Technical Guide

on strategies to enhance phytomanagement efficiency at metal(loid)-polluted/degraded sites: planting patterns, bioinoculation and soil organic amendments

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(*Demonstration of improving edaphic biodiversity, functionality, and ecosystem services in contaminated and degraded land through the phytomanagement within the Interreg Sudoe region*)

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Figure on front page: Mine tailings in Borralha mine (Portugal; ©Helena Moreira)

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PHYTOMANAGEMENT OF CONTAMINATED SOILS – AN OVERVIEW

Metal(loid)s (so-called trace elements in Biogeochemisty and Life Sciences, TE; hereafter referred as TE), mineral oils (e.g., diesel fuel) and polycyclic aromatic hydrocarbons (PAHs) are among the most widely spread contaminants affecting European top soils (Panagos et al., 2013; Van Liedekerke et al., 2014; Tóth et al., 2016; Ballabio et al., 2018). These are also what regulators are looking for first. Xenobiotics such as organochlorines, paraquat, glyphosate or similar compounds and their derivatives are probably subject also to a widespread diffuse contamination (Béranger et al., 2018; Bonvallot et al., 2020). Activities such as mining, smelting and metallurgy, electronics, agriculture, traffic and the use of fossil fuels discharge a considerable amount of TE contaminants into soils, whilst accidental spills of petroleum-based products used for transportation (typically diesel-type fuels) are the principal cause of contamination with organic compounds (Barrutia et al., 2011). Soil contamination is often complex since these contaminants frequently occur simultaneously (Agnello et al., 2016).

Over the last few decades various Gentle soil Remediation Options (GRO) have been developed to (phyto)manage contaminated soils (Vangronsveld et al., 2009; Kidd et al., 2015; Mench et al., 2009, 2010, 2018; Schroeder et al., 2018; Thijs et al., 2018;

Burges et al., 2020; Kolbas et al., 2020). GRO include *in situ* stabilization ("inactivation") and plant-based ("phytoremediation") options. Conventional methods of remediation are based on civil engineering techniques (e.g. encapsulation, vitrification, soil washing, etc.), which have a high environmental impact (destroying soil structure and function) and elevated cost (Liu et al., 2018). GRO offer alternatives, which are considered to be less invasive, more cost-effective and more sustainable (Cundy et al., 2016).

Phytoremediation was initially proposed (early 1990s) as plant-based methods to remediate contaminated environments, and alternatives to conventional civil engineering-based techniques. In the case of organic pollutants, plants and their associated microorganisms are used to degrade the contaminants to non-toxic metabolites, either within the plant tissues (phytodegradation) or in the root-soil interface or plant rhizosphere (due to microbial activity or release of enzymes from plants: rhizoremediation). In TE-contaminated sites, GRO aim to decrease the labile ("bioavailable") pool and/or total content of metal(loid)s in the soil through their uptake and accumulation in harvested plant parts (e.g. phytoextraction), or to progressively promote *in situ* inactivation of TE by combining the use of TE-excluding plants and soil amendments (e.g. phytostabilization). Both strategies have been subject to much discussion regarding their intrinsic limitations, such as the long time required to effectively extract TE from medium to highly contaminated sites (although this can be overcome by considering "bioavailable contaminant stripping"). As a result, the concept of phytomanagement (Fig. 1) evolved which combines sustainable site management with GRO leading to the reduction in pollutant linkages alongside the restoration and/or generation of wider site services (Cundy et al., 2016; Burges et al., 2018). Phytomanagement options promote the use of GRO (based on the interaction between plants, microorganisms, soil amendments and agro-ecological practices) within an integrated, mixed, site risk management solution or as part of a "holding strategy" for vacant sites. The use of profitable plants and the manipulation of the soil-plant-microbial system can control the bioavailable pool of soil contaminants, while maximizing economic and/or ecological revenues but minimizing environmental risks. Potential benefits include water runoff/drainage management, green space provision, soil erosion prevention, renewable energy and material generation, restoration/rehabilitation of plant, microbial and animal communities, greenhouse gas mitigation and carbon sequestration, recovery of land values, amenity and recreation, etc. (Evangelou et al., 2015; Kidd et al., 2015; Cundy et al., 2016; Simek et al., 2017; Touceda-González et al., 2017a; Xue et al., 2018).

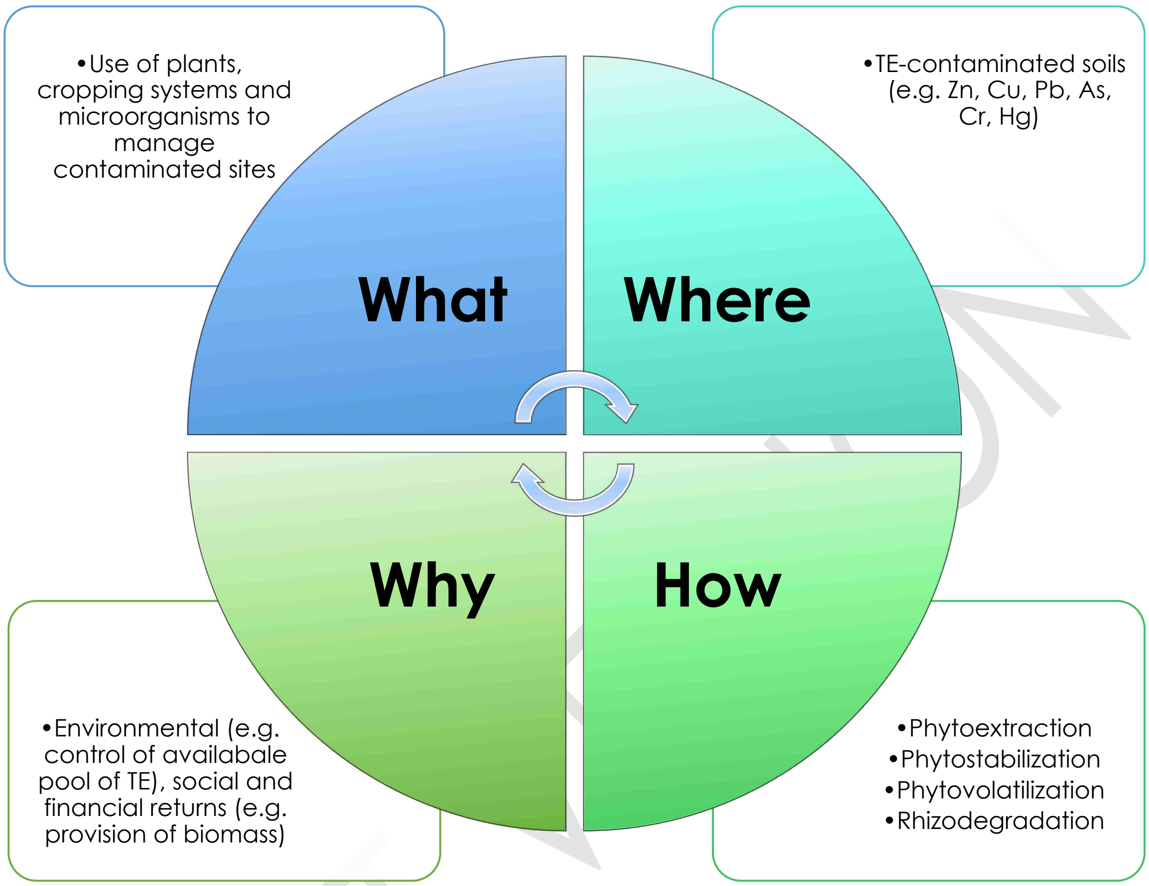


Figure 1: Overview of Phytomanagement options of TE-contaminated soils.

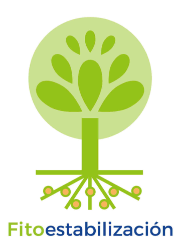
In recent years, phytomanagement has moved from a bench-scale level to full-scale deployment in the field. However, the long-term effects of various soil phytomanagement options on soil functionality, biodiversity, ecological functions and ecosystem services have been poorly assessed and reported. The objective of the PhytoSUDOE project was to increase our understanding, and to provide evidence from long-term field sites, of the effects of phytomanagement on soil functionality and provision of ecosystem services.

1.1. Phytomanagement Options

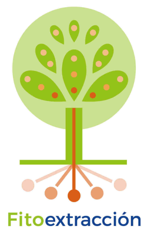
Gentle remediation options have been developed as eco-friendly alternatives to traditional, civil-engineering methods of soil remediation (Kidd et al., 2015). These remediation options include *in situ* stabilization (inactivation) and plant-based (generally termed as phytoremediation) options and are addressed to decreasing the labile (bioavailable) and/or the total content of contaminants (Cundy et al., 2016). These techniques are mainly based on the use of plants, soil microorganisms and amendments, also aided by agronomic management, which effectively reduce pollutant linkages while preserving the soil resource and remediating ecological functions (Vangronsveld et al., 2009). The use of contaminated land for the production of valuable biomass (such as the production of timber, bioenergy crops, biofortified products, ecomaterials, etc.) falls within the concept of phytomanagement (Robinson et al., 2009) and is considered essential for the commercial success of these phytotechnologies (Conesa et al., 2012).

Different options for the phytomanagement of contaminated soils are described below:

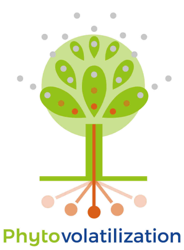
- Phytostabilization uses tolerant plant species with a TE-excluder phenotype to establish a vegetation cover and progressively stabilize and/or reduce the availability of soil pollutants (Mench et al., 2006; Ruttens et al., 2006a, 2006b; Vangronsveld et al., 2009; Dary et al., 2010). The incorporation of amendments into the soil or use of microbial inoculation (aided phytostabilization; Mench et al., 2010) can further decrease the bioavailability and phytotoxicity of pollutants located in the root zone, while improving plant establishment. Phytostabilization does not lead to the actual removal of contaminants but reduces pollutant bioavailability and transfer to other environmental compartments. The mechanical action of the plant roots reduces soil erosion and transport of soil particles through natural agents, while evapotranspiration minimizes leaching during the growing season and therefore contaminant dissemination. In addition, the adsorption, precipitation, and accumulation of the contaminants in the rhizosphere (in collaboration with microorganisms associated with plant roots) entail their immobilization (Mench et al., 2010).



- Phytoextraction is based on the use of TE-tolerant plants that take up contaminants (in general two or three TE, rarely more) from the soil and accumulate them in excess in their harvestable aboveground biomass as compared to their common ranges (Vangronsveld et al., 2009). Phytoextraction can be aided by soil amendments, chemical agents and soil microorganisms (aided phytoextraction). When marketable TE (such as Ni, Au, etc.) is recovered from the plant biomass (bio-ores) it is known as phytomining (Chaney et al., 2007). Another option is to pyrolyse/calcine such TE-rich biomass and to use the biochar or ashes as ecocatalysts in the biosourced fine chemistry (Escande et al., 2014; Clavé et al., 2016).



- Phytovolatilization exploits the ability of plants to transform pollutants into volatile compounds either outside or inside some plant parts after uptake or to absorb and transport volatile compounds from the soil to the aboveground biomass where they can then be released to the atmosphere (Wenzel, 2009). When the contaminant is transformed and released directly from the soil surrounding plant roots (rhizosphere), it is usually termed as rhizovolatilization (Zhang and Frankenberger, 2000).



- Rhizodegradation, Phytodegradation or Phytotransformation uses plants (and their associated microorganisms) to degrade organic contaminants to non-toxic metabolites having at their concentrations less or no toxic effect (Weyens et al., 2009). When the degradation takes place in the rhizosphere of plants (due to microbial activity or release of enzymes from plants), terms such as phytostimulation or rhizodegradation are more correct (Becerra-Castro et al. 2013).

- Rhizofiltration is based on the use of aquatic plants to absorb in and/or adsorb on their roots the contaminants present in water, sediments or aqueous wastes in their roots. The use of aquatic macrophytes as biofilters in natural and constructed wetlands and wastewater treatment facilities has gained interest due to their well-known bioaccumulation properties (Marchand et al., 2010; Salem et al., 2014).

1.2. Advantages and Constrains

The remediation of contaminated soils by phytotechnologies is considered an environmentally friendly, aesthetically pleasing and economically viable alternative to harsher civil engineering-based methods (Kennen et al., 2015). Moreover, they can be applied *in situ* and on a large scale. Establishing an extensive plant cover prevents the dispersion of contaminated soil particles by wind and/or water erosion and can decrease contaminant availability and mobility through root accumulation, rhizosphere-induced adsorption and precipitation and/or degradation (Vangronsveld et al., 2009). However, these techniques do present a series of limitations and still require optimization before they can become fully implemented on a wide scale. In addition to the inherent problems associated with any agronomical practice (such as the dependence on climate and season, water supply, outbreaks of pests or disease, etc.), a major problem associated with these techniques is the length of time required for the clean-up process (of particular concern in phytoextraction). Several authors have suggested that to be realistically viable the clean-up time should not exceed 10 years (Robinson et al., 2009; Vangronsveld et al., 2009).
The time length required can also be significantly reduced if the target values are based on the available pool of contaminants and the pollutant linkages instead of total soil contaminant concentration. As mentioned above, the shift from phytoremediation strategies to phytomanagement options, in which remediation strategies are combined with sustainable site management options, result in a net gain (or at least no gross reduction) in soil functions and ecosystem services, as well as achieving effective risk management (Cundy et al., 2016). The provision of ecosystem services may compensate some of the limitations of the remediation process. In this context, (aided) phytostabilization should be considered as a management strategy for contaminated sites, which offers economic, environmental, and societal benefits (Cundy et al., 2016). Climatic conditions pose a crucial and obvious limitation to the success of phytomanagement. Temperature controls transpiration, water chemistry, growth and metabolism of plants, and therefore directly affects both contaminant uptake and their fate in plant parts and other ecosystem compartments (Bhargava et al., 2012). Soil moisture affects both plant growth and contaminant transport in soil, and GRO implemented also needs water management, especially in arid and semi-arid areas that undergo relatively long periods of drought and heatwaves. Prolonged drought induces stress which enhances plants' sensitivity to pathogens or herbivory and, more importantly, reduces plant

growth with negative implications on the phytoremediation success. Additional site-specific problems concern mining areas, tailings and sandy soils, where soils are often characterized by a low water retention capacity (Kidd et al., 2015).

