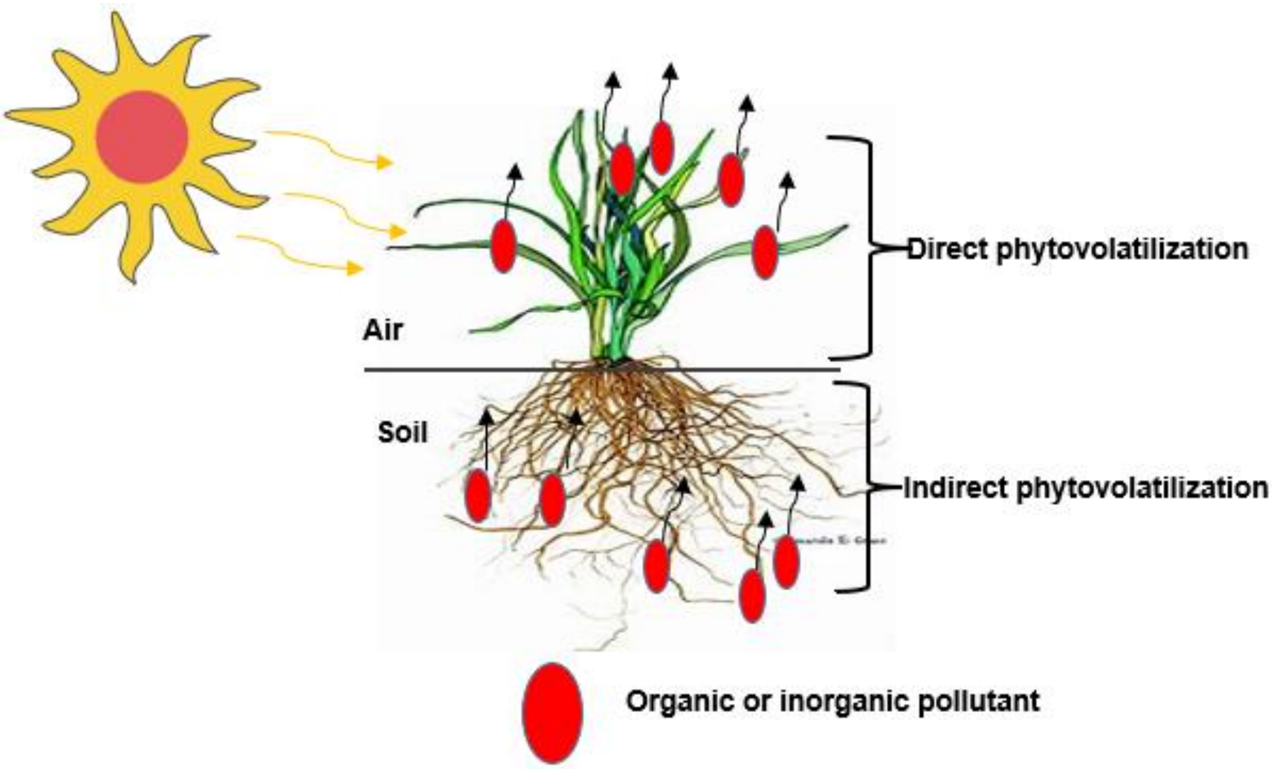

Figure 5.1- Indirect and direct phytovolatilization processes.

5.1.2 Indirect volatilization

Indirect phytovolatilization refers to the significant changes in subsurface chemical fate and transport caused by volatile pollutants from plant root activity and plants with high water movement rates as shown in Figure 5.1. These processes increase the pollutant flux by various mechanisms, including increased soil permeability, advection with groundwater towards the surface, lowering the water table, chemical transport via hydraulic redistribution, and advection with gas fluxes (Limmer and Burken, 2016). From the mechanisms of indirect phytovolatilization mentioned above, the mechanism of volatilization of organic and inorganic pollutants via plant removal of the subsurface by lowering the water table and increasing the magnitude of the vadose zone is the most dominant process. The reason for this is that the volatile pollutant transport is faster through the air than through water, resulting in higher fluxes due to plant removal. Especially if the source area is exposed to the vadose zone, or is deeper in the saturated zone, where diffusion to the capillary fringe often limits mass transport. Lowering the water table decreases the saturated zone thickness, decreases diffusion distances, and increases the fluxes (Limmer and Burken, 2016). Moreover, diel fluctuations in plant water removal also led to groundwater elevation changes that can increase vapor fluxes, oxygen influx, rhizodegradation, and the advection of volatile pollutants from the vadose zone (Limmer and Burken, 2016).

Root activity influences pollutant transport in the subsurface by changing soil texture throughout growth and senescence. Root turnover generates low-tortuosity routes, which intensifies pollutant volatilization. Living roots can disperse groundwater via hydraulic redistribution, allowing water to travel from saturated to dry locations in soil (Limmer and Burken, 2016). Organic pollutants passively traverse root membranes, causing subsurface redistribution of pollutants due to low transpiration stream concentration factors. Phytovolatilization systems are designed to intercept rainfall, effectively preventing volatile organic compounds (VOCs) from infiltrating the vadose zone. This process subsequently leads to a reduction in soil moisture content and an enhancement of effective diffusion coefficients in the vadose zone (Limmer and Burken, 2016).

5.2 Practical application

Sakakibara et al. (2010) performed a greenhouse pilot on soil pollutants with As using *Pteris vittata* plants and phytoremediation through the direct phytovolatilization process (Figure 5.2). Vapour samples were collected to quantify the phytovolatilization of As compounds from its fronds. in soil contaminated with arsenic Inductively coupled plasma mass spectrometry (ICP-MS), high-performance liquid chromatography (HPLC), and an HPLC/ICP-MS system were used to determine the content of arsenic in trap samples. *P. vittata* eliminated over 90% of the total arsenic from arsenic-contaminated soils in the greenhouse, under subtropical conditions. However, if the fern discharged sufficient arsenic into the atmosphere under field conditions, the procedure could have resulted in secondary arsenic poisoning of the surrounding soils.

Figure 5.2- *Pteris vittata* in an arsenic volatilization experiment, which includes a transplanted fern (A), a growing fern (B), a volatilizing frond (C), and a vapor collecting experiment (D). (Sakakibara et al., 2010)

Another feasibility study by Ma et al. (2004) investigates the phytovolatilization of methyl tert-butyl ether (MTBE) in the process of phytoremediation in hydroponic systems. The result shows that hybrid poplar cuttings exhibited uptake of MTBE and subsequent volatilization of the pollutant into the atmosphere via both stems and leaves. The exponential decrease in MTBE concentration within the transpiration stream as a function of height indicates the significance of stem volatilization and uptake as key processes for MTBE removal. There were no detectable metabolites of MTBE with volatile properties, and the woody stems from previous growth exhibited the highest concentration of MTBE. The findings of this study indicate that the concentration of MTBE in plant tissues remains constant, and there is no discernible mechanism of build-up that could result in higher amounts compared to the amounts in groundwater.

Direct phytovolatilization measurements at field sites provide useful information regarding the amount of phytovolatilization fluxes in field settings (Limmer and Burken, 2016). An example of such a study is the pilot study conducted by Doucette et al. (2003). The authors investigated trichloroethylene (TCE) phytovolatilization in willow and Russian olive trees. They discovered that trees near a contaminated seep emitted 1.1± 0.97 mg TCE per liter of transpired water, but plants in another site emitted 0.2 ±0.15 mg TCE per liter. Other pollutants directly phytovolatilizing from phytoremediation sites have not been observed, but indirect evidence exists in various cases. In another study, for example, Ferro et al. (2013) conducted a phytoremediation test plot that indicated that the recovery rate for 1,4-dioxane was just 18%, whereas a bromide tracer exhibited a significantly higher recovery rate of 86%. The cause of this loss was ascribed to phytovolatilization; however, no explicit substantiation was shown. The comparability of direct phytovolatilization rates is hindered by multiple issues, which can be effectively addressed by the utilization of a modelling technique (Limmer and Burken, 2016).

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6 PHYTOMINING

6.1 Description of the technique

Phytoextraction uses plants that can grow in high-mineral conditions to remove metals from the soil substrate. The two main applications of phytoextraction are: (i) phytoremediation, in which contaminating metals are stabilized or recovered for safe disposal; and (ii) phytomining, in which economically valuable metals like gold (Au), platinum (Pt), and tellurium (Tl) are retrieved by cropping [1]. Conventional mining commonly relies on ores containing a substantial amount of the desired metal and demands substantial initial funding. Such mining requires ore deposits of a substantial size to be economically feasible. Conventional mining is a threat to the environment through emissions via the air and the production of hazardous residual waste products. However, mining sub- or low-grade ores and recovering secondary metal resources have been under focus recently for their role in improving the supply of critical raw materials (CRM) [2] and restoring soil health (E.g. Biochar) [3,4]. Both objectives can be achieved by phytomining, which can recover metals from low-grade ore bodies, mineralized (ultramafic) soils, metal-contaminated soils, mine tailings, and industrial sludge [5].

Phytomining involves cultivating hyperaccumulator plants, which have the unique ability to absorb and concentrate metal ions from the soil into their aboveground biomass. When the plants reach maturity, they are harvested, dried, and burnt. The resulting ash contains a high concentration of the targeted metals, which can then be processed to extract valuable resources. A variation of phytomining called agromining involves growing high-biomass crops, known as metal crops or metallophytes, on metal-rich soils. Although they are not hyperaccumulators, they have the capacity to accumulate metals in their above-ground parts and/or compensate their lowest metal accumulation capacity by their high biomass yield. Once harvested, the plants are processed to extract metals, and the remaining biomass can be used for various purposes, such as bioenergy production or soil improvement. Phytomining and agromining are innovative approaches for the extraction of valuable metals from soil using plants, and they are both referred to as phytomining hereafter. These sustainable practices provide an eco-friendly alternative to conventional mining methods. This report focuses on their advantages, disadvantages, and areas for improvement.

The identification of fast-growing, high-biomass hyperaccumulator species is necessary for efficient phytomining. Hyperaccumulators generally have low biomass and are adapted to take up specific metals, which makes phytomining a relatively slow process. Hence, the success of this technology is limited by: the annual harvestable biomass produced, the bioconcentration factor (BCF) and translocation factor (TF), metal concentration in the soil or waste, and metal phytoavailability, from a chemical, biological and physical perspective [6].

Van der Ent et al. [3] described the various steps of the phytomining technique, which are also illustrated in Figure 6.1. The authors suggest that screening for locally adapted hyperaccumulators should occur in the pre-mining stage because the species which evolved on low-grade ore outcrops, metal-containing wastes and soils provide significant genetic resources. Indeed, the conservation of native species is important as they are a resource for the mineral industry for site rehabilitation following strip mining. Candidate species are then chosen based on their yearly biomass production and uptake and accumulation capacities and translocation in the aboveground tissues, and target metal. Many metals, including nickel (Ni), cadmium (Cd), and manganese (Mn), among others, have naturally occurring hyperaccumulating plants. In some high biomass plant species (such as *Brassica juncea*), this phytoaccumulation phenomena can also be stimulated by adding compounds that solubilize metals like gold (Au), lead (Pb), zinc (Zn), and uranium (U) and make them available for plant absorption. On-site pilot tests and agronomical practices such as improving soil fertility with NPK fertilizer, increasing water-holding capacity and improving soil structure by applying organic matter, buffering the pH, and raising Ca levels by liming are subsequently performed [6]. Phytomining is ideally suited to be developed on the mined land that is left over after the extraction of resources by strip mining and on the substrates that are below the cut-off grade. The topsoil and overburden can be used directly for the restoration of the ecosystem, while the tailings are best used in rehabilitation, because their extremely poor fertility makes them extremely difficult to re-vegetate [7]. After resource exhaustion occurs, land that was formerly used for phytomining, rehabilitated land, and restored ecosystems can all be employed in the post-mining phase.

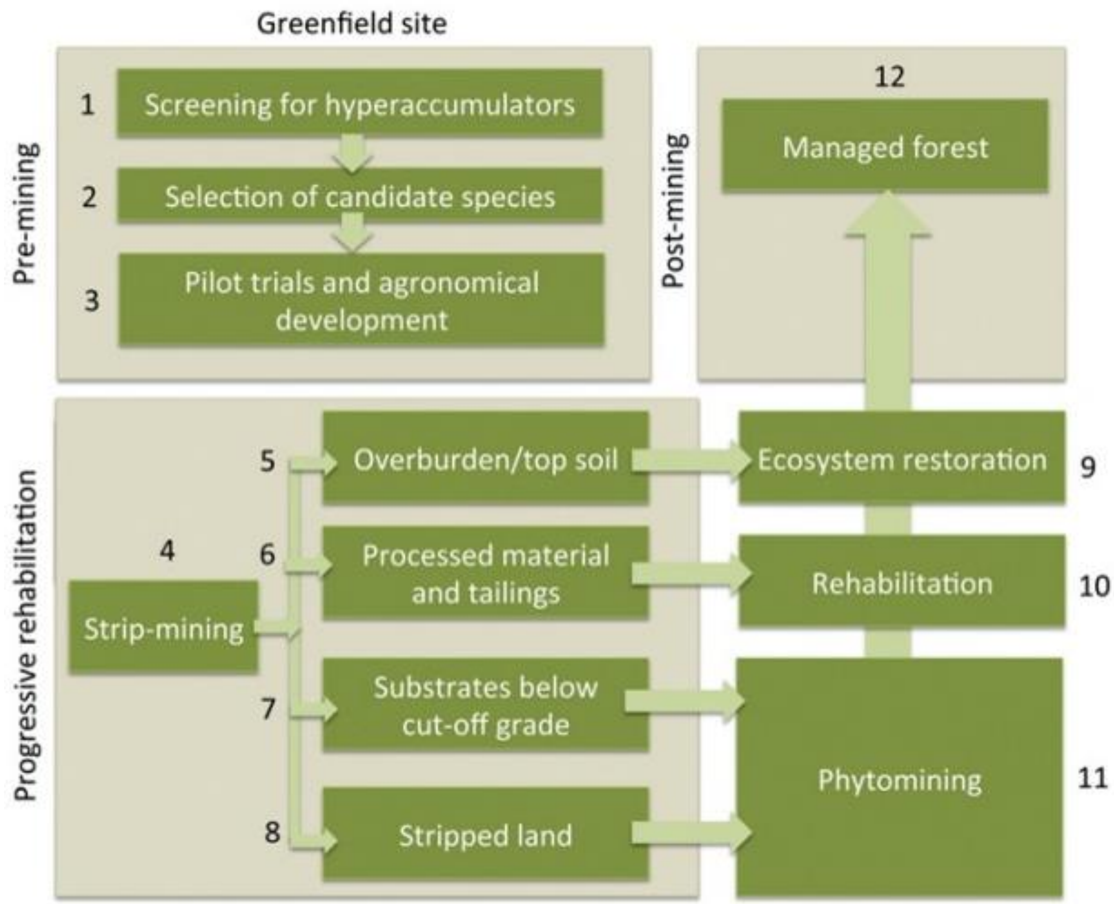

Figure 6.1- The role of phytomining in the progressive rehabilitation of mining sites, from van der Ent et al. [6]

Strip-mined land should be re-vegetated following an approach which strives to resemble natural succession and regeneration. The successional series is started by native species growing in places where topsoil was not removed, which is why it is important to avoid complete stripping to the bedrock [6]. Removing the topsoil and then using it to cover bare rock and leaving sufficiently large patches of vegetation intact can conserve local germplasm and drive the re-colonisation of cleared land by native species after mining. Nevertheless, native ecosystem preservation is always preferred over ecosystem reproduction afterwards. Natural succession can be accelerated by utilizing native plants, but their re-vegetation is still limited by a series of environmental factors, such as low fertility and low nutrient input, reduced water holding capacity and increased vulnerability to erosion.[8]

Metal extraction from hyperaccumulator biomass is an important aspect for developing phytomining technology. Different methods can be used for metal recovery: (1) leaching involves using liquid solvents to selectively dissolve and extract metals from the plant ash; (2) bioleaching is an eco-friendly method where microorganisms, which can enhance metal solubilisation and facilitate the separation of metals from plant ash, are employed to assist in metal extraction; (3) electrowinning is an electrochemical technique where metal ions in solution are deposited onto electrodes through the application of an electrical current; (4) hyperaccumulation is an alternative method for metal uptake in plants, where metals are solubilized in the soil solution, enabling passive uptake by plants.

Hyperaccumulator plants are first picked and dried, then ashed to obtain the necessary metal phase and mineralized organic matter without volatilizing metals. The processing method for Ni bio-ore proposed by van der Ent et al. [4] is illustrated in Figure 6.2. After drying, the biomass can either follow the hydrometallurgical (leaching) flow directly or go through a pyrometallurgical (ashing) phase. Several hyperaccumulators are known to uptake 1-3% Ni in dry biomass and to contain 12% to >20% Ni in the ash. After ashing, Ni can be smelted in a high temperature reactor to obtain metal Ni, or extracted by leaching, thus recovering the bio-ore to yield high-value Ni compounds. For example, in a study published in 2012, Barbaroux et al. [7] studied Ni phytomining by the hyperaccumulator plant *Alyssum murale* and found that nickel ammonium disulfate salt ($Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$) can be obtained by leaching the ashed biomass of this plants. Indeed, comparing the phytomining technology to conventional hydrometallurgical procedures, it can be noted that phytomining increases the production of Ni salt while lowering the initial capital outlay. Moreover, since ashing of the dried biomass is exotermic, this reaction generates energy which could be recovered [4], thus further establishing phytomining as a sustainable solution by including this technology in a circular economy action plan.

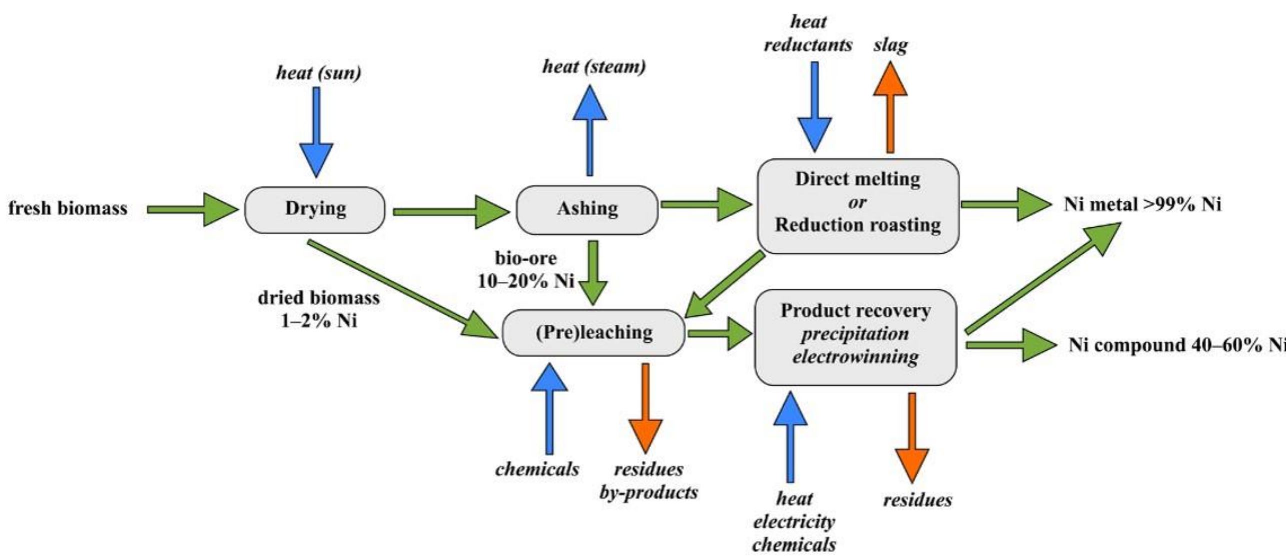

Figure 6.2- Flow sheet of bio-ore processing options, from van der Ent et al. [4]

The net economic gain of a phytomining operation in a steady-state was modelled by Robinson et al. [6] as follows:

$$G = [V_{met} \cdot Y_{met}] - C$$

where G – Net economic gain (€/(ha·year))

C – Operating costs e.g., labor, fertilizers and amendments (€/(ha·year))

V_{met} – Current metal value (€/kg)

Y_{met} – Total metal gain (kg/(ha·year))

$$\text{with } Y_{met} = F_{met} \cdot Y_{bio}$$

where F_{met} – Average metal fraction in hyperaccumulator biomass

Y_{bio} – Biomass yield of hyperaccumulator (kg/(ha·year))

Y_{met} – Total metal gain (kg/(ha·year))

Phytomining offers several other economic benefits that make it an attractive alternative to conventional mining methods. Conventional mining involves significant expenses related to exploration, excavation, transportation, and processing, while phytomining, due to its simpler extraction process and lower energy requirements, can significantly reduce these costs, while also minimising the need for large-scale infrastructure, heavy machinery, and energy-intensive operations. Extracted metals can be further processed into products such as high-purity metals, alloys, and specialized materials used in various industries [9]. These value-added products command higher prices in the market compared to raw ores, potentially increasing the overall economic returns from the extraction process. Nonetheless, the following known technical and economic factors must be taken into account: (1) relatively large time frame; (2) the cost of construction and maintenance for the processing facility and related infrastructure; (3) the cost of power, reagents, labour, and other operational costs; (4) the size and value of the product(s); (5) the cost of disposing of waste materials; (6) the availability of skilled labour to ensure that the process can be operated according to design specifications; and (8) the availability of a reliable market for the product [8].

Phytomining aligns with sustainability goals, making it attractive to environmentally conscious consumers and investors. Companies adopting these practices might gain a competitive edge by attracting investors interested in sustainable and responsible resource management. Phytomining has the potential to reclaim degraded land and contaminated soils. By extracting metals from these soils, these practices improve soil quality, making them suitable for other forms of land use over time. This can contribute to the rehabilitation of unproductive land and increase its overall economic value. While the primary focus is on economic benefits, it's important to note that the reduced environmental impact of phytomining can indirectly lead to economic advantages as well. Conventional mining often results in long-term environmental liabilities and high remediation costs. The minimized ecological footprint of green extraction practices reduces the financial burden associated with environmental clean-up and mitigation efforts.

Phytomining can be implemented in regions that have metal-rich soils wastes or low-grade metal ores but lack other natural resources (ultramafic/ serpentine soils can be mainly found in temperate (e.g., Alps, Balkans, Turkey, California) and tropical regions (e.g., New Caledonia, Cuba, Brazil, Malaysia, Indonesia). This creates opportunities for economic development in areas that might otherwise be marginalized. Local communities can benefit from job creation in activities such as planting, harvesting, processing, and even research and development related to optimizing the extraction processes. Agromining, in particular, provides a dual benefit by producing both valuable metals and biomass resources. This diversification of income streams can help farmers and communities to become less reliant on traditional agricultural products and open up additional revenue sources. The sale of metal crops and extracted metals can provide supplementary income during periods of fluctuating crop prices [11].

While the economic, environmental, and social benefits of phytomining are promising, it is important to consider the potential challenges related to the long timeframe needed. Careful planning, investment in research and development, and collaboration between various stakeholders, including governments, researchers, local communities, and industries, are essential for maximizing economic advantages while addressing potential drawbacks and ensuring responsible resource management [12,13].

6.1.1 References

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6.2 Practical application

6.2.1 Scope

With the increase in anthropogenic impacts, there is a growing burden on the environment caused by the accumulation of metals, which disrupt the ecosystem. Metals such as Cd, Pb, Zn, Cr, Ni, noble metals[i], and rare earth elements[ii] when present in high concentrations in the soil, can pose hazards to plants growing in that area. This can affect the plant's metabolism and overall growth. The bioaccumulation of metals in plants represents a risk to both humans and animals (Shan and Nongkynrih, 2007). The removal of excess metals from the soil can be achieved through various chemical or biological methods. Numerous agronomic experiments have been undertaken with early field trial studies, dating back to the 1980s and 1990s, and these studies have substantially advanced our understanding on phytomining agronomy. As described in section 5.1, phytomining entails cultivating a metal-hyperaccumulating plant species, harvesting its biomass, and then burning it to create a bio-ore (van der Ent et al., 2018; Jally et al., 2021; Laubie et al., 2021; Tognacchini et al., 2021; Zhang et al. 2021; Dihn et al., 2022a; Dihn et al., 2022b). According to Anderson et al. (1999), there are approximately 300 species of Ni-hyperaccumulators, along with 26 species of Co-, 24 species of Cu-, 19 species of Se-, 16 species of Zn-, 11 species of Mn-, 2 species of Tl-, and one species of Cd- hyperaccumulators (as indicated in Table 6.1). Initially considered scientific curiosities, these plants gained significance when Chaney (1983) and Baker and Brooks (1989) suggested their potential use in phytoremediation to extract pollutants from soils.

Table 6.1- Specific hyperaccumulators (natural and induced) that could be used for phytomining (Anderson et al., 1999).

Element *(in alphabetic order)*	**Species**	**Mean metal concentration (mg/kg $_{DW}$)**	**Biomass (t/ha)**
Cadmium	*Thlaspi caerulescens*	3,000 (1)	4
Cobalt	*Haumaniastrum robertii*	10,200 (1)	4
Copper	*Haumaniastrum katangense*	8,356 (1)	5
Gold[a]	*Brassica juncea*	10 (0.001)	20
Lead	*Thlaspi rotundifolium sub sp.*	8,200 (5)	4
Manganese	*Macadamia neurophylla*	55,000 (400)	30
Nickel	*Alyssum bertolonii*	400 (2)	9
	Berkheya coddii	17,000 (2)	22
Selenium	*Astragalus pattersoni*	6,000 (1)	5
Thallium	*Biscutella laevigata*	13,768 (1)	4
	Iberis intermedia	4,055 (1)	10
Uranium	*Atriplex confertifolia*	100 (0.5)	10
Zinc	*Thlaspi calaminare*	10,000 (100)	4

d.w. D dry weight.
a Induced hyperaccumulation using ammonium thiocyanate.
NB: values in parentheses are mean concentrations usually found in non-accumulator plants.

In their peer-reviewed article, Anderson et al. (1999) introduced an economic model for phytomining, illustrated in Figure 6.3. The model applies to both natural and induced hyperaccumulation. Factors influencing the economics include the plant's metal content, annual biomass production, and the potential for recovering and selling energy from biomass combustion.

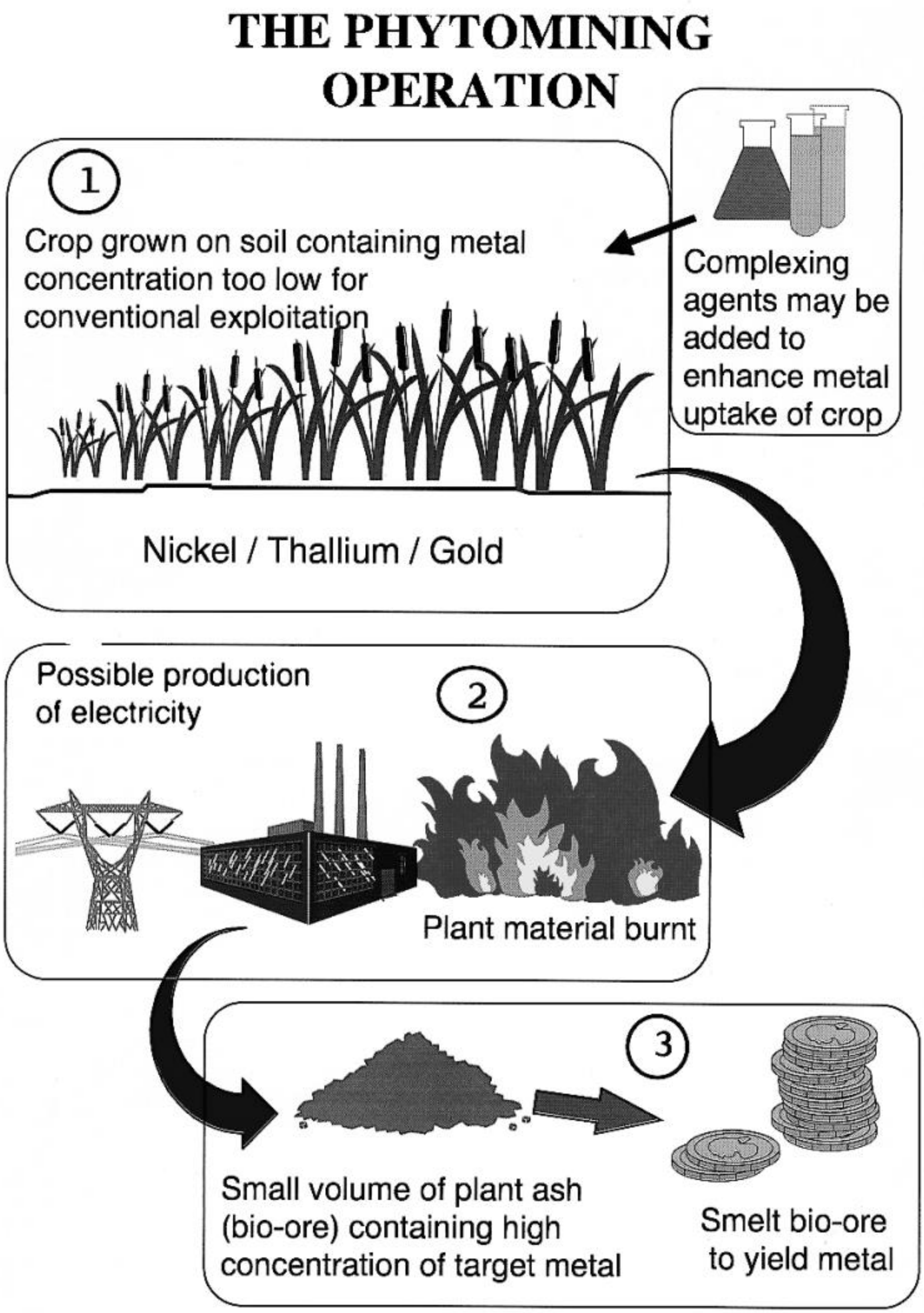

Figure 6.3- Economic model of a proposed system for phytomining for metals (Anderson et al., 1999).

In the following subsection, we assess the phytomining potential of three promising candidates: nickel, gold, and thallium. The selection of nickel (Ni), gold (Au), and thallium (Tl) for phytomining potential is driven by a combination of economic value, environmental concerns associated with conventional mining methods, and the unique ability of hyperaccumulator plants to extract and accumulate these metals. This approach aligns with sustainable resource management and offers the potential to reshape how these metals are sourced and recovered. In this section, we highlight their suitability and applications in the phytomining process, through a selection of reported case studies. Additionally, we discuss two possible scenarios for the future advancement of phytomining beyond theoretical and pilot plant stages.

6.2.2 Case studies of phytomining for nickel

*Alyssum murale (*Alpine Penny-cress) has been extensively studied for its remarkable ability to hyperaccumulate nickel. Field trials conducted by researchers in serpentine soil, a nickel-rich substrate, have demonstrated that *Alyssum murale* can accumulate high concentrations of nickel in its biomass (Chaney et al., 2007). This plant shows promise as a potential candidate for commercial-scale phytomining of nickel. Albania's ultramafic soil presents phytomining potential with *Alyssum murale*. A five-year field experiment by Bani et al. (2015) on an ultramafic Vertisol aimed to optimize cost-effective nickel phytoremediation, using *Alyssum murale* adapted to the Balkans. They studied plant phenology, element distribution, nutrition, fertilization, plant cover, weed control, and planting techniques on 18-square-meter plots. The mid-flowering stage was identified as the optimal harvest time, maximizing nickel concentration and biomass yield. N, P, and K fertilizers, specifically split 100-kg/ha N application, increased *Alyssum murale* density, shoot yield, and maintained biomass production. Graminaceous weed control required anti-monocots herbicide in natural stands. However, optimized fertilization and harvest minimized the benefits of weed control. Cultivating sown *Alyssum murale* outperformed enhancing native stands, resulting in higher biomass (0.3 to 9.0 tons/ha) and phytoextraction yields (1.7 to 105 kg/ha).

Berkheya coddii, a plant native to South Africa, has also been investigated for its exceptional nickel hyperaccumulation properties. Research conducted on *Berkheya coddii* has highlighted its ability to efficiently accumulate nickel (Robinson et al., 1997 and Robinson et al. 2003). The plant's capacity to extract and accumulate high levels of nickel in its foliage makes it a valuable species for phytomining efforts in nickel-rich areas. Keeling et al. (2003) investigated *Berkheya coddii*, a high-biomass Ni hyperaccumulator, for phytoextraction of Co and/or Ni from metalliferous media. Higher total metal concentrations in single-element substrates increased the bioaccumulation coefficient. *Berkheya coddii* readily accumulated Co with or without the presence of Ni, but equal Co concentration hindered Ni uptake. Bioaccumulation coefficients for Ni and Co (1000 µg/g total metal concentration) were 100 and 50, respectively. Co exhibited phytotoxicity above 20 µg g^{-1} total concentration, reducing biomass production without affecting bioaccumulation. In mixed Ni-Co substrates, bioaccumulation coefficients for both metals were 22. Phytotoxicity occurred above 15 µg g^{-1} total Co concentration. The coexistence of Ni and Co reduced bioaccumulation coefficients, indicating competition for root binding sites. The interference between Ni and Co uptake suggests limitations to phytomining when both metals are present.

In California, the initial phytomining trials involved the use of the Ni-hyperaccumulator plant species *Streptanthus polygaloides*. These experiments yielded 100 kg of sulphur-free Ni per hectare. The research group by Anderson et al. (1999) applied the same technique to assess the phytomining potential of Ni-hyperaccumulators *Alyssum bertolonii* originating from Italy and *Berkheya coddii* originating from South Africa. In Tuscany (Italy), they conducted in situ experiments to examine the impact of various fertilizer treatments on the growth of *Alyssum bertolonii*. The results showed that the plant's biomass could be increased nearly threefold (from 4.5 t/ha to 12 t/ha) without significant loss of Ni concentration (7600 mg/kg_{DW}) in the plant. Similar experiments were carried out using *Berkheya coddii*, which achieved a biomass yield of over 20 t/ha, although the Ni concentration was not as high as in *A. Bertolonii*. Nonetheless, the overall yield was considerably greater.

The discovery of Rinorea niccolifera in the Philippines has drawn attention due to its remarkable ability to accumulate high levels of nickel in its leaves. *Rinorea niccolifera* shows potential for utilization in phytomining operations within nickel-rich areas (Fernando et al., 2014). *Rinorea niccolifera* accumulates to >18,000 µg g-1 of nickel in its leaf tissues and is thus regarded as a Ni hyperaccumulator.

Odontarrhena chalcidica (synonym *Alyssum murale*), found in the Balkan, is known for its capacity to accumulate high concentrations of nickel. Research studies have evaluated its potential for phytomining in nickel-contaminated soils, highlighting its efficiency in nickel extraction. Northwestern Greece holds potential for phytomining with ultramafic Cambisols, while these soils in Spain and Austria are underutilized (Bani et al., 2021). *Odontarrhena chalcidica*, a Ni-hyperaccumulator, thrives widely on Balkan ultramafic soils, often as a spontaneous weed among crops. Recent field studies in the context of two recent EU-funded projects, Agronickel and LIFE-Agromine, examined *Odontarrhena chalcidica* and native species *Bornmuellera emarginata* and *Bornmuellera tymphaea* outside the Mediterranean (Bani et al., 2021). Comparison was made with local hyperaccumulator plants *(Noccaea goesingense* in Austria and *Odontarrhena serpyllifolia s.l.* in Spain). Between 2016 and 2021, project sites in Albania, Austria, Greece, and Spain annually imported 0.5 to 2 tonnes of hyperaccumulator biomass to the Lorraine University lab in France for the purpose of the LIFE-Agromine project (LIFE-Agromine, accessed 19 July 2023). The biomass was burned in a heat reclamation system boiler, providing a substantial portion of the laboratory's heating needs during winter months (excluding lockdown periods). Despite the biomass have an average calorific value, it proved sufficient for heating purposes. Approximately 100 kg of ashes were recovered from the burned biomass, yielding 12-15 kg of extracted nickel (LIFE-Agromine, accessed 19 July 2023). While potassium was identified as the most valuable by-product, its re-use was deemed cost-ineffective. The Agronikel project has as well demonstrated the significance of Ni availability in ultramafic soils, highlighted the effectiveness of organic manure fertilization, optimized density, and harvesting patterns, and achieved improved yields of 150-200 kg Ni per hectare per year. The project also ensures compatibility with EU regulations regarding energy recovery from biomass and the generation of by-products suitable for use as potassium fertilizers (Agronickel, accessed 19 July 2023). These studies aim to optimize Ni phytomining by developing soil and crop management practices, exploring fertilization regimes, crop selection, cropping patterns (with agroecological practices), and bioaugmentation using plant-associated microorganisms. Rosenkranz et al. (2019) conducted a field-scale test on the phytomining potential of *Odontarrhena chalcidica* and *Noccaea goesingensis*. The field experiment took place in the serpentine area of Eastern Austria in the province of Burgenland, starting in October 2016. *Odontarrhena chalcidica* achieved the highest Ni yield, reaching 55 kg Ni/ha in the sulphur treatment. *Noccaea goesingensis* attained its maximum yield of 36 kg Ni/ha in the high-density treatment. Further measures are necessary to optimize the Ni yield on this site. These measures include improving agronomic practices such as the selection and application of fertilizers, watering, and weed management.

Phyllanthus balgooyi, a Ni hyperaccumulator native plant known in Sabah, Malaysia, on the island of Borneo, has displayed promising nickel hyperaccumulation abilities. It contains over 16% Ni in its phloem sap, making it one of the highest concentrations of Ni in any living material worldwide. In a study by Mesjasz-Przybylowicz et al. (2016) nuclear microprobe imaging was used to examine the distribution of Ni and other elements in different parts of *Phyllanthus balgooyi*. The results revealed that Ni concentrations were exceptionally high in the phloem of stems and petioles, while significant enrichment occurred in major vascular bundles of leaves. The preferential accumulation of Ni in vascular tracts suggests its presence in a metabolically active form. This elemental distribution in *Phyllanthus balgooyi* differs from many other Ni hyperaccumulator plant species, where Ni is primarily accumulated in leaf epidermal cells. Research by Mesjasz-Przybylowicz et al. (2016) indicates that it can accumulate significant amounts of nickel in its shoots, suggesting its potential application in phytomining operations.

These case studies present examples of diverse Ni hyperaccumulator plants that exhibit potential for nickel phytomining. However, it's important to note that these plants' feasibility and commercial viability for large-scale phytomining operations are still subjects of ongoing research and development. Nkrumah et al. (2016) identified significant challenges and key research priorities for the commercial development and implementation of Ni phytomining, as outlined in Table 6.2.

Table 6.2- *Major challenges and research priorities for developing Ni phytomining around the world (Nkrumah et al., 2016).*

Steps to develop	**Ni phytomining Challenges**	**Research priorities**
Selection of Ni-rich soils	Phytoavailability of Ni in soils Topography/landform of sites Size of available land area Lease of land	Identify soils where Ni phytomining could be profitable. Develop Ni phytoavailability assays to predict Ni yield in metal crops. Negotiate land ownership agreements. Undertake repeated hyperaccumulator cropping experiments to assess the number of crop years possible for profitable phytomining.
Discovery and selection of 'metal crops'	Native crops are most suitable requiring screening be present at each locality Hypernickelophore species are very rare globally	There is the need for increased surveys especially in tropical regions. Breeding of improved cultivars to optimise growth rate and biomass production.
Soil and plant management practices	The Ni uptake and biomass yield of most potential phytomining 'metal crops' remain untested at field scale	Greenhouse or growth chamber trials to assess Ni uptake and biomass yield of such crops. Test the effect of other plant management practices such as fertilization, crop rotation and mixed cropping on Ni yield.
Harvesting techniques	Different cropping systems may require different harvesting techniques	Identify appropriate harvesting techniques suitable for each cropping system.
Post-harvest processing of nickel	Nickel recovery using smelter is profitable, while other high value products such as pure Ni salts currently have limited markets	Explore more methods of producing high value Ni products with potential markets in the near future from the biomass ash. Explore the production of Ni catalysts from biomass.