As mentioned above, a major limitation of phytoextraction is the very long time required to effectively extract TE from soils, particularly in medium and highly contaminated sites (Zhao et al., 2003). However, if the aim of the phytoextraction option only aims at stripping the bioavailable TE fraction from soil ("bioavailable contaminant stripping") and not to reach total TE concentration targets established by legal frameworks, then the time required for successfully reaching this target is much shorter (Vangronsveld et al., 2009; Mench et al., 2018; Neaman et al. 2020). Also, for phytoextraction, the low biomass and slow growth of most hyperaccumulators are largely responsible for the long time required. This limitation can be overcome to some extent by using plant species that provide an added value in order to obtain economic benefit during the phytoextraction process itself. Energy crops, such as *Miscanthus* spp., *Ricinus communis* L., and *Brassica napus* L., have been proposed due to their metal tolerance and accumulation capacity along with their usefulness for biofuel production (Burges et al., 2018). Other commercial applications of plants used in phytomanagement, such as biochar production, raw materials for industries (oil, paper, bio-chemicals, essential oils, etc.) and medicinal purposes are being studied (Pandey et al., 2016). The use of fast-growing trees offers the possibility to combine metal (Cd, Zn, and Ni) extraction with production of biomass for bioenergy and other end-products (e.g., timber, resin, adhesives, etc.) (Schroeder et al., 2018). Recovery of high-value metals or strategic elements, from metal(loid)-rich plant biomass is another means of increasing the economic viability of phytoextraction (in this case termed as phytomining), while simultaneously addressing the need for disposal of the metal(loid)-rich biomass. Chaney et al. (2007) demonstrated that phytomining of Ni can be highly profitable in Ni-contaminated soils (van der Ent et al., 2015; Kidd et al., 2018; Lopez et al. 2019).

Additional aspects that should be considered include the degree of soil contamination, the bioavailability and accessibility of the contaminants, and the capacity of the plants and their associated microorganisms to adsorb, accumulate and/or degrade the contaminants (Vangronsveld et al., 2009). Assisted phytoextraction using chelates has been proposed as a means of increasing metal bioavailability, but an important limitation of chelate-induced phytoextraction is the possibility of promoting metal leaching to other environmental compartments (e.g., groundwater; Burges et al., 2018).

The establishment and growth of plants on contaminated sites are other major obstacles (Tordoff et al., 2000, Mendez and Maier, 2008). In addition to the phytotoxic effects of available pollutants in excess, contaminated soils usually present edaphic conditions, which can severely limit plant growth (e.g. nutrient deficiency, poor soil structure, low organic matter, etc.). The careful selection of tolerant and resilient plant species is vital for the long-term success of phytomanagement options (Batty 2005, Clemente et al., 2012; Parraga-Aguado et al., 2014). The efficiency of phytotechnologies can also be enhanced by incorporating agronomic practices. For example, plant cropping patterns (rotation, intercropping) can improve plant growth and performance and, depending on the phytotechnology, can be designed so as to enhance or mitigate metal(loid) availability, uptake and accumulation (Kidd et al., 2015). Intercropping, traditionally used in agriculture to increase crop yield, can pair phytoextracting plant species with other crops, in order to promote remediation while providing economic benefits (Burges et al., 2018). The use of deep-rooting plants, mycorrhizal plants or bioinoculants can enhance plant growth and GRO efficiency (Kidd et al., 2009, 2015). The use of organic and inorganic amendments may optimize plant growth and performance by improving soil physicochemical properties, fertility and microbial activity and diversity (Bolan et al., 2011; Pardo et al., 2014a, 2014b, 2014d; Xue et al., 2018; Burges et al., 2020). In addition, amendments directly or indirectly influence the availability and mobility contaminants through the modification of soil physico-chemical and biological conditions (pH, redox conditions, concentration of chelating and complexing agents, cation exchange capacity, and biological activity) (Pérez-De-Mora et al., 2005; Kidd et al., 2015; Pardo et al., 2016). Depending on site characteristics a selection of the most appropriate phytomanagement options will be necessary; in some cases, the implementation of several approaches may be needed. The combination of different options can be more effective in site remediation than using a single approach.

1.3. Current Status

Phytomanagement requires the use of appropriate agronomic and crop management practices and can be assisted through the application of soil amendments. However, long-term field experiments are crucial for monitoring the efficiency and sustainability of phytomanagement options over time. A growing number of studies under field conditions can be found in the literature and these should contribute towards reaching full-scale deployment of these techniques. Such field studies have shown that phytostabilization can effectively reduce TE mobility by altering speciation, as well as to improve soil physicochemical properties and fertility, increase microbial diversity and restore functionality in the long-term (Kumpiene et al., 2009; Clemente et al., 2012; Zornoza et al., 2012; Pardo et al., 2014c, 2014d, 2016, 2017; Quintela-Sabarís et al., 2017; Xue et al., 2015, 2018; Mench et al., 2018; Kolbas et al., 2020). At any given site, it will be necessary to implement a long-term monitoring programme so as to ensure that any reduction achieved in metal toxicity and improvement in soil quality are maintained (Epelde et al., 2014). The last few years have seen a growing interest in the influence of microorganisms on plant growth and contaminant bioavailability and degradation. Rhizosphere and endophytic organisms that have received much attention because of their beneficial effects on plant growth health and resistance to stress are the plant growth-promoting bacteria (PGPB), mycorrhizal and endophytic fungi (Mendes et al., 2013; Coninx et al., 2017). Microorganisms can increase the availability of essential plant nutrients, such as nitrogen (N_2-fixing organisms), phosphorus (by solubilization or mineralization through the production of organic acids and/or phosphatases) or iron (by releasing Fe(III)-specific chelating agents or siderophores). Plant growth-promoting bacteria can also directly influence plant growth and physiology through the production of phytohormones (e.g., IAA or by reducing stress ethylene levels in plants through the production of the enzyme 1-aminocyclopropane-1-carboxylate deaminase). Some bacteria can inhibit or reduce plant diseases indirectly by competing for nutrients and space (niche exclusion), producing antimicrobial compounds or through the induction of plant defense mechanisms (Goswami et al., 2016; Guo et al., 2020).

Several field-based trials implementing phytostabilization in metal-contaminated soils have shown the benefits of organic-based amendments for recovery of soil biological fertility. Microbial biomass and soil enzymatic activities were higher in acidic mine soils amended with pig manure/sewage sludge/marble waste than in the untreated mine tailings (Zanuzzi et al., 2009; Zornoza et al., 2012). Pardo et al. (2014d) successfully used olive-mill waste compost as a soil amendment to promote the growth of a native legume (*Bituminaria bituminosa* (L.) C. H. Stirt.) in a mine-affected soil from a semi-arid area (Southeast Spain) contaminated with trace elements (As, Cd, Cu, Mn, Pb, and Zn).
However, the use of amendments has to be carried out with caution as amendments can have undesirable effects: for instance, an inappropriate use of organic amendments can result in underground water contamination by nitrates, antibiotics, hormones, and loss of soil biodiversity, posing a risk to environmental and human health (Goss et al., 2013; Burges et al., 2016,

2018). Organic and inorganic amendments can induce other negative effects like destruction of soil structure, addition of potentially toxic compounds, immobilization of essential nutrients, etc. (Alkorta et al., 2010). Moreover, although amendments have demonstrated to aid revegetation, plant roots may not extend readily from a fertile layer into underlying non-amended contaminated soil (Pulford and Watson, 2003), limiting the potential of this phytotechnology to the top layer of soil.

1.4. Legal and Regulatory Framework

Key concerns regarding the increasing loss of soil quality through degradation or contamination of soils led the European Commission to develop a Soil Framework Directive (EC, 2006) which presented a Thematic Strategy toward soil protection considering eight main threats to European soils: (1) erosion, (2) loss of organic matter, (3) contamination, (4) compaction and other physical soil degradation, (5) salinization, (6) decline of biodiversity, (7) soil sealing by infrastructure, and (8) floods and landslides (EC, 2006). Unfortunately, this Thematic Strategy was not accepted by all EU Member Countries. The Global Soil Partnership (GSP) was established in 2012 by the Food and Agriculture Organization of the United Nations (FAO) in order to develop interaction and enhanced collaboration amongst all relevant stakeholders (from land users to policy makers) towards the development of soil legislation and sustainable soil management measures. This proposal was very important and promoted a discussion on how to translate soil science into environmental policies (Bouma et al., 2017). The Intergovernmental Technical Panel on Soils (ITPS), which was established at the first Plenary Assembly of the GSP in 2013, published the first-ever comprehensive report on the State of the World's Soil Resources (SWSR; FAO and ITPS, 2015). Major threats to soil functions at a global scale were identified as soil erosion, loss of soil organic carbon, nutrient imbalance, and salinization and sodification. Requirements for soil protection are also often included in other EU policies, such as the Nitrates Directive and the Water Framework Directive, and in the national legislations of various European countries, specifically addressing water, waste, and mining regulations. Although these policies consider soil contamination and contribute indirectly to soil protection, they only feature soil as a secondary objective.

The legislation available in many industrialized countries, regulating local soil contamination, and guidelines for assessing potentially contaminated soils, is based on total contaminant concentrations (Reinikainen et al., 2016; Ramon and Lull, 2019). However, negative effects of metal(loid)s on soil functioning is known to be related to mobile/bioavailable elemental pools rather than total metal concentrations (Kumpiene et al., 2009). Therefore, the site-specific approach based on conceptual model, pollutant linkages and risk assessment are more and more adopted in European countries (e.g. France, UK, Germany; Mench et al., 2020). On the other hand, it is often the case that bioavailable concentrations show no correlation with total concentrations (Burges et al., 2015). There is a general consensus that metal(loid) bioavailability is more important for environmental protection and risk assessment than total metal(loid) concentrations because it represents the labile fraction subject to leaching and uptake by soil organisms (Madejón et al., 2006). In recent years, more sophisticated risk-based approaches to deal with the local effects of soil pollution have been developed, which include the concept of pollutant linkages (contaminant-receptor-pathway). Decision makers and regulatory organizations have accepted that bioavailability of soil contaminants is a key variable to be taken into consideration in risk assessment, regulation policies and soil remediation (Bolan et al., 2014; Naidu et al., 2015). These risk- orientated policies focus on the abandonment of policies aimed at restoring soils to their original 'clean' state. Some national trigger values classifying soils as contaminated or requiring remediation now have bioavailability explicitly (e.g., in the UK, Belgium, Switzerland; Mench et al., 2020) or implicitly (trigger values set according to the main influencing soil physicochemical properties, e.g., soil pH, granulometry, organic matter content) embedded within them. Several phytomanagement options are aimed at removing the bioavailable contaminant fraction ("bioavailable stripping"), a target, which significantly reduces the length of time required for rehabilitation (Herzig et al., 2014; Li et al., 2014; Lillo et al., 2020; Neaman et al., 2020).

There is now an emerging consensus in the broad frameworks and approaches for sustainable remediation (Bardos, 2014), which is culminating in the drafting of international standards by ISO and ASTM International. The fundamental basis of sustainable remediation is to promote the use of more sustainable practices during environmental clean-up activities, with the objective of balancing economic viability, conservation of natural resources and biodiversity, and the enhancement of the quality of life in surrounding communities. In broad terms, concepts of sustainable remediation are based on achieving a net benefit across a range of environmental, economic, and social concerns that are judged to be representative of sustainability. This is a key goal in land remediation and land regeneration, given the large global contaminated land legacy and the overall resource and financial cost required to bring this land back into beneficial use. The implementation of the International Organization for

Standardization (ISO) Standard on Sustainable Remediation is now at an advanced stage (Bardos et al., 2016). Remediation begins with an option appraisal that short lists strategies that could deliver the required reduction in risk. A remediation strategy comprises one or more remediation technologies that will deliver the safe and timely elimination and/or control of unacceptable risks. The ISO standard will help assessors identifying the most sustainable among the shortlisted, valid alternative remediation strategies.

This guide intends to give an overview of the profitable plants that can be used in phytomanagement projects as well as strategies that will improve its efficiency in the recovery of contaminated sites with TE. These strategies include the use of crops systems that maximize plants productivity and overall improvement of soils; the use of beneficial microorganisms that promote crops establishment and stress resistance; and the use of soil amendments, specifically organic, that immobilize TE and help plants growth.