6.2.3 Case studies of phytomining for gold

Gold phytomining seems closer to practical application compared to other precious metals, and induced hyperaccumulation has been the primary approach in gold phytomining experiments. Gold has been extensively studied as promising candidate for phytomining. Many studies (Girling & Peterson, 1980; Warren and Delavault, 1950; Anderson et al., 1998) have demonstrated the ability of plants to accumulate gold, with certain plants considered hyperaccumulators if they accumulate more than 1 mg/kg_{DW} of gold. Although scientists have been intrigued by the ability of plants to uptake gold for over a century, no reliable natural hyperaccumulator species for gold has been reported, mainly due to its low solubility in soil. Induced hyperaccumulation is an alternative method for metal uptake in plants, where metals are solubilized in the soil solution, enabling passive uptake by plants. This technique, initially developed for phytoremediation using EDTA to solubilize heavy metals like lead in contaminated soils (Blaylock et al., 1997), allows several plant species to reach high concentration levels, up to 1% in dry tissue. In 1998, Anderson et al. first reported induced hyperaccumulation of gold by plants using a similar approach. The process of gold uptake by plants is complex, involving several steps (Sheoran et al., 2013): 1) solubilization of metal from the soil matrix, 2) uptake into the roots, 3) transport to the shoots, detoxification, and sequestration (see Figure 6.4).

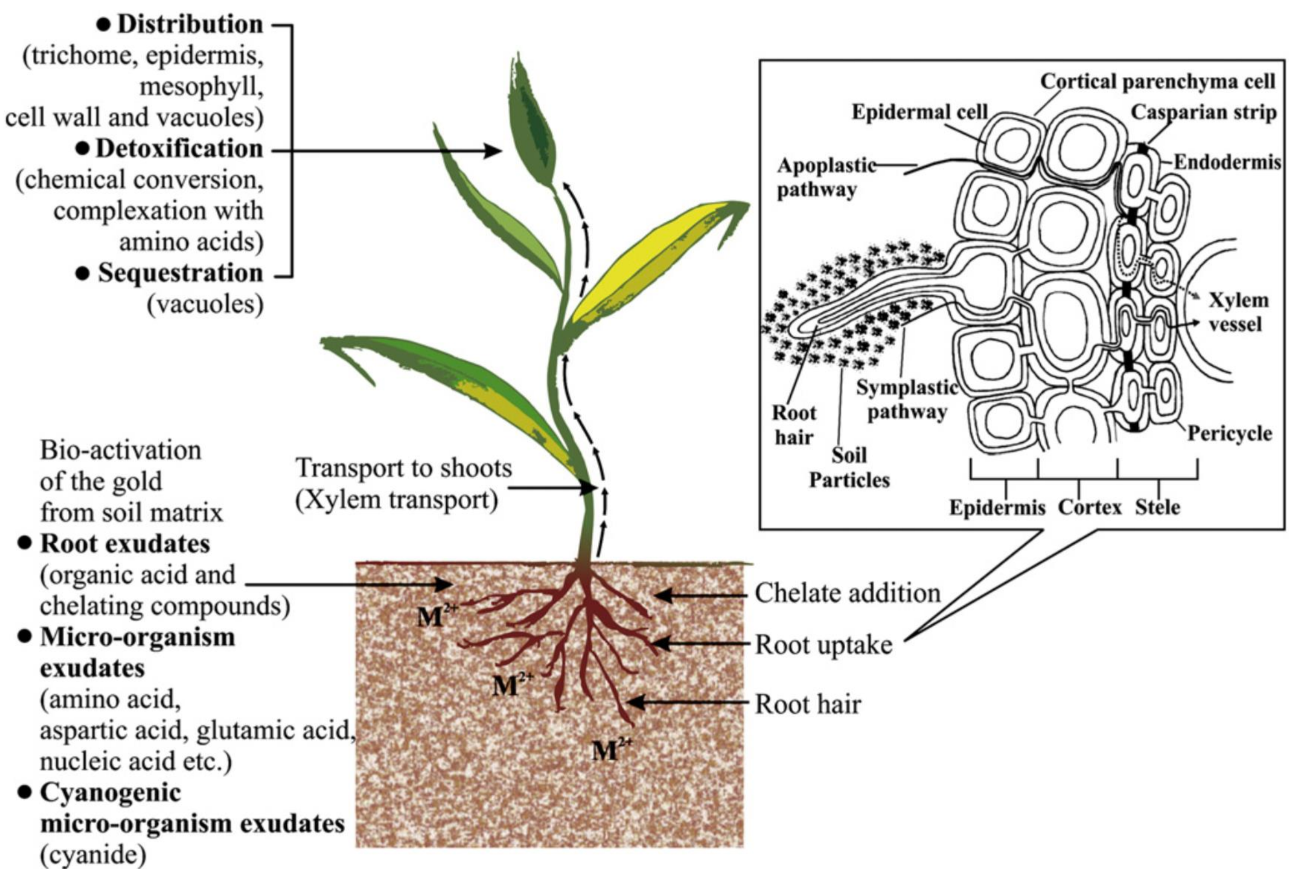

Figure 6.4- Mechanism of gold uptake by Sheoran et al. (2013).

In 2014, the gold accumulation ability of three plant species was tested: *Cyperus kyllingia* (nut grass), *Lindernia crustacea* (Scrophulariaceae), and *Paspalum conjugatum* (carabao grass) using cyanidation tailings containing 1.68 mg/kg_{DW} Au. To induce accumulation, sodium cyanide NaCN (1 g/kg_{DW}) and ammonium thiosulfate (NH4)2S2O3 (2 g/kg_{DW}) were added. However, *Paspalum conjugatum* only reached a maximum gold concentration of 0.602 mg/kg_{DW} in the shoot under the amendment of ammonium thiosulfate (Handayanto et al., 2014).

In 2016, another phytoextraction field trial (Krisnayanti et al., 2016) used Tobacco grown on cyanidation tailing substrate with 1.03 mg kg−1 Au and 18.2 mg kg−1 Ag, treated with 0.05 g kg−1 of NaCN (sodium cyanide). In the field conditions, mean gold and silver concentrations in Tobacco reached 1.2 and 54.3 mg kg−1, respectively.

In 2018, González-Valdez et al. evaluated *Brassica napus* (Rapeseed) for gold extraction from mine tailings with 0.5164 mg kg−1 Au. Under the effect of NH_4SCN (ammonium thiocyanate), the gold concentration in the stems reached 1.5 mg kg−1, and in the roots, it was approximately seven times higher than in the shoots of the plant.

In 2003 and 2005, Anderson et al. conducted preliminary research revealing the promising potential of certain local terrestrial wild cultivars of plants to accumulate substantial amounts of gold, up to 30-40 mg/kg_{DW} of plant dry weight (based on unpublished data). This accumulation was facilitated by using chelating agents known to enhance gold availability to plant roots. The induced gold concentration in a plant depends on the gold content in the soil where it is grown. Experimental findings by Anderson et al. (2003) suggested that plants can accumulate approximately 20% of the total available gold within the root zone, influenced by the specific chelating agent employed. This 20% recovery trend has been observed across various tested plant species. A study at the Fazenda Brasileiro Gold Mines in Brazil by the Anderson et al. (2003 and 2005) showcased the cost-effectiveness of phytomining technology. *Brassica* sp. and *Zea mays* plants acted as hyperaccumulator plants in ore with an extraction grade of around 1.5 g/t. The results demonstrated promising outcomes, affirming phytomining's potential as a viable and economical technology.

6.2.4 Case studies of phytomining for thallium

Thallium, known for its extreme toxicity, has diverse applications ranging from rat poison and ant control to its use in the electronics industry for semiconductors, switches, and fuses. Because of this uses and potential to be agromined, thallium has perhaps the greatest potential to be economically successful. Despite this promise, thallium has received relatively little attention. Certain plant species have the remarkable capacity to efficiently absorb and accumulate thallium, making them valuable for metal recovery purposes (Anderson et al., 1999; LaCoste et al., 2001). In a study by Anderson et al. (1999), it was found that whole *Iberis intermedia* and *Biscutella laevigata* plants from the *Brassicaceae* family contained thallium levels of 4 kg/t and 15 kg/t (dry weight) respectively. These findings highlight the potential of phytomining for future mining of low-grade metal ores. Leblanc et al. (1999) discovered high thallium hyperaccumulation in Iberis intermedia and *Biscutella laevigata* plants growing on mine tailings in France. *Iberis* had up to 4000 mg/kg_{DW} thallium with a biomass of 10,000 kg/ha, while *Biscutella* had over 14,000 mg/kg_{DW} thallium with a biomass of 4000 kg/ha Similar results were found in New Zealand. This exceptional Tl accumulation holds significance for animal and human health, phytoremediation of contaminated soils, and Tl phytomining.

In the late 1990s, Anderson et al. (1999) presented the economics of Thallium phytomining. Hyperaccumulator plants yielded bio-ore with 8 kg of thallium per hectare, valued at 2200 EUR (world price of $US 300/kg). To be economically viable, phytomining should achieve 460 EUR/ha, regardless of revenue from biomass incineration. Iberis intermedia, with a biomass of 10,000 kg/ha, would need at least 170 mg/kg_{DW} thallium, achievable with this plant. Biscutella laevigata, with a biomass of 4000 kg/ha but higher thallium concentration, would need approximately 425 mg/kg_{DW} thallium, with 39% of plants exceeding this threshold. Biomass incineration could add 120 EUR/ha for Iberis and 49 EUR/ha for Biscutella, based on assumptions by Nicks and Chambers (1998).

6.2.5 Future developments of phytomining

The potential of phytomining can be enhanced by identifying fast-growing plants with high biomass and the ability to accumulate metals in harvestable parts and through plant breeding. Metal accumulation, translocation, and sequestration in plants involve multiple genes, and introducing these genes into candidate plants through genetic engineering is a viable strategy for improving phytoremediation traits (Chaney et al., 2007).

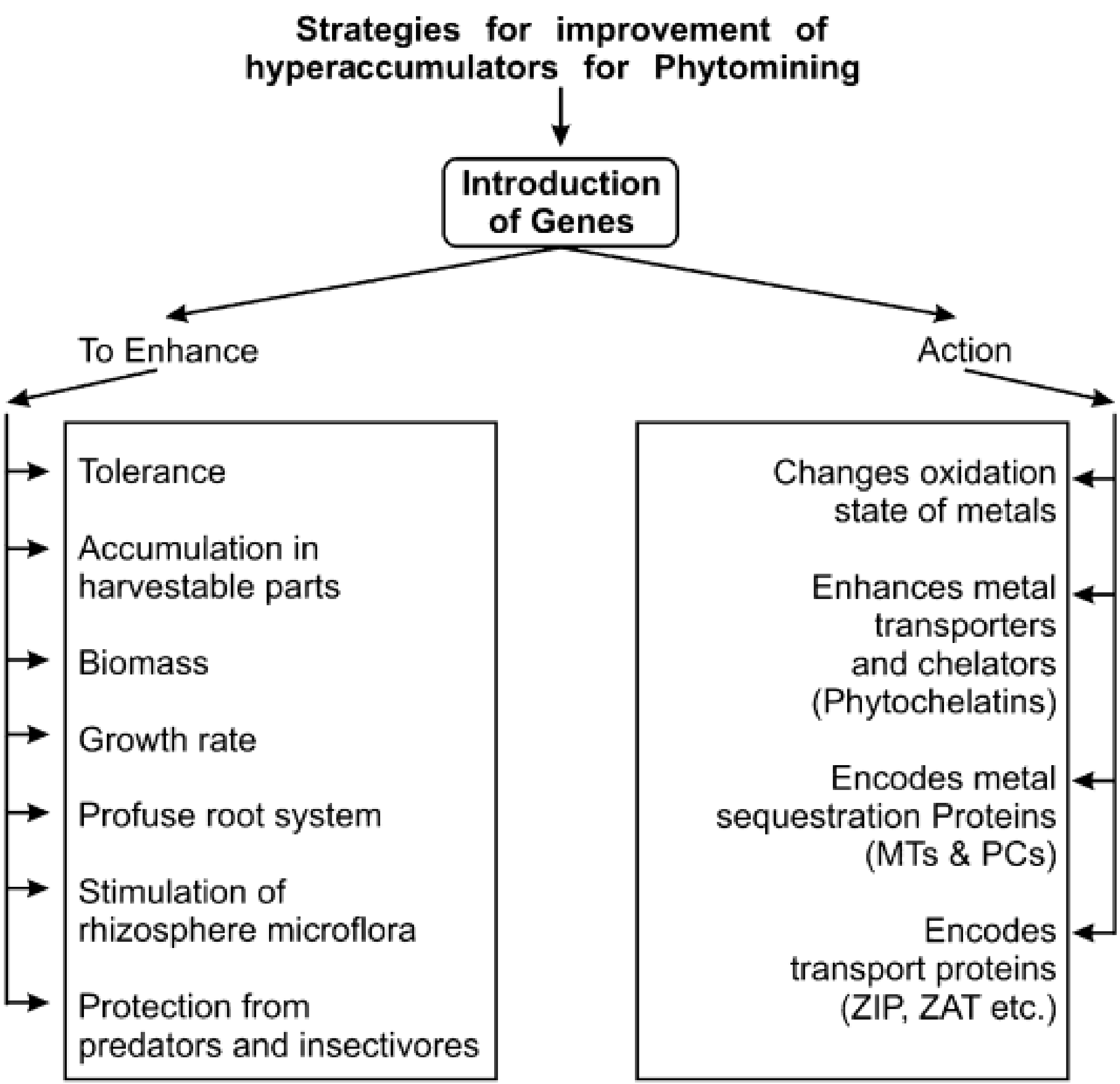

Figure 6.5- Strategies for improvement of hyperaccumulators using genetic engineering (PCs-phytochelatin, MTs-metallothioneins) by Sheoran et al. (2009).

Selecting individuals with genetic traits for high metal content, high biomass production, and superior tolerance to soil heavy metal content not only improves metal crop yields, but also provides genetic material that can be transferred to other plant species. Genetic engineering is currently employed to enhance metal hyperaccumulation in plants by modifying metal oxidation states, improving metal transporters and chelators, and encoding metal sequestration proteins such as encoding metal sequestration proteins (MTs and PCs), and encoding transport proteins such as ZIP family proteins (zinc–iron permease), and ZAT (Zn transporter) Sheoran et al. (2009). Further research is needed to understand the forms of metal complexes within plants.

If phytomining advances beyond theoretical and pilot plant stages, two possible scenarios can be envisioned according to Sheoran et al. (2009) as depicted inFigure 6.5. The first scenario involves large-scale commercial projects, spanning several square kilometers of low-grade metalliferous soil. The second scenario, which is more promising, entails phytomining being decentralized to small-scale land owners in the region. Peasant farmers could cultivate a few hectares of the plant material, harvest it, and process it in proximity to urban areas where industrial equipment can be utilized for plant processing. This process could generate steam to produce local electricity supplies. Locations with sub-economic metal mineralization and ultramafic soils are ideal for the small-scale farmer's scenario (Brooks et al., 2001).

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7 REMEDIATION TRAIN

Remediation train: ReSoil® + phytomanagement

Remediation train ReSoil® + phytomanagement (Gluhar et al., 2021b) is used to enhance biodegradation of organic pollutants in toxic metal and metalloid contaminated post-industrial soils and improve the quality of remediated soil. Remediation train is composed of a physico-chemical method and phytomanagement to remove pollutants (metals and metalloids and organic pollutants) from multiple pollutant-contaminated soils. According to Robinson et al., (2009) phytomanagement describes the manipulation of soil-plant systems to affect the fluxes of pollutants in the environment to remediate contaminated soils, recover valuable metals and metalloids, or increase micronutrient concentrations in crops. Phytomanagement includes all biological, chemical, and physical technologies employed on a vegetated site. After ReSoil®, soil chemical and biological properties of the soil are largely preserved. Adding nutrient-rich organic substrates to the slurry phase during the ReSoil® process can stimulate the indigenous microbial population of washed soil to enhance biodegradation of organic pollutants. The two-stage process can be effective in the removal of both, metals and metalloids and organic pollutants.

7.1 Description of the technology

7.1.1 Two-stage remediation

The technique is designed as a two-stage remediation; two remediation technologies are incorporated in one remediation train (Figure 7.1). In the first stage (Stage I), a sustainable soil extraction technology, ReSoil®, is used to efficiently remove toxic metals and metalloids from contaminated soils. ReSoil® preserves the soil as a natural substrate. In Stage I, some special supplements such as detergents, oil absorbents, etc. can be used to also remove some of the PH and PAH. In the second stage (Stage II), organic pollutants (e.g. PAHs) are removed and healthy soils are created by "green" technologies: bioremediation and phytomanagement. This two-stage remediation technology ensures fully functional and healthy soils without potentially harmful residues (metals and metalloids, and organic pollutants) in the remediated soils.

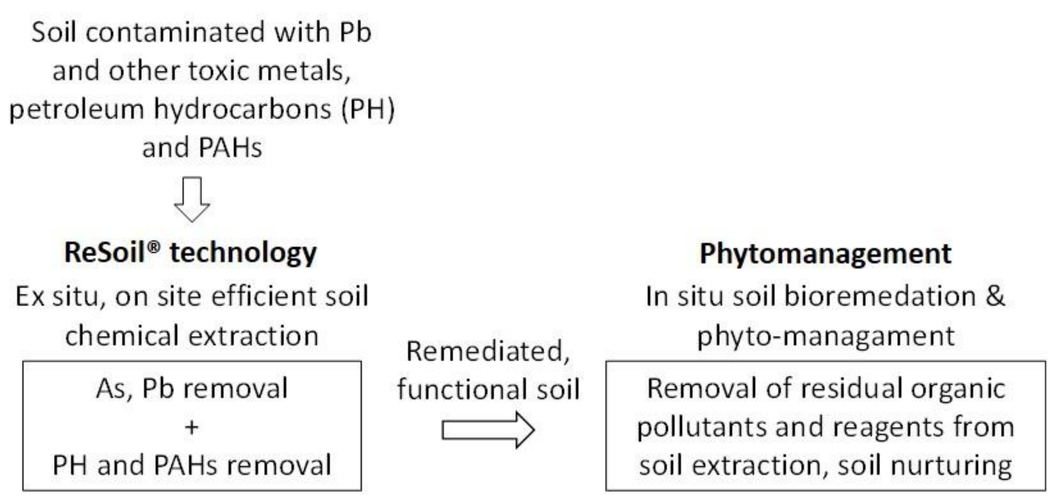

Figure 7.1- The remediation train is comprised of two stages, the ReSoil® technology + phytomanagement.

7.1.2 ReSoil® technology description

Removal of As, Pb and other pollutants from soil:

EDTA is the most efficient and tested chelator. Toxic metals such as Pb form strong water-soluble complexes (chelates) with EDTA and are thus removed from the solid phase. The anionic metalloids, such as As, do not interact directly with EDTA. Therefore, simultaneous removal of As and toxic metals from contaminated soils is very challenging; they cannot be removed by the same chemical mechanism. The main sinks for As in soil are Fe oxides-hydroxides. In ReSoil®, As bound in amorphous Fe oxides-hydroxides is extracted by oxalic acid; to extract As from crystalline Fe oxides-hydroxides, simultaneous reductive dissolution with Na-dithionite is required. As part of the same process, EDTA is used to chelate the released Fe, preventing precipitation of a new Fe oxide-hydroxide phase and re-adsorption of As.

The simultaneous removal of As and Pb and other toxic metals from used washing (uWS) and other process solutions (uRS) and the reuse of EDTA and process waters is one of the most innovative features of ReSoil® Stage I. The process produces no wastewater. During soil washing the As-containing amorphous and crystalline Fe oxide-hydroxide soil fractions are dissolved by oxalic acid and Na-dithionite, respectively. The released Fe is chelated with EDTA, which prevents precipitation of a new Fe oxide-hydroxide phase and readsorption of As. The As and Fe-EDTA appeared in used washing and other process solutions along with Pb-, Zn-, and Cd-EDTA chelates. After alkalinisation to pH > 12.5 with quicklime (CaO), Fe leaves EDTA chelate and precipitates as hydroxide. The solid precipitates are removed from the treated process solution by filtration as solid waste, which is safely disposed. The selected process solution (rinsing solution) is acidified to pH 2 after alkaline phase by adding H_2SO_4 to precipitate and recover the remaining EDTA in acidic form by filtration. At the same time, the excess Ca^{2+} and SO_4^{2-} ions are precipitated as insoluble gypsum ($CaSO_4$), which is removed with the remediated soil as a soil conditioner beneficial for soil re-aggregation. The recovered EDTA and treated process solutions are reused in the next in the series of batches.

Oxalic acid is not present in used washing and other process solutions. Oxalic acid precipitates during washing of (calcareous) soil or with Ca^{2+} after alkalinisation of process solutions with CaO and is removed from solution after solid/liquid separation in a filter press. Oxalic acid forms a highly insoluble salt, Ca-oxalate, with Ca over a wide pH range. The Ca-oxalate mineral is also naturally present in soils formed by fungi and in the rhizosphere from plant exudates of oxalic acid. It is used by saprotrophic microbes and some mesofauna as a source of energy and C. Likewise to oxalic acid, Na-dithionite is not detected in the used washing and other process solutions. Na-dithionite is a labile compound that is rapidly disproportionated in aqueous solutions. Under oxidative conditions during ReSoil® soil extraction, it converts to sulphite (HSO_3^-) and sulphate (HSO_4^-) and finally precipitates as gypsum, which is removed with the remediated soil after solid/liquid separation.

The ReSoil® recycles EDTA mainly in the form of Ca-EDTA (and approx. 20 % as acidic H_4EDTA). The chelation of toxic metals by Ca-EDTA is kinetically hindered relative to Na-EDTA, resulting in long soil extraction time (> 12 h). In ReSoil®, oxalic acid added to the treated washing solution shortens the required soil extraction time to < 1 h. The reason is the stability of Ca-EDTA chelate, which decreases with the acidity of the solution, while oxalic acids forms strong chelates with Ca. Oxalic acid therefore captures Ca from Ca-EDTA and forms insoluble Ca-oxalate, thus activating EDTA.

In ReSoil® (Figure 7.2), zero valent Fe (ZVI) is added to the soil slurry immediately before solid/liquid separation, effectively containing toxic emissions from the remediated soil and immobilising pollutants that could not be removed by washing and remain in the remediated soil.

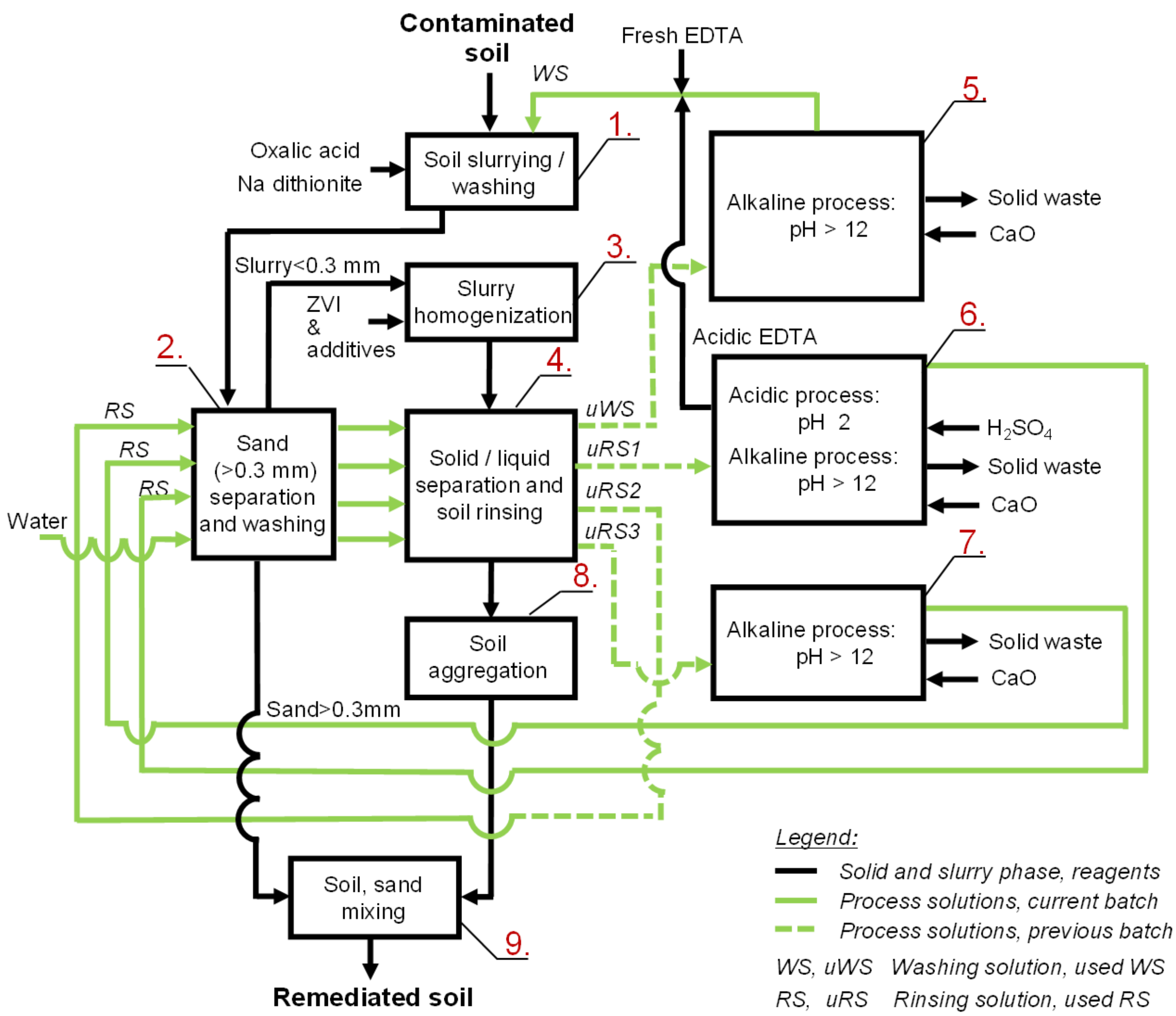

Figure 7.2- The ReSoil® process steps

After addition, a highly adsorptive oxide-hydroxide shell is formed around the ZVI core. The As is forming inner-sphere complexes with the oxide-hydroxide shell, with both As reduction and oxidation occurring in parallel and independently in the oxide-hydroxide shell and the metallic core of ZVI. The absorbed As impregnates into the solid phase by immobilisation mechanism involving adsorption, reduction, oxidation, and complex formation. The oxide-hydroxide shell of ZVI also provides the sites for metal cation adsorption, while the Fe core provides a reducing force for immobilization of adsorbed metals. This dual property of adsorption and reduction endowed ZVI a superior ability to sequestrate toxic metals such as Pb with a more positive standard redox potential than Fe. Pb is initially bound to the oxide-hydroxide shell of ZVI by physical sorption, then strongly bound by chemisorption, and finally some parts of the adsorbed Pb are reduced to Pb^0 and strongly immobilised. Zn and Cd have a more negative standard redox potential than Fe, and the reduction reactions are not involved in the ZVI-based sequestration process. Zn and Cd can only be adsorbed on the ZVI oxide-hydroxide shell, which is mostly positively charged at alkaline pH and attracts anions. Toxic metals are therefore also adsorbed as chelates with EDTA, which are negatively charged over the wide pH range. Surface complexation at the outer sphere is the dominant adsorption mechanism of EDTA and EDTA chelates, but surface complexation at the inner sphere (bidentate dinuclear adsorption on goethite and monodentate adsorption on hematite) was also reported.

7.1.3 Phytomanagement description

Phytomanagement (Stage II) includes active and passive bioremediation, which is conducted after the soils are treated in Stage I and returned to the excavation site. In the active phase, fast-growing, short-season crops (which can be sown from spring to late summer) such as buckwheat (*Fagopyrum esculentum*) and rapeseed (*Brassica napus*) are sown as the first crop and then mulched as green manure. Buckwheat and rapeseed have branching root systems that reach deep into the soil and improve aggregation of the remediated soil (with lost natural structure) through an extensive network of fine roots. In this phase, earthworms, vermicompost, compost, and manure can be added to boost soil microbial activity in the soil to enhance the biodegradation of organic pollutants that remain in the soil after soil extraction (Stage I). The active phase is followed by the passive, post-remedial natural attenuation phase. The reason for post-remedial phase is that some beneficial remedial effects can be expected even after the active operations have been completed. For example: it is known that intensive microbial processes in the plant rhizosphere during phytomanagement promote the degradation of various environmentally harmful xenobiotics.

Figure 7.3- Preparation of soil for vegetable production by growing buckwheat as the first crop after remediation (raised bed 7), harvesting (raised bed 8) and mulching the buckwheat biomass (raised bed 9).

7.2 Practical application

7.2.1 Large-scale study on the ReSoil® process (Stage I) and preliminary study on the use of remediated soil as a substrate for phytomanagement - growing plants (Stage II)

The sustainability of the ReSoil® soil extraction remediation technology, which is an important part of this remediation train, has been demonstrated in numerous articles. The main study case was conducted in the demonstration gardens established in the town of Prevalje (Slovenia) near the demonstration plant, where 1 t of contaminated soil per batch can be treated. The area of the Meza valley in Slovenia is a site of more than 300 years of Pb and Zn mining and smelting. The surface soil (0–30 cm) was excavated from grassland on the banks of the Meza River in the town of Prevalje (14°93’73” E and 46°54’57” N). The site was contaminated by river sediments after occasional flooding. In situ investigations using a portable X-ray fluorescence spectrophotometer (XRF, see below) showed a strong concentration gradient of Pb contamination from the riverbank. The excavated soil (approximately 35 m3) was homogenised in situ and then transported to a nearby remediation facility for soil washing using ReSoil® technology. The technology Readiness Level of the plant operation was TRL 7 (EU, NASA methodology). For this study, the contaminated soil was remediated in a series of 16 batches, washing a total of 16 t of soil. The average concentrations of toxic metals were 1854.0 ± 69.4 mg kg^{-1} Pb, 3833.2 ± 77.8 mg kg^{-1} Zn and 21.2 ± 0.7 mg kg^{-1} Cd in the original soil and 545.1 ± 9.6 mg kg^{-1} Pb, 2743.4 ± 69.4 mg kg^{-1} Zn and 9.9 ± 0.2 mg kg^{-1} Cd in the remediated soil. On average, remediation reduced the concentration of Pb, Zn and Cd by 71, 28 and 54%, respectively. Zn removal was characterized by lower extractability, likely due to the predominant Zn association with non-labile soil fractions.

The vegetable garden with 9 raised beds was planted in July 2018 (Figure 7.4). Each raised bed (4 × 1 × 0.5 m) was filled with approximately 1.75 t of soil. The soil was fertilized with 120 g m^{-2} NPK (15:15:15) and 40 g m^{-2} MnSO4. Six beds (Nos. 2, 4, 5, 6, 8, and 9) were filled with remediated soil, and three (No. 1, 3, and 7) with non-remediated (original) soil, which served as controls. The beds with original (Orig) and remediated soil (Rem) were randomly selected. Fast-growing buckwheat (*Fagopyrum esculentum*) was the first crop sown as green manure on 19 July 2018. After 6 weeks of growth, 4.4 kg (wet weight) m^{-2} of the mulched buckwheat biomass was buried in the soil with a shovel. Immediately after green manuring, three Rem beds were amended by adding 3.1 t (dry weight) ha^{-2} of vermicompost containing approximately 0.008 kg^{-1} of *Eisenia fetida* earthworms, 0.11 kg (dry weight) m^{-2} of rhizosphere soil with indigenous mycorrhizal fungi, and 18 species m^{-2} of grey earthworms *Aporrectodea caliginosa* to obtain a remediated and vitalized soil (Rem+V). Vermicompost was obtained from a local farmer. It was produced with *Eisenia fetida* earthworms fed with kitchen waste. Rhizosphere soil was prepared by chopping the rhizosphere soil with roots from local grassland (but not in the contaminated area) dominated by mycorrhizal plant species. Vermicompost and rhizosphere soil were carefully dug into top 5 cm of the soil with a rake (Gluhar et al., 2021).

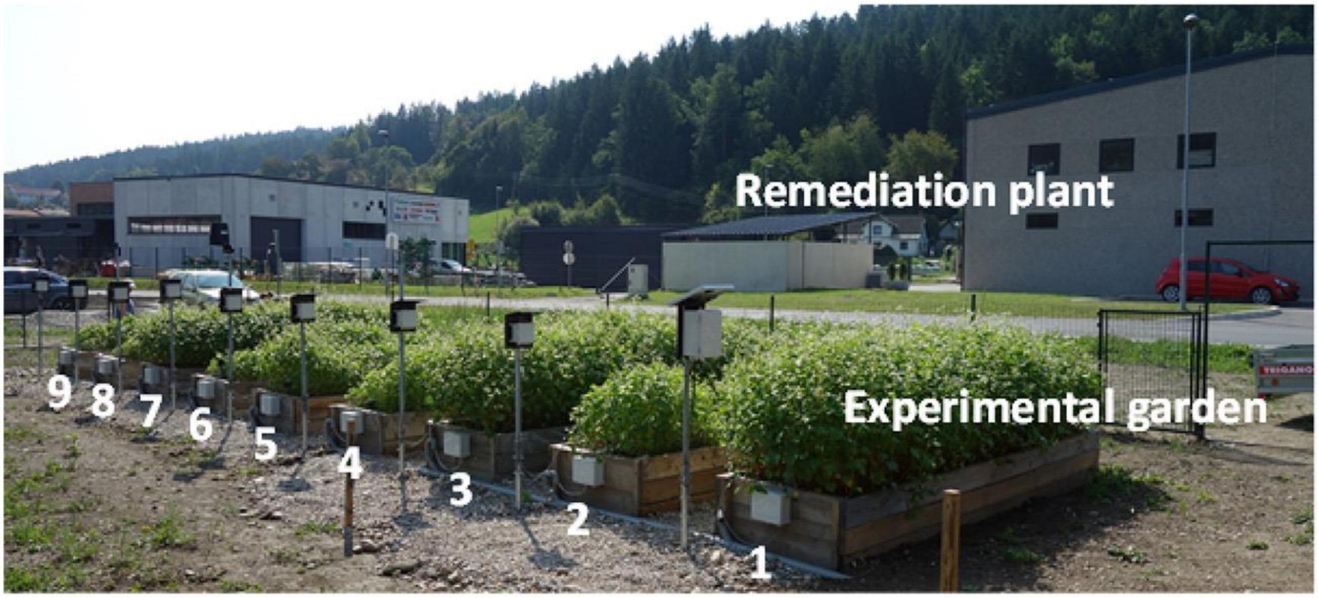

Figure 7.4- The experimental vegetable garden with nine raised beds constructed as lysimeters in the vicinity of demonstrational remediation plant with ReSoil® technology.

In this study, we also evaluated the effects of washing Pb-, Zn-, and Cd-contaminated soil using EDTA-based technology (ReSoil® - Stage I) on soil biological properties by measuring some of the most commonly used/sensitive biological indicators of soil perturbation. We estimated the temporal dynamics of soil respiration, the activities of soil enzymes (dehydrogenase, β-glucosidase, urease, acid and alkaline phosphatase), and the effects of the soil washing on arbuscular mycorrhizal (AM) fungi in original (Orig), remediated (Rem) and remediated vitalized (Rem+V) soils during a more than one-year garden experiment. ReSoil® technology initially affected the activity level of soil microbial respiration and all enzyme activities except urease, and reduced AM fungal potential in the soil. However, after one year of vegetable cultivation and standard gardening practices, soil microbial respiration, acid and alkaline phosphatase in the Rem and Rem+V reached similar activities as in the Orig. Only the activities of dehydrogenase and β-glucosidase remained lower in the remediated soil compared to the Orig. The frequency of arbuscular mycorrhiza in the root system, arbuscular density in the colonized root fragment, and the intensity of mycorrhizal colonization in the colonized root fragments in the remediated treatments increased with time. At the end of the experiment, no consistent differences in these parameters of mycorrhizal colonization were found among the treatments. Our results suggest a restored biological functioning of the remediated soil after one year of vegetable cultivation. In general, no differences were found between the Rem and Rem+V treatments, indicating that simple common garden practices are sufficient to restore soil functioning after remediation or sustainable metal extraction (Kaurin et al., 2021).

7.2.2 Pilot study on the modified ReSoil® process (Stage I) and preliminary study on the use of remediated soil as substrate for phytomanagement - growing plants (Stage II)

The second case study was conducted on a pilot scale. The same soil was used as in the larger scale study explained/demonstrated above, but this time it was additionally artificially contaminated with As. For the pilot-scale operation, the novel technology was embodied in modified ReSoil® process. ReSoil®, originally developed to remove toxic metals from contaminated soils, was modified by adding oxalic acid and Na-dithionite to the soil slurry. This enabled shorter washing times and removal of metalloids. 15 kg of air-dry soil per batch was washed for 1 h with 100 mM oxalic acid, 50 mM Na-dithionite, and approximately 90 mM EDTA. The contaminated soil was washed in 9 consecutive batches. This removed 55–65% of As, 74–80% of Pb, 26–33% of Zn, and 47–57% of Cd.

Standard pedological analysis was used to assess the chemical and mechanical properties of Orig and Rem + ZVI. Sustainable metal an metalloids extraction had minor effects on soil organic C (SOC) content, available K content (measured as K2O), soil carbonates, and soil texture. The average pH was higher in Rem + ZVI, which could be attributed to the use of quicklime in the treatment of process solutions. Soil washing decreased the total N (TN) content, which slightly increased the soil C/N ratio. The concentration of available P (measured as P_2O_5) was 2.7 times lower in Rem + ZVI compared to Orig. Overall, the remediation technology did not irreversibly impaired soil quality. For example, N and P are essential elements for plants and soil life, but their loss and reduced availability in remediated soils were easily amended by soil fertilization. Since late season (October 16, 2020), winter crop rapeseed (*Brassica napus*) was sown to evaluate the effects of remediation on plant growth and toxic metal uptake. Biomass yield was significantly higher under Rem + ZVI at 0.37 ± 0.01 kg dry biomass m^2 than under Orig at 0.20 ± 0.01 kg m^2. The difference in biomass yields can be partly explained by differences in plant preferences for soil properties such as pH, which changed after remediation. ReSoil® extraction reduced the concentration of Pb in the green parts of rapeseed 5.0 times, Zn 2.6 times, and Cd 9.0 times. As concentration in plants grown on Rem + ZVI was below the detection limit (LOQ = 0.2 mg/ kg_{DW}), while 5.36 mg/kg_{DW} of As was detected in rapeseed grown on Orig. Six enzymes were measured after the rapeseed harvest (June 4, 2021). Soil washing increased dehydrogenase activity by 3.2 times compared to Orig. FDA hydrolysis was not affected. Dehydrogenases and FDA hydrolysis are involved in microbial degradation of organic substances and are used as indicators of soil microbial activity. Dehydrogenases represent immediate metabolic activities of soil microorganisms, and it appears that more active microbes were present in Rem + ZVI than in Orig. This could be linked to the higher plant biomass in Rem + ZVI, as microbial activity is closely related to root exudates and plant residues. The β-glucosidase activity, a C cycling enzyme, was significantly affected by remediation and was on average 1.8 times lower than in Orig. Alkaline phosphatase activity was also reduced in Rem + ZVI compared to Orig (1.4 times on average), while no statistically significant differences were found for acid phosphatase. Acid phosphatase activity was lower than alkaline phosphatase activity, which predominates in calcareous soils with near alkaline pH. Urease activity, which is related to N cycling, was on average 1.3 times higher in Rem + ZVI than in Orig, but the differences were not statistically significant due to the large standard deviation in Orig. In general, FDA hydrolysis, dehydrogenase, acid phosphatase, and urease activities recovered after remediation, whereas β-glucosidase and alkaline phosphatase activities remained significantly lower. It has been shown that microbial and enzyme activity takes some time to recover after soil washing and can be shifted back to the original structure by simple agricultural practices such as fertilization and planting, as shown in previous studies (Morales Arteaga et al., 2022).