2

Plant Selection and Cropping Systems

Phytomanagement uses plants that can withstand adverse levels of TE, while providing environmental gains and financial returns for stakeholders (Cundy et al., 2016). The increased attention given to this approach during the last decade fostered laboratory and field research aimed at finding plant species and associated microorganisms with abilities to restore TE-contaminated areas (Fig. 2). Some valuable crops arose as promising candidates in adding value to those areas by e.g. generating biomass-based products, feedstock for fibers, essential oils, pharmaceutical products, ecocatalysts, platform-chemicals for green chemistry and ecomaterials/insulation, while lowering or stabilizing pollutants in soil.

Figure 2: Poplar (*Populus* sp.) growing in soils contaminated with Cu, Cd, and Zn (Borralha Mine, Portugal; photo ©Helena Moreira)

The selection of a crop suitable for a specific contaminated site is therefore critical for the success of phytomanagement options, and depends on the following factors (Kidd et al., 2015; Cundy et al., 2016):

i) type, concentration, chemical speciation, and bioavailability of the contaminants;
ii) physico-chemical characteristics of soil (e.g. structure, compaction, fertility, moisture, pH, OM);
iii) pollutant linkages;
iv) water availability;
v) climatic conditions (e.g. temperature, precipitation, wind, altitude);
vi) local facilities for processing biomass (as transport is not possible, and this biomass must be merged with larger batches).

Screening through appropriate literature and experiments at similar locations can help determining whether certain types of crops are suitable for the target site. Scanning surrounding, undisturbed areas can also help to find plants with potential to grow in the target site, given that growth conditions may be similar or comparable. Furthermore, there are some phytotechnology online databases (e.g., https://www.iret.cnr.it/en/phytoportal; http://phytosociety.org) that can help selecting the adequate crops. However, preliminary plant survival and growth trials are advisable prior to their large-scale employment, for ensuring the success of the phytomanagement action.

The selection process must also consider potentially invasive/aggressive species that may compete with local endemic species and/or act as ecological disruptors and are, therefore, not advisable. Additional traits such as growth rate, life cycle (perennial, annual, and biennial), type (deciduous or evergreen), growth habit (e.g. herb, graminoid, shrub, and tree), water requirements and resilience to climate changes, root depth, susceptibility to diseases/pests, and contaminant (TE, xenobiotics or both) tolerance should also be considered to attain the specific goals of the phytomanagement action at the target site, and maximize its success.

Finally, the phytomanagement goal is to phytoextract or phytostabilize TE, and/or to promote xenobiotic biodegradation when there is a mixed contamination of soils, this will also condition plants' selection. The behavior of contaminants in the candidate plant should be carefully considered. Information describing the processes and pathways by which TE interact with specific plants can also be found in dedicated databases and in the literature. However, TE uptake is element and plant host-specific, which means that it can be highly variable even within plants' species and depends on plant nutrition and TE bioavailability in soil.
Besides selecting the most suitable crop(s), designing the appropriate cropping system, i.e., the type of management and crop's spatiotemporal organization is critical and can increase phytomanagement efficiency in a given area. An optimal cropping system will maximize soil fertility, promote an efficient use of nutrients and water and enhance crop's yield. It can also affect TE mobility and availability.

Cropping systems comprise three components: i) crop succession, which dictates the sequence of crops across years (e.g. monocultures, crop rotation, co- and inter-cropping, and fallowing); ii) cropping patterns, which comprises the spatial arrangement and yearly sequence; and iii) management practices (e.g. tillage system, irrigation practices, and nutrient management (Bégué et al., 2018; Figs. 3, 4).

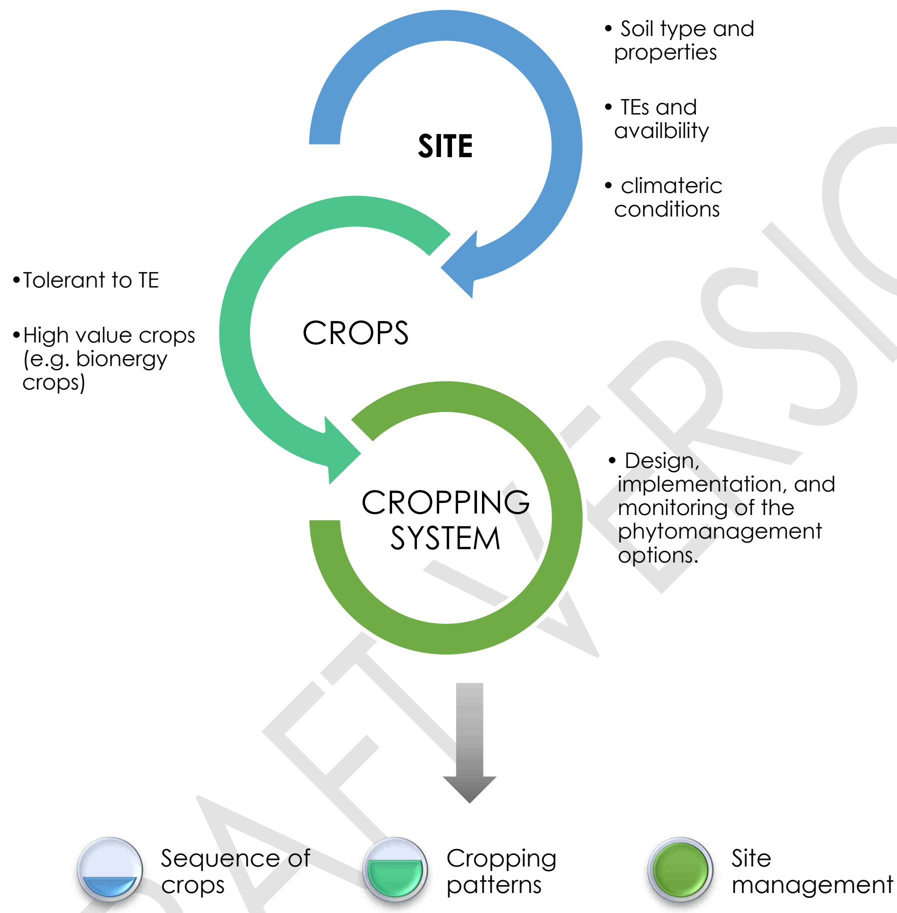


Figure 3: Schematic diagram showing the different steps for phytomanaging a targeted contaminated site.

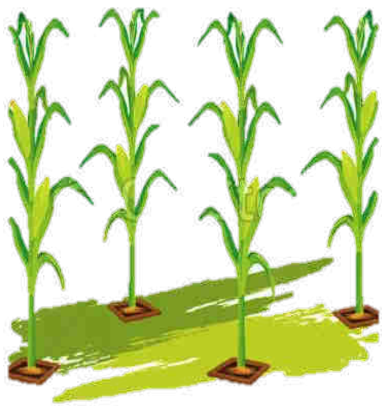
Cover crops can also be included in phytomanagement options to improve their success. These crops are mostly planted for improving soil fertility, and not for biomass or energy production purposes.

This section summarizes the main traits, tolerance to TE and phytomanagement applications of some economically valuable crops. It also highlights the most relevant cropping patterns and crop successions to be applied in TE-contaminated sites in order to enhance the success of phytotechnologies.

Figure 4: Poplar (*Populus* sp.) plantations in Borralha Mine (northern Portugal) intercropped with alfalfa (*Medicago sativa;* photo ©Helena Moreira).

2.1. Economically valuable crops

Phytomanagement of TE-contaminated sites uses plants able to overcome abiotic and biotic stresses, while offering economic benefits, such as biomass for energy production, or/and other profitable products such as biofuel, oil, fiber and timber.
The main criteria for selecting these crops are:



HIGH VALUE CROPS

- 1. Fast-growth rate
- 2. Tolerant to TE concentrations
- 3. High biomass yield
- 4. High rooting depth and density
- 5. Easy to cultivate and harvest
- 5. Biomass quality (e.g. for biomass-processing technologies)

A wide range of fast-growing plants, varieties, cultivars or hybrids, has the potential to be used in TE-affected sites. However, although tolerant to TE, these crops can suffer from toxicity at early stages of their growth development.

The global crop characteristics, TE tolerance/accumulation, main potential products and phytotechnology details of the most promising annual and perennial energy crops are described in the next sections.

2.1.1. Annual Crops

2.1.1.1. Sunflower (*Helianthus annuus* L.)

A. CHARACTERISTICS:

- **Family**: *Asteraceae*
- **Growth habit**: herb
- **Plantation:** mid spring *in situ*; an early start can be made by sowing seeds in pot in greenhouse in early spring
- **Harvest**: September (in western Europe)
- **Water usage**: medium
- **Cultivation details**: prefers moist and well-drained soils; suitable for various soil pH; it can grow in semi-shade or no shade; tolerant to drought

photo ©Helena Moreira

B. TRACE ELEMENTS (tolerance/accumulation):

Al	**As**	Ag	**Cd**	Co	**Cr**	**Cu**	Fe	Mn	Mo	**Ni**	**Pb**	**Zn**	Sn	Tl
	⏏		⏏		⏏	⏏				⏏	⏏	⏏		

C. POTENTIAL PRODUCTS:

Livestock forage; biomass; biofuel (bioethanol, biodiesel and biogas); seeds oil; insulating materials; fibers.

D. PHYTOTECHNOLOGY DETAILS/APPLICATIONS:

Sunflower is a TE tolerant high-yield crop with several potential uses. This crop can be used to phytoextract bioavailable TE (Cd, Zn, and Cu) in contaminated soils and provide financial revenues from the biomass processing (Kolbas et al., 2011; Herzig et al., 2014; Kidd et al., 2015; Mench et al., 2010, 2018). Sunflower shoots and oilseed are relevant raw feedstock for several biomass processing technologies, such as: insulation material, hydrothermal processing which converts raw materials such as lignocellulosic materials into bioenergy and high added-value chemicals (Ruiz et al., 2013), fatty acids for supporting microbes, oil, production of bioethanol and biogas (Camargo and Sene, 2014; Hesami et al., 2015), fibers to reinforce plastic products (Liu et al., 2017) and briquettes (Alaru et al., 2013). Several sunflower mutant lines obtained by chemical mutagenesis phytoextract more TE (Cu, Zn, Cd, and Pb) than their mother lines in field conditions (Herzig et al., 2014; Kolbas et al., 2011, 2014, 2018), and show an increased antioxidant status at high TE exposure (Nehnevajova et al., 2012). As water supply and its distribution during crop cycles is a limiting factor for crop production. Sunflowers' ability to resist to more frequent heatwaves and long drought periods due to the climate change is an advantage (Kidd et al., 2015). To avoid the pest occurrence, a crop rotation is often mandatory.

Figure 5: Sunflower grown at Saint-Médard-d'Eyrans (France; photo ©M. Mench).

2.1.1.2. **Tobacco** (*Nicotiana tabacum* L.)

A. CHARACTERISTICS:

- **Family:** *Solanaceae*
- **Growth habit:** herb
- **Plantation:** seed – surface sow in greenhouse before transplanting
- **Harvest:** July-October
- **Water use:** medium
- **Cultivation details:** prefers well-drained and fertile moisty soils; suitable for different soil pH; sunny areas (12-13 h of light during its vegetative growth); prefers temperatures of 20-30 °C and atmospheric humidity of 80-85%

photo Joachim Süß by Pixabay

B. TRACE ELEMENTS (tolerance/accumulation):

Al	As	Ag	**Cd**	Co	**Cr**	Cu	**Fe**	**Mn**	Mo	Ni	**Pb**	**Zn**	Sn	Tl
			⏏		⏏	⏏	⏏	⏏			⏏	⏏		

C. POTENTIAL PRODUCTS:

Oil; biomass; biofuel (biodiesel, biogas); biochar and activated carbons; livestock feed; pellets.

D. PHYTOTECHNOLOGY DETAILS/APPLICATIONS

Tobacco is able to tolerate and accumulate several TE such as Cd, Zn, and Cu, especially in leaves. In the past decade, tobacco has gathered interest within the energy sector as a bioenergy crop by having the potential to generate up to 170 tons of biomass per ha (Barla and Kumar, 2019). Phytomanagement of TE-contaminated soils using tobacco and processing of its biomass are reported in several research studies (Gonsalvesh et al., 2016; Hattab et al., 2016; Thijs et al., 2018; Burges et al., 2020; Kolbas et al., 2020). Tobacco shoots can be used in various conversion processes, i.e. vacuum and oxidative pyrolysis, synthesis of hydrogen fuel, biofuel and activated carbons, hydrothermal oxidation, gasification, among others (Usta, 2005; Maskos and Dellinger, 2008; Andrianov et al. 2010; Syc et al., 2011). Some tobacco cultivars show particular tolerance to Cu (Keller et al., 2003; Rout and Sahoo, 2007) and Cd (Mench et al.,1989; Vangronsveld et al. 2009). Metal-resistant somaclonal variants of tobacco have also been tested in Switzerland and Belgium (Lyubenova et al., 2009; Vangronsveld et al., 2009; Herzig et al., 2015) and comparing with parental lines, the best variants had a higher shoot TE concentration. Such tobacco variants are candidates for non-food crop rotation on metal-contaminated soils, but their productivity and shoot metal removal in southwest Europe, notably at sites with Cu-contaminated soils, is still underexplored.

Figure 6: Tobacco plants in a former wood preservation site contaminated with Cu and PAHs (Saint-Médard-d'Eyrans, France; photo ©M. Mench).

2.1.1.3. Industrial Hemp* (*Cannabis sativa* L.) *Low TCH content-cultivar

A. CHARACTERISTICS:

- **Family:** *Cannabaceae*
- **Growth habit:** herb
- **Plantation:** seed – sow in early spring in greenhouse or *in situ* in mid spring.
- **Harvest:** September-October
- **Water use:** high
- **Cultivation details**: very adaptable to soil and climatic conditions; prefers fertile and well-drained soils; optimal soil pH 6.3-6.8; requires more than 12-13 h of light during vegetative growth; growth period of 2-10 months, depending on latitude

photo NickyPe by Pixabay

B. TRACE ELEMENTS (tolerance/accumulation):

Al	As	Ag	**Cd**	Co	**Cr**	**Cu**	Fe	Mn	Mo	**Ni**	**Pb**	**Zn**	Sn	Tl
			⏏		⏏	⏏				⏏	⏏	⏏		

C. POTENTIAL PRODUCTS:

Fibers; biofuel (bioethanol, biodiesel and biogas); livestock forage; animal bedding; paper; compost; paint; oil; composited materials.

D. PHYTOTECHNOLOGY DETAILS/APPLICATIONS:

Industrial hemp can grow in multi-contaminated and low fertility soils, and it can be cultivated in several EU countries (with permission). It has about 1 m deep and dense roots, which can be also used to produce organic compost. This crop is able to establish and grow with very low agrochemical inputs and can be used for a wide range of applications, including building materials, textiles and for biofuel production (Angelova et al., 2004; Alaru et al., 2013; Kumar et al., 2017). Hemp has a high cellulose content, which is a key factor for bioethanol production (Kumar et al., 2017).
The use of hemp for phytoremediation has been poorly explored but it is seen as one of the most profitable annual bioenergy crops for managing TE contaminated soils.
Hemp is able to tolerate high concentrations of Cu, Cd, Pb, Zn, Cr and Ni in soils (Ahmad et al., 2016), and depending on cultivars and edaphic conditions, it can be used either to phytostabilization or phytoextraction (ex. Zn; Malik et al., 2010). In the case of Cu, hemp can highly concentrate it in plant tissues (Angelova et al., 2004; Arru et al, 2004; Elisa et al., 2007). This crop is also able to absorb Se and could be used for biofortification purposes (edible seeds; Stonehouse et al., 2020).

2.1.1.4. MAIZE (*Zea mays* L.)

A. CHARACTERISTICS:

- **Family:** *Poaceae*
- **Growth habit:** graminoid
- **Plantation:** spring
- **Harvest:** September-October
- **Water usage:** high
- **Cultivation details:** versatile crop; suitable pH: acid and neutral soils; does not grow in shadow; dependent on soil moisture; sensitive to drought and frost; high fertility requirements

photo © Sofia Pereira

B. TRACE ELEMENTS (tolerance/accumulation):

Al	**As**	Ag	**Cd**	Co	**Cr**	**Cu**	**Fe**	**Mn**	Mo	**Ni**	**Pb**	**Zn**	Sn	Tl
⏏	⏏		⏏		⏏	⏏	⏏	⏏		⏏	⏏	⏏		

C. POTENTIAL PRODUCTS:

Biofuel (biodiesel, bioethanol and biogas); biomass; livestock forage; charcoal.

D. PHYTOTECHNOLOGY DETAILS/APPLICATIONS:

Energy maize is a high biomass and fast-growing crop, with long roots, that can be used for power generation and for biofuel production. Its nutritional requirements and other management issues in its plantation are well established, which facilitates its adoption for phytoremediation. However, as it is considered a first-generation bioenergy crop, and is seen as a competitor with food crops for fertile soils, its cultivation for energy purposes is not consensual. Nonetheless, there are several energy cultivars on the market that are able to grow in contaminated sites, tolerating a broad range of TE such as Cr, Cu, As, and Al. Maize can accumulate high concentrations of Cd, Pb, and Zn above phytotoxic concentrations without showing any symptoms of toxicity. This crop can be used for both phytoextraction (Kimenyu et al., 2009; Pereira et al., 2007) and phytostabilization (Moreira et al., 2016a), depending on genotype, TE and soil chemical characteristics.

The kernel usually presents the lowest TE concentration. This results from plant defense mechanisms to prevent non-essential elements to reach the reproductive organs (Meers et al., 2010).

Figure 7: Energy maize at the Cd/Zn-contaminated Balen site, Belgium (Vangronsveld et al.; photo ©M. Mench) – FP 7 EU Greenland project (see Thijs et al. 2018)

2.1.2. Perennial Grass Crops

2.1.2.1. Silvergrass (*Miscanthus* spp.)

A. CHARACTERISTICS:

- **Family:** *Poaceae*
- **Growth habit:** graminoid
- **Plantation:** spring; propagated by seeds and rhizome dispersion
- **Harvest:** late winter or early spring (SRC)
- **Water use**: medium
- **Cultivation details:** adaptable to a wide range of soils (sandy to organic) and pHs; some clones grow on low winter temperatures (-14°C); low nutrient input requirements


photo ©M. Mench

B. TRACE ELEMENTS (tolerance/accumulation):

Al	As	Ag	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn	Sn	Tl
⏏	⏏		⏏		⏏	⏏				⏏	⏏	⏏	⏏	

C. POTENTIAL PRODUCTS:

Pulp and fiber; livestock bedding and forage; biomass; biofuel (bioethanol); biochar; paper; pellets and briquettes.

D. PHYTOTECHNOLOGY DETAILS/APPLICATIONS:

Miscanthus sp. is a high-biomass crop with a deep and extensive root system that is highly tolerant to a broad range of TE, which are accumulated mainly in belowground tissues (roots and rhizome; Pandey et al., 2016). *Miscanthus* is highly recommended for phytomanaging mining, smelting sites and marginal lands (Van Ginneken et al., 2007; Balsamo et al., 2015; Nsanganwimana et al., 2014, 2016) due to its promising results when tested in those areas. Shoot biomass of *Miscanthus* species can be converted to energy, fuels, and chemical feedstock including lignocellulose for bio-products (McKendry, 2002; Angelini et al., 2009; Brosse et al., 2012; Murphy et al., 2013; Van der Weijde et al., 2013; Nsanganwimana et al., 2014). From the several species, only four have economic value: *Miscanthus sinensis*; *M. sacchariflorus*, *M. floridulus*, and *M.* x *giganteus*, which have the potential of being harvested twice a year. The later, *M.* x *giganteus,* is the one that is mostly studied for phytomanagement of contaminated soils because it is sterile and yields the highest biomass, reaching over 4 m height (Pidlisnyuk et al., 2014; Kolodziej et al., 2016; Burges et al., 2018). *M.* x *giganteus* is affected by only a few pests (Stefanovska et al., 2017), which prevents yield losses.
This crop can further decrease soil disturbance and planting costs when comparing with annual crops (Nsanganwimana et al., 2014). The rhizome system of *Miscanthus* sp. favors the cycling of nutrients between the below ground and aerial plant parts, which minimizes fertilizers inputs (Lewandowski et al., 2000). *Miscanthus* sp. can also highly contribute to soil C sequestration (Clifton-Brown et al., 2007; Christensen et al., 2016) and soil health.

Figure 8: *M.* x *giganteus* in Saint-Médard-d'Eyrans(France; photo ©M. Mench).

Figure 9: *M.* x *giganteus* around the Metal Europe site (Nsangawimana et al., ISA-Yncrea, Lille, France; photo ©M. Mench).

2.1.2.2. **Vetiver** (*Crysopogon zizanioides* (L.) Robert)

A. CHARACTERISTICS:

- **Family:** *Poaceae*
- **Growth habit**: graminoid
- **Plantation:** transplantation of plantlets (with a good root system) to field
- **Water use:** medium
- **Cultivation details:** prefer sandy-loam soils; highly adaptable to a wide pH (3.3 – 9.5) and temperature (-10°C – +55 °C) range; sensitive to shade; low nutrient input requirements; very tolerant to salinity and drought.

photo WikiImages by Pixabay

B. TRACE ELEMENTS (tolerance/accumulation):

Al	**As**	Ag	**Cd**	Co	**Cr**	**Cu**	Fe	Mn	Mo	**Ni**	**Pb**	**Zn**	Sn	Tl
⏏	⏏		⏏		⏏	⏏				⏏	⏏	⏏		

C. POTENTIAL PRODUCTS:

Handicrafts; essential oil; livestock fodder; fiber; biomass; biofuel (bioethanol).

D. PHYTOTECHNOLOGY DETAILS/APPLICATIONS:

Vetiver is a widely adaptable high-yield plant that can reach 2-3 m height. This crop has a very extensive and thick root system (over 4 m), which allows a larger contact surface with TE, decreasing its leaching. It has a very fast growth and is very effective in fixing sediments and controlling erosion (Cazzuffi et al., 2006; Prakasa et al., 2008). Generally, only sterile cultivars of the species *C. zizanioides* are used in phytomanagement (Wilde et al., 2005), which have no negative impact on native species (Wilde et al., 2005). Vetiver has high tolerance to As, Cd, Cu, Pb, and Zn (Chen et al., 2004, 2020) and can be used in multi-contaminated sites. Low TE concentrations in the shoots limit their transfer into the food chain (Yang et al., 2003). Vetiver has been assessed for e.g. the assisted phytostabilization of Pb/Zn tailings, both *in situ* and *ex situ* (Pang et al., 2003; Yang et al., 2003; Rotkittikhun et al., 2007 and for the phytoextraction of Pb, Cu, Zn, and Cd (Chen et al., 2004, Chiu et al., 2005, 2006; Antiochia et al., 2007; Angin et al., 2008) with very good results. Only few studies have been performed with vetiver on the aided-phytostabilization of Cu-contaminated soils (Chiu et al., 2006; Saidi, 2000; Geiger et al., 2019). Vetiver is also able to increase soil quality (nutrients and microbial populations; Chen et al., 2020).

Figure 10: Vetiver with 3 years of growth – Cu stabilization - in St. Médard d'Eyrans (France; photo ©M. Mench)

2.1.3. Woody Crops

2.1.3.1. **Poplar** (*Populus* sp.)

A. CHARACTERISTICS:

- **Family:** *Salicaceae*
- **Growth habit:** tree
- **Sowing:** mainly propagated by cuttings
- **Water need:** medium
- **Cultivation details:** require direct sunlight; prefer moisty soils rich in OM; pH 5–7.5; adapted from fine to coarse textured soils; high drought tolerance; sensitive to waterlogging

photo Doris Jungo by Pixabay

B. TRACE ELEMENTS (tolerance/accumulation):

Al	As	Ag	**Cd**	Co	**Cr**	**Cu**	Fe	Mn	Mo	**Ni**	**Pb**	**Zn**	Sn	Tl
⏏			⏏		⏏	⏏				⏏	⏏	⏏		

C. POTENTIAL PRODUCTS:

Biomass; biofuel (bioethanol); timber; paper; paper board; pulp.

C. PHYTOTECHNOLOGY DETAILS/APPLICATIONS:

Poplar is a widely adaptable crop, which can be cultivated in multi-contaminated sites, including with organic contaminants. The *Populus* genus comprises several species and cultivars and can be grown in TE contaminated areas both as a forest crop in long rotations (10–20 years) and as short rotation coppices (SRC) (2–5 years; Krzyżaniak et al., 2019). It has been successfully used in long-term field experiments, especially under SRC, due to its high biomass production, deep-rooting system and high tolerance to TE (Kidd et al., 2015; Quintela-Sabarís et al., 2017; Thijs et al., 2018; Xue et al., 2018; Chalot et al., 2020). With a deep root system, poplar can be used for phytoextraction (e.g. Cd, Zn) or phytostabilization (Cu, Pb). However, poplar cultivars and clones show great disparity TE tolerance and accumulation patterns (Ruttens et al., 2011; Van Slycken et al., 2013).

Poplar yields have been estimated from 2 up to 25 Mg ha^{-1} $year^{-1}$ DW, but vary according to several factors such as climate, soil, cultivar, planting density and rotation (Krzyżaniak et al., 2019). Due to its fast growth and high yield, poplar also highly contributes to CO_2 emission mitigation and soil C sequestration.

Figure 11: Poplar SRC at the Zn/Cd contaminated Balen site, Belgium (managed by Vangronsveld et al.; photo ©M. Mench)

2.1.3.2. **Willow** (*Salix* sp.)

A. CHARACTERISTICS

- **Family:** *Salicaceae*
- **Growth habit:** tree
- **Plantation:** cuttings (late November to late February/early March)
- **Water need:** medium-high
- **Cultivation details:** adapted to a wide range of soils, from fine to coarse textured soils; adapted to moist and flooded soils; prefers sunny areas.