7.2.3 Pilot study of combined modified ReSoil® process (Stage I) and phytomanagement (Stage II, active phase)

For the third case study, soil from the second case study was used, and was additionally artificially contaminated with Cu, pyrene (model for polyaromatic hydrocarbons, PAHs), and mineral oil (model for petroleum hydrocarbons, PEs). Some of the artificially contaminated soil (15 kg) was slurryed in a polymer-coated vessel (80 L) with 22.5 L of washing solution (WS) recycled from previous in series of batches. The WS contained approx. 100 mM of EDTA. Oxalic acid (100 mM), Na-dithionite (50 mM) and 0.5% of a surfactant mixture (SDS and Tween 80) were added to the slurry. The slurry was washed by mixing for 1 h. Then, the sand fraction (> 2 mm) was separated from the slurry by wet sieving in a newly constructed trommel and washed with the three rinsing solutions (RS) recycled from the previous in series of batches, and with fresh water. The slurry (< 2mm) was mixed with 1% (w / w) of zero-valent Fe (ZVI, < 0.5 mm granules) and 1% of rapeseed oil treated sawdust. The slurry was transferred to a chamber filter press where the washed soil was separated from the used washing solution (uWS). The washed soil in the press was rinsed with three RS and water from the sand-washing step. Blocks of washed and rinsed soil from the filter press were milled to obtain artificial soil aggregate grains, approx. 5 mm wide, and mixed with washed sand to constitute the final product of the soil washing process. The phytomanagement experiment was conducted in a 2 x 2 m wide and 1.5 m high greenhouse made of wood and PTE foil, containing 9 pots (23 x 23 cm wide and 20 cm high) constructed as lysimeters (Figure 7.5), surrogates of lysimeters raised beds.

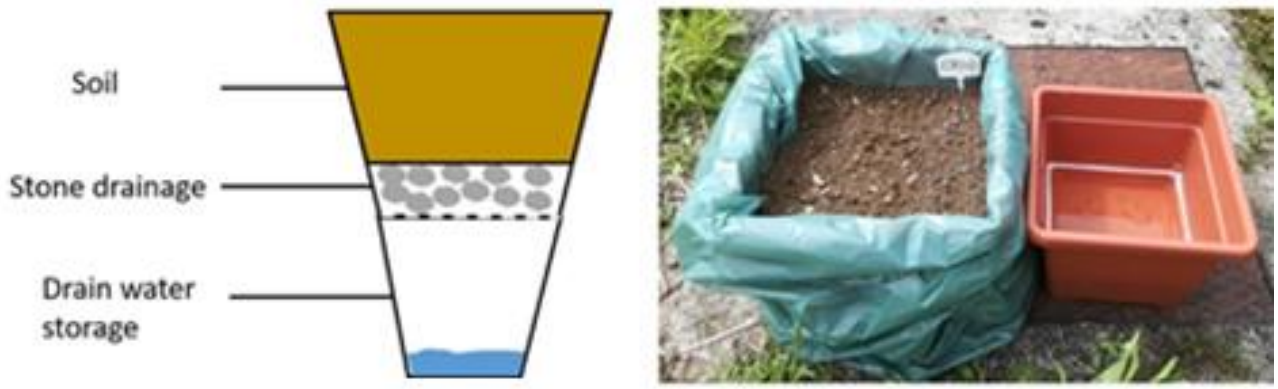

Figure 7.5- Experimental lysimeter pots, 23 x 23 x 20 cm, constructed to demonstrate functioning of remediated soil as a plant substrate and natural habitat and to demonstrate no-emissions from remediated soil and safety of ReSoil® technology.

The concentration of pollutants in the leachates of soil remediated with ReSoil® was quite low during the phytomanagement process. The As was not even detected. As explained above, phytomanagement comprises of active and passive bioremediation. The active phase includes biodegradation of organic pollutants (especially in plant rhizosphere), transformation and immobilisation of residues, and improvement of soil properties through plant-promoted soil reaggregation. The first crop sown was the fast-growing short-season rapeseed (*Brassica napus*) (Figure 7.6), which was then mulched as a green manure. There was no visible evidence of any effect of remediation on plant germination and development.

Using this remediation train (ReSoil® + phytomanagement) we demonstrated effective simultaneous removal of toxic metalloids, toxic metals and organic pollutants from (partly) artificially contaminated soils on a pilot scale. We removed up to 64% of As, 84% of Pb, 33% of Zn, 68% of Cd, 69% of Cu, 68% of pyrene (model for PAHs, after 2 weeks of phytomanagement) and 37% of mineral oil (model for PHs, after one week of phytomanagement). It is expected that the contamination levels of organic pollutants can be further reduced if the duration of active (biodegradation) and passive (phytomanagement, natural attenuation) bioremediation processes is longer.

Figure 7.6- ***The collection of leachates and phytomanagement of ReSoil® remediated soil with rapeseed (Brassica napus).***

7.2.4 ReSoil® technology reach

Applications suitable for ReSoil® technology

Soils contaminated with toxic metals (e.g. Pb, Zn, Cd, Cu) and toxic metalloids (As, Sb) from various gaseous and liquid industrial emissions (e.g. smelters, foundries, dumping or burning of lead batteries), traffic emissions (e.g. leaded gasoline), peels of external lead-based paint, heavy metals containing pesticides (i.e. lead arsenate, copper sulphate), fertilizers (i.e. Cd in phosphates), ammunition (i.e. shooting grounds), the fallout from the discharge of community waste incinerators, soil contaminated by old plumbing and lead and zinc roofing (i.e. burning of Notre Dame in Paris).

Applications not suitable for ReSoil® technology

Ores, tailings, ashes, sludges…and other solid materials from mining, smelting, and other industries, where heavy metals are present and entrapped in mineral forms (i.e. silicates) and not accessible by EDTA and also not bio-accessible/-available. ReSoil® is not a metallurgical process.

7.3 References

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8 CONCLUSIONS

This report summarizes the latest information on phytoremediation, that could help the distinct stakeholders such as site owners, surrounding community, project managers, contractors, regulators, and other practitioners to understand all the information emanating from each remediation project. Phytoremediation is the general technique that applies the use of plants to remediate selected pollutants partially or substantially in contaminated soil, sludge, sediment, groundwater, surface water, and waste water, using a variety of plant biological processes and the physical characteristics of plants. Generally, phytoremediation is considered as a low-cost remediation technology, requiring a relatively long time to be effective. As a positive side effect, of phytoremediation involves greens areas, which has a positive impact on human health and well-being.

The application of phytoremediation and the selection of appropriate plants depend on a series of site-specific characteristics. Moreover, application requires knowledge from different disciplines, i.e., plant physiology, ecology, pedology, chemistry, and physical sciences. There is potential to use phytoremediation beneficially under a wide variety of site conditions. Type of sites at which phytoremediation has been applied or evaluated includes: pipelines; industrial and municipal landfills; agricultural fields; wood treating sites; military bases; fuel storage tank farms; gas stations; army ammunition plants; sewage treatment plants; and mining sites. Phytoremediation is often applied at brownfield sites, mostly in case of the combination of large areas and low pollutant concentrations, with the purpose of redevelopment of the brownfield.

A specific type of phytoremediation is **phytoextraction**. Several studies and experiments suggested and sometimes proved that phytoextraction is an effective remediation technology, by reducing the concentrations of metals in soils, due to the ability of plants (herbs, shrubs, and trees) to take up pollutants and move them to aerial parts, also to leaves. Some plants have a high potential in extracting specific metals, but are ineffective for other metals. So, different types of metals require different vegetal species. In general, phytoextraction is a slow process that could take years or even decades. The use of hyperaccumulator plants combined with high biomass accumulating plants, however, can accelerate the process. In addition to cleaning up, the principal advantage of using phytoextraction techniques is the same of other phytomanagement applications: no need to move the soil off-site to save the soil resource and the increase of green areas also in order to remove CO_2 from the atmosphere. More feedback from real situation including land use scenarios is needed to make operational this technology.

A specific type of phytoremediation is **phytostabilization.** It is one of the operational phytoremediation technologies, already implemented at real scale, in particular at mining sites. This technology does not remove pollutants from the site, but decreases the global risk associated to large surfaces of polluted soils or waste dumps. The process consists in the assisted development of a vegetal cover on the soil /waste surface that induces the physical and chemical immobilization of the pollutants in the plant rhizosphere. The phytostabilization process decreases the dispersion of pollutants in their particulate and dissolved forms. Preliminary studies must demonstrate that the global risk for human health and ecosystems will be decreased by the implementation of the phytostabilization technology. This technology can be included in the global management plan of polluted lands or sites; however it should be carefully framed by preliminary investigations and evaluation of the risk/benefits associated with different scenarios (no action versus phytostabilization), feasibility studies including up-scaling from laboratory to pilot experiments, and long-term monitoring plan. The site's long-term management should consider the fate of the biomass, potentially containing pollutants. The site maintenance plan, included in the life cycle assessment of the technology, should be adapted according to this constraint. The long-term evolution of the phytostabilized sites remains a subject of research, associated with the need of feedback and long-term monitoring data acquisition and processing. A special case of phytostabilisation is the hydraulic control of infiltrating water and the hydraulic containment.

A specific type of phytoremediation is **phytodegradation**. This is a promising and sustainable approach to remediate soil pollution using plants and their associated microorganisms. The technique excels in scenarios where its merits outweigh its limitations, such as low to moderate contamination levels and bioavailable pollutants.

Successful implementation requires careful site assessment, plant selection, and long-term management. Phytodegradation offers aesthetic and ecological benefits and is a cost-effective and long-term solution. While the technique may be slower than some conventional methods, it transforms pollutants into less harmful forms while conferring ecological benefits.

A specific type of phytoremediation is phytovolatilization. This is a phytoremediation technology where plants absorb pollutants, convert them to less hazardous forms, and release them through transpiration in the atmosphere. This approach is practical for detoxifying volatile organic pollutants and heavy metals such as selenium, mercury, and arsenic. *Brassicaceae* family members are effective as selenium volatilizers, whereas mercury is an easily volatilized liquid element. As an advantage over other phytoremediation technologies, phytovolatilization removes pollutants from contaminated sites without requiring plant harvesting and disposal. However, it does not completely remove pollutants, as they remain in the environment. Instead, it transports pollutants from the soil to the atmosphere, where toxic, volatile pollutants can contaminate the air. Additionally, these pollutants may be redeposited in the soil by precipitation, necessitating a risk assessment such as metabolism within larger and smaller plants since larger plants in the environments are expected to transpire more water, depending on temperature, wind speed, and light intensity. It is recommended to apply intermediate-scale tests and mathematical models before being applied on a pilot scale.

Pollutant volatilization pathways have been established through phytovolatilization research. However, the significance of these elimination mechanisms remain unknown, particularly for fewer researched pollutants. Pollutants with an n-octanol-water partition coefficient higher than 5 are unlikely to be translocated in plants due to their organic matter partitioning. Less hydrophobic pollutants are more likely to be translocated, influenced by molecular weight and hydrogen bonding.

Field trials lack indirect phytovolatilization measures, making it difficult to differentiate between direct soil volatilization and indirect phytovolatilization processes. Phytovolatilization is critical for a variety of chemicals that are highly mobile in the subsoil and plants, such as ammonia and ethylene.

A specific type of phytoremediation is **phytomining**. Phytomining involves the in-situ removal of metals from sub-economic ore bodies or contaminated mine sites, with the objective of recovering economically significant amounts of metals from plants. Soils contaminated with high concentrations of heavy metals and metalloids offer opportunities for critical raw materials and provide a greener alternative to environmentally destructive open-cast mining practices. Phytomining capitalizes on the natural properties of hyperaccumulating plants, which can tolerate metals, transport them from roots to aerial parts, and achieve high biomass while accumulating high metal concentrations. This technology holds potential applications in the metal and minerals industry for low-grade metal and mineral mining, as well as metal recycling from polluted soil.

Mining operations traditionally focus on high-grade ores, requiring significant investments. However, low-grade ore bodies, especially in ultramafic deposits, are more abundant but pose economic challenges for conventional extraction methods. These ultramafic or serpentine soils contain elevated levels of metals and rare earth elements, making them potential sources of critical raw materials. Flora adapted to these soils has evolved mechanisms for metal accumulation or tolerance, making the exploitation of such areas for revenue generation through metal extraction increasingly important.

The connection between minerals and plants has been recognized for centuries, and advancements in the 20th century have enabled the analysis of metal concentrations in plant tissues. The rhizosphere, the micro-ecosystem around plant roots, plays a crucial role in soil-plant interactions. Metal uptake by plants occurs through root absorption and transport to above-ground biomass. Some plants are sensitive to high metal concentrations, while others develop resistance and tolerance, resulting in the accumulation of metals in their tissues. Plants that accumulate metals 100 times more than normal plants are termed hyperaccumulators.

Hyperaccumulators effectively extract metals from metalliferous soils and transport them to above-ground tissues. After harvesting, plants are dried and reduced to ash, which can be further processed using conventional metal refining methods to recover metals.

Phytomining reduces the negative impacts associated with conventional mining, while also contributing to land restoration, reduced pollution, and conservation efforts, thus aligning with the sustainable development goals. This technique provides remarkable results in the extraction of valuable metals from soil with significantly lower energy consumption compared to conventional mining methods. This energy-efficient approach not only reduces greenhouse gas emissions but also contributes to overall sustainability and resource conservation. By harnessing the power of natural processes and eliminating the need for energy-intensive steps, this green extraction method contributes to a more sustainable and responsible approach to resource extraction, benefiting both the environment and society as a whole. Incorporating this green practice into resource management strategies will help society move closer to a more sustainable and harmonious relationship with the environment.

An innovative type of phytoremediation is the **remediation train**. A pilot-scale study was conducted, utilizing a remediation train (ReSoil® + phytomanagement) to successfully demonstrate the simultaneous removal of toxic metals and metalloids, and organic pollutants from soils that were partially artificially contaminated. ReSoil® is a soil extraction process that allows us to efficiently remove toxic metals and metalloids from the soil, leaving soil functionality to serve as a substrate for phytomanagement. Phytomanagment comprises active and passive bioremediation. In the active phase, fast-growing, short-season crops (which can be sown from spring to late summer) such as buckwheat (*Fagopyrum esculentum*) and rapeseed (*Brassica napus*) are sown as the first crop and afterwards mulched as green manure. Buckwheat and rapeseed have branching root systems that reach deep into the soil and improve aggregation of the remediated soil (with lost natural structure) through an extensive network of fine roots. Earthworms, vermicompost, compost and manure can be added in this phase to boost soil microbial activity to enhance biodegradation of organic pollutants that remain in the soil after soil extraction. The active phase is followed by the passive post-remedial natural attenuation. The reason for the post-remedial phase is that some beneficial remedial effects are expected even after the active operations have been completed. For example, it is known that intensive microbial processes in the plant rhizosphere during phytomanagement promote the degradation of various xenobiotics that are harmful to the environment.

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[i] Noble metals (NMs) such as silver (Ag), gold (Au), and platinum group metals (iridium, osmium, palladium, platinum, rhodium, and ruthenium) are known for their resistance to corrosion and oxidation, even in humid air when heated (Cotton, 1997). These metals are rare and occur in low concentrations in the Earth's crust.

[ii] Rare earth elements (REEs) are a group of 17 chemically similar metallic elements in the periodic table, including scandium (Sc), yttrium (Y), and 15 elements known as lanthanides, from lanthanum (La) to lutetium (Lu). They are also referred to as rare earths (REs) or rare earth metals or minerals (REMs). REEs are categorized into two groups: light rare earth elements (LREEs), which include lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), and scandium (Sc), and heavy rare earth elements (HREEs), which consist of gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu), and yttrium (Y) (Schüler et al., 2011).

European Union Network for the Implementation and Enforcement of Environmental Law

Annex 1

Phytoremediation – Case studies

IMPEL Project no. 2023/11

1. Contact details - CASE STUDY: Phytoremediation n.1

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1.7 Phone number	

2. Site background

2.1 History of the site

Kolleberga Plantskola

The site is a previous tree nursery operation in Southern Sweden (Ljungbyhed) where cultivation and selling of pine and spruce plants was carried in many different agricultural fields that covers ca. 23 hectares in total.

Figure 1 - Site area

Since its initiation in 1950s, different types of pesticides, including DDT, were used to control weeds, fungus and pest related damage. DDT was used by both dipping the plants in barrels of liquid dissolved DDT as well as spraying across the field. The use of DDT was discontinued in the 1970s but concentrations of DDT exceeding the soil guideline value in Sweden for less sensitive land use (1 mg/kg) are still detected across a large area of the site and hotspots with levels exceedingly even 100 mg/kg have also been detected during site investigation. The agricultural fields are currently unused and are managed by periodically cutting then plowing the grass back into the soil to prevent regrowth. The primary risks at the site, as determined during risk assessment, are primarily to the local environment due to uptake in earthworms and potential secondary poisoning as the DDT is spread further in the food chain if predators were to feed at the site. A pilot-scale field experiment has been established at Kolleberga to test different gentle remediation option (GRO) strategies to i) manage the DDT contamination in-situ and ii) preserve the good quality of the agricultural soil by maintaining or improving soil functioning. Aided phytostabilisation with biochar and grasses or willow, phytoextraction with pumpkin, and phyto/rhizodegradation with clover/alfalfa are being tested and evaluated to achieve these objectives.

2.2 Geological setting

The site geology is glacio-fluvial sediment consisting of fine-medium sand mixed with small amounts silt, clay, small gravel and larger stones (shown in Fig.2). The soil has low levels of organic carbon and is nutrient poor. The groundwater depth is unknown.

Figure 2 - Test pit for soil

Figure 3 - Soil map: Green is glacio-fluvial sediment, orange is postglacial sand, tan is peat, blue is sandy glacial till, pink is flood sediment (sand). The site lies within the blue-ringed area

2.3 Contaminants of concern

The contaminants of concern at the site are DDT and its metabolites DDE and DDD, which in Swedish regulation are considered together as ΣDDT. Total concentrations were measured using GC-MS and the freely dissolved bioavailable fraction in the soil porewater measured polyoxymethylene (POM) passive sampling method, which was developed as a method for measuring DDT during this study. The average total ΣDDT concentration in the pilot experiment plots at the start of the experiment was 10.5 mg/kg.

2.4 Regulatory framework

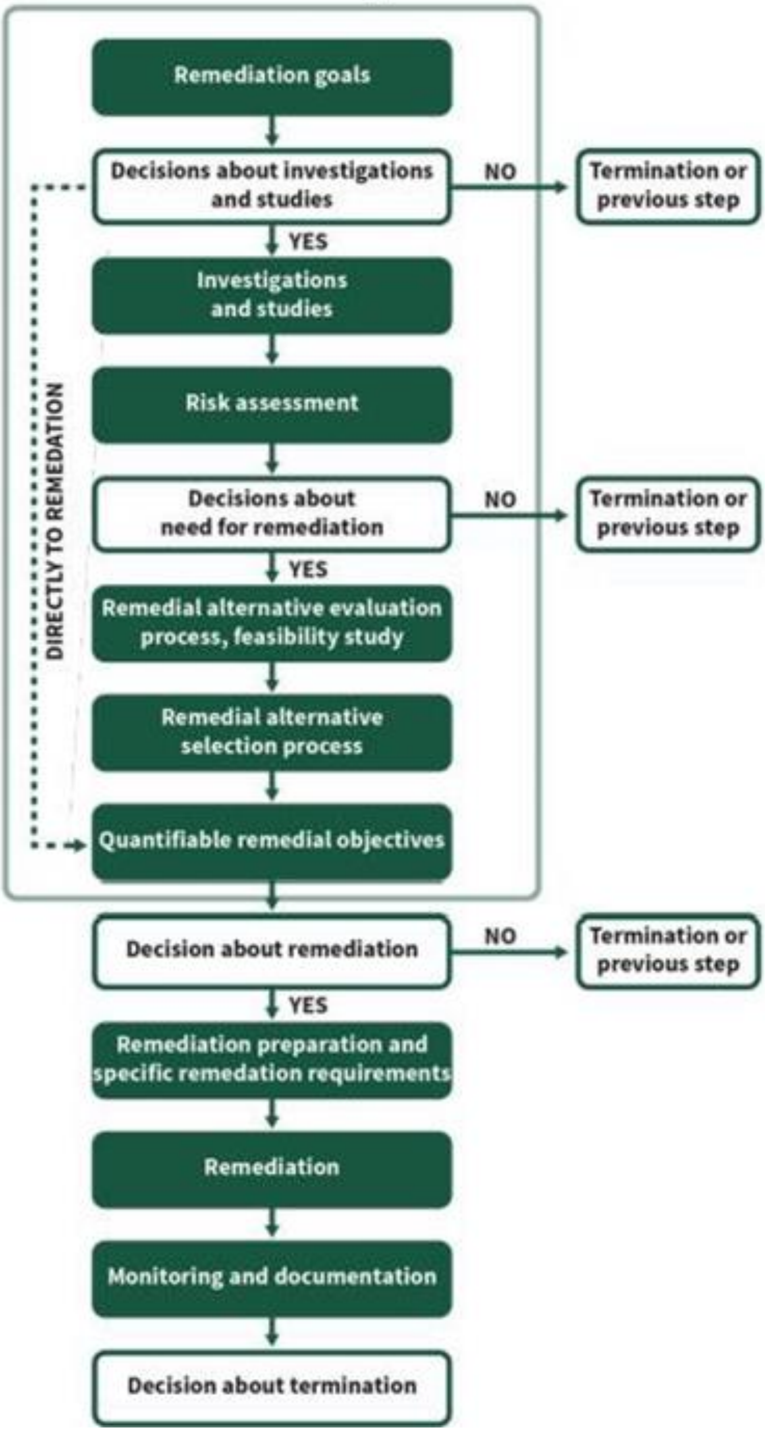

Figure 4 - A schematic illustration of the remediation process in Sweden.
From: https://www.naturvardsverket.se/en/topics/contaminated-areas/contaminated-areas-in-sweden/

The Swedish regulatory framework for contaminated land is based on a stepwise procedure as shown in the figure below. For Kolleberga, the site investigation detected concentrations of ΣDDT and the subsequent risk assessment determined that the contaminant posed risks to the local environment, soil organisms and potential spreading upwards in the food chain. This pilot project is part of a series of investigations and research initiatives to sustainably manage DDT contaminated sites in Sweden since simply excavating the entire site is not feasible (nor desirable) and there are much such sites across the country. The status of the Kolleberga site is that the environmental regulating body for the region where Kolleberga is located has decreed that a remediation plan must be created for the locally contaminated hotspot with dangerously high levels of ΣDDT. Further investigations and an evaluation of feasible remediation alternatives for the rest of the site (multiple hectares of field) is also currently underway.

3. Pilot-scale application in field

3.1 Laboratory Study

No laboratory studies were conducted before the pilot experiment was established in the field.

The choice of phytoremediation strategy and selection of plant species was made through a combination of literature review and applying a recently derived framework for identifying suitable gentle remediation option (GRO) strategies for risk management at a particular site (see link below). Viable plants and strategies were selected to test in a pilot field application regarding their effectiveness for risk mitigation and effects on soil functions.

https://www.sciencedirect.com/science/article/pii/S004896972104955X

3.2 Treatment unit (pilot scale)

A pilot-scale field experiment was established at the Kolleberga site in Ljungbyhed (Southern Sweden) according to a randomized block design of test plots after thoroughly homogenizing the soil in a pile and mixing half of the volume of soil with biochar.

Treatments consisted of four different types of plants, with or without biochar addition to the soil, and were established in triplicate (in total 24 plots). The four different plants are aimed at different phytoremediation strategies: 1) phytoextraction (pumpkin), 2) aided phytostabilisation (Salix, grass-mixture) with biochar expected to facilitate immobilisation, and 3) phyto/rhizodegradation (nitrogen-fixing plants, such as clover and alfalfa).

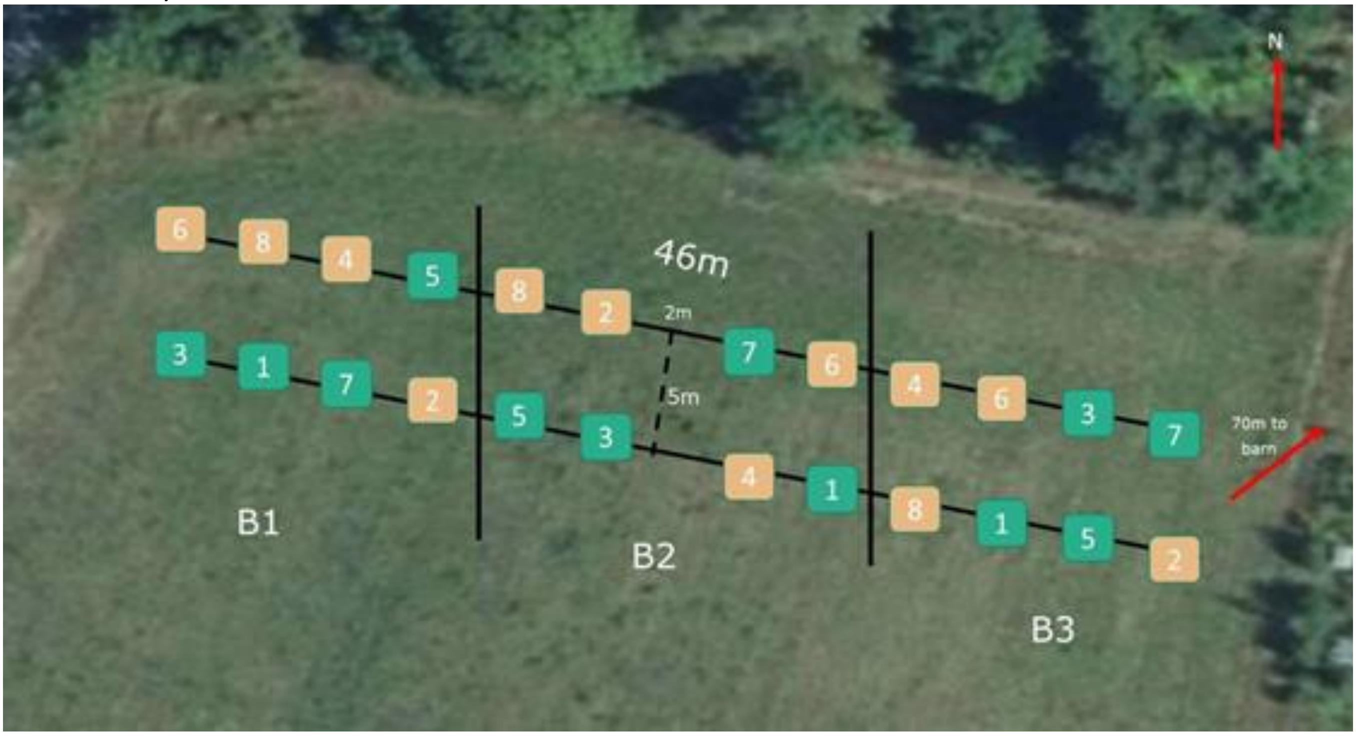

Figure 5 - Pilot treatment unit

Treatment (orange boxes in figure are with biochar):

1. Pumpkin
2. Pumpkin with biochar
3. Grass mix
4. Grass mix with biochar
5. Legume mix
6. Legume with biochar
7. Salix
8. Salix with biochar

3.3 Control parameters and verification of the applicability (pilot scale)

A battery of physical, chemical, and biological indicators was selected via a 'logical sieve' to assess the GRO strategies in two assessment tracks: 1) GRO effectiveness for risk management and 2) soil quality assessment to determine the resulting impacts on soil functions and consequent delivery of ecosystem services. Control samples were taken at three different areas in the field (see Figure 6) at the beginning of the experiment and after the 2nd year to use as reference values when assessing the effectiveness of the GRO strategies. Samples were also taken in the newly established experimental plots at the start of the experiment as separate control values and to assess the effects of soil mixing and the immediate effects of mixing biochar into the soil.

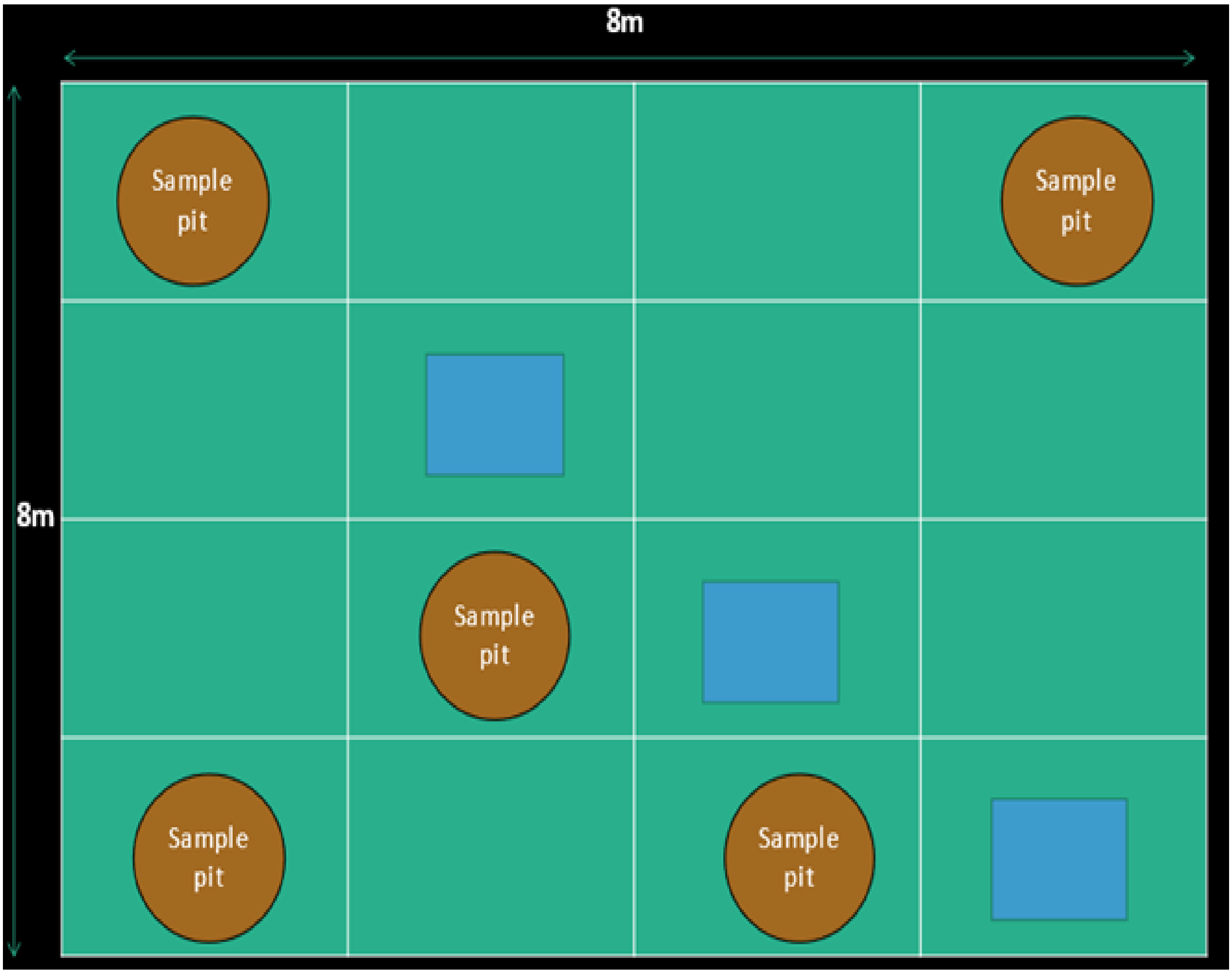

Figure 6 - Sampling protocol

Sampling protocol:

1) 5 randomly distributed sampling pits
2) Aggregate soil samples taken in a bucket to fill 8- 10 L.
3) Soil mixed in bucket to homogenize,
4) Soil distributed in plastic bags according to weight needed, marked then stored in cooling bags.

Separate occasion:

- Bait lamina strips set out in 3 groups of 8 strips (2 rows x 4 strips) – blue boxes.
- Strips checked every week for progress then taken out after 3 weeks for checking and recording.

The following parameters are measured to evaluate their effectiveness for risk management: total content of DDT in soil, bioavailable part of DDT in soil using POM, uptake of DDT in plant biomass, toxic effects on earthworms (reproduction, mortality, growth) and uptake into biomass.
The following parameters are measured to evaluate GRO's effects on soil quality: soil texture, organic matter content (TOC), pH, available nutrients (P-AL, Ca-AL, K-AL, etc.), total nitrogen, potential nitrification, bait lamina, basal respiration, microbial biomass carbon, and DNA sequencing.
The battery of analyses was performed in the beginning of the experiment (control) and after each growth season when harvesting the biomass.

4. Full-scale application

4.1 Main treatment unit

The pilot-scale field experiment is still ongoing, and it is too early to say whether it will be scaled up to a full-scale application.

4.2 Monitoring of the chemical parameters

Chemical parameters measured in the experiment include the following: Total and bioavailable (dissolved in porewater) concentrations of ΣDDT, TOC, pH, available nutrients (P, K, Ca, Mg, etc.), total Nitrogen. These parameters have been measured at the beginning of the experiment and at the end of each growth season.

4.3 Monitoring of the soil microbial community structure and functioning

Bioindicators have been selected to give a direct indication of the abundance and functional activity of soil microorganisms, which included basal respiration, microbial biomass carbon and potential nitrification. DNA sequencing (not yet available) will also provide information as to the structure and diversity of the microbial community and whether that changes because of GRO.

Physical, chemical, and biological indicators selected via a 'logical sieve' to assess the impacts on soil functions are aggregated into higher-level categories that pertain to specific soil processes and functions. These are then grouped within specific ecosystem services (ES) to assess whether there resulted an increase or decrease in a particular ecosystem service's provisioning as result of GRO application. The ES assessment results will visualize with help of a radar chart (not yet finalised).

4.4 Monitoring of plants

The monitoring of plants is done by visual inspection (survival, nutrient/water deficiency) and an assessment of the nutrient content in the plant biomass through lab analysis gives an indication of whether they are healthy or nutrient deprived (biochar may bind and render nitrogen unavailable to plants). Uptake of DDT in plants is also tested after each growth season.

5. Results

5.1 Removal rate

This pilot experiment is planned for 3 years, and data is only available for the first two years. Some results and data are not yet available either as we are still awaiting results from lab analyses, but preliminary results indicate that biochar and plants can have positive effects. In terms of risk management, preliminary results indicate that biochar for stabilisation of DDT can reduce the risks to the environment but leaves the site with DDT- concentrations as before, albeit not bioavailable to the same extent. Phytoextraction is expected to require long time horizons to reduce the concentration of DDT to below recommended guideline values. For soil quality improvement, the various treatments have also shown substantial improvements in soil functioning parameters in comparison to the Field control, and further analysis and grouping of the data to assess provision of ecosystem services is planned.

The pilot experiment is ongoing, data is still being gathered and analysed and results have not yet been published. Forthcoming articles are in progress related to the following:

- A probabilistic model estimating the time required for phytoextraction of DDT with pumpkin
- Determination of the effects of GRO for the delivery of ecosystem services by grouping the selected soil quality indicators within specific soil functions and corresponding ecosystem services which they underlie
- A case study paper evaluating the effects of GRO for the experimental site considering aspects related to both risk management and soil improvement
- Cost-benefit analysis of the GRO alternatives considered in this pilot study compared to other potential alternatives to manage the DDT contamination

6. Post treatment and/or Long Term Monitoring

6.1 Post treatment and/or Long Term Monitoring

This aspect of the project is still to be decided. The plan is to keep the project going for a longer period to assess the effects of the treatments over a long period of time. A minimum test battery, maintenance and upkeep, financing and other accompanying concerns will have to be figured out in dialogue with the landowner and relevant stakeholders. Short-term plans for the site are to restart the tree nursery operations, which may impact the pilot study, but results from this study can hopefully be useful to provide valuable decision-support to the landowners and other decision-makers

7. Additional information

7.1 Lesson learnt

Several unforeseen complications have had to be overcome in the pilot-scale field experiment so far, including:

- Difficulties with analyses – the first method chosen to assess potentially mineralizable nitrogen did not perform as expected (non-detects) so we switched to a different method for future analyses which worked better. Also, the bait lamina test was difficult to time in the season and is highly impacted by climatic conditions and the season. Water availability played an important role. Certain analyses can also be expensive and some that we would have liked to include in the experiment were out of budget or difficult to source commercially. Lesson learned: select indicators carefully and assess their sensitivity and usefulness.
- Practical concerns – a significant effort went into establishing the site with a surrounding fence to keep out rabbits and other herbivores, but then we had to contend with birds eating all the seeds and had to create impromptu scarecrows. Weeds were continuously growing on the plots and in the area that constantly threatened to take over the willow plots and posed problems for the establishment of the pumpkin plots. Pests were the decisive issue, and an invasive Spanish slug found its way to the pumpkin plots and destroyed the crop for the second growth season. It was also a significant effort (and cost) to establish an irrigation system at the site, and using an automated system proved very useful.

7.2 Additional information

There is a large difference in the effort, cost, acceptance, etc. between different GRO strategies and associated plants used to achieve a specific goal. For example, growing pumpkins to phytoextract DDT has proven to be challenging and is likely to be unfeasible for scaling up to field-scale. A continuation in this project will be to conduct a cost-benefit analysis to directly address these types of concerns and compare the different GRO strategies will other feasible alternatives.

7.3 Training need

The technical competence can be gained through 'learning by doing' but more guidance material and best practice are always appreciated. One important aspect that would become important when considering scaling up to field-scale is the dialogue, interplay, and acceptance of regulatory authorities so that becomes gradually easier to attempt projects using phytoremediation. Regulation and legislation obviously differ between countries but successful examples of interaction with such stakeholders to e.g., provide a satisfactory plan for monitoring, upkeep, financing etc. would be a valuable contribution. Also, the wider benefits of such remediation techniques and alignment with broader EU initiatives such as the Soil Health Law, Green New Deal, and others should be better emphasised going forward to justify the use of GRO.

Glossary of Terms

Term (alphabetical order)	**Definition**
Gentle remediation options (GRO)	Risk management strategies or technologies that result in a net gain (or at least no gross reduction) in soil functioning as well as achieving effective risk management
DDT	Dichlorodiphenyltrichloroethane – an organochloride compound that was an active ingredient in older pesticides that has not become a persistent organic pollutant

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2. Site background

2.1 History of the site

The site of concern is a multi-contaminant polluted soil and sediment area located in an Italian dismissed steelwork plant (metropolitan area of Naples - district of Bagnoli-Coroglio). Over the last century the site has been involved in high-impact industrial activities, from chemical research to steelwork and clinker production. The industrial shutoff occurred definitively in the middle of the ’90 when all the major facilities and plants were decommissioned.
During the following 22 years, the Bagnoli Area has been managed by the municipality of Naples to achieve the land remediation goals according to the new urban planning.
In 2015 Invitalia has been addressed by the Italian Government as the owner of the asset as well as charged of the task for land remediation and urban development.
The environmental heritage of the brownfield is very complex, ranging from wastewater pollution to sediment and soil contamination with several different toxic compounds, both organic and inorganic.

Figure 1 - Delimitation of the brownfield of Bagnoli – Coroglio (in red the inland area and in blue the marine area).

2.2 Geological setting

The study area is in the north-western sector of the Municipality of Naples, confined within the morphological unit of the Bagnoli-Fuorigrotta plain dominated, from a geological point of view, by the volcanic structure of the Campi Flegrei.

The main geological-structural element consists of the vast volcanic caldera which collapsed about 35,000 years ago following the eruption and consequent emplacement of the Campanian Ignimbrite (grey tuff from Campania). The products of this eruption constitute the main lithotypes outcropping along the scarps bordering the western and northern margins of the Flegrea depression and extending eastward along the Camaldoli- Poggioreale alignment (Orsi et al.1996), while they are absent within the area of Campi Flegrei, probably due to erosive processes or because they were covered by vulcanites from the successive eruptions of Campi Flegrei and Vesuvius and by alluvial soils.

Recent volcanic activity, ranging from 35,000 years ago to 1538 AD, can be divided into the following five main phases of activity:

- Volcanism pre-Ignimbrite Campana
- Eruption of the Campanian Ignimbrite and related caldera collapse (first)
- Volcanism between the Campanian Ignimbrite and the Neapolitan Yellow Tuff
- Eruption of Neapolitan Yellow Tuff and related caldera collapse (second)
- Post-Neapolitan yellow tuff volcanism

After the first caldera collapse, the sea entered the morphological depression; over time, the progressive filling of volcaniclastic material led to a new emergence of the area. The Yellow Neapolitan Tuff is the most typical lithoid pyroclastic deposit of this submarine eruptive phase (12,000 years ago). It outcrops along the slopes of the Posillipo hill which closes the area to the southeast, while it is located a few hundred meters below the plain of Bagnoli. The successive volcanic phases, which took place in a subaerial environment, with the works of numerous emission cones, erupted pyroclastic material alternating with paleosols in periods of eruptive quiescence.

The central area of Piana di Bagnoli takes the form of a vast depression behind the dunes bounded towards the sea by a coastal strip. This depression with depositional characteristics of the silty-marsh type was maintained as such at least from the Middle Ages until 1800 A.D., when reclamation and intense anthropization began. The urbanization and industrialization processes have led to a complete morphological transformation of the territory and consequently of the "natural" geological layer. An important blanket of fill land also formed by a component of lithoid material of

"industrial" origin mixed with reshuffled ash products covered the entire plain in a non-uniform way to form a new lithological horizon.
Based on previous studies, in the area in which the site of national interest of Bagnoli-Coroglio falls, the following lithological sequences can be identified:

- eluvial, colluvial and torrential deposits
- reworked anthropic deposits, mainly filled with old watersheds
- slope debris and landslide heaps (mainly located at the base of the Posillipo hill) characterized by a high degree of remodelling, up to the coastal strip, in the Bagnoli area, where it is present the former Italsider factory and where there are man- made deposits (processing slag mixed with remodelled natural deposits) and where sands and silts of current and recent coastal environment emerge
- stratified yellow tuffs containing pumice and scoria which constitute a modest relief (altitude of 36 m a.s.l. compared to altitude of 14 m a.s.l. of the base of the relief) which represents the wreck of a small volcano, Monte di Santa Teresa, located near the Cavalleggeri d'Aosta railway station

In the sector closest to the Bagnoli coast, for most of the former Italsider area, there are:

- current and recent coastal sands and silts. These deposits are generally not thickened, often reworked and locally coalescing with anthropic deposits consisting of processing slag. There are also deposits of marshy origin with mainly silty granulometry with peaty levels

In the whole area of the Bagnoli-Fuorigrotta-Soccavo plain, except for the areas immediately below the hills and in the immediate surroundings of Monte di Santa Teresa, the volcanic sequences are quite deep; yellow tuff is generally found in these areas at depths greater than 50 ÷ 80 m below ground level.
The stratigraphies of the boreholes analysed, which go beyond the blanket of backfill, highlight the circumstances described above. In fact, sediments of marine origin, paleo-soils and fine sediments deposited in low-energy environments of a lagoon or marshy nature (peats, silts) are found at various depths.
From a geological point of view, the soils outcropping in the study area are mostly represented by manufactured backfill consisting of very heterogeneous materials by nature, size and degree of compaction, forming a blanket of variable thickness over the entire area.
Everything can be found in man-made deposits: natural material in every grain size and origin, quarry waste, brick artefacts and fragments, residual products of industrial activity, consisting of blast furnace slag, steel mill slag, lateritic remnants mixed with volcanic soils rearranged, stratified and distributed with different thicknesses according to the transformation, over the decades, of industrial processes, resting on a substrate

mainly formed by limno-marshy and dune deposits.

At the Bagnoli-Coroglio site, the depth of the water table varies from a few centimetres near the coastline in the west to more than ten meters in the upstream areas in the east.

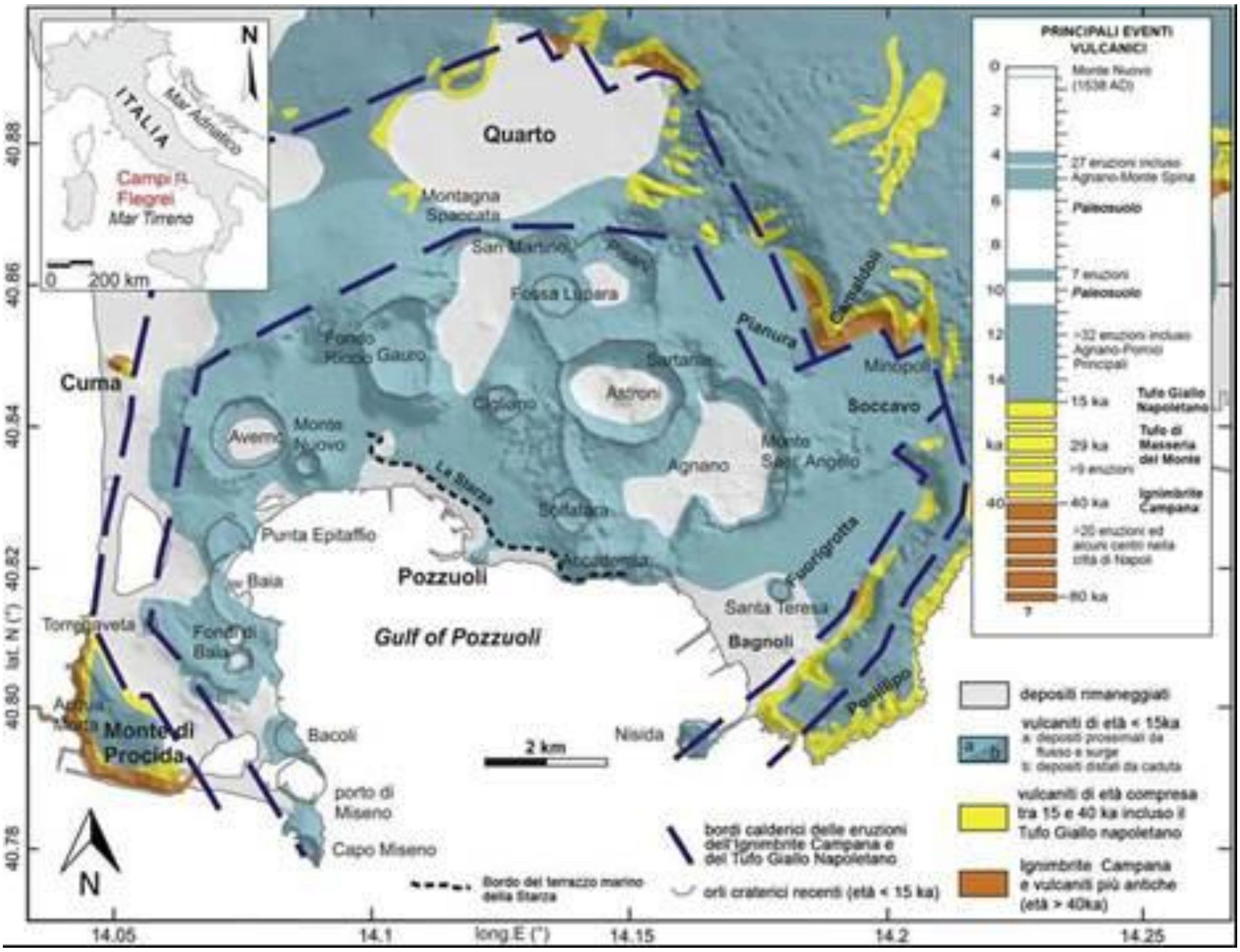

Figure 2 - Geological map of the Campi Flegrei (modified Isaia et al., 2019)

Figure 3 - Typical stratigraphy of the industrial site: fills on volcanic ash

2.3 Contaminants of concern

Environmental restoration activities are being carried out by a step-by-step approach, giving priority to eco-friendly technologies.

DETAILED SITE CHARACTERIZATION (completed)

DEFINITION OF NEW SOIL USE DESTINATION (agreed)

RISK ANALYSIS (completed)

CLEAN-UP TECHNOLOGIES FULL SCALE TEST (completed)

INTEGRATED REMEDIATION PLANNING (completed)

RESTORATION ACTIVITIES (in progress - in subareas)

TESTING/CERTIFICATION (in progress - in subareas)

In 2018 an updated investigation of the soil has been carried out, by means of 228 sub-superficial prospections and the chemical analysis of more than 900 samples collected. In the following Table 1 the highlights of the survey are presented, including the analytical set of parameters and their minimum and maximum value detected. C1, C2 and C3 refers to the average layer of soil investigated (C1: 0 to -1 m below g.l.; C2: -1 m to -2 m below g.l.; C3: -2 m to capillary zone). Some thresholds (in particular: As, Be, Pb and Zn) settled by the Italian law on remediation procedures are replaced by the relative site-specific natural background levels.

Parameter	MAX value [mg/Kg d.m.]			MIN value [mg/Kg d.m.]			Italian threshold for residential use	Italian threshold for industrial use
	C1	C2	C3	C1	C2	C3		
As	126	181	249	29	30	29	29	50
Be	-	79	-	-	79	-	9	10
Cd	220	4,1	4,5	2.3	2	2	2	15
Co	-	181	147	-	181	147	120	250
Cr	306	3.620	2820	164	155	168	150	800
Hg	8.9	6.8	4.3	1	1.1	1.3	1	5
Ni	-	-	130	-	-	130	120	500
Pb	6.782	13.379	647	105	105	114	103	1.000
Cu	379	1.766	1.046	129	122	139	120	600
Sn	87	180	317	14	14	16	14	350
V	956	1.756	233	104	105	104	100	250
Zn	1.940	2.607	1.317	165	159	159	158	1.500
HC>12	827	11.984	11.890	50	50	52	50	750
HC<12	13	27	19	13	27	19	10	250
PAHS	334	1778	76	11	11	11	10	100
PCBs	52	35	16,6	0;06	0,06	0,07	0,06	5

Table 1 - Concentration levels of pollutants from the preliminary soil investigation in the brownfield.

Analytical data have been validated by the Italian National System of Environmental Protection (SNPA) and they are considered to set a preliminary grid of potentially applicable remediation technologies. The grid underwent the examination of the technical board at the Italian Ministry of the Environment and four main technologies have been selected: soil washing, thermal desorption, in-situ chemical oxidation (ISCO) and bio-phytoremediation.

2.4 Regulatory framework

Among the different remediation technologies, an in-situ bio-phytoremediation approach has been chosen and is ongoing to clean-up soils of Bagnoli brownfield site. This bioremediation strategy uses live plants (woody or herbaceous plant) and microorganisms' populations that naturally live in the soil that are adapted to the contamination to recover the polluted areas; is non-destructive and environmentally/eco-friendly and represent an interesting research field, which results has been considerably documented over the last decades.

The application of bio-phytoremediation has been included in the overall feasibility study on the entire site, which underwent in August 2020 the examination of the scientific and control bodies to get permits and directions.

Since 2017 a cooperation with the University of Sannio – Department of Science and Technologies has been signed to have the scientific support for the full-scale application of bioremediation technology.

3. Pilot-scale

3.1 Laboratory Study

"Gentle Remediation" is mainly based on soil-plant interaction; in fact, detailed soil characterization and knowledge of the uptake of plant species are the fundamental elements to assess a priori the efficiency of any Remediation project.

Therefore, on March 27/28/29, 2017, 80 soil samples were taken at a depth of 0-50 cm chosen in relation to the old characterizations of the site (post-reclamation) for detailed characterization for the selection of areas to be used for in-situ plant-pilot testing.

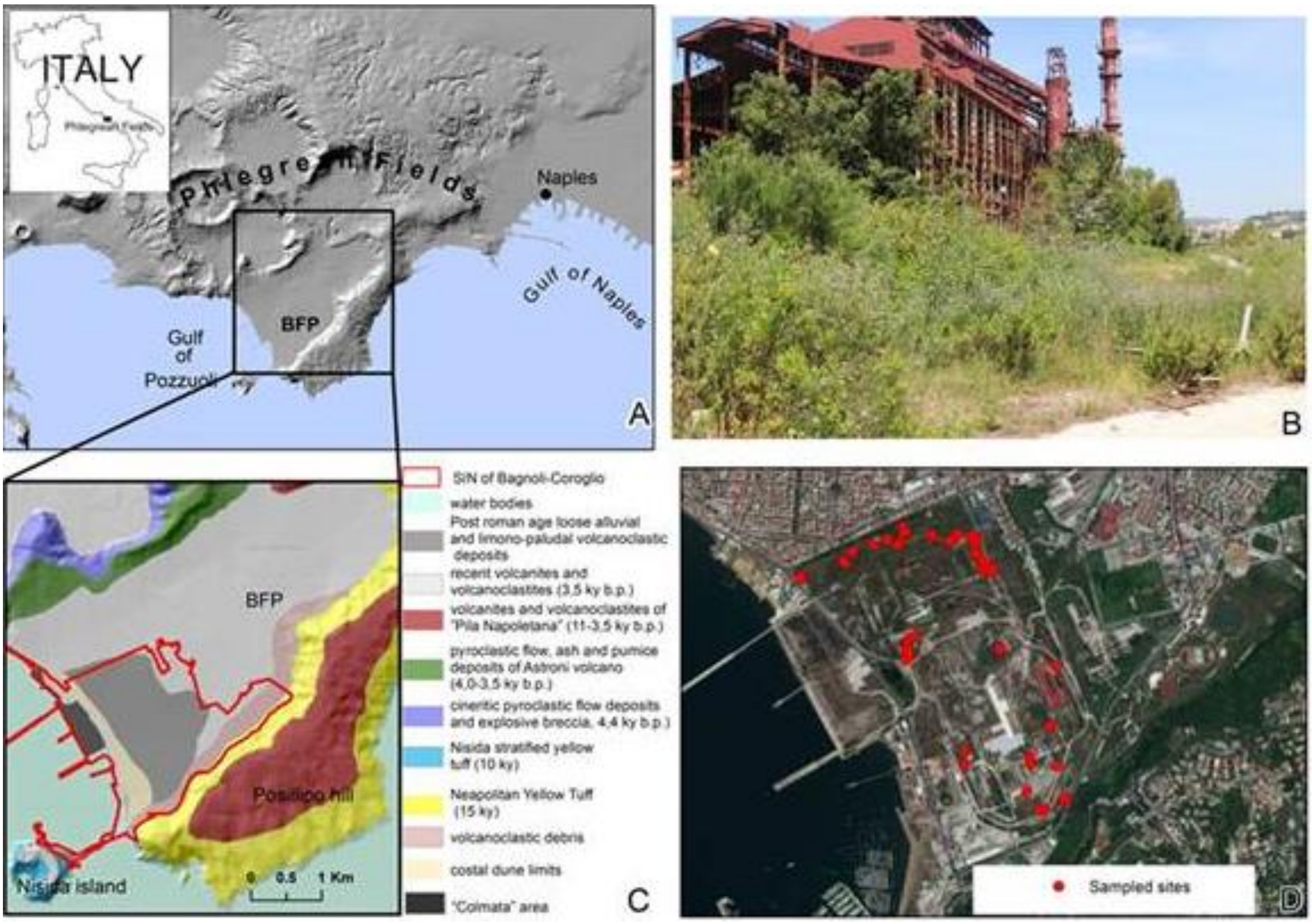

Figure 4 - Location map (A); view of one of the abandoned industrial activities (B); geological map (C)- after Russo et al., 1998, modified) and (D) sampling point maps.

Details of plant species sampling are given below. For simplicity, those for the roots are given, which are clearly like those for the aerial part. Plant tissue samples were collected from selected benchmark plants for the purpose of detecting chemical uptake.

Chorological grid mapping is a concise and effective method for indicating the distribution of individuals or populations in terms of presence/absence within a reference grid (Pedrotti, 2013). Data sampling, processing, and analysis were performed using ESRI ArcGIS 10.5. We created an ad hoc ESRI Geodatabase that includes geometry, attributes, and a spatial reference system. A polygonal vector grid function forms the class geometry. The study area was divided into 100 × 100 m cells, using Mercator's universal cross-sectional grid and WGS84 geodetic datum as reference. Within this reference grid, each polygonal cell showed the presence/absence of all plant species identified in the field surveys. A GPS receiver connected to a tablet that performed field data collection using ESRI ArcGIS 10.5. The entire study area was explored through two survey campaigns. Specimens of all plant species (and some significantly cultivated species at the site such as Populus ssp. and Eucalyptus sp.) were collected in the field and later identified in the laboratory. Once all specimens were identified, the final geodatabase was populated with presence/absence data for all taxa identified on all cells.

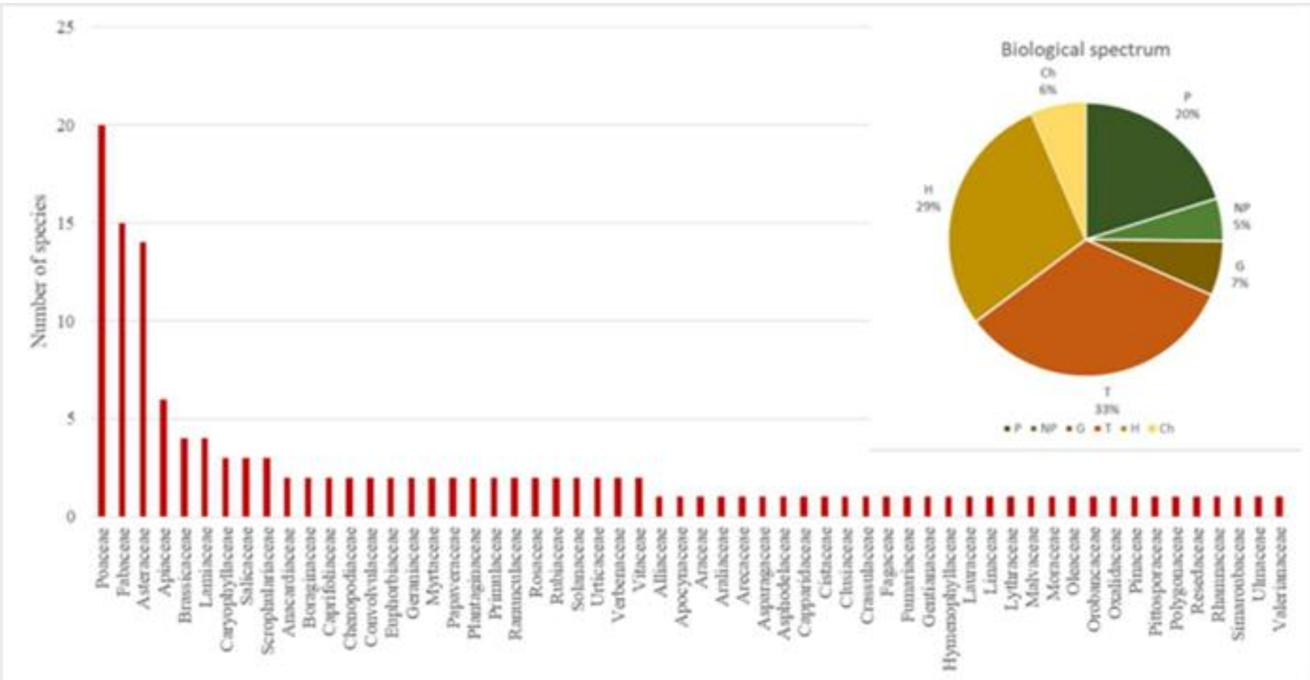

Figure 5 - Biological spectrum of plant species recorded at the Bagnoli site. Number of species per family at the Bagnoli site.

Field surveys enabled us to identify 139 plant taxa belonging to 58 families. The most represented families are Poaceae (20 taxa, 14.4%), Fabaceae (15 taxa, 10.8%), Asteraceae (14 taxa, 10.1%) and Apiaceae (6 taxa, 4.3%). The biological spectrum shows a predominance of Therophytes (33%) and Hemycriptophytes (29%), followed by Phanerophytes (20%) (Figure 5). The number of taxa identified per reference grid cell varies from 39 to a maximum of 65. As expected, a massive portion of the identified species could be considered ruderal or synanthropic. As a term of comparison, percent of the site flora is in common with the flora of Nisida (De Natale, 2003), a small neighbouring island of the Campania Archipelago, and 93.5 percent of the site flora is in common with the urban flora of Naples (De Natale and La Valva, 2000). The importance of biodiversity is increasingly being considered for the phytoremediation of metal contaminated and polluted ecosystems.

N°	Family	Plant species	Code name
1	Fabaceae	*Bituminosa bituminosa*	Bit
2		*Lotus corniculatus*	Lot
3	Asteraceae	*Dittrichia viscosa*	Dit
4		*Artemisia vulgaris*	Art
5		*Senecio vulgaris*	Sen
6	Poceae	*Festuca arundinacea*	Fes
7		*Piptatherum miliaceum*	Pip
8		*Phragmites australis*	Phr
9	Scrophulariaceae	*Verbascum sinuatum*	Ver
10	Apiaceae	*Helichrysum litoreum*	Hel
11		*Ferula communis*	Fer
12	Salicaceae	*Populus alba*	Pop
13	Plantaginaceae	*Plantago lanceolata*	Pla
14	Brassicaceae	*Brassica rapa subsp. sylvestris*	Bra
15	Myrtaceae	*Eucalyptus camaldulensis*	Euc

Table 2 - Family, species, and code name of selected native species from the Bagnoli brownfield

The criteria for selecting plant species for analysis of the contaminants of concern were as follows: the predominance of the plant species at the site, its location within the site, and the likelihood that the plant roots may be impacted the contaminated soil. In the collection of specific plant tissue samples, the following factors were considered:

1) the concentration of contaminants in leaf tissue depends on the location of leaves on trees and
2) the concentration of contaminants in roots is determined by their location within the soil at the site based on their characterization.

The most abundant species of each Taxa was sampled. From trees and shrubs were harvested roots, trunk, branches, and leaves while only leaves and roots were harvested from grasses. Each tissue harvested from 3 or more plants and combined to form one composite. The plant tissue samples, trip blanks and controls were shipped to the laboratory overnight in a 4°C cooler. Samples for contaminant analysis (metals and metalloids, PAHs, and PCBs) were collected, weighed, and stored in screw-top glass vials sealed with aluminium septa.
Fifteen species were selected in relation to their relatively high accumulation and translocation ability.

3.2 Treatment unit (pilot scale)

The mesocosm is the confined place where experimentation allows us to control some variables (soil contaminants, plant species, amount of irrigation water, and others) and let others be constrained by natural cycles (photoperiod, temperature, humidity, windiness, marine aerosol) to understand how effective the remediation system is and what factors increase its effectiveness. The species to be evaluated in mesocosm were selected based on the results of the in-situ vegetation study and from the literature data. Thus, 12 species belonging to 5 families listed in Table 2 were selected.

Family	Specie	Biological form	Spontaneous in the SIN or from Literature Sources
Fabaceae	Lotus corniculatus L.	H rep	Spontaneous
Fabaceae	Bituminaria bituminosa (L.) Stirt.	H scap	Spontaneous
Fabaceae	Medicago lupulina L.	H scap	Literature
Poaceae	Festuca arundinacea Schreb	H caesp	Spontaneous
Poaceae	Dactylis glomerata L.	H scap	Spontaneous
Poaceae	Piptatherum miliaceum L.	H caesp	Spontaneous
Poaceae	Arundo donax L.	G	Spontaneous
Scrophulariaceae	Verbascum sinuatum L.	H bien	Spontaneous
Asteraceae	Dittrichia viscosa (L.) Greuter	H scap	Spontaneous
Asteraceae	Helianthus annuus L.	T scap	Literature
Salicaceae	Salix purpurea L.	P	Literature
Salicaceae	Populus alba L.	P	Spontaneous

Table 3 - Selected species for the mesocosm

Experimentation with mesocosms was conducted at two different sites: the first inside the SIN and the other at the cold greenhouse with polyethylene covering in the Department of Science and Technology. The choice of double replication stems from the need to have results under different environmental conditions, but also to pose an alternative in case of sabotage or vandalization at the site location. The risk of possible vandalism activities was found to be non-negligible, so much so that the experimentation sites inside the SIN were under close surveillance by the in-house security service, both with inspections and CCTV cameras. Henceforth, the mesocosm set up inside the Bagnoli- Coroglio SIN will be referred to as the "Bagnoli Mesocosm," the one set up in Benevento will be referred to as the "Benevento Mesocosm". The Bagnoli mesocosm experiment was set up under the Morgan Shed (so named because at the time this set-up housed the Morgan-type rolling mill for iron rods) (Figure 3). For each of the 8 areas identified at the site, 50 pots were provided for each area for a total of 400 pots. Each pot has a volume of 30 Lt with a diameter of 40 cm and height of 33 cm. The average specific gravity of the soil is 1.1 kg/Lt, so each pot has an average amount of 33 kg of soil substrate.

Figure 6 - Location of mesocosm experimentation apparatuses

The objects of this tier of testing were to predict to the extent possible, how an existing or planned Phytoremediation system would perform on actual site material and to reveal potential problems.

The 12 selected species were tested either in purity (a single species per mesocosm) or in combination (an herbaceous species together with tree species or multiple herbaceous species per single mesocosm).

For each area and each species, 3 treatments were planned as described below:

Treatment	Typology	Quantity
Mineral and organic nutrition	• Ammonium sulfate • Ammonium phosphate • Prodigy Plus®	150 g 150 g 150 g
Bacterial/fungal consortium	PGPR bacteria and spores of ectomycorrhizal and endomycorrhizal fungi	20 g.
Compost PLOS	Substrate inoculated with Pleurotus ostreatus	400 g.

Table 4 - Treatments for each species

The choice to have three different treatments was based on the idea of testing different technological combinations to evaluate the best possible combination. The choice of Pleurotus mushroom as a biological additive referred to some literature data highlighting its ability to degrade PCBs. The rhizospheric blend of PGPRs was used for its cometabolic abilities to mitigate abiotic stresses. Soil was taken from the 8 pilot areas after removal of vegetation cover and after initial tillage for soil turning.
Soil from each area was taken at several points, collected in a dump truck for transport to the Morgan shed where it was dumped on plastic sheeting in heaps (one heap for each area), manually stirred with the help of shovels to homogenize the sample, and finally manually operated pot filling was done. The sampling was done with a screening bucket in such a way as to eliminate the presence of materials (stones, concrete mixes, bricks, etc.) with dimensions greater than 4 cm. The volume of soil used for each pilot area is about 1.5 cubic meters. At the time of set-up, the quantities of fertilizer, fungal/bacterial consortium and Pleurotus ostreatus growth medium to be provided for each mesocosm were weighed, the doses of which are given in Table 4. Fertilizers were supplemented to the soil contained in the mesocosm first, they were amalgamated with the help of garden paddles, and then the bacterial/fungal consortium was supplemented in the same way. For the integration of the inoculated straw with Pleurotus ostreatus, a layer of about 10 cm of soil was removed from the pot, the due amount of straw was added and stirred with the remaining soil, and finally, upon reaching the previously removed soil in the pot, a further stirring was given. Then the predetermined number of seed is administered on the soil surface (or a few cm below the surface in the case of Helianthus annuus) and finally the soil surface is wetted to ensure good adherence of the seed with the soil. For the planting of tree plants, part of the soil is removed, thus obtaining a cavity that will be filled with the root system of the plant, then the soil is pressed around it so that the roots are in contact with the soil and can quickly colonize it. Mesocosm establishment activities were conducted:

- In the Bagnoli mesocosm: October 2017 (start of activity) - July 2018 (end)
- In the Benevento mesocosm: October 2017 (start of activity) - July 2018 (end).

Figure 7 - Mesocosm Bagnoli in the pre-sampling period

Figure 8 - Mesocosm Benevento in the pre-sampling period

The water needs of the plant species are met by an automated drip irrigation system with a control unit. The mesocosm located in the SIN is served by two reservoirs of 5000 litres each. These are filled on need by tanker truck, given the considerable distance between the area to be served and the supply point. Irrigation water is taken from the municipal drinking water network. Electricity (24 V DC) to power the pump is provided by a battery that is recharged by two photovoltaic panels. The irrigation system of the mesocosm set up in the Benevento greenhouse differs only in the presence of a 500-liter reservoir that is filled daily from the nearby supply point consisting of a well.

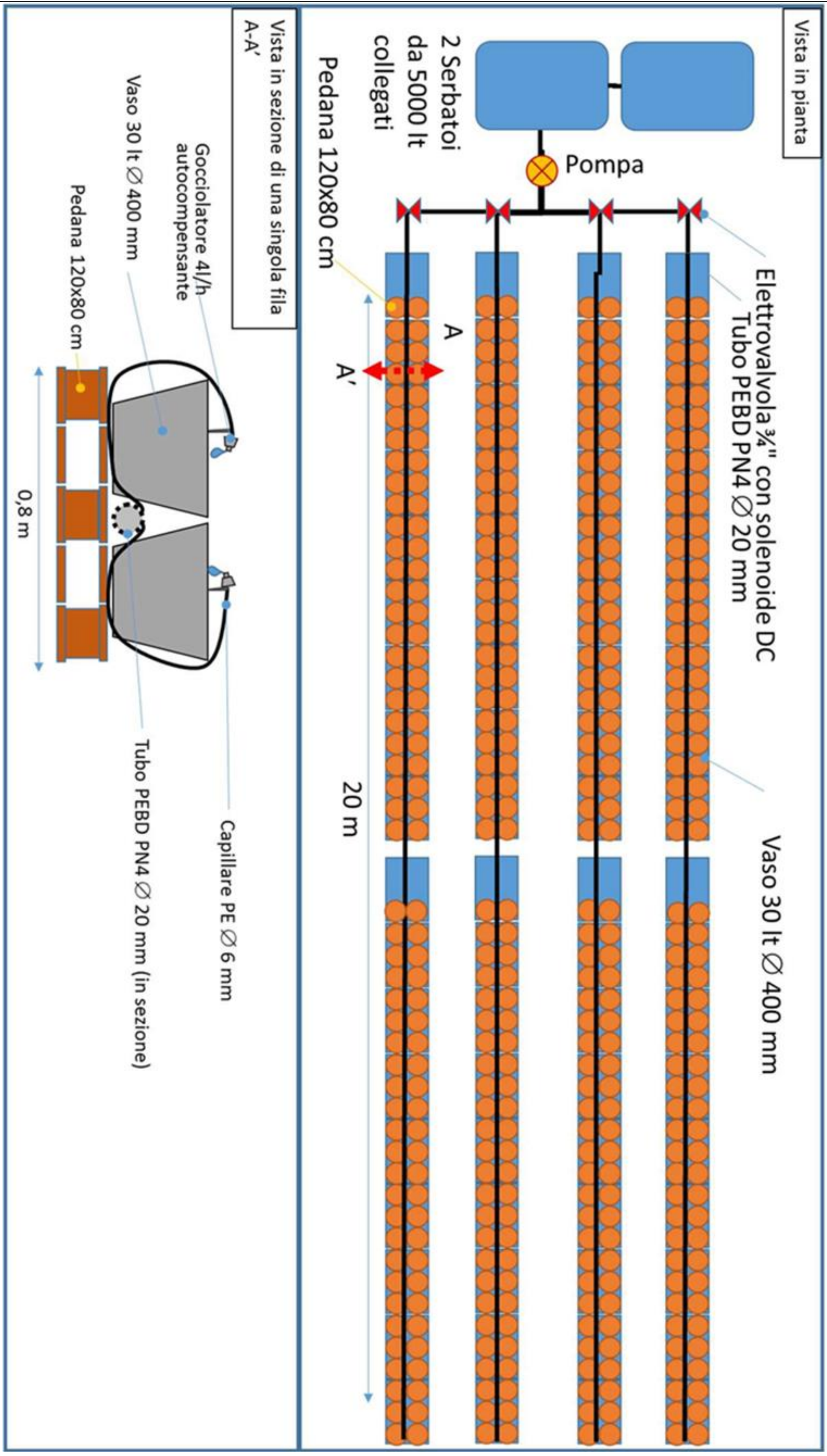

Figure 9 - Schematic diagram of the mesocosms irrigation system.

3.3 Control parameters and verification of the applicability (pilot scale)

Monitoring and field sampling program to adequately monitor the effectiveness of the three treatments:

- characterize the microbial community (fungi and bacteria) already present on the site, in different conditions of soil contamination with Metagenomic analysis of rhizospheric soils
- isolate from the site microorganisms (bacteria and fungi) that can be cultivated and therefore potential candidates for in-situ bioremediation
- select among the cultivable microorganisms, those with known and significant degradative capacity towards the target contaminants
- classify from a taxonomic point of view the microbial strains of potential interest for the bio-remedy approach
- validate the degradative capacities of microorganisms and selected consortia in tests with microcosms.

Soil analysis showed that within Proteobacteria, Alpha proteobacteria and Gammaproteobacteria are the taxa with higher proportions, both also containing PAH degrading species and Actinobacteria populations. Firmicutes and Deltaproteobacteria are relatively less than all other groups. The investigated rhizosphere soils showed Gammaproteobacteria as the most abundant class with the family Pseudomonadaceae. In the literature, the genera of Pseudomonas employed in the study of bioremediation on PAH-polluted soils carried out using a pool of bacteria with different activities and ability to use hydrocarbons as a source of carbon and energy is known. Since endophytic communities are found mainly in the phylum Proteobacteria, and our identified bacteria belong 82% to the phylum Proteobacteria, they act as endophytes. In Table 2 there are the main genera of bacteria that function as endophytes, Pseudomonas, Bacillus, Burkholderia, Rhizobium and Microbacterium. A good method to reduce PAH contamination is the process of biodegradation by cometabolism of different microorganisms therefore, cometabolism is one of the most important mechanisms in the transformation of PAHs in soil. Plant roots release exudates such as sugars, organic acids, fatty acids, secondary metabolites, nucleotides, and inorganic compounds that play an important role in establishing and determining the microbial population of the rhizosphere. These exudates directly or indirectly determine and regulate the activity of biodegradable microorganisms. Therefore, the ability of individual bacterial strains to produce indole-3- acetic acid (IAA), siderophores, exopolysaccharides and ammonia was evaluated. Thirty- nine of the bacterial isolates can produce IAA. Paenibacillus polymyxa is the best producer of IAA. In both Poaceae and Plantaginaceae rhizosphere soil, Rhizobium leguminosaurum produced the highest amount of IAA. Mycobacterium sp. MOTT36Y, on the other hand, produced only in the Poaceae family the highest amount of IAA. The rhizosphere of Plantaginaceae and Fabaceae has Pseudomonas cichorii as a good producer of IAA. Plantaginaceae and Fabaceae showed strains with high IAA of Paenibacillus sp. JDR-Z, Bacillus cereus group, Klesbiella aerogenes for the former and Rhizobium etli bv mimosae for the latter, respectively. In addition, 60% of the isolates were found to have siderophore production capacity. Rhodococcus jostii produced high amount of siderophores in all rhizosphere soils; only in Plantaginaceae, Bacillus cereus group also showed siderophore activity. Three-rhizosphere soils showed 50% isolates capable of producing EPS. The high amount of EPSs production was present in Burkholderia cenocepacia, Burkholderia ambifaria AMMD, Bacillus cereus group, Sinorhizobium fredii, Sinorhizobium meliloti. Only 38% of the isolates showed ammonia production. The high amount of ammonia was in Burkholderia pseudomallei NCTC 13178 and Bacillus cereus group (Table 5).

Bacterial isolates	**Production IAA**	**Siderophore release**	**EPSs production**
Bradyrhizobium japonicum USDA6	++	-	-
Bradyrhizobium japonicum	+++	+++	+++
Bradyrhizobium diazoefficiens	++	++	++
Bradyrhizobium oligotrophicum	++	++	+
Bradyrhizobium oligotrophicum S58	+	-	+
Nitrobacter hamburgensis	-	-	-
Nitrobacter hamburgensis X14	-	-	-
Rhizobium leguminosaurum	+++	+++	+++
Rhizobium leguminosarum bv trifolii	++	++	++
Rhizobium leguminosarum bv trifolii WSM2304	++	++	++
Rhizobium etli CIAT652	+	+	+
Rhizobium etli bv mimosae	-	++	-
Sinorhizobium fredii	+++	+++	+++
Sinorhizobium meliloti	-	++	-
Mesorhizobium ciceri	-	-	-
Achromobacter xylosoxidans	++	++	-
Burkholderia cenocepacia	+	-	-
Burkholderia ambifaria AMMD	-	+	+
Burkholderia pseudomallei NCTC 13178	+	-	-
Burkholderia gladioli	+	-	-
Burkholderia glumae BGR1	-	-	-
Paraburkholderia rhiroxinica	-	-	++
Paraburkholderia xenovorans	+	+	-
Pseudomonas aeruginosa	+	+	-
Pseudomonas aeruginosa PA7	-	++	-

Bacterial isolates	Production IAA	Siderophore release	EPSs production
Pseudomonas stutzeri	-	+++	-
Pseudomonas mendocina NK-01	++	++	-
Pseudomonas resinovorans	++	++	-
Pseudomonas resinovorans NBRC 106553	+++	-	-
Pseudomonas fluorescens	+++	+++	+++
Pseudomonas fluorescens Pf0-1	-	-	-
Pseudomonas fluorescens SBW25	-	++	++
Pseudomonas fluorescens F113	-	++	++
Pseudomonas poae	-	+	+
Pseudomonas putida	-	+	++
Pseudomonas putida H8234	-	+	++
Pseudomonas putida NBRC 14164	-	-	-
Pseudomonas putida GB-1	-	-	-
Pseudomonas putida HB3267	+++	-	-
Pseudomonas fulva 12-X	+	-	-
Pseudomonas stutzeri DSM 10701	+	-	-
Pseudomonas stutzeri DSM 4166	+	+	+
Pseudomonas stutzeri A1501	+	+	-
Pseudomonas syringaepv.tomato	+ +	++	++
Pseudomonas brassicacearum	++	++	++
Pseudomonas pertucinogena group	++	++	++
Pseudomonas cichorii	+++	+	++
Enterobacter aerogenes	++	-	+++
Geobacter bemidjiensis	++	-	+++
Rhodococcus opacus B4	++	-	-
Rhodococcus jostii	+	++	+
Rhodococcus pyridinivorans sb3094	++	++	++
Mycobacterium abscessus subsp bolletii	++	+	++
Paenibacillus mucilaginosus K02	++	++	++
Paenibacillus mucilaginosus 3016	+	+	+
Paenibacillus sp JDR-2	-	-	-
Paenibacillus polymyxa	+	++	++
Bacillus cereus group	+++	++	++
Bacillus thuringiensis serovar finitimus	++	-	-
Deinococcus proteolyticus	-	-	-

Table 5 - The ability of individual isolated bacterial strains to produce indole-3-acetic acid (IAA), siderophores, exopolysaccharides and ammonia

In contrast to the heterogeneity of bacterial structure taxonomy (82%) in three different rhizosphere soils, there are important alterations in the abundance of genes encoding enzymes for PAH degradation (Figure 5). Our results showed high gene abundance of several oxidoreductases involved in PAH metabolism as a test of the potential for PAH degradation by native soil microorganisms. Indeed, genes in the dioxygenase family (naphthalene 1,2-dioxygenase, estradiol dioxygenase, benzoate 1,2-dioxygenase, protocatechuate 4,5-dioxygenase (alpha and beta chain) and 1, 2-dihydroxynaphthalene dioxygenase) are the most abundant in PAH-contaminated soils, particularly in the rhizosphere soil of Plantago lanceolata compared to Piptatherum miliaceum and Lothus corniculatus (Figure 5). Therefore, from our data, several modes of PAH degradation can be hypothesized, although all of them could start with an initial oxidation by laccases followed by the activity of dioxygenases and decarboxylases, promoting further degradation of PAHs; assuming that natural soil bacterial populations are able to use specific enzymes and common pathways to degrade PAHs.