WikimedialImages by Pixabay

B. TRACE ELEMENTS (tolerance/accumulation):

Al	As	Ag	**Cd**	Co	**Cr**	**Cu**	Fe	Mn	Mo	**Ni**	**Pb**	**Zn**	Sn	Tl
			⏏		⏏	⏏				⏏	⏏	⏏		

C. POTENTIAL PRODUCTS:

Biomass, biofuel

D. PHYTOTECHNOLOGY DETAILS/APPLICATIONS:

Willows are spread worldwide and species such as *Salix vimialis, Salix caprea* and *Salix smithiana* (Adler et al., 2008; Puschenreiter et al., 2013; Hoefer et al., 2015) are used in phytotechnologies due to its high biomass production, deep-rooting system, and tolerance to TE. Willows are usually Cd/Zn accumulators in the leaves but have a Cu-excluder phenotype. They have been assessed for phytomanaging former wood preservation sites (Mench et al., 2010; Kidd et al., 2015) and long-term Cd and Zn-contaminated areas across Central Europe (Unterbrunner et al., 2007). Similar to poplars, willows are usually used in SRC (Utmazian and Wenzel, 2007; Ruttens et al., 2011; Dimitriou et al., 2006, 2012). A large number of willow cultivars and clones show great variation in biomass production, TE tolerance and accumulation patterns (Ruttens et al., 2011; Van Slycken et al., 2013). Some species or clones of willow have high bioconcentration factors for Cd (up to 27) and Zn (up to 3) (Dickinson and Pulford, 2005; Wieshammer et al., 2007). It is possible to select the best-performing clones based on their TE tolerance, and uptake efficiency (accumulating clones for phytoextraction vs. excluding clones for phytostabilization), TE translocation from roots to shoots, and biomass production (Pulford and Dickinson, 2006; Pourrut et al., 2011). Clones can also be selected for their ability to accumulate selected TE (Cd and Zn) while at the same time immobilizing elements such as Cu or Pb (French et al., 2006).

Willow has also a significant role in soil C sequestration and enhances the diversity and activity of soil microbial communities (Xue et al., 2015).

Figure 12: *Salix smithiana* after 4 years of growth in Pb/Zn tailings of Rubiais Mine (Spain; photo © Petra Kidd)

Figure 13: Willow SRC at the Zn/Cd contaminated Balen site, Belgium (managed by Vangronsveld et al.; photo ©M. Mench)

2.2. Cover crops

Cover crops are plants that are usually grown between cropping seasons, or simultaneously with the main crop to improve soil fertility and prevent soil erosion (Blanco-Canqui et al., 2015). In some cases, only these plants are planted, taking the role of main crop.

They are also occasionally designated "catch crops" due to their capacity to uptake and hold nitrogen (N) and other nutrients that might otherwise leach out of the rooting zone and be lost to deeper soil profiles, and potentially to underground water (Poeplau et al., 2015).

They can present several benefits in TE contaminated sites, including:

COVER CROPS

- 1. improve soils' fertility (e.g. N) and health
- 2. protect soil against erosion
- 3. prevent nutrient leaching
- 4. increase main crop yields
- 5. improve SOM
- 6. improve biodiversity (e.g. beneficial insects and pollinators)
- 7. improve soils moisture

Using a mixture of different cover crops is often the best option for contaminated sites because they can have complementary benefits. However, choosing compatible crops and adjusting seeding rates is key to prevent them from overlapping each other.

Legumes and grasses are the most commonly used, but brassicas and some tree species have also proven to be effective cover crops.

2.2.1. Legumes

One of the main purposes of selecting legumes as cover crops is their ability to fix nitrogen (N_2) from the atmosphere. These plants contain N_2-fixing bacteria (rhizobia) within nodules in roots, which deliver N to the plant, assisting its' growth. Once the plant is dead, the N is released to soil, enhancing soils' fertility. For this reason, these cover crops are often called green manure. Due to its N_2-fixation capacity, legumes can survive on N-depleted soils, which is an advantage for restoring degraded areas using phytotechnologies.

Legumes tend to nodulate freely, but generally seeds available on the market are already inoculated with rhizobia strains to insure efficient nodulation. However, most of these strains may not be tolerant/resistant to TE, which limits the success of symbiosis. The use of tolerant strains could improve N fixation in contaminated sites (Cleyet-Marel et al., 2001; Mengoni et al., 2001).

Several types of legumes can be used, including:

- **Fava bean** (*Vicia faba* L.; Fabaceae)
  annual plant; grows best in near neutral to alkaline pH (5.4-8.0), well-structured soils; prefers well drain loam to clay soils; susceptible to boron toxicity; low tolerance to salinity; able to survive to waterlogged conditions.

- **Hairy vetch** (*Vicia vilosa* L.; Fabaceae)
  annual plant; good winter crop for northern climates (moderately winter-hardy species); grows well on a wide range of soil types; better option for sandy soils; residues decompose rapidly, releasing nitrogen faster than most other cover crops; high tolerance to drought.

- **Alfalfa** (*Medicago sativa* L.; Fabaceae)
  perennial with new growth from crown each year; cold, low-shade and drought-tolerant; deep-rooted. It is commonly interseeded with oats, wheat, and barley, and it grows after the grain is harvested.

- **Red clover** (*Trifolium pratense* L., Fabaceae)
perennial; short-lived cold-tolerant; low tolerance to waterlogging, drought and salinity; moderate tolerant to soil lime; slightly acid to neutral pH; prefers sunny sites; better suited for short crop rotations.

- **White clover** (*Trifolium repens* L., Fabaceae)
perennial; short-lived; optimal pH 5.6-6.5; prefers well-drained soils; low tolerance to drought and waterlogging. Tolerates shading better than other legumes and may be useful as orchard-floor covers or as living mulch.

Figure 14: White clover growing in a Cu-rich mining soil (Borralha mine, northern Portugal; photo ©Helena Moreira).

2.2.2. Grasses

Grass cover crops include annual cereals (e.g. wheat), annual or perennial forage grasses (e.g. ryegrass), and warm-season grasses (e.g. sudangrass). Their benefits include fast establishment and extensive root system, which is very effective in preventing soil erosion while increasing OM due to the leftover of their residues (Restovich et al., 2012). These plants are highly effective in retrieving nutrients, namely N, left in soil from a previous crop. However, if grasses are grown to maturity to achieve the maximum amount of residue, the available nitrogen for the next crop may be reduced. This shortcoming can be prevented by taking out the grass early or by seeding a legume-grass mix.

Some grasses that have been used in TE contaminated sites are:

- **Colonial bentgrass** (*Agrostis capillaris* L.; Poaceae; Fig. 11)
  Perennial; can be sown at any time of the year; better by vegetative propagation by rhizomes and stolons; prefers freely drained or fairly dry soils but can grow in poorly drained soils; adaptable to fine to coarse textured soil; acidic to neutral soil pH; tolerant to drought, high and low temperatures.

Figure 15: *Agrostis capillaris* cv. Highland in Touro Mines' tailings (Spain) after seven years of growth (photo © Petra Kidd).

- **Ryegrass** (*Lolium* sp.; Poaceae)**:**
  Winter annual (*L. multiflorum* Lam.) or perennial (*L. perenne* L.); grows well in the fall if established early enough; cold-tolerant, fast growth-rate; high lime, low drought and low salinity tolerant.

- **Sudangrass** (*Sorghum × drummondii* (Steud.) Millsp. & Chas; Poaceae) and other sorghum-sudan hybrids annual grasses)
  Summer annual; fast-growing crop; very helpful for loosening compacted soil; livestock forage; drought tolerant; can suppress weeds; high-biomass crop; water- and nutrient-use efficient.

2.2.3. Other crops

Other types of crops that can also be used include:

- **Buckwheat** (*Fagopyrum esculentum* Moench; Polygonaceae):
  summer annual; fast-growing crop; can reseed if flowers matures; grows well in low-fertility soils; can suppress weeds; reported to suppress important root pathogens, including *Thielaviopsis* and *Rhizoctonia* species; potential for growing multiple buckwheat crops per year in many regions; moderate drought and shade tolerance; good for soil aggregation; cold-sensitive.

- **Brassicas** (include mustard (e.g. *Brassica juncea* (L.) Czern.; rapeseed (*B. napus* L.); forage radish (*Raphanus sativus* L.); and camelina (*Camelina sativa* Crantz) Brassicaceae):
  used as winter or rotational cover crops; can alleviate soil compaction, suppress weeds, improve SOM, reduce nitrate leaching and control soil erosion; mustard can act as a bio-fumigant; rapeseed is sensitive to harsh cold and to high concentrations of copper; forage radish is a fast-growth crop in late summer; camelina can be used to produce an important seed oil for industrial purposes and pharmaceuticals.

2.2. Cropping Systems

2.2.1. Sequence of crops

Crop successions include monoculture, crop rotation and fallowing.
Monoculture (monocropping) occurs when a site is cultivated with a single crop over multiple consecutive years; crop rotation is the sequential growth of different crops on the same field in alternate seasons or years.
Legumes are often included in the rotation because of their ability to fix N_2 (Anglade et al., 2015; Stagnari et al., 2017; Uzoh et al., 2019).

Based on the successional time period, crop rotations can be classified in short-rotation (2-5 years) and extended rotation (> 5 years). As addressed before, an interesting and common crop rotation in TE-contaminated sites is short rotation coppice (SRC), i.e., some fast-growing trees, like poplars and willows, can be coppiced while in dormancy, and produce several new stems in the next growing season. In this case, a high planting rate is advised.

2.2.2. Cropping patterns

Cropping patterns (Fig.16) comprise single- and multiple cropping. In single cropping (either annual or perennial) the cropping pattern refers to the spatial arrangement of a single crop. Multi-cropping refers to the growth of two or more crops in the same area and includes its yearly sequence and spatial arrangement. It comprises sequential cropping and intercropping, which can be subdivided in several forms (see below).

Cropping patterns may affect phytoextraction of TE from the soil because coexistence of multiple plant species may change rhizosphere microorganisms, soil enzymes activities and abiotic micro-environment, and thus may affect the TE bioavailable in rhizosphere soil (Tang et al., 2012; Zeng et al., 2019a; Zeng et al., 2019b; Brereton et al., 2020).

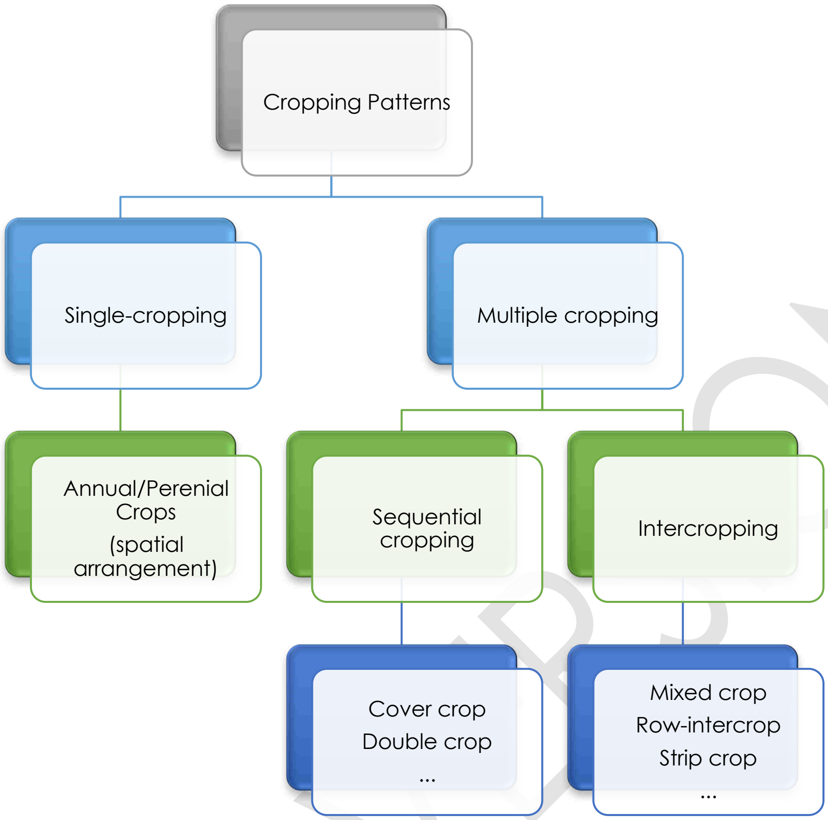


Figure 16: Diagram showing the different types of cropping patterns.

2.2.2.1. Types of cropping patterns

1. Single Cropping

The spatial arrangement of crops, i.e. orientation, inter and within-row spacing depends on the selected species and on the pretended plants' density.

2. Multiple cropping

A multiple cropping pattern is considered as an efficient strategy for soil conservation and restoration but requires a thorough planning that takes the type of soil, climate, crops, and used crops' varieties, planting and maturity rates into account.

Multiple cropping includes i) sequential cropping and ii) intercropping:

i) Sequential cropping: growing of different crops sequentially in the same area. It can be subdivided in:
   - Cover crops: planted after a crop that is harvested and is terminated before the subsequent crop is planted, e.g. winter cropping.
   - Double cropping: growing two crops in sequence.
   - Triple/quadruple/(...): Cropping with three/four or more crops in sequence

ii) Intercropping: Simultaneous growth of multiple crops in the same area. In intercropping is particularly important to select crops with minimum competition potential either for physical space, nutrients, water, or sunlight. As above-mentioned, legumes are excellent partner crops in intercropping systems because they provide N and induce soil acidification, and thus increase the bioavailability of P and other otherwise immobile elements (Noble et al., 2008).

Intercropping can be further classified as:

- Mixed intercropping – Simultaneous growing of two or more crops (including cover crops) with no distinct rows or divisions arrangement;
- Row intercropping – Simultaneous growing of two or more crops in rows;
- Strip intercropping – Simultaneous growing of two or more crops for part of their life cycles, i.e., planting the second before the first has been harvested).

Figure 17: Mixed stand of trees, alfalfa and grassy species in year 1 at the Parc aux Angélique site, Bordeaux, France (Marchand et al.; photo ©M. Mench).