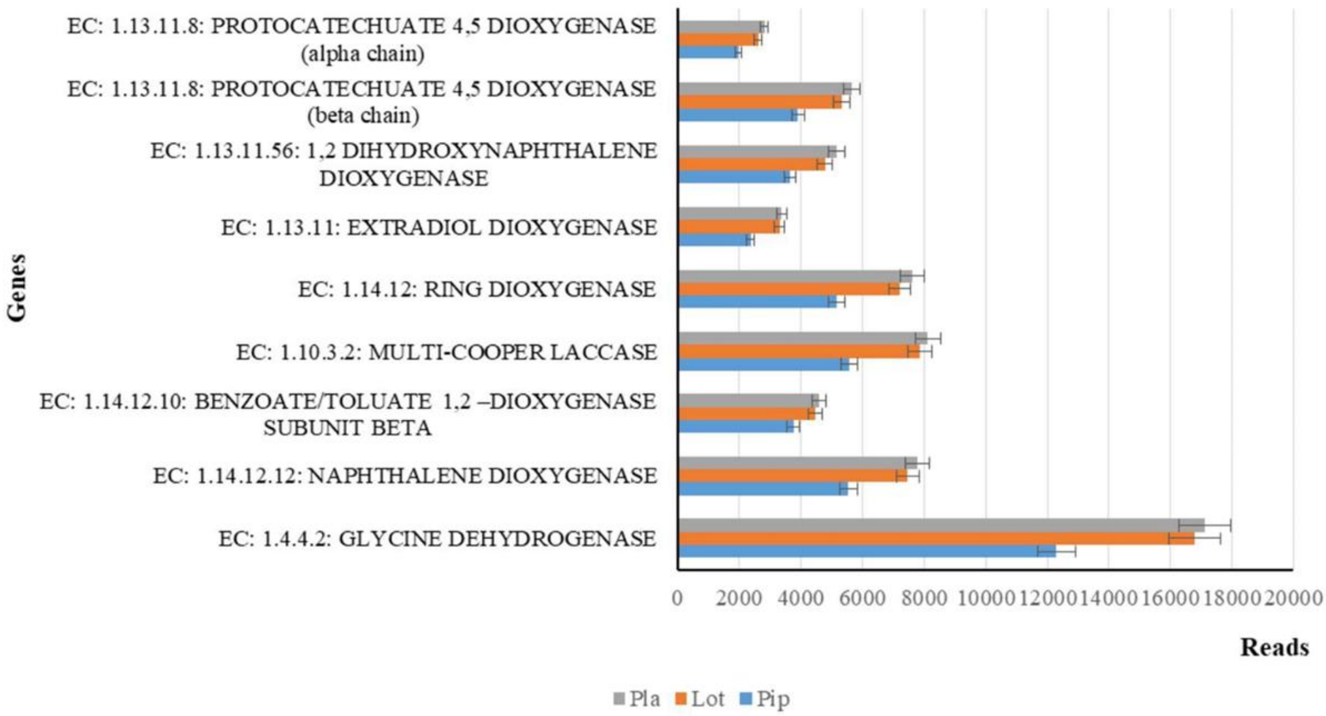

Figure 10- Genes for PHA degradation from soil metagenomes of Piptatherum miliaceum [Pip], Lothus corniculatus [Lot], Plantago lanceolata [Pla].

WATER AND LAND

IMPEL

4. Full scale application

4.1 Main treatment unit

Considering the indications of the legislation with particular regard to the aspects of environmental sustainability, the proposed remediation intervention is based on the combined and synergistic use of two remediation approaches on biological bases: bio and phytoremediation. The feasibility of this approach was verified with:

- a bibliographic verification of similar case studies
- site-specific experiments with microcosms on soil samples taken from the site

The specific objective of bioaugmentation is to activate the degradation processes of interest for remediation, through the addition of microorganisms selected from the site and specialized in the biodegradation of target contaminants:

- The specific objective of phytoremediation is to interrupt the migration paths of contaminants, to favour the development of complex and resilient microbial communities through the creation of favourable rhizospheric microhabitats, to activate further degradative processes mediated by microorganisms present in the internal of plant tissues (endophytes) and metabolic detoxification activity.
- Further environmental positives determined by the planting of plant organisms relate to the reactivation of higher-scale ecological processes (ecosystem services) such as: the removal of atmospheric CO2, the reduction of the heating effect associated with urbanization, the improvement of the quality of air, the regulation of hydrological processes, the increase of biodiversity. In addition to these environmental benefits, there are also aesthetic-landscaping and more generally the well-being of the population.

RESULT FROM MICROCOSMS showed:

1. The degradation processes naturally active in the site are not sufficient to produce a significant degradation of the tested contaminants (Landfarming - R1 – Treatment)
2. The permanence of optimal conditions for microbial activity must be guaranteed in-situ during the thermally favourable seasons and the addition of carrier supports the vitality of the indigenous mushrooms (Natural Attenuation R2 – Treatment)
3. The addition of the mushroom Pleurotus ostreatus and the bacterial/fungal consortium demonstrated very interesting degradation capacities for PAHs but apparently more limited for linear hydrocarbons (Bioaugmentation – assistited-

R3- Treatment).

INOCULUM IN PRACTICAL

1. The inoculum of the selected bacterial/fungal consortium is distributed in liquid suspension by spraying the cell suspension directly on the excavation walls and on the excavated ground, during the re-insertion phase.
2. The microbial density of the inoculum, defined in units of spores per unit mass of soil to be treated, was that identified as effective in the microcosm testing phase (Table 6).

Group	Specie	Quantity per kg of mix
Endomycorrhiza	Glomus clarum	≈2000 spore/kg
	Glomus etunicatum	
	Glomus intraradices	
	Entrophospora columbiana	
Ectomycorrhiza	Pisolithus tinctorius	110 million di spore/kg
Beneficial bacteria in the rhizosphere	Bacillus licheniformis	172 million UFC/kg
	Bacillus megaterium	
	Bacillus polymyxa	
	Bacillus subtilis	
	Bacillus thuringiensis	
	Paenibacillus azotofixans	

Table 6 - Composition of the fungal/bacterial consortium

A total of 8 areas have been built, for a total of about 3500 m^2. The areas were defined by placing the vertices of the quadrilaterals in the field, the topographic survey of which was conducted by total station. An initial provisional delineation of the areas was followed, after preliminary soil work, by a final one made with wire mesh.

No. 8 pilot areas of varying area were identified. Table 7 shows the codes, the coordinates of the vertices that identify them and the areas, and in Figure 11 they are located within the perimeter defined in the 2017 Characterization Plan (PdC).

It should be noted that the interventions described below were conducted for all areas, with the sole exclusion of the construction of the irrigation system for area No. 6, which was established during construction due to logistical difficulties related to the considerable distance from the water supply point. The lack of the irrigation system in this area made it impossible to conduct sowing since the dry season was underway.

However, for this area all the work and treatments performed for the others were conducted.

Codice area pilota	Vertici	COORDINATE Longitudine WGS 84	COORDINATE Latitudine WGS 84	SUPERFICI (mq)
1	A	14° 10.619	40° 48.859	566
	B	14° 10.629	40° 48. 864	
	C	14° 10.644	40° 48. 849	
	D	14° 10. 631	40° 48. 845	
2	A	14° 10.500	40° 48.855	691
	B	14° 10.484	40°48.849	
	C	14° 10.492	40° 48.836	
	D	14° 10.509	40° 48.842	
3	A	14°10.3843	40°48.6244	420
	B	14°10.3970	40°48.6312	
	C	14°10.4051	40°48.6232	
	D	14°10.3959	40°48.6157	
4	A	14°10.5291	40°48.7059	240
	B	14°10.5389	40°48.7099	
	C	14°10.5440	40°48.7019	
	D	14°10.5354	40°48.6985	
5	A	14°10.6831	40°48.6931	741
	B	14°10.7041	40°48.6983	
	C	14°10.7077	40°48.6847	
	D	14°10.6909	40°48.6797	
6	A	14°10.8646	40°48.2295	335
	B	14°10.8746	40°48.2373	
	C	14°10.8845	40°48.2298	
	D	14°10.8735	40°48.2243	
7	A	14°10.5343	40°48.8859	330
	B	14°10.5227	40°48.8801	
	C	14°10.5171	40°48.8895	
	D	14°10.5271	40°48.8939	
8	A	14°10.3493	40°48.8807	330
	B	14°10.3594	40°48.8851	
	C	14°10.3664	40°48.8770	
	D	14°10.3550	40°48.8713	

Table 7 - Pilot areas: vertex coordinates and surfaces

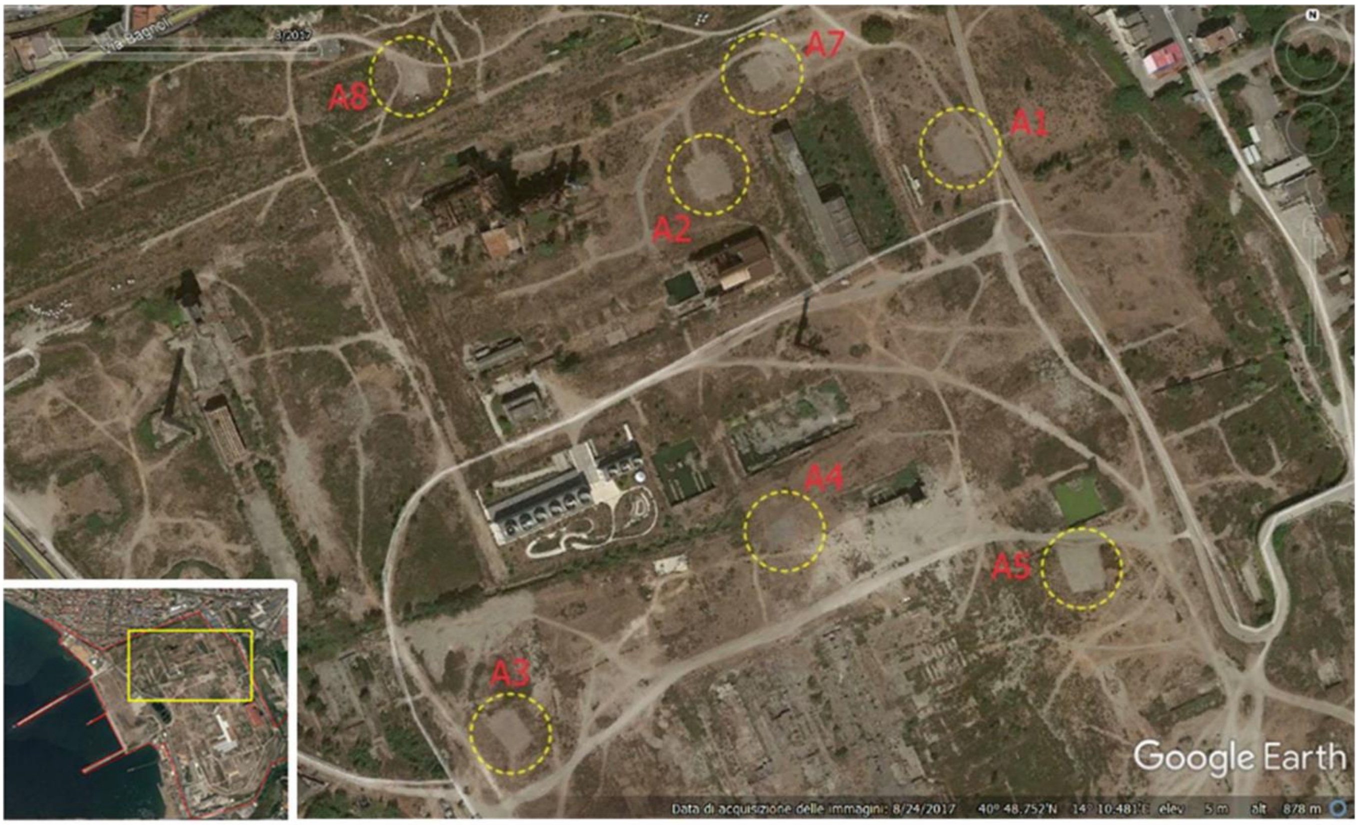

Figure 11 - Northern zone of the SIN: Location of pilot areas

TRENCH IN PRACTICAL

1. opening of the trench with positioning of the excavated soil at the edges, separating the first superficial layer (about 20 cm) from the deep ones
2. spraying the bacterial/ fungal spores' suspension on the excavation walls
3. placing an organic carrier layer (fine sand) on the bottom and spraying the bacterial and fungal spores' suspension
4. spraying the bacterial and fungal spores' suspension on the soil during reintroduction (a layer of about 50 cm)
5. fertilization with Ammonium sulphate, Monoammonium phosphate and organic fertilizer (Prodigy Plus®)
6. positioning of the last layer of organic carrier at a depth of 20 cm
7. arrangement of the two lines of driplines along the lateral edges of the excavation, directly in contact with the last layer of organic carrier
8. positioning of the plants in the centre of the trench (where foreseen) and covering it with the surface soil previously closed
9. tilling of the ground at the base of the plants.

SPECIES

The ground cover of the experimental plots consists of species belonging to the Fabaceae and Poaceae families also tested in mesocosms. This choice is supported by the verified ability of each of them to accumulate heavy metals and especially their direct involvement in PAH degradative processes at the rhizospheric level. The species used to belong to the Fabaceae family are **Lotus corniculatus L., Medicago sativa L. and Bituminaria bituminosa (L.) C.H. Stirt**. The selected Fabaceae were used to set up three pilot monoculture areas i.e. a single species in each area.

The species used from the Poaceae family are: **Piptatherum miliaceum L., Dactylis glomerata L., Festuca arundinacea Schreb**. These species are used in intercropping in four pilot areas as shown in Table 19. At the time of planting, a mixture containing the seeds of the three species is prepared according to a specific percentage (Table 19). It must be said that these three species are microtherms, i.e., they have an optimum of development under conditions of not high temperatures such as summer, so they ensure complete cover and lushness during the winter months, while in the summer months, they may suffer stress from high temperatures. To overcome this limitation, the species **Cynodon dactylon (L.) Pers**. was also introduced in the intercropping, which, in addition to possessing a number of favourable ecological characteristics for development in open field conditions during the summer months including tolerance to high temperatures, shows the following peculiarities: tolerance of salinity and drought, deep root system, very low incidence of diseases, high expansion and colonization of soil due to the ability to produce stolons. These characteristics are combined with phytoremediation capabilities like those of the other previously mentioned species.

Two tree species were used in the experimental phase: white willow (**Salix alba L.**) and white poplar (**Populus alba L.**). Noting the ability of species belonging to the genera Salix and Populus to tolerate and accumulate heavy metals, the great contribution in the use of these two species for phytoremediation is related to their ability to develop a wide and deep rhizosphere that provides an excellent habitat for the proliferation of microorganisms directly involved in the degradation of organic contaminants and PCBs.
Thus, the close interconnection between the plant and the fungal-bacterial consortium magnifies the remedial effect. Plants of both tree species will be planted in November according to good arboricultural standards.

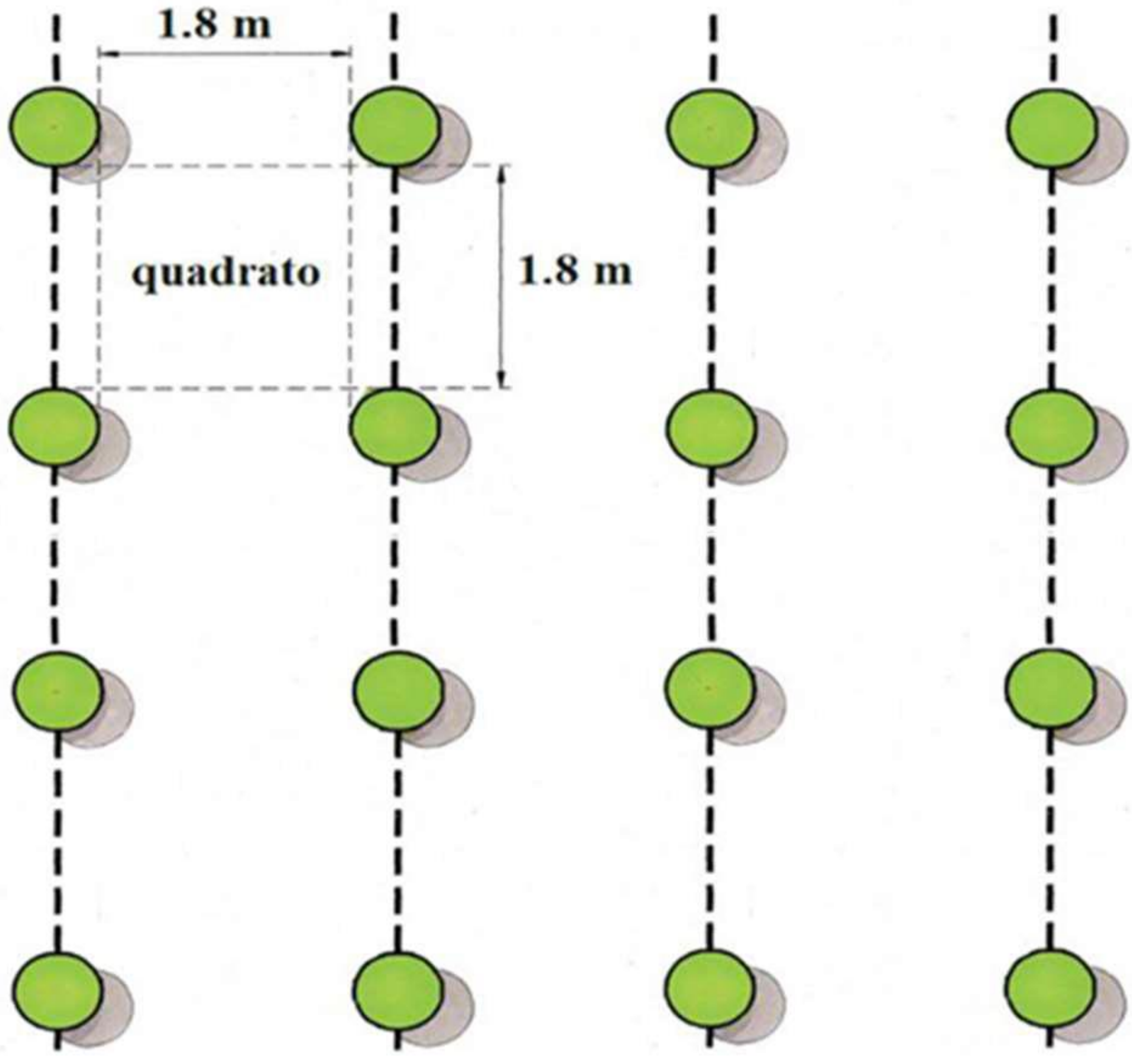

Figure 12 - Arrangement of Populus alba and Salix alba planting sites

Among the eligible poplars, black and white poplars have similar edaphic requirements, preferring deep, rich soils, but they can also adapt well to growing on poor, gravelly substrates. Of the two, white poplar is opted for because it is the more heliophilic and thermophilic of the two species, thus the one that best meets the soil and climate requirements of the location. Internally to the P. alba species, the "Villafranca" clone showed good accumulation of heavy metals in the roots, as well as excellent adaptation to local environmental conditions. The species Salix alba clone "Levante" showed to be more tolerant in the presence of heavy metal-contaminated substrates and with the ability to accumulate Cd. Tree plants have a stem circumference of 8 cm in the case of poplars and 6 cm in the case of willows according to the product categories of the nursery industry. The planting distances for both poplars and willows were 1.8 m on the row and 1.8 between rows. Wooden stakes of 3 m in length and 8 cm in diameter driven at least 50 cm into the ground shall be placed along each row. The distance between the stakes is 6 m along the row, and the stakes are connected to each other by galvanized wire placed at a height of 120 cm. The plants were bound to the wire with rubber agricultural-use ties to support them during the rooting stage.

Fig. 13 - Area 1: Lotus corniculatus L., Populus alba L. clone “Villafranca”, Salix alba L. clone "Levante"

Figure 14 - Area 2: Medicago sativa L., Populus alba L. clone “Villafranca”, Salix alba L. clone "Levante"

Figure 15 - Area 3: Bituminaria bituminosa L., Populus alba L. clone “Villafranca”, Salix alba L. clone "Levante"

Figure 16 - Area 4: Mix of grasses, Populus alba L. clone “Villafranca”

Figure 17 - Area 5: Mix of grasses, Salix alba L. clone "Levante"

Figure 18 - Area 6: lack of irrigation system

Figure 19 - Area 7: Mix of grasses, Salix alba L. clone "Levante"

Figure 20 - Area 8: Mix of grasses, Salix alba L. clone "Levante"

4.2 Monitoring of the chemical parameters

Property analysis was determined on rhizospheric soil samples according to All.5 Tab.1 Part Four D. Lgs. 152/06 and the main agronomic analyses at different monitoring times. Below are the parameters with the analytical methods used in the following Table 9:

In addition, the Bioavailability values of major metals and metalloids such as As, Cd, Cr, Ni, Cu, Pb, Tl, Zn were determined according to (i) Hudson-Edwards et al. (2004) for Arsenic (NH4OAcetate extraction), (ii) Martens and Lindsay (1990) for Cadmium, Copper Lead and Zinc (DTPA extraction) and (iii) Al-Najar et al. (2005) for Thallium (NH4OAcetate extraction). The bioavailable fraction of Hg in soil was determined based on a sequential extraction with H2O, 0.5 M HCl and 0.2 M NaOH followed by a final step with 0.27 M ammonium thiosulfate (AT) (Guarino and Sciarrillo, 2017).

Parametro	Metodo	CSC - D.lgs 152/2006 Parte IV All.5, Tab.1 mg/Kg ss Colonna A	CSC - D.lgs 152/2006 Parte IV All.5, Tab.1 mg/Kg ss Colonna B	Valori fondo naturale mg/Kg ss
ARSENICO	EPA3051+EPA6010	20	50	29
CADMIO	EPA3051+EPA6010	2	15	
MERCURIO	EPA7473	1	5	
PIOMBO	EPA3051+EPA6010	100	1000	103
VANADIO	EPA3051+EPA6010	90	250	100
ZINCO	EPA3051+EPA6010	150	1500	158
IDROCARBURI C>12 (C12-C40)	LINEE GUIDA 75/2011 ISPRA ARPA APPA	50	750	
IDROCARBURI C≤12 (6<C<12)	EPA5035+EPA8015	10	250	
BENZO(a)ANTRACENE	EPA3545+EPA8270	0,5	10	
BENZO(a)PIRENE	EPA3545+EPA8270	0,1	10	
BENZO(b)FLUORANTENE	EPA3545+EPA8270	0,5	10	
BENZO(k)FLUORANTENE	EPA3545+EPA8270	0,5	10	
BENZO(g,h,i)PERILENE	EPA3545+EPA8270	0,1	10	
CRISENE	EPA3545+EPA8270	5	50	
DIBENZO(a,e)PIRENE	EPA3545+EPA8270	0,1	10	
DIBENZO(a,l)PIRENE	EPA3545+EPA8270	0,1	10	
DIBENZO(a,i)PIRENE	EPA3545+EPA8270	0,1	10	
DIBENZO(a,h)PIRENE	EPA3545+EPA8270	0,1	10	
DIBENZO(a,h)ANTRACENE	EPA3545+EPA8270	0,1	10	
INDENOPIRENE	EPA3545+EPA8270	0,1	5	
PIRENE	EPA3545+EPA8270	5	50	
SOMMATORIA IPA (da 25 a 34)	EPA3545+EPA8270	10	100	
SOMMATORIA PCB (da calcolo)	EPA3545+EPA8270	0,06	5	
SCHELETRO	D.M. 13/09/99 SO			
RESIDUO A 105 °C	D.M. 13/09/99			

Parametro	Metodo
BORO	DM 13/09/1999 Met.XVI.2
CALCIO SCAMBIABILE	DM 13/09/99 XIII.5
FERRO (INDICE DI DISPONIBILITÀ)	DM 13/09/1999 Met.XII.1
FERRO	EPA3051+EPA6010
MANGANESE (INDICE DI DISPONIBILITÀ)	DM 13/09/1999 Met.XII.1
MANGANESE	EPA3051+EPA6010
ARGILLA	DM 13/09/1999 Met. II.6
AZOTO TOTALE KJELDAHL	DM 13/9/1999 Met XIV.2+ XIV.3 DM 25/3/02
CALCARE ATTIVO	DM 13/09/1999 Met.V.2
CALCARE TOTALE	DM 13/09/1999 Met.V.1
CARBONIO ORGANICO (TOC)	DM 13/09/1999 Met.VII.3
CONDUCIBILITÀ	DM 13/09/1999 Met. IV.1
FOSFORO ASSIMILABILE	DM 13/09/1999 Met.XV.3
LIMO FINE	DM 13/09/1999 Met. II.6
LIMO GROSSO	DM 13/09/1999 Met. II.6
MAGNESIO SCAMBIABILE	DM 13/09/99 XIII.5
pH	DM 13/09/1999 Met. III.1
POTASSIO SCAMBIABILE	DM 13/09/99 XIII.5
SABBIA FINE	DM 13/09/1999 Met. II.6
SABBIA GROSSA	DM 13/09/1999 Met. II.6
SODIO SCAMBIABILE	DM 13/09/99 XIII.5
CAPACITÀ DI SCAMBIO CATIONICO	DM 13/09/1999 Met.XII.2
RESIDUO A 105 °C	D.M. 13/09/99 Met II.2
SCHELETRO	D.M. 13/09/99 SO n°185 GU n° 248 21/10/99 Met II.1

Table 9 - Monitoring parameters and methods

4.3 Monitoring of the soil microbial community structure and functioning

The molecular identification of native of bacteria in soil was described by Guarino et al. (2019c). Brief, two grams of soil selected from 3 different points of each area, were used for the isolation of genomic DNA using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., USA) from following the manufacturer's instructions. Extracted DNA preparations were quantified and quality checked using a Nanodrop 1000 Spectrophotometer. Universal eubacterial primers (F27a: AGAGTTTGATCCTGGCTCAG;

R1492a: GGTTACCTTGTTACGACTT) were used as template for16S rRNA gene amplification. Polymerase chain reactions (PCR) were performed with GoTaq® Polymerase (Promega) according to the supplier's instructions. PCR-amplified DNA was sequenced with a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc. USA) using an automated DNA sequencer (ABI model 3500 Genetic Analyzer). Nucleotide sequences were edited and assembled with Lasergene version 11.2.1 (DNASTAR®) and subjected to homology comparison (BLAST analysis) at the National Center for Biotechnology Information (NCBI) server (www.ncbi.nlm.nih.gov/blast/Blast.cgi).

4.4 Monitoring of plants

X ray soil and root

Elemental distribution maps of soil and root samples were acquired using a benchtop micro X-ray fluorescence spectrometer (μXRF; M4 Tornado, Bruker Nano GmbH, Germany), equipped with a Rh tube with policapillary optics (50 kV, 600 μA, 30 W, spot size of 25 μm), and two 30 mm2 XFlash® silicon drift detectors. An aliquot of 2 mm-sieved soil was preliminary embedded in epoxy resin (L.R. White Resin, Polyscience Europe GmbH, Germany) to obtain a thin section (30 μm thickness), as described by Allegretta et al. (2018). Root samples were carefully washed with deionized water and air- dried. Two root portions of approximately 2 cm length were cut, one from the basal trait and the other from the apex and placed between two thin polypropylene membranes (Premier Lab Supply, USA). The membranes were well-tightened and fixed on the top of a plastic X-ray fluorescence sample cup (Fluxana, Germany), to keep the sample in position while scanning. X-ray maps of soil and root samples were collected under vacuum (20 mbar), using both detectors simultaneously and setting up a step size of 15 μm, an acquisition time of 10 ms per pixel, and five acquisition cycles.
Soil mapping was performed using an AlTi 100/25 sandwich-filter to reduce the background and enhance the detection of potentially toxic elements (PTEs) of greater interest, namely Pb, As and Zn. X-ray fluorescence hyperspectral data were processed using Bruker M4 software.

Stress markers and antioxidant enzymes

Stress markers and antioxidant enzymes activities in leaves of the treated (plants grown on contaminated soil with microbial consortium) and control groups (plants grown on uncontaminated soil) were analysed at each time point. Thirty fresh leaves (250 g) of plant species cultivated in monoculture and polyculture were homogenized under liquid nitrogen into a fine powder and were centrifuged at 19,000g for 30 min at 4 °C. The activities of stress marker (Glutathione S-Transferase, Phenylalanine Ammonia Lyase, Proline content, and Lipid peroxidation) were evaluated as described by Zuzolo et al. (2021), Guarino and Sciarrillo (2017). Glutathione S-Transferase (GST) was expressed as µM/min/µg protein. Phenylalanine Ammonia Lyase (PAL) was expressed as µg t-cinnamic acid/h/µg protein. Proline content as µmol/g fw and Lipid peroxidation (MDA) as µmol/g fw were expressed. The antioxidant enzyme activities (Superoxide dismutase, Catalase, Ascorbate peroxidase, Guaiacol peroxidase) were determined as described by Guarino et al. (2018). Superoxide dismutase (SOD) activity was expressed as unit mg−1protein. Catalase (CAT) activity was expressed as nmol of H_2O_2 mg^{-1}protein min^{-1}. Ascorbate peroxidase (APX) activity was assessed as mmol of ascorbate mg−1protein min^{-1}. Guaiacol peroxidase (GPX) activity was measured as nmol of guaiacol mg^{-1}protein min^{-1}.

4.5 Other monitoring

Seventy-six samples of selected species were sampled into two sections (root system and above ground biomass), thoroughly washed with tap water to remove dust particle, sand and carefully washed with deionized water. Plant samples were then acid-digested according to the USEPA method (USEPA, 1996). Total content of PTEs in plant extracts were determined by an ICP-OES (Varian Inc., Vista MPX) following (Guarino et al., 2019a).

5. Results

5.1 Removal rate

Soil sampling was performed from 2017 to 2022. Sampling was conducted at four points in each area as marked in the following Figure 21.

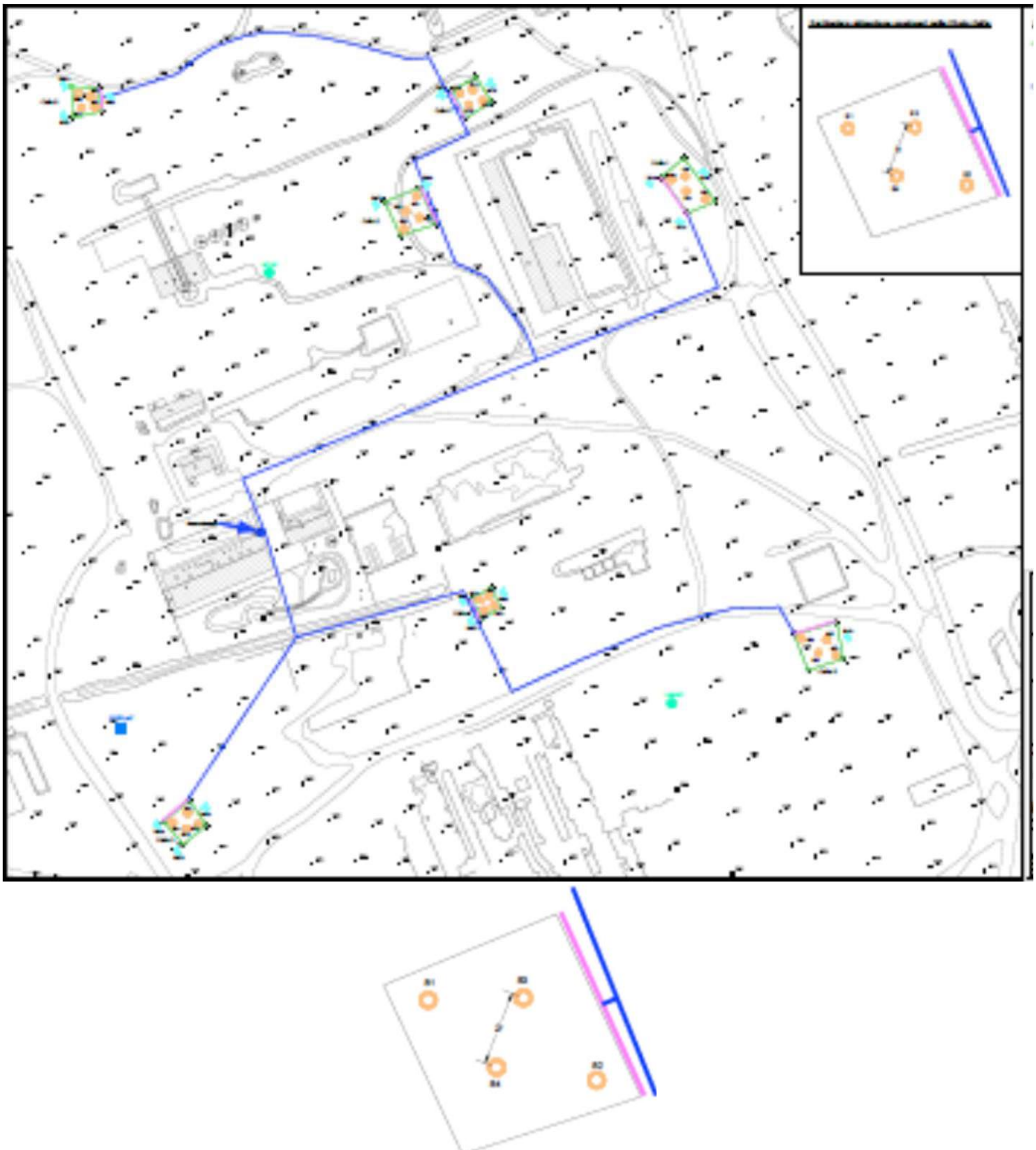

Fig. 21 - Soil sampling locations

At each of the four sampling points, three samples with a diameter of 1 m were taken with a garden shovel at a depth of 25-50 cm. In addition, the individual samples were combined into one composite sample.

Figure 22 - Soil profile colonized by the root systems of Lotus corniculatus. The depth reached is more than 40 cm.

Calculations and Statistics

Statistical analyses were performed using R Statistical Software (team and R Core Team, 2015). A statistical Two-Factor without Replication - ANOVA analysis was adopted in order to conclude if there were significant differences between the removal from soil of the considered PTEs by the plant species, experimental area (PTEs concentration in soil) and the dependency of phytoremediation on type of plants association (monoculture and polyculture). It was considered as most appropriate significance level a value p = 0.05, which correspond to the probability of rejecting the null hypothesis when this is true. The homogeneity of variance assumption was checked. For ANOVA significant results, Tukey's

HSD post-hoc tests was run to find out which specific groups' means differ. ANOVA and post-hoc analyses were performed using the R stats package (team and R Core Team, 2015).

Besides, a PCA biplot was performed to detect general trends in the relationships between plants and antioxidant response over time. The biplot analysis has been widely used to obtain a 2D graphical representation for multivariate data sets to describe and display the relationships between observations and variables (Zuzolo et al., 2020). PCA was performed using R stats package and its output (PCA biplot) was conducted by factoextra package which provides ggplot2- based visualization (Wickham, 2009).

The function prcomp (stats package) calculates the principal components with singular value decomposition (SVD) on the data matrix. The data were standardized (centred and scaled to have unit variance and zero mean).

Figure 23 - Profile of soil colonized by Medicago sativa

Removal Rate

The total concentrations of the various contaminants in the soil before and after treatment shown below.

The efficiency of PTEs removal process from contaminated soil have been evaluated by the following equation (Khandanlou et al., 2016):

$$\text{Efficiency of elemental removal(ER)}\% = \frac{C_0 - C_f}{C_0} \times 100$$

Where C_0 is the initial contaminant concentration (mg/kg) and C_f is the final contaminant concentration (mg/kg) in soil.

SOIL	Plants	Elemental removal performance (%)						Multi-element removal performance (%)
		% ER As	% ER Cd	% ER Hg	%ER Pb	% ER V	% ER Zn	
A_1	*Salix purpurea*	21.9^{a}	6.8^{a}	7.3^{a}	17.4^{a}	16.1^{a}	29.6^{a}	16.5^{a}
	Lotus corniculatus	2.8^{a}	19.0^{a}	46.3^{a}	15.3^{a}	12.9^{a}	6.4^{a}	17.1^{a}
	Festuca arundinacea	15.6^{a}	8.4^{a}	48.8^{a}	30.3^{a}	36.6^{a}	13.6^{a}	25.5^{a}
	Piptatherum miliaceum	2.9^{a}	10.7^{a}	17.1^{a}	47.4^{a}	19.4^{a}	28.3^{a}	21.0^{a}
	Dactylis glomerata	18.8^{a}	3.0^{a}	36.6^{a}	1.3^{a}	33.3^{a}	22.1^{a}	19.2^{a}
	Medicago lupulina	18.8^{a}	11.1^{a}	31.7^{a}	36.6^{a}	33.3^{a}	6.7^{a}	23.0^{a}
	P1 (*Salix purpurea, Festuca arundinacea, Piptatherum miliaceum, Dactylis glomerata*)	43.8^{b}	28.6^{c}	54.9^{a}	48.2^{a}	55.9^{b}	50.0^{b}	46.9^{b}
	P2 (*Salix purpurea, Lotus corniculatus*)	37.5^{b}	17.0^{ab}	57.6^{a}	45.7^{a}	50.5^{b}	46.7^{b}	42.5^{b}
	P3 (*Salix purpurea, Medicago lupulina*)	34.4^{b}	20.8^{bc}	60.0^{a}	42.1^{a}	54.8^{b}	47.6^{b}	43.3^{b}
A_5	*Salix purpurea*	10.6^{a}	58.8^{a}	80.9^{ab}	56.2^{a}	50.0^{a}	26.5^{a}	47.2^{a}
	Lotus corniculatus	10.6^{a}	50.6^{a}	81.8^{ab}	64.7^{a}	61.6^{a}	43.0^{a}	52.1^{a}
	Festuca arundinacea	51.2^{a}	58.8^{a}	83.6^{ab}	73.3^{a}	68.6^{a}	53.0^{a}	64.7^{a}
	Piptatherum miliaceum	2.4^{a}	29.4^{a}	76.4^{ab}	1.2^{a}	64.0^{a}	30.5^{a}	34.0^{a}
	Dactylis glomerata	2.4^{a}	29.4^{a}	87.3^{ab}	70.5^{a}	69.8^{a}	38.6^{a}	49.7^{a}
	Medicago lupulina	21.1^{a}	45.9^{a}	67.3^{ab}	62.8^{a}	66.3^{a}	38.9^{a}	50.4^{a}
	P1 (*Salix purpurea, Festuca arundinacea, Piptatherum miliaceum,Dactylis glomerata*)	55.4^{b}	27.1^{ab}	90.0^{a}	81.8^{a}	79.1^{b}	69.5^{b}	67.1^{a}
	P2 (*Salix purpurea, Lotus corniculatus*)	42.6^{ab}	10.0^{b}	80.9^{ab}	80.2^{a}	70.9^{ab}	68.2^{b}	58.8^{a}
	P3 (*Salix purpurea, Medicago lupulina*)	40.0^{ab}	7.1^{b}	77.3^{b}	79.5^{a}	75.6^{ab}	64.5^{b}	57.3^{a}
A_8	*Salix purpurea*	14.2^{a}	62.0^{a}	92.9^{ab}	83.9^{a}	2.3^{a}	41.7^{a}	49.5^{a}
	Lotus corniculatus	4.8^{a}	7.6^{a}	91.4^{ab}	66.4^{a}	7.0^{a}	3.6^{a}	30.1^{a}
	Festuca arundinacea	48.7^{a}	56.0^{a}	83.6^{ab}	90.6^{a}	27.9^{a}	72.7^{a}	63.2^{a}
	Piptatherum miliaceum	40.7^{a}	62.5^{a}	91.4^{ab}	81.9^{a}	23.3^{a}	48.9^{a}	58.1^{a}
	Dactylis glomerata	42.5^{a}	62.0^{a}	92.1^{ab}	75.2^{a}	30.2^{a}	58.3^{a}	60.0^{a}
	Medicago lupulina	16.8^{a}	62.0^{a}	92.9^{ab}	83.2^{a}	20.9^{a}	56.1^{a}	55.3^{a}
	P1 (*Salix purpurea, Festuca arundinacea, Piptatherum miliaceum, Dactylis glomerata*)	71.2^{b}	35.7^{a}	96.4^{a}	94.0^{a}	48.8^{b}	82.0^{a}	71.4^{a}
	P2 (*Salix purpurea, Lotus corniculatus*)	61.4^{b}	15.7^{a}	92.1^{a}	92.6^{a}	46.5^{b}	81.3^{a}	64.9^{a}
	P3 (*Salix purpurea, Medicago lupulina*)	59.6^{ab}	12.9^{a}	85.0^{b}	91.9^{a}	44.2^{b}	79.9^{a}	62.2^{a}

Table 10 - Removal performance of PTEs in pilot areas. The selected plant species performance grown as monoculture and polyculture (P) in three areas (A1, A5 and A8) are shown. Different letters indicate significant differences (Tukey Posthoc analysis, $p < 0.05$) between monocultures and polycultures in each of the soil types.

The removal rates (% removal rate) for each area regarding organic contamination specifically heavy hydrocarbons (C12-C40). is shown in the table below.

Area%	Removal Rate
Area 1	44.77
Area 2	27.95
Area 3	30.92
Area 4	60.77
Area 7	53.31
Area 8	63.25

Table 11 – Removal rates of each area

As far as PCB degradation is concerned, Pilot Area No. 2 is, for the time being, the only one in which there has been such tangible evidence for PCB degradation (%RR 66.5). As is well known, PCB degradation occurs through the integrated action of the plant-fungus-bacteria biological system that has been introduced in the pilot areas, but the direct degradation action is operated by the exoenzymes produced by the fungus Pleurotus ostreatus. Therefore, given the encouraging results of the mesocosm, since a second inoculation was carried out in January, which is the most suitable period for the proliferation of fungal mycelium, and following this there was a strong proliferation of basidiomes on all the pilot areas, it is plausible that in the next cycle the results will be collected on the other areas as well.

6. Post treatment and/or Long Term Monitoring

6.1 Post treatment and/or Long Term Monitoring

A post treatment and long-term monitoring will be carried out after the implementation of the system in the 8 hectares area mentioned in the chapter 7.1.

For the contaminants that eventually will not reach the target remediation concentration, a number of testings will be set:

- leaching test
- bioavailability test

The results will be processed in site-specific risk assessment to evaluate if the concentration were acceptable for the conceptual site model scenario.

7. Additional information

7.1 Lesson learnt

In the following list the main key findings and lessons learned about the site of Bagnoli-Coroglio will be described as bullet points:

- Difficulties and weaknesses - In the field activities some stressors not encountered at laboratory or greenhouse scale affected phytoremediation: an uneven distribution of contaminants and the heterogeneity of the soil structure most affected the final outcomes.
- Successes and strengths – a preliminary survey of the investigated site has been necessary to define the proper sub-areas where bio-phytoremediation should be applied, especially in terms of depth and degree of contamination.
- Keystones – the laboratory must work under QA/QC procedures and using Certified Reference Materials.
- Rooms for improvement – the full-scale application on an approximately 8 hectares sub-area will confirm, in full or partially, the achieved outcomes and the restoration trends.
- The heterogeneity of the site required an extreme fragmentation of agronomic practices. In fact, the pH fluctuation, and the presence of a granulometric structure of the soil required a customization of some procedures. For example, an acid fertilization based on phosphorus sulphates has been prepared to increase the bioavailability of heavy metals. Furthermore, mycorrhizal consortia and their affinity for phosphorus have been exploited to create arsenic uptakes.
- Genetically modified organisms were not used both in the laboratory and in the mesocosm, as it was not possible to transfer them in the field. Therefore, our choice was oriented towards the construction of a microbial consortium (fungi and bacteria) specific for the problems of Bagnoli and which amplified the power of plants to absorb heavy metals and the power of the rhizosphere in the degradation of organic pollutants.

7.2 Additional information

The main clues and evidence referable to the success of remediation are the following:

- Monitoring in the long run - Based on the extrapolations of data obtained from laboratory and mesocosm experiments, the preliminary interpretations can lead to an unproper evaluation of the full-scale trend.

- Certification activities – At the end of the scheduled time a cross-examination with the control bodies is necessary to understand if the restoration goals have been achieved. There is no specific guideline or operative protocol to define the best practices for such activities.
- It is necessary to better understand the complex interactions between contaminants, soil, plant roots, and microorganisms (bacteria and mycorrhiza) in the rhizosphere. A large amount of knowledge is now available on the biochemical processes involved in the detoxification of pollutants inside plant cells. One of the most important challenges is to use this basic scientific information to improve the efficiency of phytoremediation in the field.

7.3 Training need

The most effective training tool from the technical, procedural, organizational point of view is a mix of different items such as workshops, training on-the job, webinars, and e-learning.
Now we have published several technical documents and papers, and we've attended important workshops and seminar. A list follows.

Scientific papers

- C. Guarino, D. Zuzolo, M. Marziano, G. Baiamonte, L. Morra; D. Benotti; D. Gresia; E. Robortella Stacul; D. Cicchella, R Sciarrillo 2018 Identification of native-metal tolerant plant species in situ: environmental implications and functional traits. Science of the Total Environment, Feb; 650 (2), pag. 3156-3167.
- E. Robortella Stacul, D. Benotti, L. Morra, D. Gresia. 2019 Applicazione su scala pilota e reale di tecnologie di bonifica biologiche, chimiche e fisiche in un SIN: vantaggi di un approccio integrato – Geologia dell'Ambiente, 2/19, 258-261
- C. Guarino, D. Zuzolo, M. Marziano, B. Conte, G. Baiamonte, L. Morra; D. Benotti;
- D. Gresia; E. Robortella Stacul; D. Cicchella, D. Cicchella & R Sciarrillo 2019 Investigation and Assessment for an effective approach to the reclamation of Polycyclic Aromatic Hydrocarbon (PAHs) contaminated site: SIN Bagnoli, Italy. Scientific Reports – Nature Research, 2019 9:11522
- Teani, F. Saraceno, E. Robortella Stacul, D. Benotti, L. Morra, D. Gresia. 2020 Tecnologie di bonifica applicabili per il risanamento di siti dismessi, test di laboratorio e prove industriali per la progettazione di interventi efficaci e sostenibili: il caso del SIN di Bagnoli-Coroglio. Proceedings del Convegno HUB Tecnologica Campania – Remtech
- Ca. Ferone, M Ponticelli, S. Micheli, Ce. Ferone, E. Robortella Stacul, D. Benotti, L. Morra, D. Gresia. 2020 La sostenibilità delle bonifiche ambientali: test pilota di Bio- Phytoremediation sul SIN di Bagnoli – Coroglio. Proceedings del Convegno HUB Tecnologica Campania – Remtech
- E. Robortella Stacul, L. Morra, D. Gresia, C. Fiore, 2021 Applicazione della norma ISO 18504 "Soil Quality – Sustainable Remediation" ad un intervento di bonifica su scala industriale mediante

tecnologie di trattamento chimico-fisiche e biologiche. Monografia di Geologia Ambientale "Le bonifiche ambientali nell'ambito della transizione ecologica" 240 – 248.

- Iacobini, D. Baldi, S. Vinci, R. Mangolin, C. Fiore, E. Robortella Stacul 2021 WEB GIS per l'organizzazione, l'elaborazione e la condivisione dei dati ambientali: l'esempio del SIN di Bagnoli – Coroglio. Monografia di Geologia Ambientale "Le bonifiche ambientali nell'ambito della transizione ecologica," 263 – 268
- D. Zuzolo, C. Guarino, A. Postiglione, M. Tartaglia, P. Scarano, A. Prigioniero, R. Terzano, C. Porfido, L. Morra, D. Benotti, D. Gresia, E. Robortella Stacul, R. Sciarrillo (2021) Overcome the limits of multi-contaminated industrial soils bioremediation: Insights from a multi-disciplinary study. J. Haz. Mat. 421(5):126762

Oral presentations

- Workshop SICON_2020 – Special Session "Aspetti e criticità emergenti nella bonifica di siti contaminati" Roma, 12–14 febbraio 2020
- Seminar "Bonifica dei siti inquinati – Analisi e soluzioni di problemi complessi." Napoli, 17 febbraio 2020 Workshop "Siti contaminati e bonifiche eco-compatibili". Napoli, 12 aprile 2022
- Workshop "Il territorio campano tra specificità geochimiche ed emergenze ambientali." Napoli, 22 aprile 2022 Workshop "Il Programma di Risanamento Ambientale e Rigenerazione Urbana "PRARU" del Area di Rilevante Interesse Nazionale di Bagnoli Coroglio". Napoli, 05 maggio 2022
- Workshop "Area di Rilevante Interesse Nazionale di Bagnoli Coroglio – Lo stato di attuazione degli interventi di risanamento ambientale." Napoli, 22 maggio 2022
- Workshop SICON_2023 Plenary Session "Aspetti integrati di risanamento ambientale e rigenerazione urbana. Le linee di intervento di Invitalia Roma, 8–10 febbraio 2023
- Policy Briefing_Life Sedremed_Expert Roundtable - Classification and management of sediments in the EU "Presentation of IT legislation on management of contaminated sediments and necessary policy developments, the Bagnoli case-study," Bruxelles, 9 febbraio 2023

1. Contact details - CASE STUDY: Phytoremediation n.3

1.1 Name and Surname	María Jesús Mallada
1.2 Country/Jurisdiction	ESPAÑA / La Rioja
1.3 Organisation	Directorate General for Environmental Quality
1.4 Position	Environmental coordinator
1.5 Duties	In this project, contracts, following up on the evolution of the pilot test
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2. Site background

2.1 History of the site

Soils contaminated by chromium from tanneries that use chromium compounds in the leather dyeing and tanning process. Very high chromium concentrations were detected (maximum 17500 mg/kg), exceeding the established intervention levels (500 mg/kg in the Basque Country, 700 mg/kg Catalonia, 380 Dutch regulations).

During 2001 and 2002, a pilot test was carried out to assess the applicability of phytoremediation as a decontamination technique for chromium-contaminated soils.

Around 110 m^3 of the soil near the industry located in the village centre was moved to a previously prepared plot of land measuring 20x20 m, where it was treated by seeding it different plant species. The pilot test consisted of developing a nearby plot of land, moving part of the land with chromium and sowing different plant species: alfalfa, lupin, wheat, barley, green beans, tomato, corn, and mustard.

2.2 Geological setting

The soil was excavated and moved out of the urban area.

The pilot test site was insulated from the ground with a polyethylene sheet.

2.3 Contaminants of concern

Chromium

2.4 Regulatory framework

At the time of the proceedings there was no regulation in force. Therefore, the intervention levels established in the Dutch regulation (380 mg/kg) were taken as a reference.

3. Pilot-scale application in field

3.2 Treatment unit (pilot scale)

Start of operations: Year 2000

Location of contaminated soil: Drainage ditch in Santo Domingo de la Calzada.

Location of the Pilot Test: Municipal District of Santo Domingo de La Calzada. Plot 788a. Site: "Las Tejeras".

Approximately 110 m^3 of soil from the irrigation ditch was transferred to a previously prepared 20x20 m plot, where it was treated by planting different plant species.

Species selected in Autumn: Alfalfa, Lupin, Wheat, and Barley.

Cultivation work conducted:

1. First tillage pass
2. Mulching
3. Inorganic fertilisation. 40 kg. of NPK
4. Preparation of the seedbed (mulching).
5. Sowing
6. Treatment with HCl, 0.01 M and 0.02 M to mobilise chromium.

Figure 1 - Waterproofing

Figure 2 - Installation of drainage ditch and water collection pit


Figure 3 - Sowing

Figure 4 - State of crops 40 days after autumn sowing (Wheat and Barley)

Activities in Spring

Chemical reactions

Chromium concentration data in the plot

- Chromium III: 99.69%
- Chromium VI: 0.3%
- Bioavailable chromium, 0.008%

Chromium bioavailability is increased through complexation of soluble organic acids that keep it in solution at low pH values.

Cr redox reactions

Reduction in the presence of organic matter and Fe minerals. At pH between 5 and 7 Cr+3 predominates in the form of $Cr(OH)_3$

Cr 6+ ____________________Cr 3+

Oxidation, in the presence of strong oxidants e.g. MnO_2. At pH higher than 7, CrO_4^{2-} chromates are formed. The dichromate ion predominates at pH below 6.

4. Full-scale application

4.4 Monitoring of plants

Plant sampling

- Bare-root plant sample after cleaning of adhering soil.
- Several replicates per crop
- Transport to the laboratory in an airtight bag under suitable storage conditions.
- Once in the laboratory, differentiation of the different parts of the plant (root, stem, leaves and fruit) to establish the preferred organ for accumulation.

Total chromium extraction (mg Cr), autumn crops.

	Alfalfa (8 meses)	Altramuz (6 meses)	Trigo (6 meses)	Cebada (6 meses)
Raíz	8420	805	2784	2088
Tallo	3190	76,8	2602,62	3013,56
Hojas		270,98		
Fruto	-	0.32	14.55	0
TOTAL	11610	1153	5401,13	5101,56

Table 1 -Total chromium extraction (mg Cr), autumn crops.

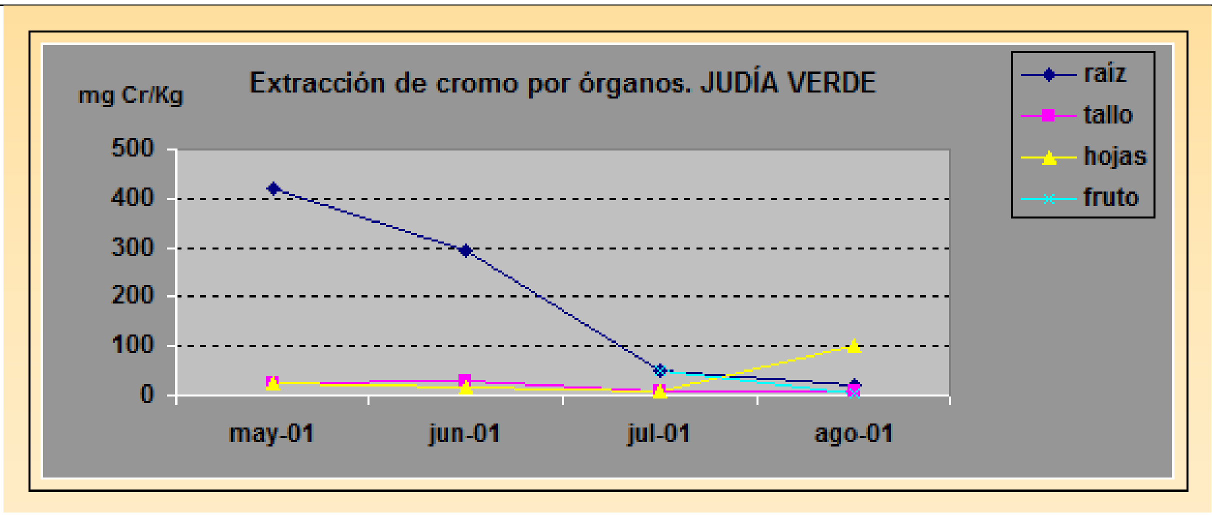

Figure 5 - Extraction rates for green beans

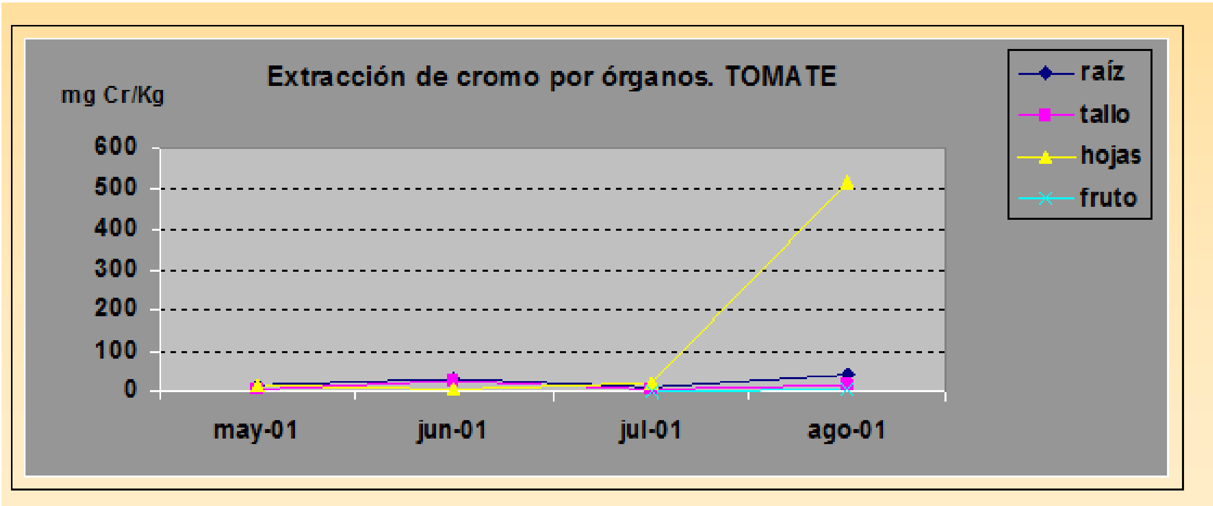

Figure 6 - Extraction rates for tomato

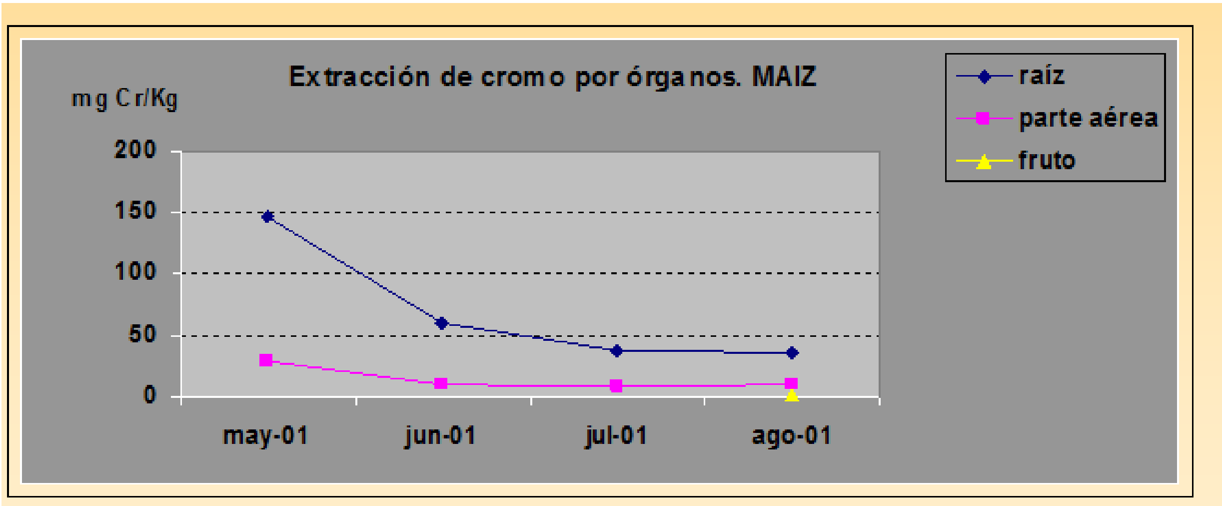

Figure 7 - Extraction rates for corn

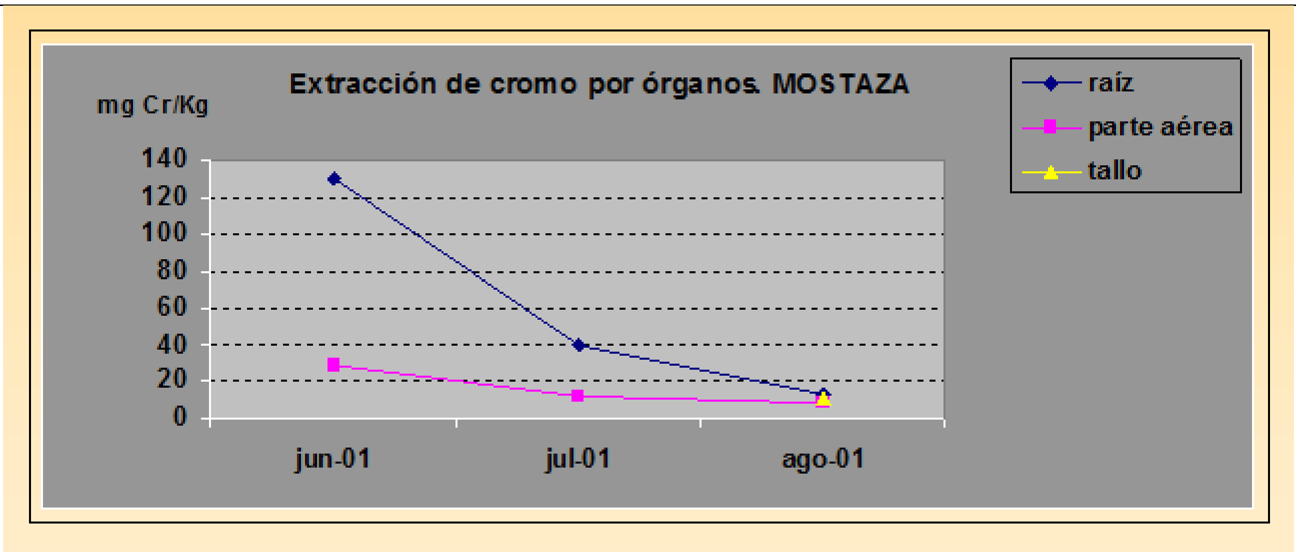

Figure 8 - Extraction rates for mustard

5. Results

5.1 Removal rate

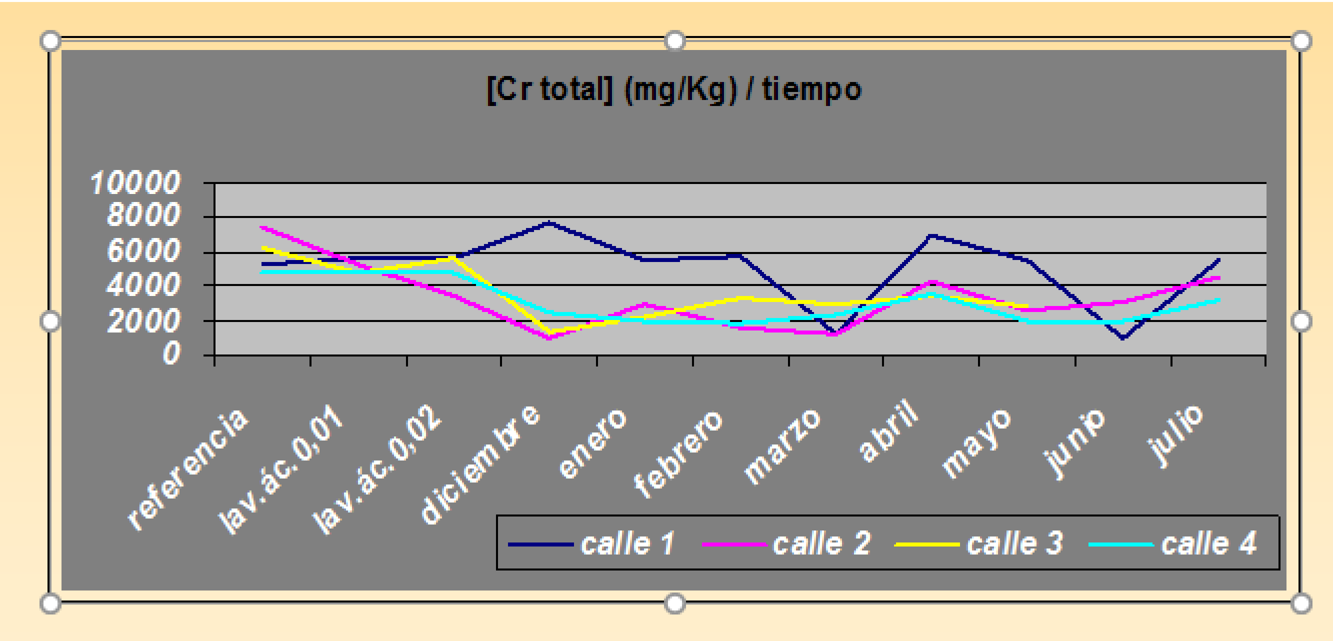

Figure 9 -Removal rate of Cr total

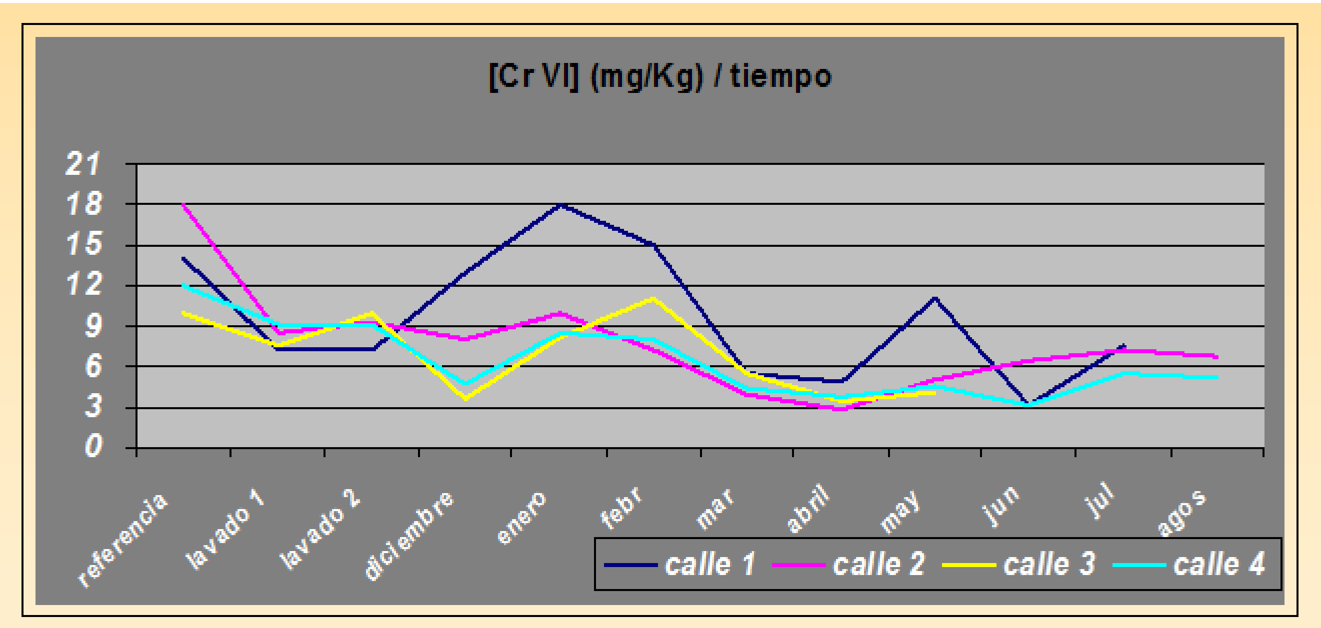

Figure 10 - Removal rate of Cr VI

7. Additional information

7.1 Lesson learnt

- The decision to move the land to another site was based on a decision to move the population away from land with high chromium content, it would have been more efficient to carry out the treatment on site.
- All the species planted in the plot successfully completed their agronomic cycles, with no growth problems, so the high levels of contamination observed in the soil of the plot do not represent a serious problem for plant development. It should be noted that the concentration of chromium biologically available to plants is very low.
- The chromium uptake rates experienced by the crops were, in all cases, very satisfactory in relation to the concentrations of bioavailable chromium in the soil (potentially extractable by phytoremediation).
- Autumn species, mainly cereals, have achieved excellent decontamination performance through root and stem organs, with minimal accumulation in leaves and fruit.
- Spring species show similar accumulation rates to autumn species, with a difference in the preferred organs of accumulation, as they show translocation of the pollutant to leaves and fruit.
- The treatment of soils contaminated by chromium by means of phytoremediation through cereal crops (specifically wheat) is effective for the removal of the bioavailable fraction of chromium, as a medium-term treatment.
- It is also an environmentally compatible and economically viable treatment, as it does not produce any alteration of the environment and its costs, for this type of crop, are very low. However, in highly contaminated soils, it is a relatively slow process.

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2. Site background

2.1 History of the site

The site is a former industrial area where various chemicals were produced mainly for agriculture. After the decommissioning of the industrial activities, the site was the subject of various interventions for the disposal of processing residues, the dismantling of plants and the demolition of buildings. Subsequently, the site underwent emergency safety measures for the land implemented by covering and waterproofing all the contaminated areas and groundwater (construction and activation of a hydraulic barrier with a water treatment plant).

Figure 1 - Picture from the affected area

2.2 Geological setting

The geognostic investigations have shown that the surface part is mainly made up of fill material and medium-fine sands, even if, in some points, the presence of gravels can also constitute the entirety of this first interval. Some marginal sectors present evident differentiations, such as those due to the presence of a discrete pelitic blanket. This first layer is indicatively estimated at about 3-7 m. The domain immediately below, which extends to depths ranging between 23 and 29 metres, corresponds to a succession consisting essentially of gravels in a sandy matrix with subordinate and discontinuous silty, silty-sandy horizons, mainly present at altitudes between 10 and 15 meters. From a hydrogeological point of view, the land hosts a low aquifer gradient, the subjacency of which is stationed on average at 2 meters from the ground level.

2.3 Contaminants of concern

The contamination is due to arsenic (As) and lead (Pb) deriving from chemical plant activities and is very heterogeneous. On this basis, the area was divided into Thiessen polygons, and the most significant soil samples were chosen to verify the feasibility of phytoextraction and determine which plant species, among those chosen, were the most suitable. On the considered soil samples (three for each selected polygon), the total concentrations of As and Pb were determined, together with their speciation, by sequential extraction.

2.4 Regulatory framework

The operational reclamation project envisaged, in addition to the excavation and disposal of unsaturated soils (up to 2 m from the ground level), also in situ phytoremediation interventions aimed at the reclamation of soils of the first meter of depth, contaminated by As and subordinately by Pb.

3. Pilot-scale

3.1 Laboratory Study

The experimental framework of the laboratory activity included **metal speciation, mesocosm tests** and **microcosm tests with the addition of autochthonous PGPB**.

Metal speciation

The main contaminants Pb and As, are characterised by different chemical natures. Therefore, the potentially bioavailable concentrations of the two metals were determined by means of two separate tests.

The speciation of As, whose solubility in water is lower than the detection limit, was performed by sequential extraction with 0.05M$(NH_4)_2SO_4$ and 0.05MK_2HPO_4, according to the procedure suggested by Wenzel et al. (2001).

A sequential extraction procedure with H_2O, 1 M KNO_3 and 1% EDTA (Pedron et al., 2009) was instead applied to determine the chemical forms of Pb in the soil. The first two steps (H_2O and KNO_3) reflect the amount readily available to plants, while the third indicates the maximum amount of lead that can be displaced into the liquid phase from the soil by treatment with EDTA, the additive chosen for phytoextraction assisted. For the samples that had the most significant values of lead contamination, another Pb complexing agent, EDDS, was tried as an alternative to EDTA due to its greater biodegradability compared to the latter, which in case of proven effectiveness, would make it preferable to use it in real applications.

Mesocosm test

The following species were selected for the experimentation:

Lupinus albus, Helianthus annuus, Brassica juncea and Zea Mays.

The mesocosm tests were conducted in a greenhouse using 5 kg for each pot. Each mesocosm was prepared using 12 seeds for L. albus, 9 seeds for H. annuus and 0.5 g for B. juncea.

Experiments were set up for each plant species with a control (in duplicate) and a treatment (in triplicate); 75 vessels (5 per species, on each of the five soil samples investigated) for the As investigation, and 36 vessels in the Pb case (6 per species, on each of the two soil samples, investigated).

For each pot, the collection of the leachate was planned (through a silicone tube which connected the base of the pot with a container on the ground) and carried out before starting the treatment and at the end of the test, at the time of harvesting the plants. The test lasted 60 days. The mean daily volume of irrigation water was 200 mL per pot.

The treatment with mobilizing agents (assisted phytoremediation) began about 30 days after sowing.
The mobilizing agents were 0.1M K_2HPO_4 (0.82 g kg^{-1} soil) for As and 2 mM EDTA (0.04 g kg^{-1} soil) for Pb. Furthermore, EDDS 10 mM was also investigated as a mobilizing agent for lead contamination.
To minimize possible phytotoxic effects, the solutions were added by dividing the total doses over five days, providing a daily volume of 50 mL per mesocosm, diluted in 150 mL of water to satisfy the water requirement of the culture.
No fertilizer has been added so as not to introduce further variables into the system. The growing conditions in the greenhouse were: 24-19 °C for the day/night period and about 65% humidity.
The harvesting procedure began on average about ten days after the end of the treatments with mobilizing agents, leaving the plants in the pots as long as possible, based on their state of health. The plants were inspected to carefully separate the aerial parts from the roots.
Samples were washed with deionized water; for the roots, a further washing in an ultrasonic bath was carried out for 10 min to remove any soil particles still present. The samples were then dried in a ventilated stove at 40°C up to constant weight, and their dry weight was determined gravimetrically. Plant samples were digested and analysed to determine contaminant concentrations after digestion with a 2,5:1 HNO_3/H_2O_2 mixture using a microwave system with pulsed mode emission in Teflon vials. After digestion, plant samples were made up to 25 mL with Milli-Q water and then analysed. Analyses were conducted using IPC for arsenic and atomic absorption for lead.

Microcosm test with PGPB

Selection of arsenic-tolerant bacteria

The isolation of arsenic-tolerant and putative PGPB was conducted from the rhizosphere of Z. mays (which has shown the best performance), as well as the bulk of pots containing contaminated soil. A collection of five isolates belonging to three different phyla: Alphaproteobacteria (Beijerinckia fluminensis), Firmicutes (Bacillus megaterium, Paenibacillus lautus) and Actinobacteria (Rhodococcus ruber, Microbacterium oxydans) and able to grow in the presence of Na_3AsO_4, 200 mM and $NaAsO_2$, 10 mM was obtained. The identified strains were subjected to a series of in vitro assays to assess their plant growth-promoting potential. The production of auxin indole-3-acetic acid (IAA), siderophore molecules, alkaline proteases, ammonia, exopolysaccharides (EPS) and in vitro biofilm formation (pellicle) was examined.
The isolates were grown in LB medium, and cell pellets were pooled, resuspended in a suitable protective medium (1% sodium glutamate and 7% sucrose), frozen, and vacuum-treated in small aliquots. About 108 colony-forming units (CFU) per gram of soil

were used in microcosm trials.
Microcosm experiments were carried out on soil samples taken from one polygon. Tests were conducted in a climatic chamber using 500 g for each pot. Plants' growth conditions were as follows. Daytime: 14 h at 24 °C and 200 µmol m^{-2} s^{-1} illumination; Nighttime: 10 h at 19 °C; constant humidity of 65%.
Each microcosm was prepared by using 4 seeds for H. annuus, 0.3 g for B. juncea and 6 seeds for Z. mays. For each plant, fourteen trials were arranged: two controls (in triplicate) and three treatments (two in triplicate using phosphate and one in duplicate using thiosulfate). Total duration: 30 days. This time is sufficient to reach a complete development of plant species in the first stage of growth.
10 mL of irrigation water were provided daily per pot. Irrigation was carried out by spraying water on the soil surface to avoid disturbing the seeds.
PGPB inoculations were done immediately after seed germination.
Treatment with a mobilising agent (10 ml of 0.05 M K_2HPO_4/microcosm) began after 15 days after sowing. The total dosage was subdivided and provided over 5 days to minimise the possible phytotoxic effects (i.e. a daily volume of 2 mL per microcosm, diluted in 8 mL of water).
Regarding PGPB inoculations, three controls and three phosphate microcosms were treated by adding the PGPB consortium.
The plant harvest started on average about 10 days after the end of the treatment with mobilising agent. Plants were inspected to accurately separate the aerial part from the roots and subjected to the preparation and analysis with the procedures previously described.
The PGPB addition led to significant increases in arsenic in the aerial part of H. annuus that doubled the absorption. Arsenic accumulation in the roots, on the other hand, clearly showed a positive contribution of PGPB, as the contaminant content almost doubled for all plant species compared with control microcosms. A further increase was obtained by combining the PGPB addition with mobilising agents supply. For example, in the case of B. juncea, the arsenic uptake increased by almost 4 times with phosphate, compared to the control; the As value at the root level raised up about 8 times in microcosms treated with phosphate and PGPB. Improvements were also observed for the other plants, for which the combined effect of phosphate and PGPB was less evident yet still remarkable, with an increase of root biomass over the controls of about 80% for Z. mays and 230% for H. annuus.
Another aspect emerging from these results, which is particularly relevant for an application in the field, is that all treatments increased the As total uptake, although in different ways and with different effectiveness. In fact, an increase in total uptake was also found in microcosms treated with PGPB but not with mobilising agents, suggesting that plants were better-responding thanks to the presence of PGPB.

In summary, though further investigation at the field scale is needed to evaluate the effect of environmental conditions on plant-microbe interactions in contaminated soils, the obtained results confirm the key role of PGPB in supporting and enhancing plant activities. The combined use of a mobilising chemical (potassium phosphate) and PGPB led to significant increases in arsenic extraction.

REFERENCES

- Wenzel et al. 2001_ Arsenic fractionation in soils using an improved sequential extraction procedure, Analytica Chimica Acta, Volume 436, Issue 2, https://doi.org/10.1016/S0003-2670(01)00924-2
- Pedron et al. 2009_ Strategies to use phytoextraction in very acidic soil. Chemosphere 75, https://doi.org/10.1016/j.chemosphere.2009.01.044

3.2 Treatment unit (pilot scale)

The pilot test was planned directly in the contaminated site with the primary purpose of the reduction of the bioavailable fraction of the main contaminants (arsenic and lead) present in the soil.
The objective of the phytoremediation test in the field was the full-scale verification of the results obtained from the tests in a greenhouse, in which treatments with mobilising agents and PGPB have favoured the removal of bioavailable fractions of contaminants by the plant species tested, which have a good growth capacity on the contaminated soil.
The test included the following activities:

- preliminary activities in the field (setting up, demolition, stripping and cleaning of the area, setting up a shed, construction of piezometers and irrigation system)
- preparation of the phytoremediation test (stone removal, ploughing, preparation of the bed for sowing, hydraulic-agricultural arrangement, sowing of herbaceous species)
- agronomic management of the test field (fertilisation, treatment with mobilising agents and PGPB, grass cutting and disposal)
- monitoring and laboratory analyses
- dismantling of the phytoremediation module
- full-scale executive design.

3.3 Control parameters and verification of the applicability (pilot scale)

- Construction of piezometers: to allow the monitoring of groundwater and possible leaching of the contaminants, 2 piezometers will be built (upstream-downstream) within the area of the worksite.
- Irrigation system: an automatic drip irrigation system will be built; the spacing and the flow will be selected according to the climatic trend of the area, the type of crop and the type of soil to provide suitable quantities of irrigation for optimal plant growth.
- Monitoring and laboratory tests to verify the progress of the ongoing phytoremediation process and its efficiency, periodic monitoring of the environmental matrices (soil, water, plant species and air) involved in the remediation process has been arranged. In particular
- The total concentration of As and Pb
- The bioavailable concentration of As and Pb
- Analysis of the quality of irrigation water
- Biometric parameters: total fresh weight (g), length of the epigeal and hypogeal apparatus (cm).
- Concentrations of airborne pollutants

4. Full-scale application

4.1 Main treatment unit

A field test has not yet been performed after the laboratory and greenhouse phases.

6. Post treatment and/or Long Term Monitoring

6.1 Post treatment and/or Long Term Monitoring

- Bioavailable concentration of As and Pb
- Total content of As and Pb in the groundwater continuous analysers (in gas and off gas)

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WATER AND LAND

2. Site background

2.1 History of the site

The Abbaretz site is a former Sn mine (Pays de la Loire Region, France) with about 4 million m3 of tailings and waste rock. Most of the 45 ha of the site are still bare (without vegetation) more than half a century after the end of mine exploitation (1957). At this site, the aim of phytostabilisation pilot tests was to find a natural and affordable solution to tackle the intense erosion.

Natural soil elevation is 55 m in the south and 37.5 in the north. Tailings elevations are between 58 and 6 m above natural soil elevation.

An inventory of native plants was made and included: birch, bruyere and some herbaceous plants (agrostis, fescue and lotus). Native vegetation repartition is very sparse mainly due to the poor quality of soil and to intense erosion.