3

BIOAUGMENTATION

Phytoremediation processes are ruled by interactions between three key players: soil, plants, and microorganisms. Plants release substances such as sugars and amino acids through their roots into the soil, building up a particularly life-prone environment in their vicinity – the rhizosphere – which harbors a very high concentration of soil microorganisms (Glick, 2010). Some of these microorganisms can positively impact plant growth and health and can modify TE bioavailability. However, the presence of high TE levels in soils generally leads to shifts in microbial community structure of soils (Touceda-González et al., 2017; Xue et al. 2015, 2018; Burges et al., 2020), and to the selection of certain groups like TE-resistant microorganism. TE-affected soils often have lower microbial density and activity, which often results in a decline of soil functionalities.

Bioaugmentation in contaminated areas can help mitigate these negative effects. The introduction of selected microorganisms (bioinoculants) can help promote plants' establishment, growth and health, but can also transform contaminants to a less toxic form or reduce their availability in the soil. Furthermore, the consequent increase in microbial activity is a prerequisite to achieve the restoration of soil functionality and the promotion of nutrient cycling. A growing body of positive results in the last decades has strengthened the crucial role of plant-associated microorganisms in achieving phytoremediation success (Sessitsch et al., 2013; Afzal et al., 2014; Lenoir et al., 2016; Thijs et al., 2016; Benizri and Kidd, 2017; Deng and Cao, 2017; Feng et al., 2017; Kidd et al., 2017; Moreira et al., 2014, 2016a, b, c, 2019).

Two types of microorganisms stand out as particularly effective for bioaugmentation in phytomanagement approaches: i) bacteria, namely PGPB; and ii) fungi, especially mycorrhizal fungi. These microorganisms have the ability to modify plant metabolic functions in stressful environments, allowing them to withstand hazardous TE levels (Sessitsch et al., 2013; Ploiniczak et al., 2020; Saran et al., 2020).

Different types of combinations of bioinoculants can be applied in bioaugmentation strategies to TE contaminated soils (Fig. 18):

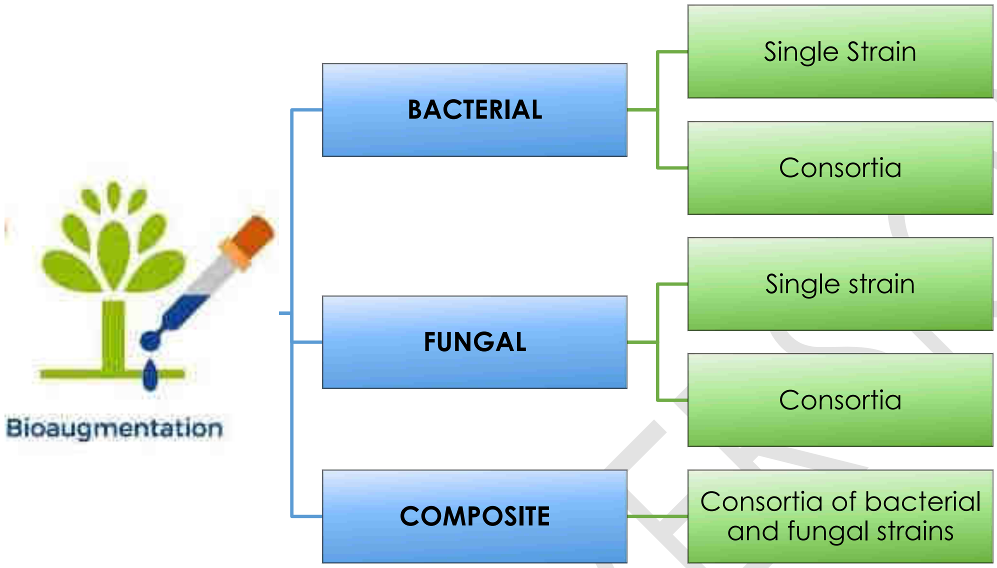


Figure 18: Types of bioinoculants that can be applied to TE-contaminated soils.

According to their origin, microorganisms used as bioinoculants can be: i) autochthonous - microorganisms collected from the site targeted for phytomanagement, grown *ex-situ* and then re-introduced in the soil; or ii) allochthonous - microorganisms collected from contaminated areas other than the one targeted for phytomanagement, and cultivated *ex-situ* for subsequent inoculation.

Commercial products can also be used. However, these may not be able to comply with the desirable outcomes if their composition does not include strains well-adapted to hazardous concentrations of TE or with the required plant-growth promoting traits.

3.1. Bioaugmentation - types of bioinoculants

3.1.1. Bacterial inoculants

Bacterial bioinoculants are mostly composed by PGPB, which include free-living bacteria, such as rhizobacteria, and bacterial endophytes.
PGPB play a significant role in enhancing plant growth and development (Figs. 20 and 21) under stress conditions through a number of direct and indirect mechanisms (Figure 22; Goswami et al., 2016; Olanrewaju et al., 2017; Guo et al., 2020;), which can act simultaneously or successively along the different stages of the host plants' life cycle.

Phytoremediation approaches aim to take advantage of the beneficial effects of the mechanisms by which these bacteria confer increased TE tolerance to the plant and/or enhance its biomass while removing/arresting pollutants present in the soil. They can also improve the accumulation of TE in plants aboveground tissues (phytoextraction) or reduce the mobility/availability of TE contaminants in the rhizosphere (phytostabilization) via the release of metabolites or due to biochemical reactions. For instance, some bacteria can bind TE to their cell surfaces (biosorption) while others can leach them (bioleaching).
Various genera of bacteria (e.g. *Arthrobacter, Azotobacter, Bacillus Pseudomonas*, *Klebsiella*, *Serratia*) are known to cause noticeable effects on plant growth in TE contaminated soils (Table 1).

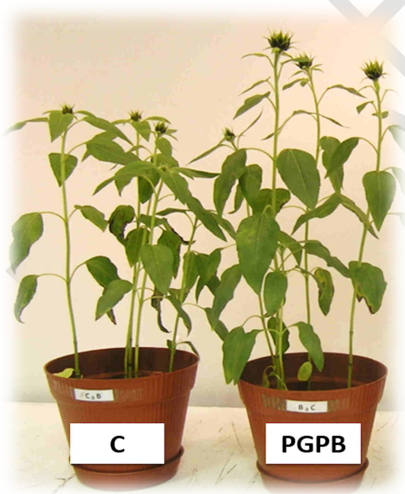


Figure 19: Effect of the inoculation of PGPB (endophyte) in sunflower grown in Cu and Cd-contaminated soil (photo ©Sofia Pereira).

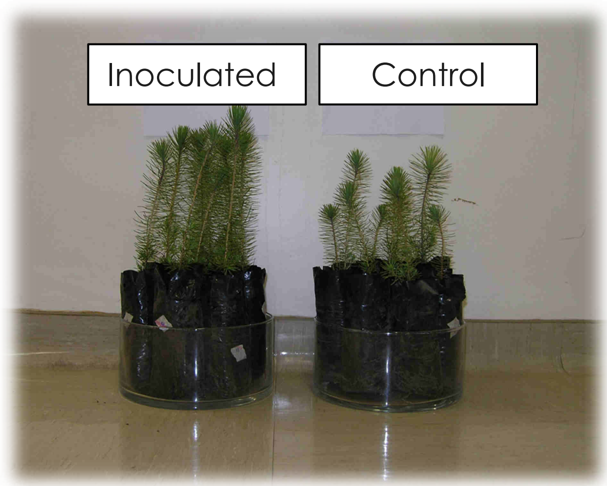
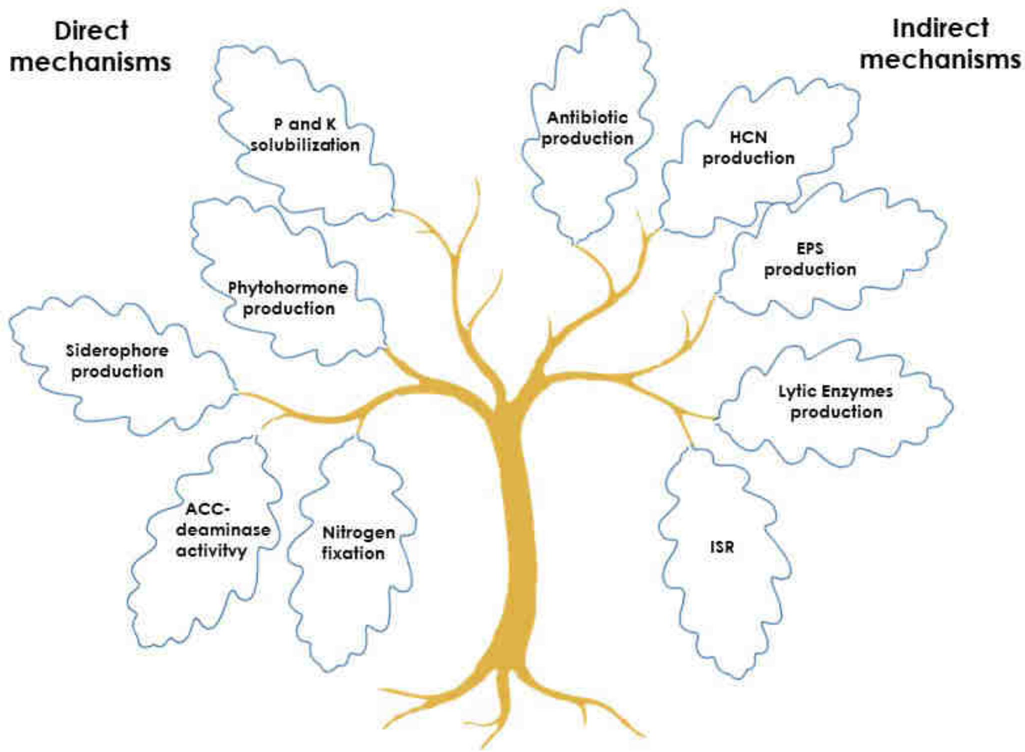


Figure 20: Effect of the inoculation of PGPB in *Pinus* pinaster (photo ©Miguel Ramos).



Direct mechanisms

- **1) Phytohormone production** - synthesis of auxins, such as indole-3-acetic acid (IAA), cytokinins and gibberellins that promote plant growth;

- **2) Siderophores production -** chelators that bind iron from soils and provide it to plants; control of phytopathogenic activity (biocontrol);

- **3) ACC[1]-deaminase activity -** decrease of ethylene levels in plants;

- **4) Phosphate (P) solubilization** – mineralization of organic phosphates and conversion of insoluble inorganic phosphates into available P forms, easily absorbed by plants;

- **5) Nitrogen fixation -** supply of nitrogen to plants through symbiotic and non-symbiotic fixation. In the first, atmospheric N_2 is fixed by bacterial in specific structures in roots - nodules, while in the second, non-symbiotic N-fixers fix N_2 in the soil.

Indirect mechanisms

- **1) Antibiotics production -** control of phytopathogenic activity (biocontrol);

- **2) HCN production -** hydrogen cyanide is a secondary metabolite that can suppress the growth of phytopathogens that can affect;

- **3) Lytic enzymes production** - inhibit phytopathogens including fungi through cell wall hydrolases.

- **4) Induction Systemic Resistance –** net of processes

Figure 21: Direct and indirect mechanism of PGPB plant growth promotion; [1]1–aminocyclopropane-1-carboxylate.

Over the past years, several studies reported the importance of PGPB in alleviating TE stress in plants. A non-exhaustive list of those studies and the main outcomes are summarized in Table 1.

Table 1: Effects of PGPB inoculation on plant growth and TE accumulation in contaminated soils.

BACTERIAL STRAIN	HOST PLANT	GROWTH PROMOTING TRAITS	EFFECTS ON HOST	REFERENCE
***Pseudomonas tolaasii* ACC23** ***Pseudomonas alcaligenes* ZN4** ***Pseudomonas fluorescens* ACC9** ***Mycobacterium* sp. ACC14**	*Brassica napus*	ACC-deaminase activity IAA and siderophore production P solubilization	↑ root elongation ↑ plant dry biomass ↑ total Cd accumulation	Dell'Amico et al., 2008
***Burkholderia* sp. J62**	*Brassica juncea* *Zeas mays* *Lycopersicon esculentum*	IAA and siderophore production ACC-deaminase activity P solubilization	↑ root, shoot biomass (*Z. mays; L. esculentum*) ↑ Cd and Pb uptake (*Z. mays; L. esculentum)*	Jiang et al., 2008
Pseudomonas aeruginosa ***P. fluorescens*** ***Ralstonia metallidurans***	*Zea mays*	Siderophore production	↑ shoot biomass ↑ Cr and Pb accumulation in shoots ↓ total metal uptake ↑ translocation factor	Braud et al., 2009
***Psychrobacter* sp. SRA1, SRA2** ***Bacillus cereus* SRA 10**	*Brassica juncea*	ACC-deaminase activity IAA and siderophore production P solubilization	↑ fresh/dry biomass ↑ Ni bioavailability ↑ Ni accumulation ↑ vigour index	Ma et al., 2009
***Bradyrrizobium* sp. 750**	*Lupinus luteus*	N_2 fixation	↑ plant biomass, N content ↑ Zn accumulation (roots, shoots	Dary et al., 2010
Bacillus pumilus* ES4** ***B. pumilis* RIZO1** ***Azospirilum brasiliense	*Atriplex lentiformis*	N_2 fixation P solubilization	↑ seed germination, root length ↑ root, shoot dry biomass	de-Bashan et al., 2010
Bacillus megaterium	*Brassica juncea* *Abutilon theophrasti*	P solubilization Organic acid release	↑ total biomass (*B. juncea)* ↑ Cd accumulation ↑ Cd bioavailability	Jeong et al., 2012
***Burkholderia* sp. GL12** ***B. megaterium* JL35** ***Sphingomonas* sp. YM22**	*Zea mays*	IAA and siderophore production ACC-deaminase activity	↑ root, shoot dry biomass ↑ Cu accumulation (roots and shoots) ↑ Cu bioavailability	Sheng et al., 2012
***Chryseobacterium humi* ECP37** ***Ralstonia eutropha* 1C2**	*Helianthuus annus*	IAA production ACC-deaminase activity HCN and NH_3 production	↓ Zn shoot accumulation (*C. humi*) ↔ Cd accumulation	Marques et al., 2013
***Pseudomonas putida* (MH3, MH6, MH7)** ***P. fluorescens* (MH9, MH15)**	*Sinapsis alba*	IAA and siderophores production ACC-deaminase activity HCN production P solubilization	↑ shoot and roots fresh/dry biomass ↑ Zn, Cd, Cu accumulation (roots and shoots) ↑Cd translocation	Plociniczak et al., 2013
Bacillus* sp.** ***Klebsiela* sp.** ***Stenotrophomonas	*Triticum aestivum*	IAA production ACC-deaminase activity	↑ plant dry biomass ↓ Cd root accumulation	Ahmad et al., 2014

sp. ***Serratia* sp.**	*Zea mays*	EPS and siderophore production		
***R. eutropha* 1C2, *C. humi* ECP37, *P. fluorescens* S3X, *Rhizobium radiobacter* EC1B, *P. reactans* EDP28**	*Zea mays*	IAA, Ammonia, HCN and siderophore production ACC-deaminase activity	↑ root dry biomass (EDP28) ↑ shoot dry biomass (ECP37, S3X; EDP28) ↑ Cd, Zn shoot accumulation ↓ Zn root accumulation	Moreira et al. 2016 b
***Bacillus methylotrophicus* SMT38, *Bacillus aryabhattai* SMT48, *B. aryabhattai* SMT50, *Bacillus licheniformis* SMT51**	*Spartina maritima*	Nitrogen fixation P solubilization Biofilm-forming capacity IAA and siderophore production	↑ belowground biomass ↑ total As, Cu, Cd, and Pb accumulation in roots ↓ root respiration ↑ soluble protein content in roots and shoots ↓ catalase, superoxide dismutase, guaiacol peroxidase activities in roots	Mesa-Marín et al., 2018
***Microbacterium oxydans* JYC17, *Pseudomonas thivervalensis* Y1-3-9, *Burkholderia cepacia* J62**	*Brassica napus*	ACC-deaminase activity IAA and siderophore production	↑ plant biomass ↑ total Cu accumulation ↑ Cu water soluble fractions Cu in soil (Y1-3-9; J62) ↑ ascorbic acid and glutathione content ↓ lipid peroxidation	Ren et al., 2019

3.1.2. Fungal inoculants

Another group of microorganisms that has demonstrated great potential in assisting the recovery of areas contaminated with TE are mycorrhizal fungi.

According to their morphology, mycorrhizal fungi can be divided into several groups (Selosse and Tacon, 1998), with arbuscular mycorrhizal fungi (AMF) being the most common. AMF are known to establish symbiosis with 80% of the plant species and are unable to complete their life cycle without a plant host (Garg and Chandel, 2010). These fungi form branched structures – arbuscules –, vesicles and hyphae in the cortical root cells of the host plant. These inside-plant structures are named intraradical mycelium (IRM). AMF also spread a hyphal network – the extraradical mycelium (ERM; Miransari, 2011) – to the surrounding soil, structure that is responsible for the translocation of nutrients to the roots. Both symbiotic partners, fungus and plant, benefit from the association: the host plant provides carbon to the fungus and receives water and nutrients from it (Smith and Read, 2008).

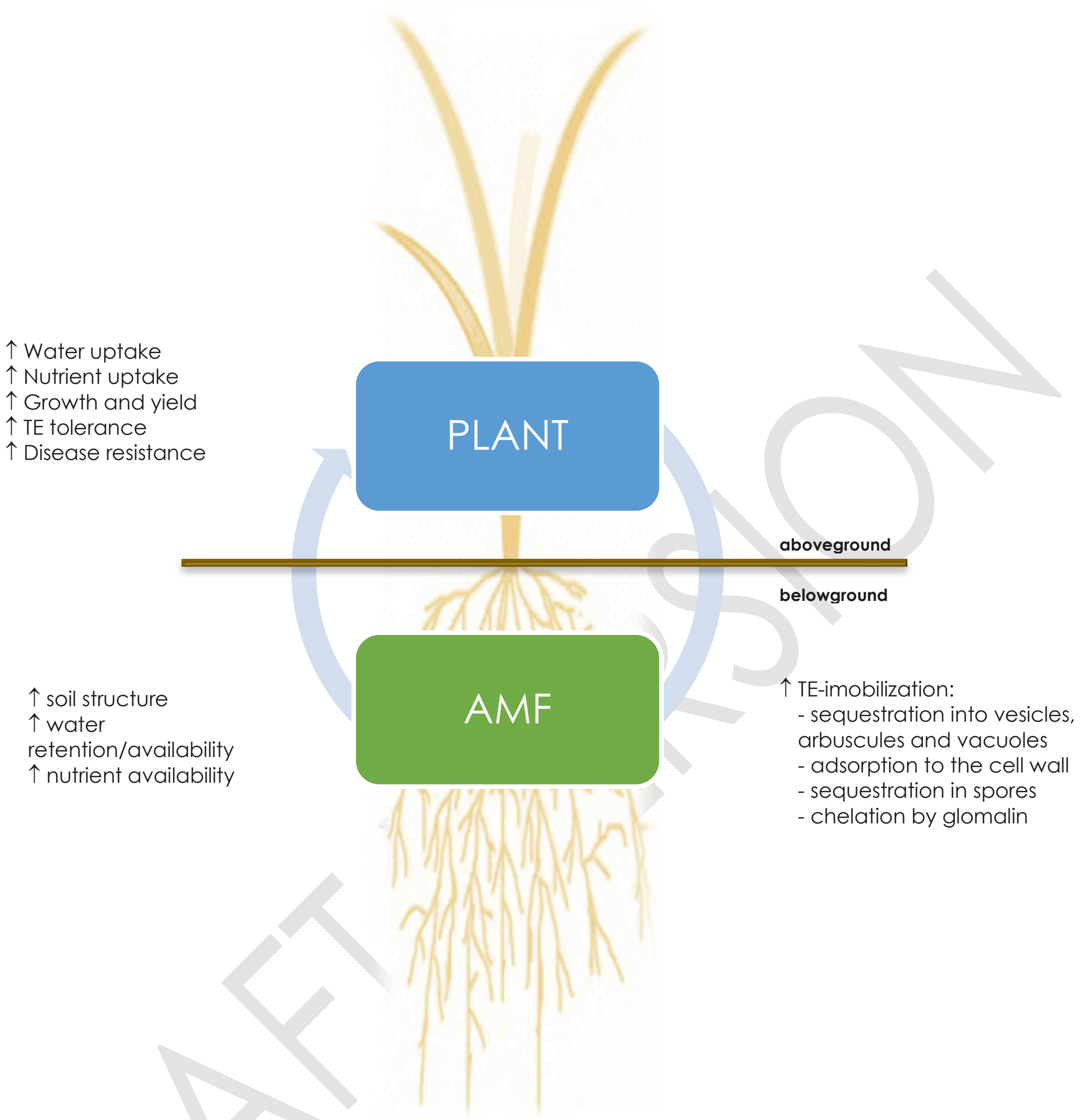


Figure 23: Mechanisms involved in stress-tolerance of AMF-mycorrhized plants grown in TE- contaminated sites.

AMF can contribute to the establishment and growth of plants in TE-affected areas by:

i) Inducing nutrient acquisition, especially P, but also N, K, Fe, and Ca through their ERM;
ii) Restraining the dissemination of pathogens;
iii) Changing antioxidant enzyme activities;
iv) Improving water acquisition.

AMF also improves soil aggregation by producing a glycoprotein – glomalin –, which prevents water erosion (Smith and Read, 2008).

Soils severely affected by TE are known to host several TE-resistant AMF, which, under such adverse conditions, are particularly helpful for the development of plants (Adewole et al., 2010; Bhosale and Shinde, 2011). Their capacity to thrive in contaminated areas is linked to the presence of molecular mechanisms that evolved to confer resistance and survival (Khan, 2005). However, the richness and diversity of AMF in TE-polluted soils are still relatively low when compared to that observed in non-disturbed sites (Sudová et al., 2008)

Given the above-described benefits to the host plants and generalized resistance to TE perturbations, AMF bioinoculation emerged as a promising biotechnological approach. Similar to bacterial inoculants, autochthonous AMF may be more suitable than allochthonous species for bioinoculation.

Arbuscular mycorrhizal fungi can play a key role in phytostabilization by inducing TE immobilization either in IRM and ERM mycelia. The mechanisms involved in this TE immobilization include: i) binding to biopolymers (e.g. chitin and chitosan) in the cell wall; ii) binding to the plasmatic membrane; iii) intracellular sequestration (Clemens, 2001; Hall, 2002; Göhre and Paskowski, 2006; Fig. 23).

However, some studies have shown that AMF also promote phytoextraction, causing an increase in TE translocation to the shoots. It was suggested that AMF promote phytoextraction in situations when the TE concentration in the soil is low, while phytostabilization prevails at high TE concentrations (Audet and Charest, 2007). Mycorrhizal fungi have been employed in phytoextraction actions coupled with hyperaccumulator plants, although symbiotic relations were only achieved with a limited number of species (Miransari, 2011).

The following table summarizes the main benefits of the inoculation of plants with AMF for phytoremediation of TE-contaminated soils.

Table 1: Effects of AMF inoculation in plant growth and TE accumulation in contaminated soils.

MYCORRIZAL SPECIES	PLANT	EFFECTS ON HOST	REFERENCE
Glomus claroideum ***G. intraradices***	*Solanum nigrum*	↑ Zn accumulation	Marques et al., 2007
G. mosseae ***Glomus sp.***	*Z. mays*	↑ root and shoot biomass ↓ Pb, Zn, Cd accumulation	Liang et al., 2009
Glomus sp.	*Z. mays*	↑ shoot length ↑ seedling biomass ↑ SOD activity ↑ Pb accumulation in roots	Zhang et al., 2010
G. mosseae ***Glomus intrarradices***	*Helianthus annus*	↑ plant yield ↑ root dry biomass ↑ Cd, Pb root accumulation	Adewole et al., 2010
Glomus clarum ***Gigaspora margarita*** ***Acaulospora sp.***	*Coffea arabica*	↑ plant growth ↑ P uptake ↑ nutrient acquisition ↓ Cu, Zn translocation	Andrade et al., 2010
G. mosseae	*Cajanus cajan*	↑ number of flowers and pods ↑ seed production, dry biomass ↑ total plant dry biomass ↓ Zn, Cd accumulation	Garg and Kaur, 2013
Acaulospora laevis ***Glomus mosseae***	*Zea mays*	↑ shoot length ↑ shoot, root biomass ↑ Cd, Cu accumulation in roots	Abdelmoneim et al., 2014
Rhizophagus fasciculatus ***R. intraradices*** ***Funneliformis mosseae*** ***Glomus aggregatum***	*Zea mays*	↑ root and shoot length ↑ Cd, Cr, Ni, Pb, Fe, Zn, Cu, and Mn accumulation in roots ↑ photosynthetic pigments ↑ proline content ↑ total P uptake in root and shoot ↑ soil enzyme activities: dehydrogenase, acid and alkaline phosphatase, β-glucosidase	Singh et al., 2019
Claroideoglomus etunicatum BEG168	*Sorghum bicolor*	↑ plant biomass ↑ Mo accumulation in roots and shoots ↑ P, N and S uptake ↑ photosystem II efficiency	Shi et al., 2020

AMF survival, colonization and activity are highly dependent on factors such as physico-chemical properties of soils and indigenous microbial communities. These factors have downstream consequences on their ability to mobilize and/or immobilize metals in host plants. Therefore, because each soil has a specific biotic and abiotic profile, AMF-induced TE uptake may vary considerably with geographic location, and with symbiotic partner species (Rajkumar et al., 2010). Information is still lacking to appropriately grasp the complexity of AMF-host plant interactions, and further research (inc. field trials) are needed to elucidate which, how and when microorganisms interact with plants to improve the success of phytoremediation strategies

3.1.2. Composite

Positive interactions can take place between PGPB and AMF, which benefit plants, not only by enhancing their growth but also by promoting TE stress tolerance (Azcón et al., 2010; Miransari et al., 2013). AMF can increase the population of bacteria in the surrounding soil by promoting root exudation, which delivers compounds required for bacterial growth (Yusran et al., 2009). Mycorrhizal fungi development is also improved by the presence of PGPB, which facilitates the colonization of plant roots (Hildebrandt et al., 2002), supporting the latter's establishment and improving its survival (Nadeem et al., 2014). For instance, Vivas et al. (2003) showed that PGPR inoculation improved mycorrhizal colonization in red clovers (*Trifolium pratense*), and its N and P uptake under Pb exposure. In a different study, Vivas et al. (2006), revealed the effectiveness of the dual inoculation, PGPR and AMF, in *T. repens* exposed to toxic levels of Zn, by inducing the decrease of metal accumulation in plant tissues while improving its' nutritional status (N and P) and enhancing AMF colonization. Also, Ázcon et al. (2010) showed the beneficial interaction between AMF and PGPR under TE multi-contaminated soils, where the dual association increased *T. repens* biomass and decreased TE accumulation. Nevertheless, the specificities and outcomes of these beneficial interactions are different among species, and the same bacterial strain may react differently with different fungal species. Co-inoculation with different microorganisms may also improve rhizosphere colonization, overcoming the negative effects of competition with native and established microorganisms (Bünemann et al., 2006).