Figure 1 - Abbaretz Mine

Figure 2 - Erosion of tailings on the Abbaretz mine

Figure 3 - Erosion of tailings deposits on the Abbaretz mine

2.2 Geological setting

Substrate is composed of waste material from mine exploitation and is mainly composed of very fine particles (more than 65% is under 80 µm) over several meters. Low soil pH is measured: from 4 to 6. Below, the geological formation is altered schists.
The depth to ground water is approximately 0.5 to 6 meters below ground surface depending on the location.

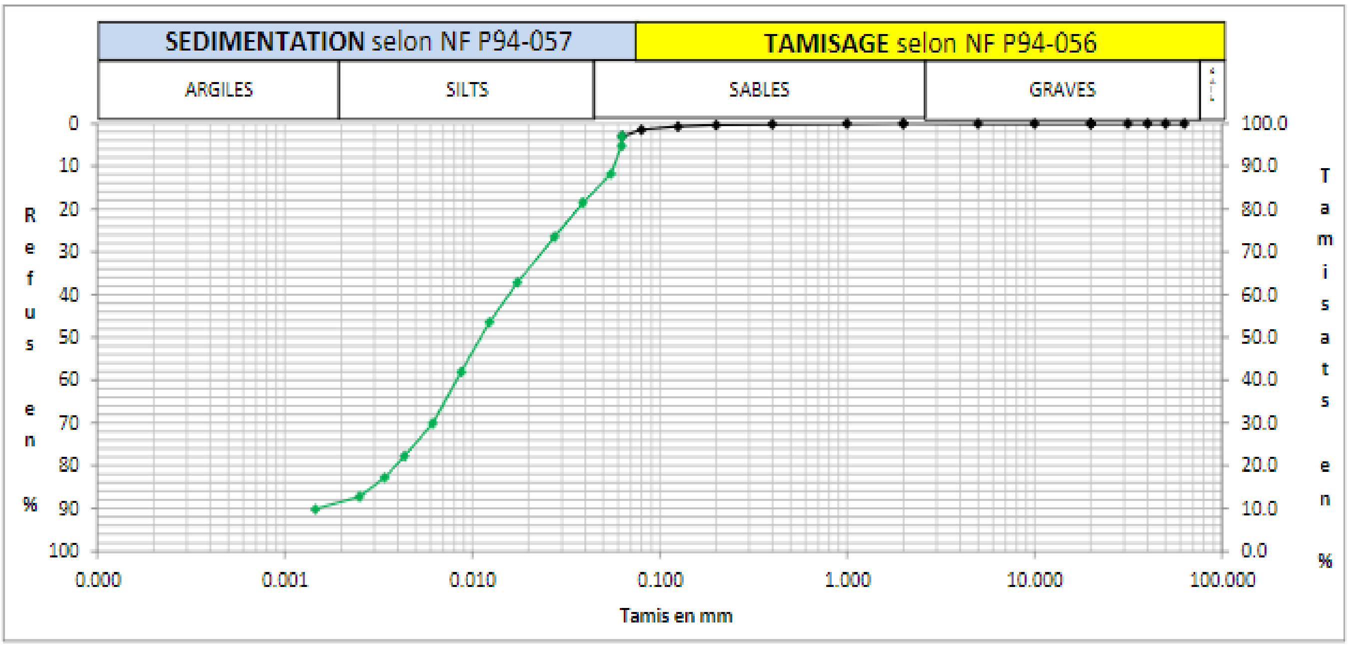

Figure 4 - Soil granulometry of tailing material

2.3 Contaminants of concern

- The main contaminant in mining wastes is arsenic (from 100 ppm up to 1400 ppm) from natural origin (contained in the exploited ore).
- The main contaminants in water are Fe, Al, Mn, As (from 100 µg/L up to 10000 µg/L), Co, Ni, Cu.

Figure 5 - On site pXRF As analysis (ppm)

2.4 Regulatory framework

This mine is an orphan site. In this situation, the site rehabilitation and environment impact monitoring are under the responsibility of the competent central administration, i.e. the Ministry of Environment and the local State representative (the Prefect) assisted by the relevant regional directorate (DREAL). Operational duties are delegated to BRGM, through a dedicated department, the Department for Mine Safety and Risk Prevention (DPSM).

DPSM is entrusted with the missions of safety enhancement work for erosion control in the capacity of delegated project owner.

WATER AND LAND

3. Pilot-scale

3.1 Laboratory Study

The aim was to obtain a dense and sustainable vegetal cover with a strong root system to mechanically stabilise tailings and tackle erosion.

Substrate analysis

Substrate analysis of tailings indicated nearly no agronomical potential:

- substrate with poor water reserve
- low pH (from 4 to 6)
- poor mineral content potentially leading to potential nutrient deficiencies
- organic matter close to zero and nearly no macrofauna
- poor exchange capacity, weak soil structure.

Selection of plant species

Plants were selected from in-situ investigations:

- Lotus corniculatus (seeds from supplier)
- Festuca rubra (seeds from supplier)
- Juncus (taken on site for pots tests).

16 weeks growth tests were performed in pots to evaluate plant growth. Criteria to assess plant performance were:

- Proportion of plants that survive
- Maximal height of plant
- Number of leaves
- Biomass of plants: roots, stems and leaves.

NB: No pots tests were performed with trees due the short duration.

Agronomical properties of substrate (organic matter, minerals, exchange capacity, pH, conductivity…) showed very poor agronomical potential.

Amendments tested were:

- Compost from water domestic treatment sludge (NF 44-095)
- Limestone (size 0-20mm)
- Iron powder.
- Microorganisms were isolated from Lotus plants from site and then inoculated to seeds.

These tests were implemented without limiting factors to minimize the number or variable elements and assess only the variability of response due to pollution and amendments.

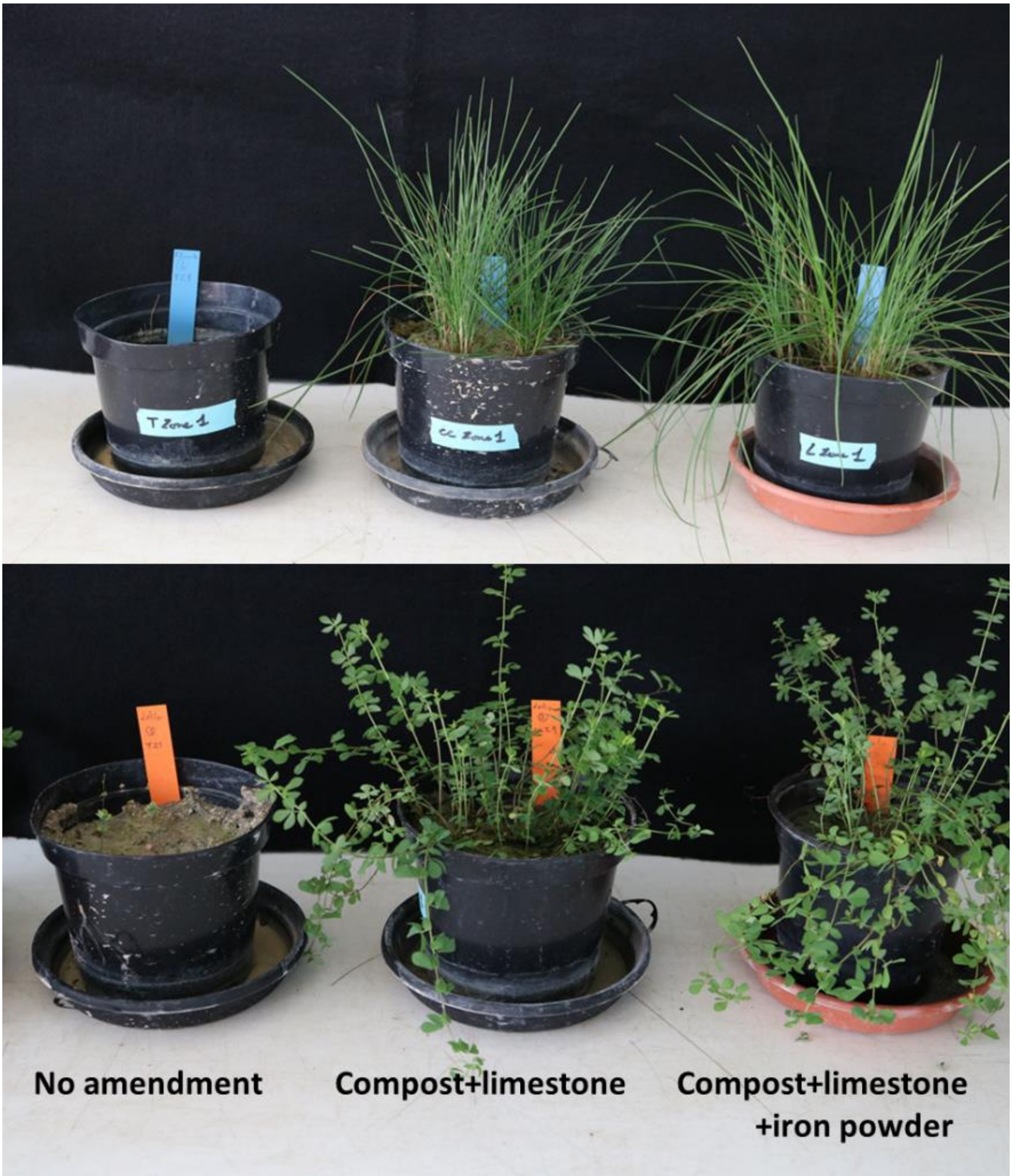

Figure 6 - Example of growth tests after 16 weeks (fescue and lotus)

<u>Simulation of the uptake / evaluation of contaminant attenuation</u>

Criteria used:

- Leaching tests (EN 12457-2) were performed to give an idea of the mobility of metal(oid)s before and after growth tests. Arsenic was reduced by a factor 3 in leachates with amendment by compost + limestone.
- Metal(oid)s content in aerial parts of plants. Arsenic was reduced by a factor 2 to 7 (depending on species) with amendment by compost + limestone.

<u>Conclusions of lab tests</u>

Growth tests showed nearly not growth without amendments. With 2 % limestone and 3 % compost, dry biomass was rather close to control (non-polluted soil). Iron powder did not increase significantly plant biomass.

3.2 Treatment unit (pilot scale)

Two 100 m² plots were selected to have pilot scale tests representative of two hydric situations (one of the main limiting factors identified on this site).

From the results of pots tests, amendments with 2% w/w limestone, 3% w/w compost were mixed within the first 20 cm of tailing. Microorganisms were inoculated with seeds.

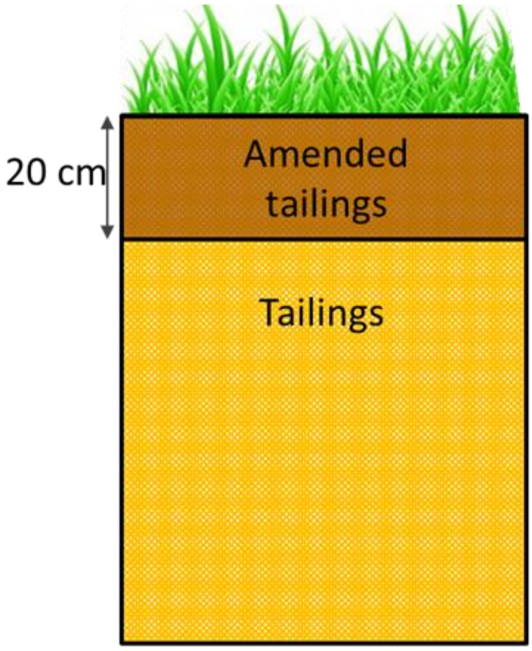

Figure 7 - Technical solution for the Abbaretz site phytostabilisation pilot tests

Protocol: One plot was situated on tailing slopes (in a rather dry zone), the other near a wet flat zone with acid drainage. Each plot was divided in 2 subplots to test: trees/without tree. Half of each subplot was covered with coco net (500 g/m²). No irrigation system was put in place. Seed mix contained the plants from lab tests (lotus and fescue) and other herbaceous plants that usually perform well on poor substrates with dry episodes (see lists and scheme below). Trees were planted on half of each plot. For the wet zone pilot, Juncus from other parts of the site were planted in addition (1 per 5 m²).

List of pilot plants	**List of pilot trees**
Cynodon dactylon	Salix purpurea
Festuca ovina	Salix viminalis
Festuca rubra	Betula verrucosa
Lotus corniculatus	Cytisus scoparius
Medicago lupulina	Caragana arborescens
Trifolium repens	
Medicago truncatula	
Dactylis glomerata subsp. hispanica	
Sanguisorba minor	
Plantago lanceolata	
Juncus	

Table 1 - List of pilot plants and trees

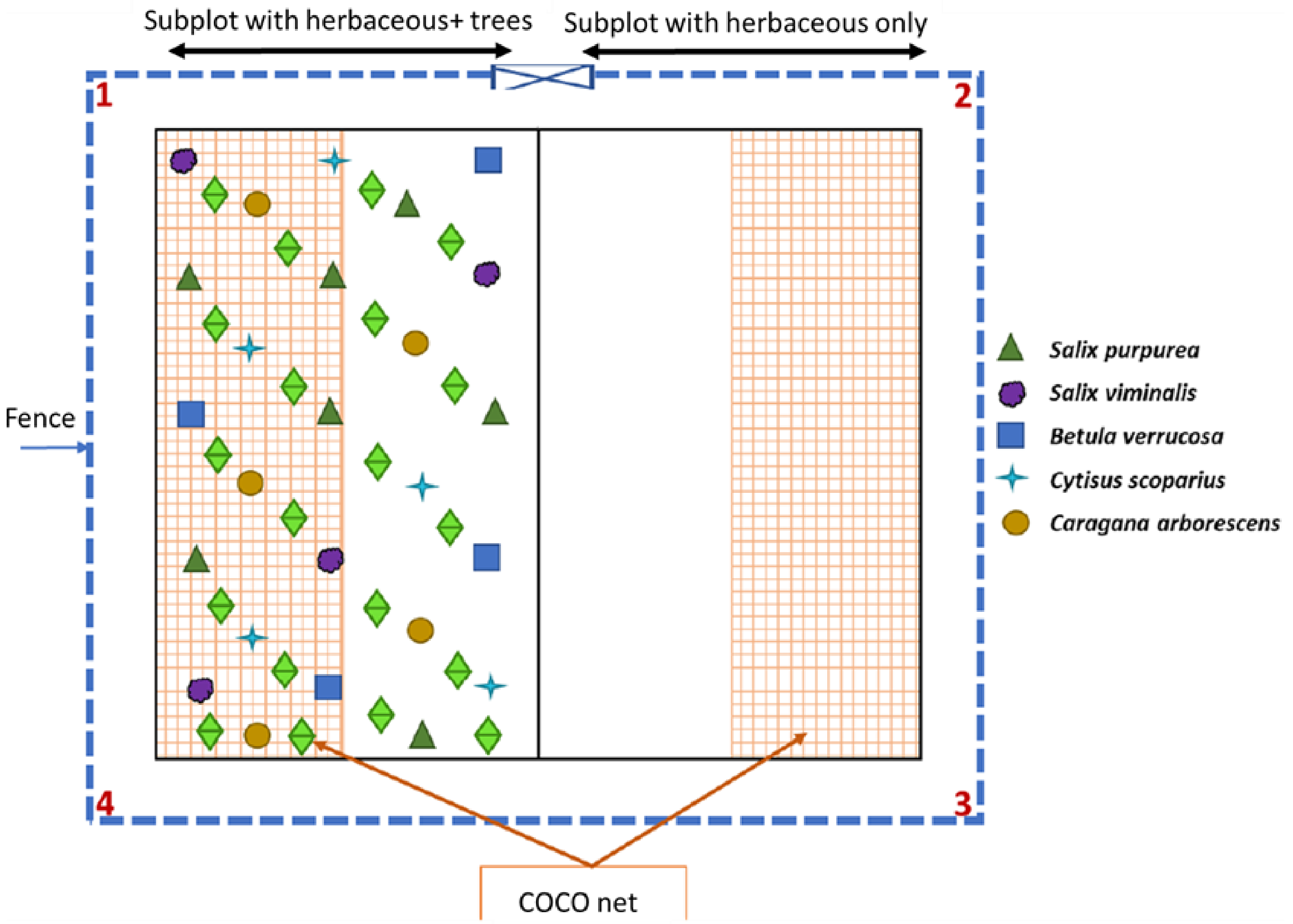

Figure 8 - Scheme of the pilot plot situated on the slope of the tailing deposit

The protocol enables the establishment of a continuous vegetal cover. A 90% vegetal cover was observed after 13 months, which overcomes the initial objective of 70%. No erosion signs were noticed on vegetized plots 2 years after seeding. From comparison of plots we concluded that availability of water and organic matter content are the key parameters to optimize phytostabilisation for site management.

2 years after seeding (summer)

Rather dry zone (Abbaretz 44)

2 years after seeding (winter)

Figure 9 - Plot on the tailing slope (rather dry and intense erosion)

2 years after seeding (summer)

Wet zone (Abbaretz 44)

2 years after seeding (winter)

Figure 10 - Plot on the wet zone (for embankments stabilisation)

3.3 Control parameters and verification of the applicability (pilot scale)

List of control parameters (herbaceous):

- Vegetal cover in %. It is the most important parameter because the objective is to cover soil to manage erosion. Estimation: on ground with quadrats method (the number of quadrats was calculated to be representative) or trough drone photo with post-treatment
- Proportion of each species
- Metal(oid)s content in aerial parts of plants (dry matter)
- Biomass of aerial parts of plants (g/m^2).

Figure 11 - Example of monitoring of vegetal cover by drone (zones with less vegetation appeared to have less amendments). Drone photo 4 months after seeding in winter

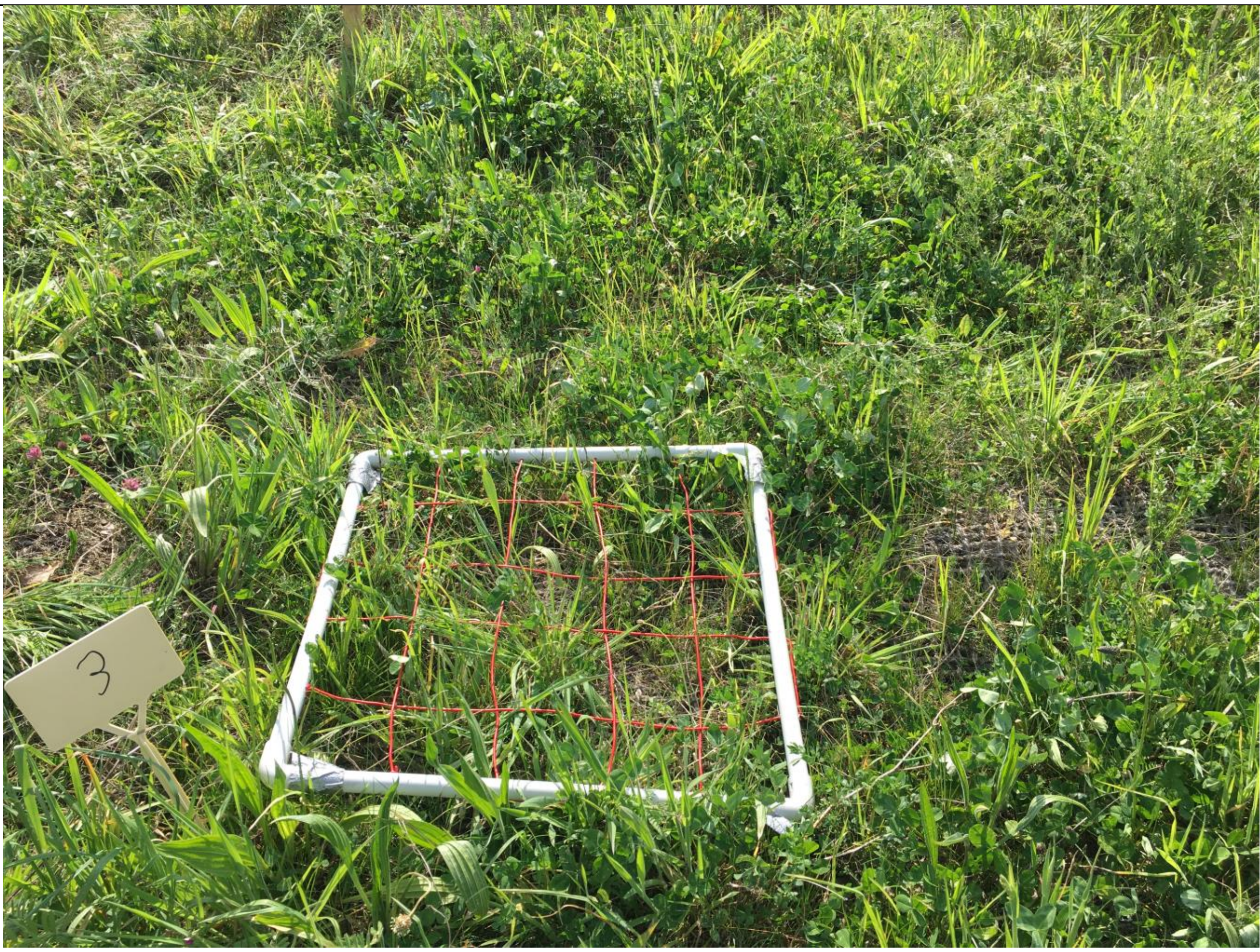

Figure 12 - Example of monitoring of vegetal cover on ground through quadrats method.

List of control parameters (trees):

- Survival % (on the rather dry zone, several trees did not survive; on the wet zone pilot 100% of trees survived)
- Vigor of trees
- Metal(oid)s content in aerial parts of plants (dry matter)
- Biomass of aerial parts of plants (g/m²).

List of control parameters (substrate):

- Visual observation of potential erosion indices
- Agronomical properties of substrate (0-20cm depth) (organic matter, minerals, exchange capacity, pH, conductivity…)
- Substrate (0-10cm depth) leaching test (EN 12457-2) were performed to give an idea of metal(oid)s mobility before and after growth tests. It was reduced by a factor 3 in leachates after 13 months (comparison with the situation before pilot setting).

Frequency of control: 4/year (each season)

4. Full-scale application

4.1 Main treatment unit

Full scale phytoremediation (several hectares) is ongoing. After pilot scale, we conclude that water availability and organic matter content are the main key parameters for successful phytostabilisation.

For full scale project it is necessary to:

- Use a wide variety of plants to obtain a continuous vegetation cover all over the year (herbaceous seeds as pilot scale, for trees only Cytisus are relevant on the rather dry plots).
- Increase the quantity of compost (minimum 5 % w/w on the first 20 cm of substrate, instead of 3 % used at pilot stage). With more compost, plants appeared more vigorous and more resistant to drought.
- Optimize amendment mixing. This step also appeared as a crucial aspect. Solutions must be developed to improve the process and reduce associated costs. On the other hand, mixing amendments should not provoke erosion through loss of tailing cohesion. Work enterprises must be challenged to suggest appropriate solutions during the rehabilitation to come.
- No irrigation system will be put in place because plants must be adapted or adapt to site conditions.
- Use coco net on slopes to increase the stabilisation of substrate after works and improve water retention.

4.2 Different areas characteristics that affect the project

The following monitoring program is proposed:

Substrate (soil):

- Agronomical properties (organic matter, mineral, exchange capacity, pH, conductivity…)
- Leaching test (EN 12457-2).

Suggested frequency: each year during spring for the first 5 years, and adjust after depending on site evolution.

4.3 Monitoring of the soil microbial-community structure and functioning

Evaluation of microbial activity of substrate (included in agronomical analysis). We are seeking for other indices (will be used once identified).
Suggested frequency: each year during spring for the first 5 years and adjust after (depending on site evolution).

4.4 Monitoring of plants

Nearly the same as for pilot scale, but less frequent (2 / year: spring, autumn) the first 5 years:

List of control parameters (herbaceous):

- Vegetal cover in %. It is the most important parameter because the objective is to cover soil to manage erosion. Estimation: on ground with quadrats method or through drone photo with post-treatment
- Proportion of each species
- Metal(oid)s content in aerial parts of plants (dry matter) to give an order of magnitude of risks of transfers to biosphere
- Biomass of aerial parts of plants (g/m^2).

List of control parameters (trees):

- % that survive (on the rather dry zone several trees don not survive)
- Vigor of trees
- Metal(oid)s content in aerial parts of plants (dry matter)
- Biomass of aerial parts of plants (g/m^2)

4.5 Other Monitoring

- Leaching of amended tailing (EN 12457-2) to evaluate metal(oid)s mobility (suggestion: 1/year the first 5 years)
- Control of erosion (visual observation, ground measurement or remote sensing) (suggestion: 2/year during the first 5 years)

5. Results

5.1 Removal rate

32 months after pilot set up:

- For the pilot plot on the slope, there is no sign of erosion on the phytostabilised area whereas several erosion marks were noted all around.
- For the pilot plot near the wetland, plants stabilised embankments (no erosion). Success to tackle erosion was validated mainly through visual observation.

In case of total or relative failure of the vegetation cover, analyses of the soil and/or environmental conditions can help to understand the failure and adapt the protocol to be applied.

When using phytostabilisation methods, it is not uncommon to have to seed several times due to unfavourable weather conditions after seeding.

6. Post treatment and/or Long Term Monitoring

6.1 Post treatment and/or Long Term Monitoring

In the medium or long term, the maintenance and monitoring of the phytostabilised zone should be minimalist: as phytostabilisation solutions will benefit from natural regeneration capacities this will limit the need for intervention. However, a light monitoring may be necessary to verify that no significant degradation occur (due to erosion, pets, drought…) and that the vegetal cover remains continuous and in good health.

One monitoring campaign each year is appropriate with:

- Assessment of vegetal cover (on ground or with drone)
- Visual inspection of erosion indices
- Research for invasive species.

Long term toxicity for plants could (should?) be also monitored, but criteria have not yet been identified.

7. Additional information

7.1 Lesson learnt
Methodology and procedures In appearance, it is a simple solution, but it requires preliminary in-depth studies and tests and a strong presence on site during works. technical aspects Management of variability. It is a key aspect: as plants are biological organisms, their responses to site conditions and stress are inherently variable. Incorporation of amendments still need to be optimized. Regarding performance Simple robust methods should be developed to evaluate the performance of phytostabilisation for erosion control. Legislative, organizational aspects Duration of preliminary test is sometimes difficult to include in a project planning. To date no legislative issue.

7.2 Additional information
The most important thing is to put in place a sustainable vegetal cover that is integrated in the environment and is resilient. Long-term data is necessary to proof that the vegetal cover could be sustainable and resilient. Use of local species already adapted to the local conditions could be a clue of success. However, associated costs should be taken into consideration.

7.3 Training need

Sharing of experience would be highly beneficial to improve phytoremediation solutions. Formation is needed on:

- The devices that are complementary to phytostabilisation to manage erosion on slopes (fascines, biodegradable nets, infiltration ponds…)
- The choice of biological indicators to assess increase in soils health thanks to phytoremediation.

7.4 Additional remarks

Mechanical stabilisation of pollutants by plants (as we try to do) seems to be an underestimated domain of phytostabilisation (most studies focus on chemical stabilisation for prevention of leaching).

Need more 1/1 scale experiment feedback.

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2. Site background

2.1 History of the site

The project deals with the pilot-scale test for the biological treatment, preliminary to a Remediation Project, related to a farming area contaminated by hydrocarbons after a spill. As the spill occurred in this area, Emergency Safety Actions and preliminary environmental investigations were immediately carried out to define a Conceptual Model and to perform a site-specific Risk Analysis.

Figure 1 - Area of the spill

Detailed pedological, chemical and microbiological analyses showed that the organic pollutants (C>12 hydrocarbons) affected the unsaturated materials up to 3 m just above the water table level in a scattered, non-continuous way. Moreover, PGPR (Plant Growth-Promoting Rhizobacteria) microorganisms and fungi potentially able to degrade hydrocarbons and to establish a symbiotic relationship with the plant roots, were detected in the site. Based on a both environmental and technical-economic sustainability and feasibility assessment, the best remediation option was an integration of two different bioremediation technologies: phytoremediation and biopiles. The choice of biological technologies was made with the aim of safeguarding as much as possible the ecosystem functions of contaminated soils by limiting the impact on the existing (agro) ecological equilibria to re-establish a safe area suitable for its traditional use in a relatively short time.

2.2 Geological setting

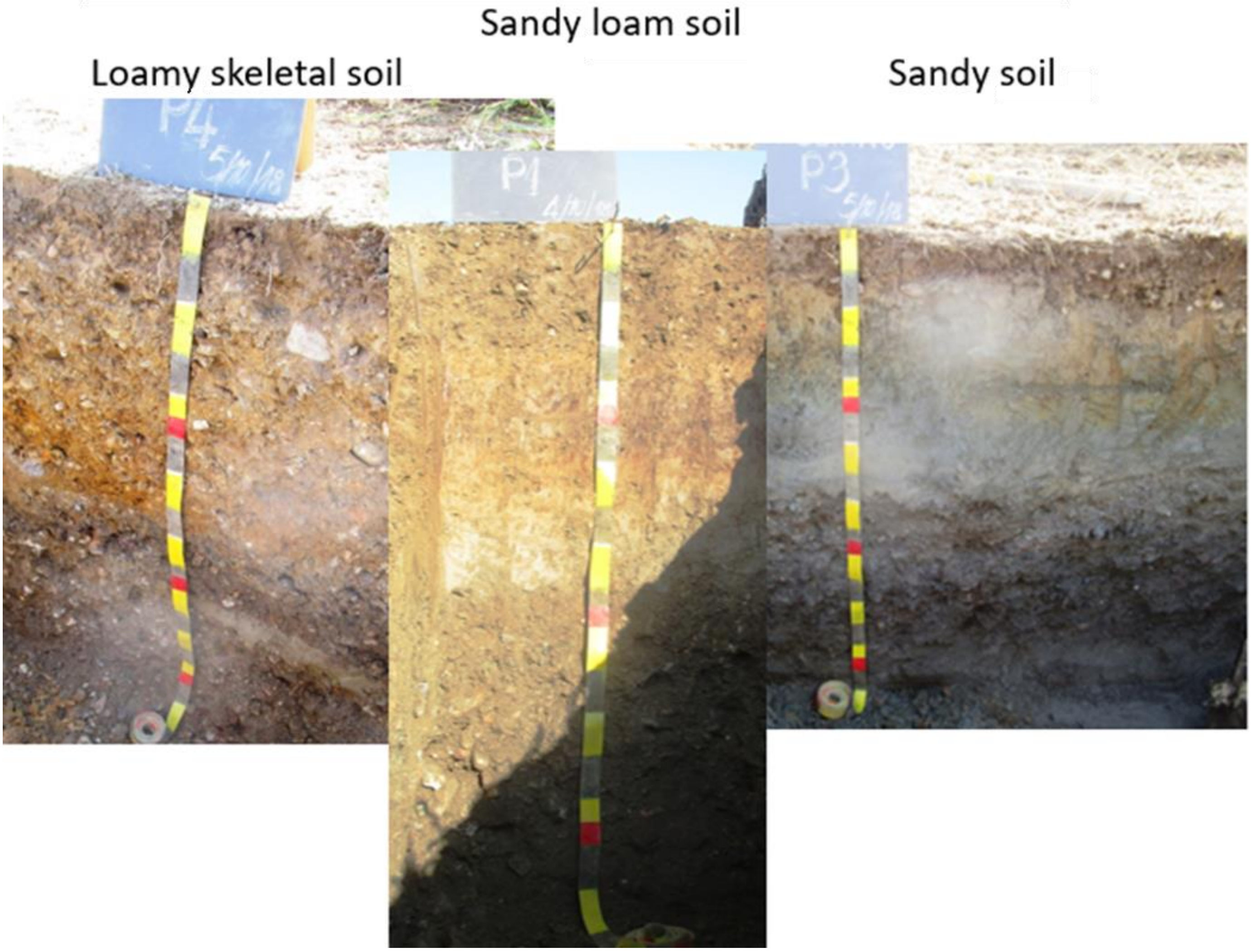

Figure 2 - General characteristic of the soil

- Water table fluctuate between 1,5-3,0 m from the surface; it flows from NW to SE with a constant gradient of 0,3%
- Stratigraphy: an agricultural horizon up to a depth of 30cm; deep soil horizons up to 100 cm; alternated grey/brownish sand and gravel up to over 10 m

According to the characteristics of the subsurface horizons (B), two pedological units have been defined:

- The GRAVELY UNIT comprising soils with a higher content of coarse fragments with a tendency to lower rooting capacity but with a better water retention due to the higher clay content. (left)
- The SANDY UNIT made up of little or non-skeletal soils at least in the first meter and a lower clay content (sometimes sandy soils): it has a better rooting ability and tends to be more sensitive to summer water stresses because of the lower water retention capacity (right)

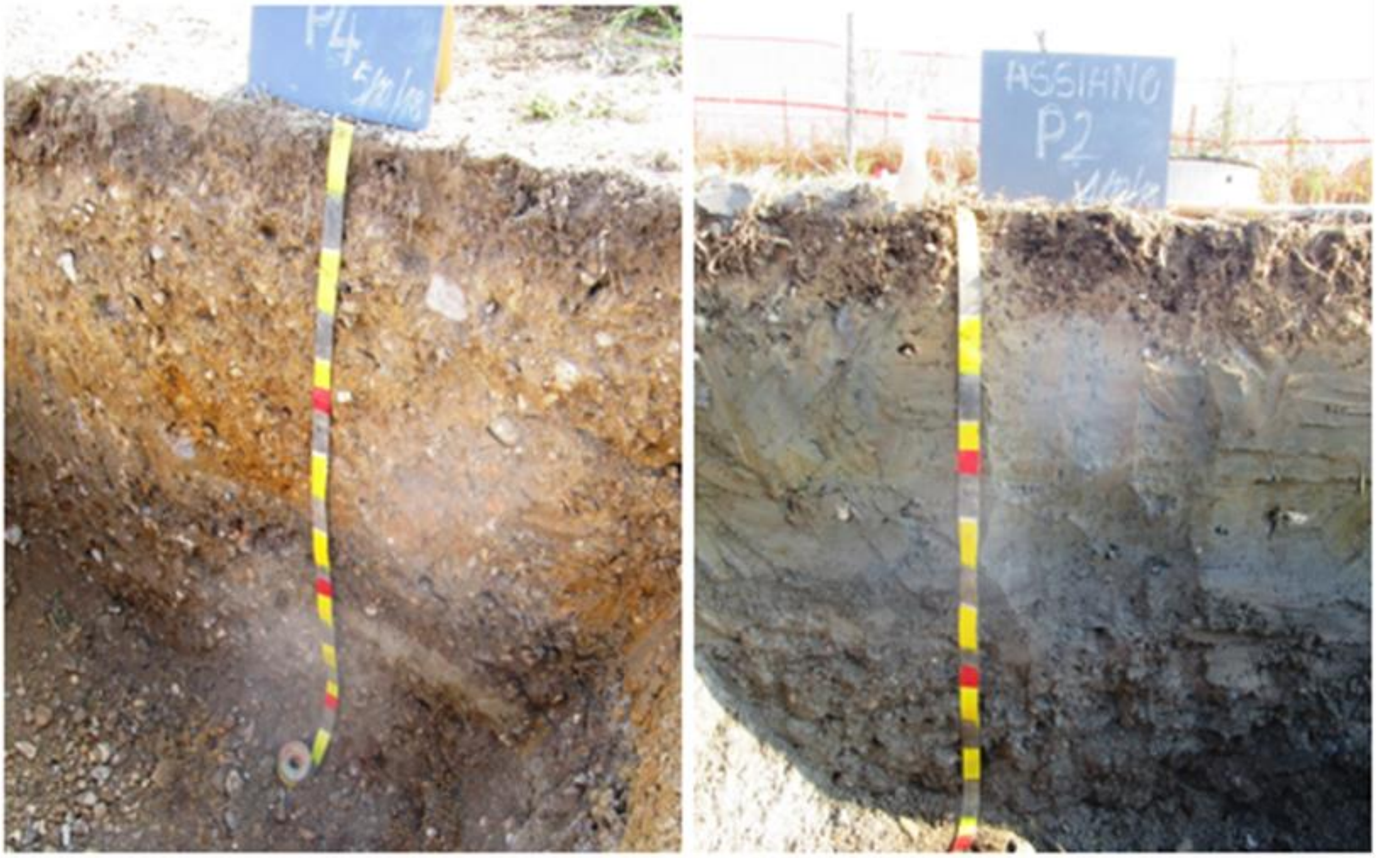

Figure 3 - Pedological units present at the site

2.3 Contaminants of concern

To define quality and quantity of pollutant according to Italian national Law (D. Lgs.152/06) the vadose zone of the stratigraphy was divided in surface soil up to 100 cm and deep from 100 cm up to 250 cm, below ground surface.

Surface soil (0-1 m. b.g.s.):

- Contaminants of concern: hydrocarbons C<12(Representative Concentration 150 mg/kg), C>12(Representative Concentration 4.000 mg/kg), Ethylbenzene, Toluene (Representative Concentration 0,8 mg/kg), Xylenes (Representative Concentration 4,1 mg/kg)
- Polluted area: 520 m^2

Deep soil (> 1m b.g.s.)

- Contaminants of concern: hydrocarbons C<12(Representative Concentration 112mg/kg), C>12(Representative Concentration 3.000 mg/kg), Xylene (Representative Concentration 2,2mg/kg)
- Polluted Area: 2300 m^2

2.4 Regulatory framework

- Due to the growing interest, sustained by the Italian environmental laws (i.e. Legislative Decree no. 152/06 and Ministerial Decree no. 46/19), to privilege the remediation technologies that are able to complete restore the site reducing waste to a minimum and maintaining the original characteristics of the threated matrix, particularly for agricultural soil, phytoremediation has an important position among biological remediation technologies.
- The project has been focused on the evaluation of phytoremediation technic and full-scale proposal, carried out after 2 years of pilot test, has been approved by Public Administration (Comune di Milano) on 06/05/2022.

3. Pilot-scale

3.1 Laboratory Study

No laboratory test has been carried out; the efficiency of a biological technology can be evaluated only considering any site-specific characteristic and condition. That is the reason why only a field trial test can show if the technology/technologies selected can be efficient for a specific site remediation.
A specific field trial test has been carried out in the site for 2 years, starting in March 2019.

3.2 Treatment unit (pilot scale)

The phytoremediation test has been implemented first in the less polluted area, as the most polluted area was meant to biopile treatment; a little part of most polluted area was anyway tested with phytoremediation.

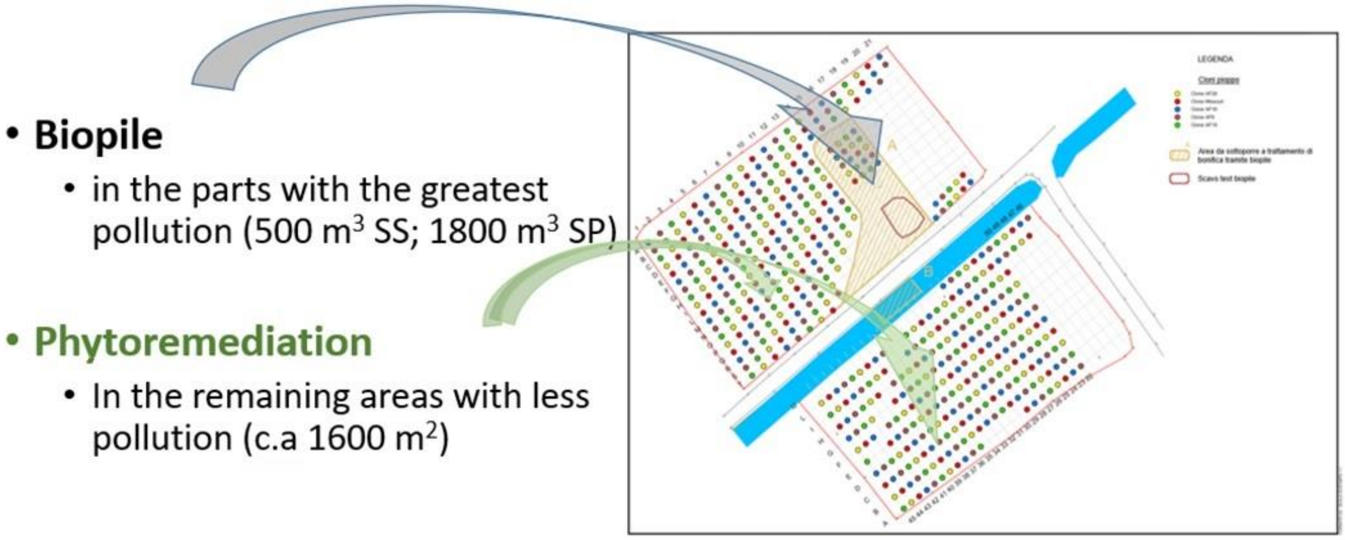

Figure 4 - Treatment units

The depth and the concentration of pollutants suggested the use of poplar (Populus spp.) as arboreal species associate with spontaneous herbaceous plants during spring-summer and with leguminous species during fall-winter time. The purpose was to develop a deep root system in the soil with a rhizosphere/endosphere rich of hydrocarbon-degrading microorganisms, supported by an intense biological activity in the upper soil horizon related to the grown of spontaneous herbaceous plants and through the nitrogen enrichment of soil by nitrogen-fixing bacteria associated with leguminous plants. Specifically, five different poplar clones were tested for their resistance to pollutants based on their above ground and root system growth. The spontaneous herbaceous plants were most from the green compost spread on surface as soil conditioner; these herbaceous species were enriched by the fall seeding of Vicia sativa appropriately inoculated with specific rhizobium bacteria to furnish N-fertilization. Because of the uneven distribution of pollutants, poplars were planted on much of the area originally affected by lower levels of pollution.

- 513 one-year-old cuttings of the 5 different clones were planted, alternating with a 3x2 m. order in March 2019.
- 80 test trees, located in the different pedological units and in potentially more or less polluted areas, were chosen for monitoring

3.3 Control parameters and verification of the applicability (pilot scale)

Field monitoring and sampling program that will adequately monitor the effectiveness of the treatment in three dimensions.
The pilot scale test lasted for 24 months, during which were monitored:

- Morpho-functional plant traits
- Chemical analyses of organic pollutants and heavy metals
- Microbiological analyses for the determination of the bacterial and fungal community structure
- Ecotoxicological analysis (germination test) were carried out every six months.

4. Full-scale application

4.1 Main treatment unit

The trial showed minor differences in the vitality of clones and in the depth of their root system due to different suitability to soil and climatic constraints rather than to pollutant content. These results suggested some improvements in planting techniques especially in less favourable soil conditions (high stoniness, lower water capacity) such as the use of longer stems and greater planting depth or a closer distance among trees to promote the deepening of the root system. In the test, a detailed monitoring plan was fundamental to optimize the remediation processes. Results of test provide all information useful for the “full scale” application of the phytoremediation technology, to reach the remediation goals.

As confirmed by trial test, the depth and the concentration of pollutants suggested the use of poplar (Populus spp.) as arboreal species associated with spontaneous herbaceous plants during spring-summer and with leguminous species during fall-winter time (Vicia). The purpose has been to develop a deep root system in the soil with a rhizosphere/endosphere rich of hydrocarbon-degrading microorganisms, supported by an intense biological activity in the upper soil horizon related to the grown of spontaneous herbaceous plants and through the nitrogen enrichment of soil by nitrogen-fixing bacteria associated with leguminous plants. Specifically, one of the five different poplar clones tested has been selected for the full-scale layout due to its resistance to pollutants based on their above ground and root system growth. The spontaneous herbaceous plants will be enriched by the fall seeding of Vicia sativa appropriately inoculated with specific rhizobium bacteria to furnish N-fertilization.

Moreover, good bacterial and fungal biodiversity in all analysed samples, with the presence of orders potentially capable of biodegrading the organic compounds present in the site, is a condition favouring the choice of rhizostimulation.

The grid chosen is 3x2m, to allow the operation vehicles to enter.

The original characteristics of the surface horizons (Ap) had been compromised by the safety and investigation activities throughout the surface (site setting up for securing of the area); due to this, the soil for planting must be prepared also according to relevant cultivation practices, such as:

- mowing of spontaneous vegetation (test start) or crop harvesting (at the end of vegetative seasons)
- surface tillage and plowing (30 cm) mixing green compost within the soil
- levelling of the area
- fertilizers distribution N-P-K

- refinement of the soil (harrowing) and preparation of the seed-bed
- planting of the poplar clone down to a hole of at least 50-100 cm

Woody species developing sufficiently deep root system over time:

- 5 poplar clones (Populus spp.)
- Fast-growing
- High biomass production
- Deep root system
- Well tolerant to hydrocarbons

Herbaceous species with more superficial roots to increase the quantity and efficiency of the microbial community in the surface soil.

- Spontaneous vegetation
- Species brought with compost
- Sowing of veccia (V. sativa, V.villosa inoculated Rhizobium)

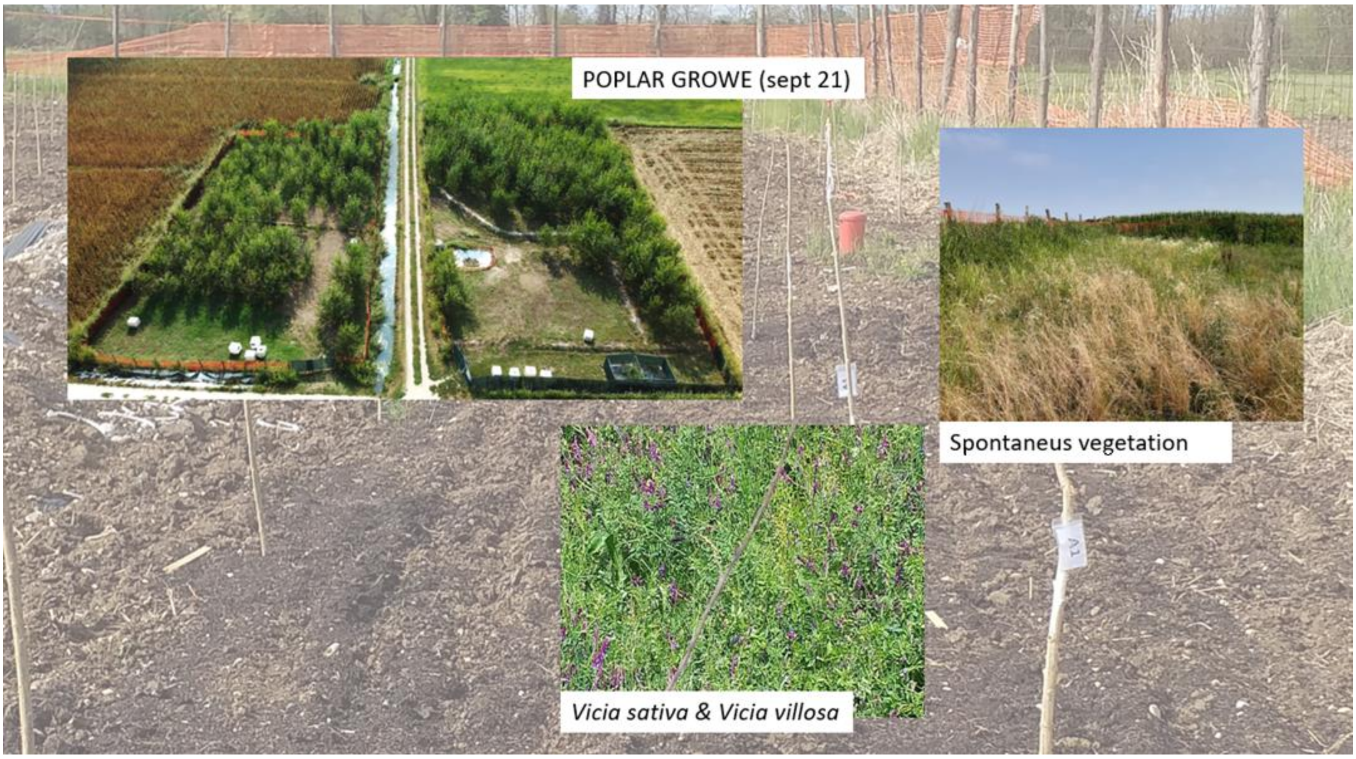

Figure 5 - Main treatment units

4.2 Monitoring of the chemical parameters

Baseline sampling is planned before the start of the treatment, and control sampling will be carried out at given times during and after treatment to assess its effectiveness; as part of the monitoring plan of the full-scale phytoremediation project, soil samples will be collected for chemical analysis to update the level of contamination.
The chemical parameters to be monitored will be defined according to the site-specific contamination characteristics.
Soil samples will be collected from borehole cores and will be analysed for the detection of:

- Hydrocarbons (C>12)
- Hydrocarbons (C<12)
- BTEXs
- fixed residue
- pH

4.3 Monitoring of the soil microbial-community structure and functioning

The biological parameters will be monitored through the determination of the tolerance of poplars in terms of functional traits, in particular biomass and deepening of the root system.
Ecotoxicological analysis will also be performed to assess the ecotoxicity of contaminants or their degradation byproducts.
Samples of rhizospheric and non-rhizospheric soil will be collected for their microbiological characterization by performing the following analyses:

- DNA extraction from an appropriately homogenized soil portion that can be representative of the material to be characterized
- Structure of the bacterial community description by sequencing the 16S rRNA gene
- Microbial community (bacteria) analysis by quantification (qPCR) of the 16S rRNA gene.

4.4 Monitoring of plants

PURPOSE:

- the tolerance of poplar clones to pollutants
- the effectiveness of the soil-plants-microorganisms system in the degradation of hydrocarbons

PARAMETERS:

- Above ground
  - Growth (height and diameter)
  - Health state (living and dead, phytopathological problems)
- Underground
  - Root system geometry and dept
  - Soil and rhizosphere microbiome

For the characterization of plant species during the growth, in addition to the estimation of viability, the dry weight differentiated in the various components will also be determined: stem and leaves, fruits or seeds.

In addition to the aerial part, it is also planned to estimate the root growth both by eradicating at least one individual per area (high contaminated area, medium contaminated area, less contaminated area), taking a large enough portion of soil to appreciate how many roots have grown, not before having observed the geometric trend and measured the depth reached along a vertical cross-section in an excavation pit/trench at the end of the growing season.

At the same time, it will be possible to take samples of rhizospheric and non-rhizospheric soil for chemical and microbiological analysis.

4.5 Other Monitoring

The parameters that will be measured for agronomic analysis on soils will be the following:

- the reaction (pH in water and neutral salt)
- organic carbon
- the major nutrients (total nitrogen, assimilable phosphorus, exchangeable potassium)
- mesonutrients (calcium, magnesium exchangeable).

5. Results

5.1 Removal rate

The vegetable consortium poplar-vetch-spontaneous vegetation resulted suitable:

- to reduce hydrocarbons in all the soil types of the area
- to be effective up to 1500 ppm of C>12 hydrocarbons in SS and even more in SP
- trial test showed the consortium to be effective over the concentration estimated as a technological limit at the beginning of the test (1500 ppm of C>12 hydrocarbons); the technological limit is shifted up to 4000 ppm of C>12 hydrocarbons.

The plant species used were found to be tolerant to hydrocarbons also thanks to the symbiosis with endophytes to increase the biodiversity of the soil microbiome and favour the development of HC degraders.

The poplar is rooting deeply even in soils that are difficult to root (skeleton and anoxia) favouring the supply of oxygen and transports the aerobic HC degrading species in depth.

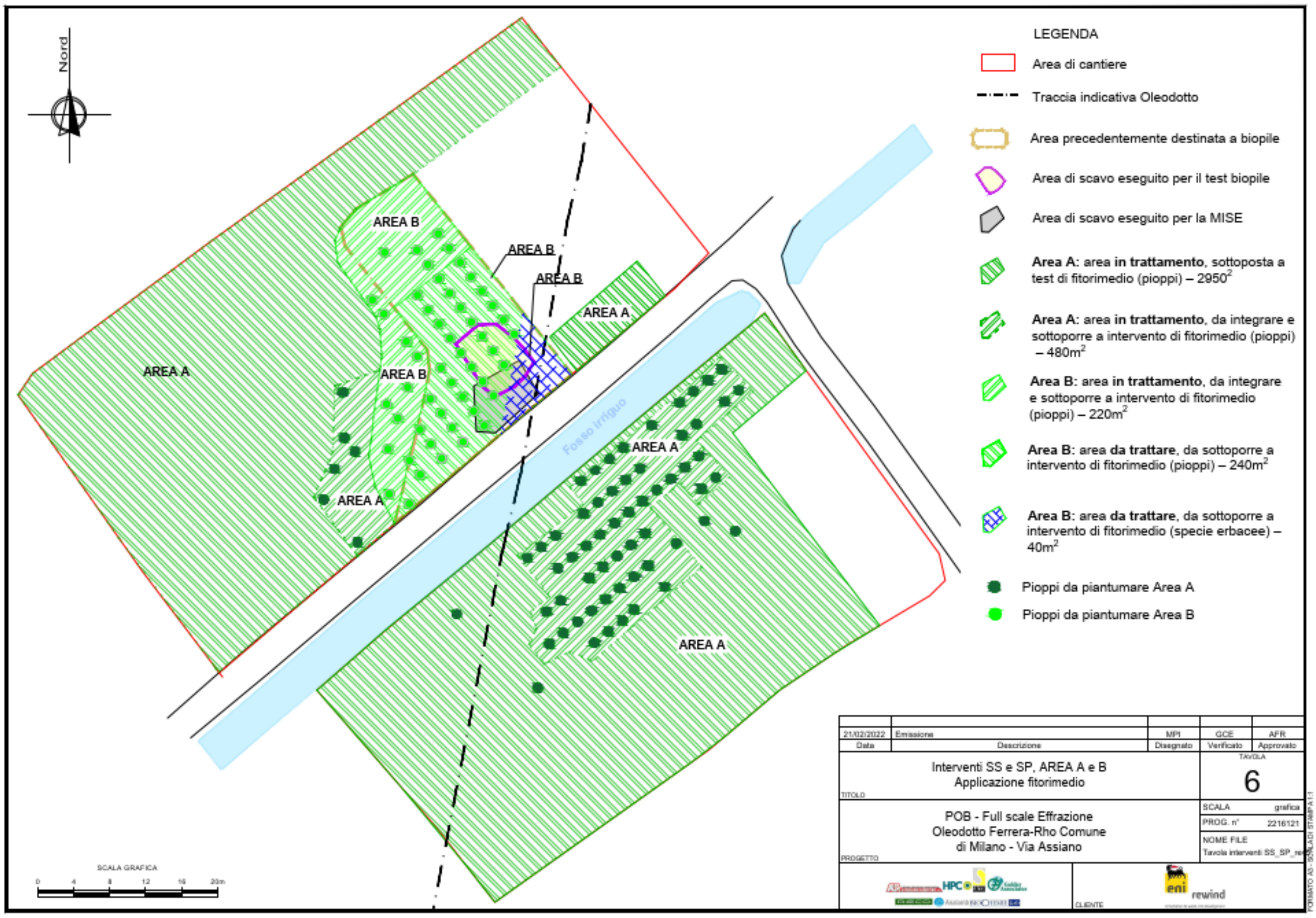

Figure 6 - Application of phytoremediation

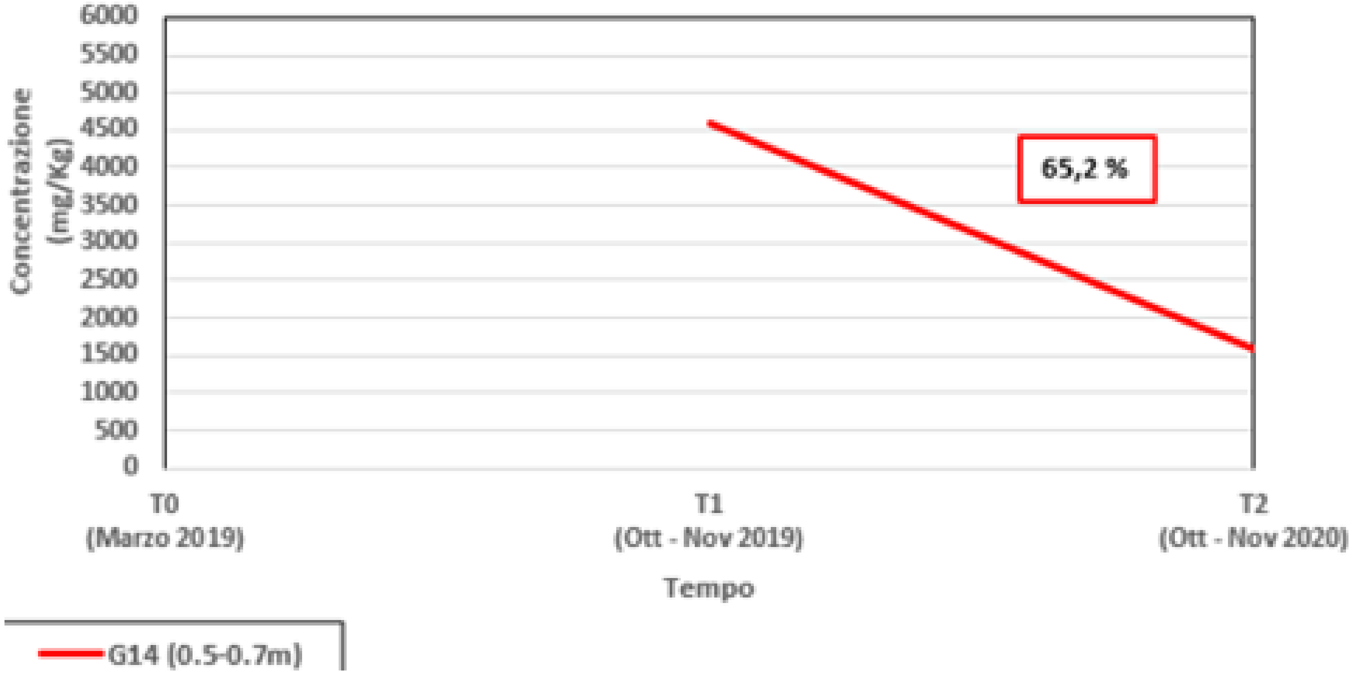

Figure 7 - Degradation of light hydrocarbons

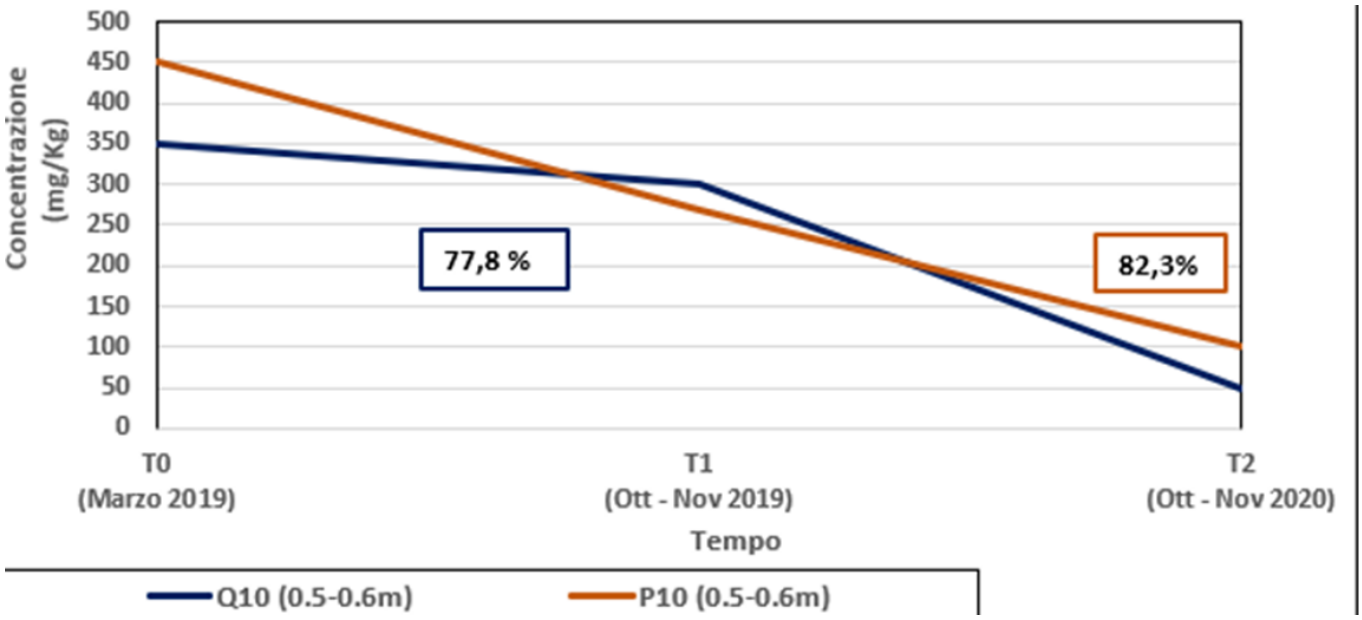

Figure 8 - Degradation of heavy hydrocarbons

REMOVAL RATE

After 2 years of treatment, hydrocarbon concentration decreased significantly in the location planted. The percentage of Hydrocarbons that was degraded after 2 years of treatment was as follows:

- C>12, 65% in the most contaminated area (reduction from 4500 to 1500 ppm) – (see graphic 1)

- C>12, 78-82% in the area characterized by medium contamination (reduction from 450 to 100 ppm) – (see Figure 7)
- C>12, 65% in the most contaminated area (reduction from 4500 to 1500 ppm) – (see Figure 8)
- C>12, 45-80% in the less contaminated area (reduction from 80 to 5 ppm)
- C<12, 95% as maximum in all area (reduction from 120 to 8 ppm)

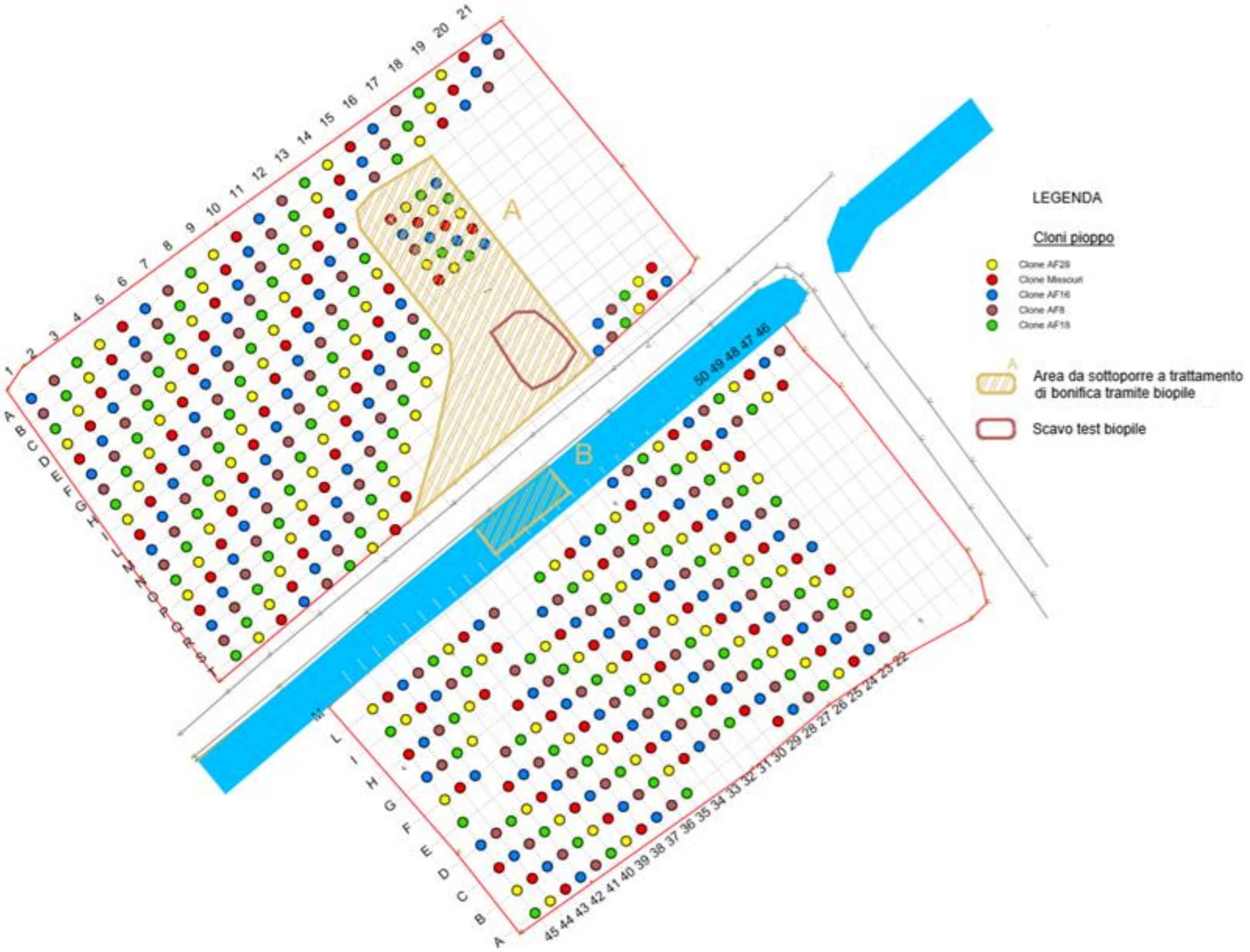

Figure 9 - Treatment area

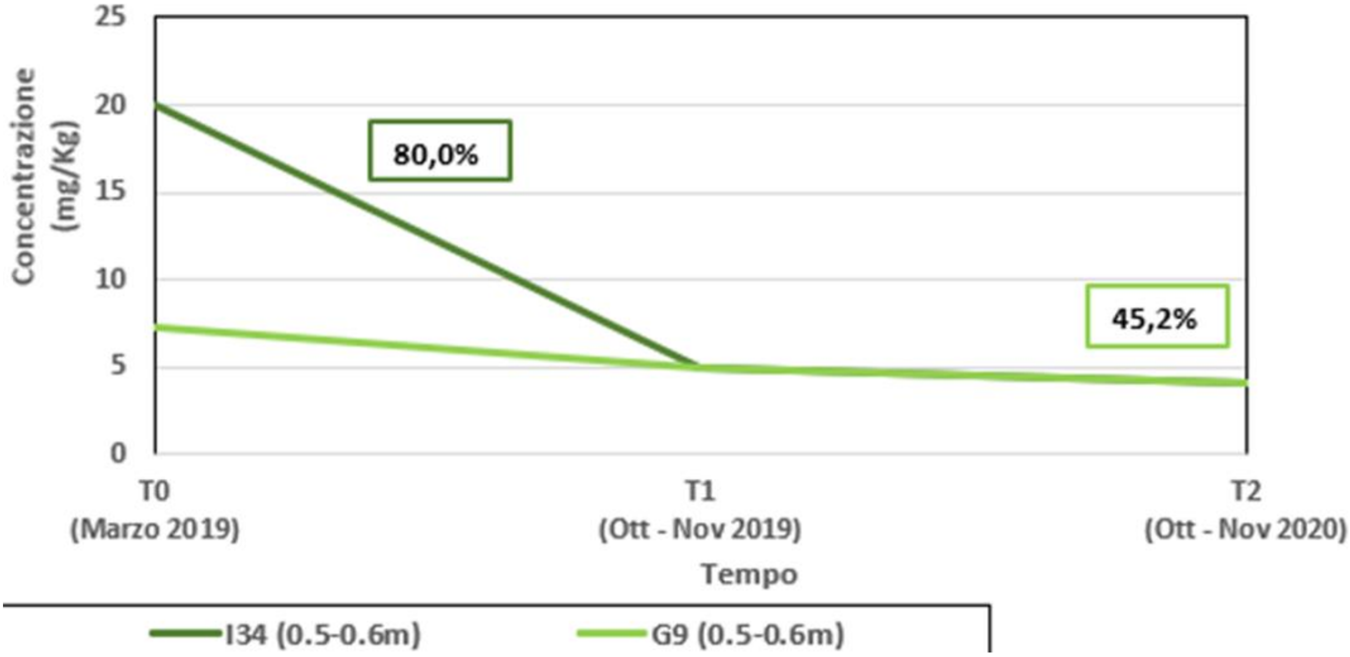

Figure 10 - Percentage of heavy hydrocarbons that reached the remediation target

6. Post treatment and/or Long Term Monitoring

6.1 Post treatment and/or Long Term Monitoring

For contaminants that will not reach the target remediation concentration, another biological treatment will be evaluated:

- enhanced bioremediation
- biopile
- new in risk assessment implemented with the residual concentrations

7. Additional information

7.1 Lesson learnt

The pilot test gave the following results and evidence:

- the planting of poplars led the diffusion of a selection of fungi and microbiological communities with Hydrocarbon degrading characteristics; there are significant differences between rhizospheric soil samples and blank samples
- Tolerance of poplars and effectiveness of the system in the reduction of hydrocarbons C>12, also in areas with higher concentrations than the technological limit initially identified, thanks to the symbiosis with endophytes
- Identification of the most tolerant poplar clones under site-specific conditions for full-scale repopulation of poplar
- Heavy hydrocarbons C>12: after 2 years of treatment in the tested area, they comply with the targets of remediation 51 points out of 58 in the 0-2m section (stretch 2-3m still to be investigated).
- Light hydrocarbons C<12: after 2 years of treatment in the tested area, they comply with the remediation targets 55 points out of 58 in the 0-2m section (stretch 2-3m still to be investigated). Degradation around 96% in 2 years.
- Good functioning of the pedo-biological system and its suitability to reduce the concentrations of pollutants present in the area
- Particularly encouraging results for the applicability of phytoremediation throughout the area, including area with high concentrations (1500-3000 mg/kg).

Operational indications

- Preferring the grafting of poplar poles in holes (> 40/50cm) to overcome the first layer of soil and accelerate the process of growth and root development in depth
- Irrigation necessary in the first vegetative season and in periods of drought
- Use of green compost improver, important for radical development in the first period (mix with the soil and insert into the holes)
- Poplars in combination with autumn-winter species (Veccia)
- Use of growth promoters (Rhizobium)
- Identification of the most suitable clone for site-specific conditions
- 3x2m or 2x2m mesh for operations logistics; telescopic mesh to be preferred over the regular one to accelerate the timing
- Monitoring of plant development in the early stages, important to make any changes in the course of work
- Evaluate the types of fertilizers also in relation to their composition.

Pros of technology:

- Costs: reduced compared to conventional remediation techniques
- Contaminants: effective for light and heavy hydrocarbons even at high concentrations (approx. 3,000 mg/kg)
- Maintenance: does not require special maintenance (apart from irrigation and pesticide actions), but only controls and monitoring to verify effectiveness
- Energy consumption: low or zero
- Landscape: high ecological value, at the same time promotes the increase of biodiversity of degraded sites

Biomass disposal: possibility of biomass reuse for packaging and/or bioenergy production

Soil conservation: the soil, not moved, retains its edaphic characteristics, essential for the return to agricultural use.

Cons of technology:

- Contaminant concentrations: up to 3500 mg/kg (testing higher concentrations)
- Depth to be reached: 3m depth
- Timing: longer than traditional systems
- Specific monitoring for plant growth assessments and development of microbial populations

Operational variables adopted:

- Poplar clones, used in combination with autumn-winter species (Veccia)
- Soil type (sandy-loamy, sandy-gravelly)
- Distribution of compost
- Use of growth promoters (Rhizobium)
- Preparation of holes of approx. 40 cm filled with compost
- Manual irrigation 1 time per week

7.2 Additional information

- To achieve the best results with the phytomeds, a preliminary chemical, microbiological, agronomical and pedological characterization of the site is indispensable. In the same way it is essential to perform an experimental field phase to test the efficiency of the system for better understand the complex interactions between contaminants, soil, plant roots, and microorganisms (bacteria and mycorrhiza) in the rhizosphere.
- Pedological and agronomic characterization is also important to determine the operating modalities for planting (for example, planting poplar at depths greater than 40-50 cm) and need and type of fertilization.
- Agronomic techniques, such as (roto)tilling and addition of lime, nutrients, organic matter, or other soil amendments, are employed prior to planting to facilitate plant growth. However, these treatments can cause changes in soil pH, oxygen content, and bioavailability of the contaminants and hence affect degradation of contaminants in a positive or negative way.

7.3 Training need

It's important to have a team that covers the various specialist areas. Indispensable comparisons based on the feedback obtained from specific activities, to align the various components under study for corrections or additions. The most performing training tool is on-the job training, which allows you to explore the various aspects, and the solutions adopted. Better, in the absence of experience, insights focused on real cases.

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2. Site background

2.1 History of the site

Fig. 1 - Site before the intervention

Fig. 2 - Site after the intervention, without vegetation yet

The site named "Landfill of Manche" is an old small municipal waste landfill located in

the municipality of “Belmonte Calabro” southern Italy. The landfill was used during the 70’s before the promulgation of the national law concerning landfill. The site was fully characterized, and no contamination was found.
The activity was born from an institutional collaboration between the “Commissario Unico per la Bonifica delle Discariche” and Arpa Umbria.

2.2 Geological setting

Natural site conformation from the top to the bottom is: alluvial deposits, dolomitic limestone, schists, and gneiss and phylladic schists.

2.3 Contaminants of concern

After carrying out a specific site risk analysis the site is turned out not contaminated, despite this, it was necessary to proceed with the renaturalization of the area by implementing a suitable intervention to regulate the rainwater giving hydraulic and geological stability to the site.

2.4 Regulatory framework

The regulatory framework is the Legislative Decree 152/06

3. Pilot-scale

3.1 Laboratory Study

We did not perform a laboratory study or a pilot scale study, because the intervention strategy is based on realization of an ET-cover (Evapotranspiration cover). The project was developed analysing the ecological data of the intervention site and selecting the most suitable species for the specific situation.

ET cover systems are designed to rely on the ability of a soil layer to store the precipitation until it is naturally evaporated or is transpired by the vegetative cover. In this respect they differ from more conventional cover designs in that they rely on obtaining an appropriate water storage capacity in the soil rather than an as-built engineered low hydraulic conductivity. ET cover system designs are based on using the hydrological processes (water balance components) at a site, which include the water storage capacity of the soil, precipitation, surface runoff, evapotranspiration, and infiltration. The greater the storage capacity and evapotranspirative properties are, the lower the potential for percolation through the cover system. (From EPA, Fact Sheet on Evapotranspiration Cover Systems for Waste Containment).

3.2 Treatment unit (pilot scale)

The selected species are Pistacia lentiscus L. and Phyllirea latifolia L., both are typical of Mediterranean scrub, perfectly adapted to coastal environments and capable of withstanding prolonged periods of summer drought. Both species chosen are evergreen sclerophylls, this one will allow to maintain a continuous and constant soil cover throughout the year.
The use of evergreen species is aimed at keeping the action carried out by the plants constant in the interception of rainwater, in order to reduce its beating effect on the ground and decrease it and slow down its outflow towards it, in parallel the evergreen species maintain a minimum of transpiring activity even in the winter months when the deciduous species are at vegetative rest.
From a morphological point of view, the selected species have thick foliage that tends to spread out and to exercise good soil cover; in case of favourable conditions, they can rise e change from shrub to tree.
As far as the edaphic needs are concerned, both species are not very demanding, capable

of adapting even to poor- and poor-quality substrates.
As far as the ecological aspect is concerned, the selected species have xerophilic behaviour, the Phyllirea latifolia L. also tolerates marine aerosol well while Pistacia lentiscus L. resists well passage of fires and grazing.

The planting will be of a mixed type, consisting of the two selected species, Pistacia lentiscus L and Phyllirea latifolia L.
The planting layout will be of the quincunx type, with a side of 2X2 meters as shown in fig. 3.

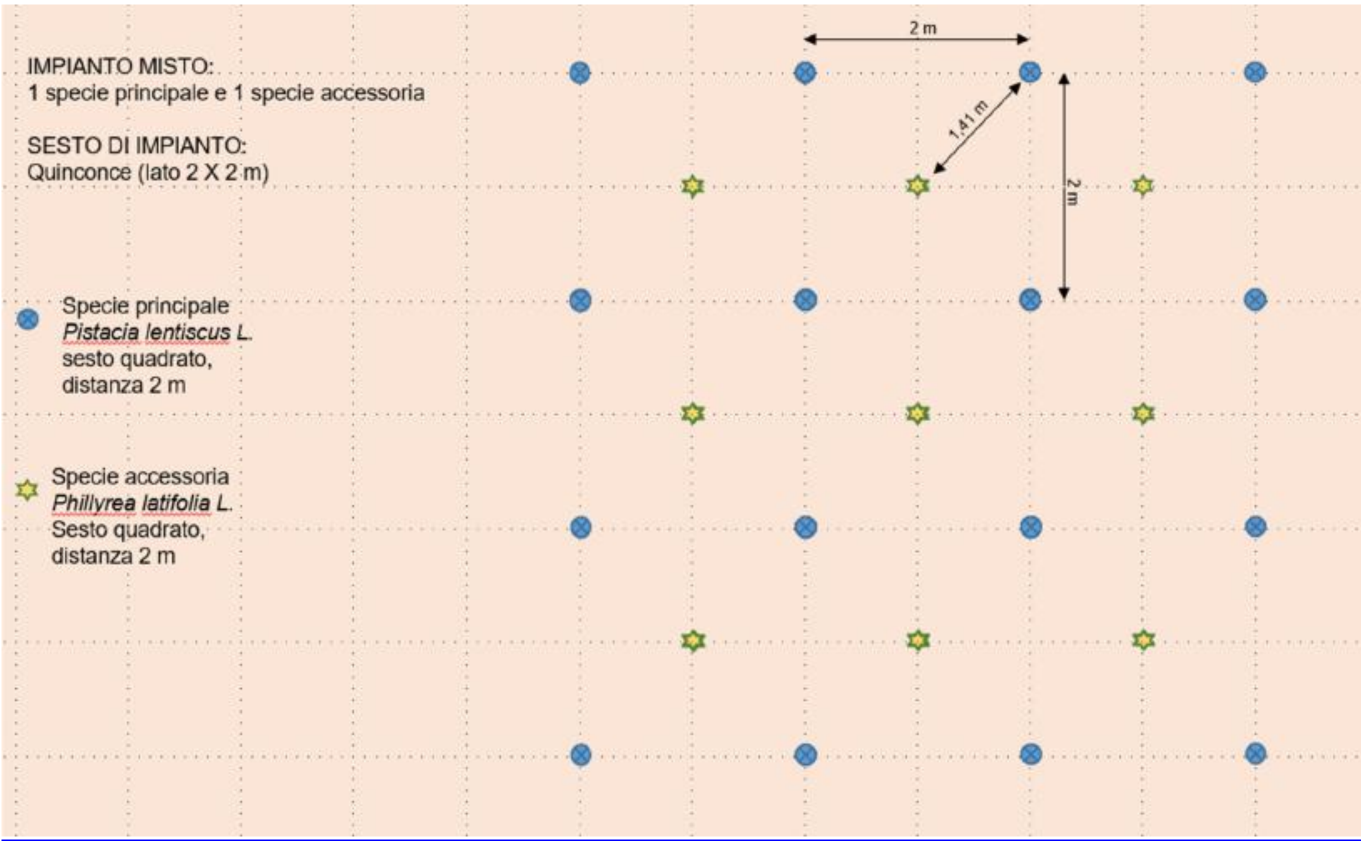

Fig. 3 – Planting layout

7. Additional information

7.1 Lesson learnt

Is it quite possible to apply ET-cover technique in old landfill site, when environmental condition is suitable, with this technique it is possible to operate for the safety of the site also realizing the renaturalization of the area.
To properly design an ET-cover it is necessary a careful ecological assessment of the site to make a correct selection of plants to be used.

7.3 Training need

It is of primary importance to organize training events to improve the technical knowledge of operators and to spread the phytotecnological approach knowledge among public administrations.
Workshops and webinars can be a helpful solution to spread knowledge about phytotechnology.

Glossary of Terms

Term (alphabetical order)	**Definition**
ET-cover	Evapotranspiration cover