3.3. Factors affecting bioaugmentation

Once introduced in soil, bioinoculants face several abiotic and biotic stressors (Fig. 24) that can reduce their effectiveness. Abiotic constrains include pH, organic matter and nutrient content, as well as TE concentrations. Biotic hurdles are mainly related to competition with indigenous microbial community, and other antagonistic interactions with protozoa and bacteriophages.

Soil, host plants and environmental factors conditions affect the efficiency of the bacterial or/and fungal inoculants used for bioaugmentation. Consequently, selecting the appropriate inoculant that allows plants to thrive in harsh conditions is critical. Preliminary trials with target plants are desirable prior to their large-scale employment, as microorganisms may have different effects depending on the plant species, soil properties and on the type/level of TE contamination.

The following issues should be addressed to guide the procedure:

1. MICROORGANISMS

Microorganisms used as bioinoculants in phytomanagement are generally retrieved from contaminated sites and tested for their *in vitro* plant growth-promoting (PGP) traits before pot and field tests. However, microorganisms differ in their susceptibility to TE type and concentrations. Disregarding this fact, microbial strains, especially bacterial, are often selected based on their *in-vitro* PGP traits in non TE-contaminated media (Abbaszadeh-Dahaji et al., 2019; Itusha et al., 2019; Saran et al., 2020; Sheng et al., 2012). However, these traits can be disrupted or changed in the presence of TE. Bacterial strains can trigger different TE resistance mechanisms such as exclusion, extracellular sequestration and enzymatic detoxification (Bruins et al., 2000), which may disturb several metabolic processes, and affect the biosynthesis of those substances with importance in alleviating TE stress in plants. Positive results in such growth-promoting tests may be meaningless,

Figure 24: Effect of combined inoculation of fungi and PGPR in *Betula* sp. (photo ©Miguel Ramos).

and therefore they should be performed under TE presence. Some bacterial strains only exhibit or activate PGP traits when exposed to TE. Conversely, some research results suggest that selecting PGP strains should be primarily be based on seedling growth promotion under TE exposure, since *in vitro* tests, even under TE exposure, lack transferability to *in vivo* conditions (Moreira et al., 2016).

Positive results in pot tests with target crops also may not have direct transference to field conditions, as they do not mimic *in situ* conditions. Nonetheless, pot trials with soil collected from the target site are currently seen as the better option to proceed for phytomanagement approaches, as robust field-based tests for bacterial and fungi specific strains are still scarce. Although field studies exist, they use different strains, which restrict any comparisons of their efficiency under different environmental conditions. This fact limits the possibility to reliably select bioinocula for application in TE-affected areas.

2. CONSORTIUM/COMPOSITE

An issue that should be considered when using a consortium or composite inocula is the compatibility between strains and absence of antagonistic interactions.
Although mixed inocula may be more adaptable to distinct environments, and some compositions are formulated to improve mutualistic interaction between all microbial intervenient, they still may fail in the field. Again, preliminary tests are advisable.

3. APPLICATION RATES

Application rates, i.e. the size of the inoculum applied and its frequency, can compromise the success of bioinocula. A limited bioinocula size may result in low colonization rates or low ability to compete with the indigenous soil populations hampering the success of the plant–microbe interaction (Compant et al., 2010). The maximization of microorganisms' promoting traits may also depend on increasing the colonization process by, among other factors, optimizing the inocula size and rates. Such optimization could further enhance plant growth or metal accumulation/immobilization and may be determinant in the efficiency of bioinoculants in TE polluted areas.

Although not without shortcomings, bioaugmentation approaches have a great potential in improving phytomanagement of TE-affected areas, as evidenced by the studies summarized in Tables 1 and 2. However, further research about the relationship between the factors involved in soil-microbial-plant interactions remains crucial for a sound understanding of these processes and to the development of innovative formulations.

3.3. Bioinoculants selection, formulations and applications

3.3.1. Bioinoculants selection

As stressed before, the selection of the appropriate strains for bioaugmentation is of utmost importance, comprising a stepwise process that cover several checking-points. Contaminated sites should be the first place where to screen for TE-resistant strains, and selection for subsequent tests.

The complete process entails several laborious and time-consuming phases; reason why frequently some stages are neglected. These steps seem essential in the case of very specific environmental, contamination and biotic characteristics.

The Fig. 25 summarizes the complete bioinoculant selection procedure, from isolation to the potential introduction into the market for a broader use of bioinoculants in phytomanagement approaches:

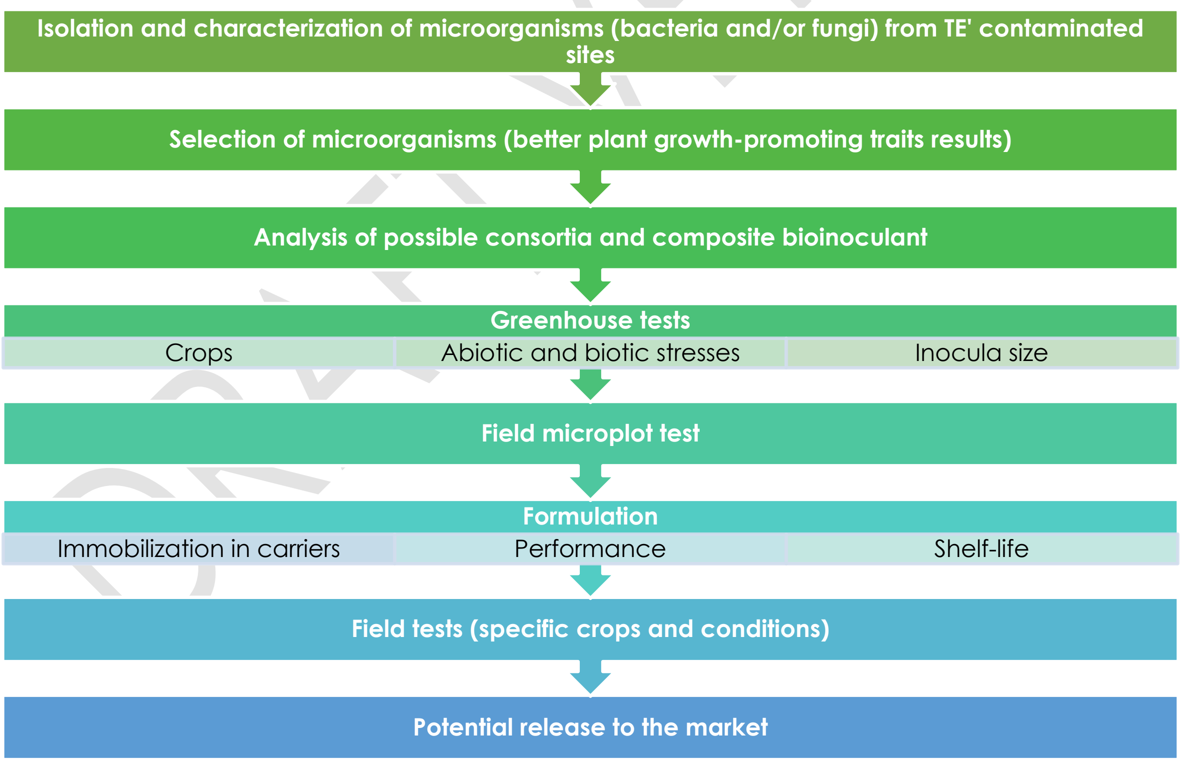


Figure 25: Process of selection bioinocula for TE-contaminated sites.

3.3.2. Types of Formulation

Bioinoculants are mostly used in research studies with no formulation. However, formulations allow large-scale applications and improve the success rate of bioaugmentation, as they provide an appropriate setting for microorganisms' to be maintained alive and even to increase their activity when inoculated in plants. Upgraded formulations with promising organisms, i.e., with demonstrated effectiveness in contaminated areas, are needed to create and commercialize products able to improve plants establishment and growth in similar conditions, within phytomanagement options.
Some commercial products are available in the market, but there is a lack of information regarding target crops in contaminated sites. This fact may hamper the success of these inoculants in these sites. Nonetheless, when using commercial inoculants, they should be used according to specifications described in the package and be of good quality at application time. The following figure summarizes the type of formulations that can be used:

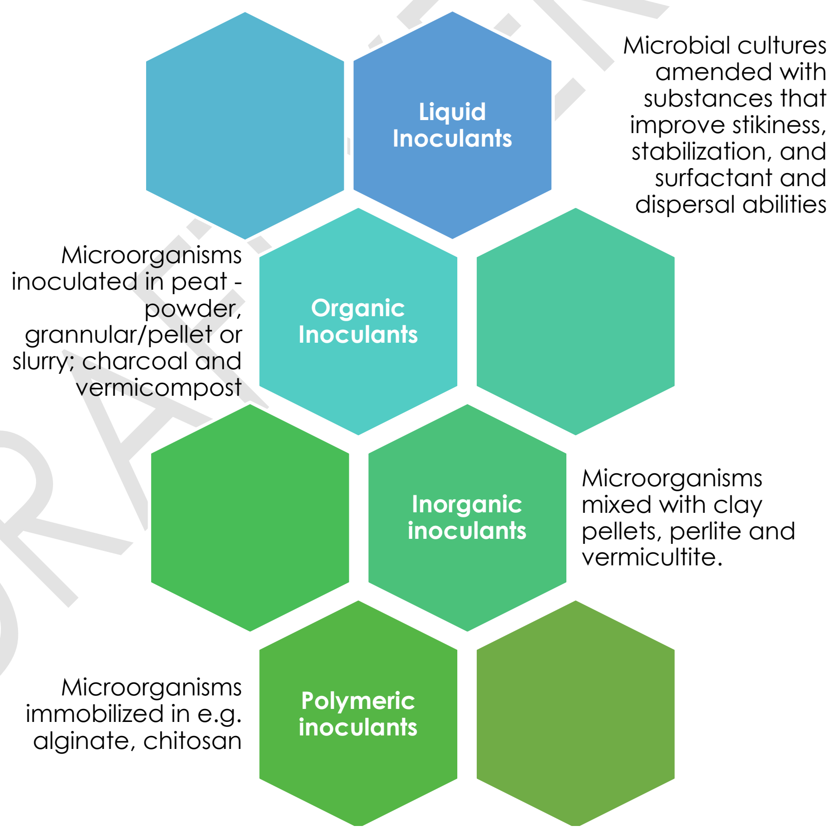


Figure 26: Type of formulations of bioinocula.

3.3.3. Application of Bioinoculants

Bioinoculants can be inoculated in liquid, granular, slurry or powder (de-Bashan et al., 2014), directly on the field or applied in plants nursery, and may be reinforced when transplanted to field.

Commonly, two application methods are used in the inoculation:

1. **direct inoculation**: the inoculant is placed in direct contact with the seed/seedling/plant (e.g. Fig. 27):

Figure 27: Inoculation with liquid formulation directly in the plants' rooting system (photo ©Miguel Ramos)

2. **indirect inoculation**: the inoculant is placed alongside or beneath the seed/seedling/plant.

   This method is usually applied when seeds are treated with fungicide or insecticide, and when high amount of inoculant is needed to outcompete the indigenous population.
   The simplest inoculation is to make the liquid formulation and spray it to the soil or directly over the seeds after placement. In this case, a higher amount of inoculant is required. Disadvantages of this method include difficulty in the distribution of inoculant.

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4

Organic Soil Amendments

Contaminated soils are frequently characterized by nutrient deficiency, a low organic matter content, poor structure and sometimes very acidic pH and/or high salinity. Soil amendments are inorganic and/or organic substances which can be added to the soil to improve its' quality for plant establishment and growth. Amendments can also strongly reduce the availability of TE to plants, thus reducing phytotoxicity and facilitating the revegetation of contaminated sites during (aided) phytostabilization. TE immobilization also limits their exposure to animals and humans (Figure 28) and decreases their leaching to superficial and groundwaters.

Commonly used soil amendments include liming agents, phosphates (H_3PO_4, triple calcium phosphate, hydroxyapatite, phosphate rock), Fe/Mn oxyhydroxides, natural and synthetic zeolites, cyclonic and fly ashes and steel shots (see reviews by Mench et al., 1998; Vangronsveld et al., 2000; Adriano et al., 2004; Ruttens et al., 2006a, b; Bolan et al., 2014), as well as organic materials (e.g. biosolids, sludge, or composts; Urra et al., 2019a). These amendments can reduce TE solubility by promoting the formation of insoluble precipitates or by increasing soil binding capacity. In general terms, retention of charged TE species by soil components can be through electrostatic attraction (non-specific adsorption) or chemical bond formation between the ions and soil surfaces (Sposito, 1984; Li et al., 2006; Zenteno et al., 2013). Numerous experiments (both bench- and field-scale) reporting amendment-induced reductions in soil TE availability and enhanced plant establishment and growth in TE-contaminated soils are described in the literature (see reviews by Vangronsveld et al., 2009; Mench et al., 2010; Bolan et al., 2014; Kumpiene et al., 2019).

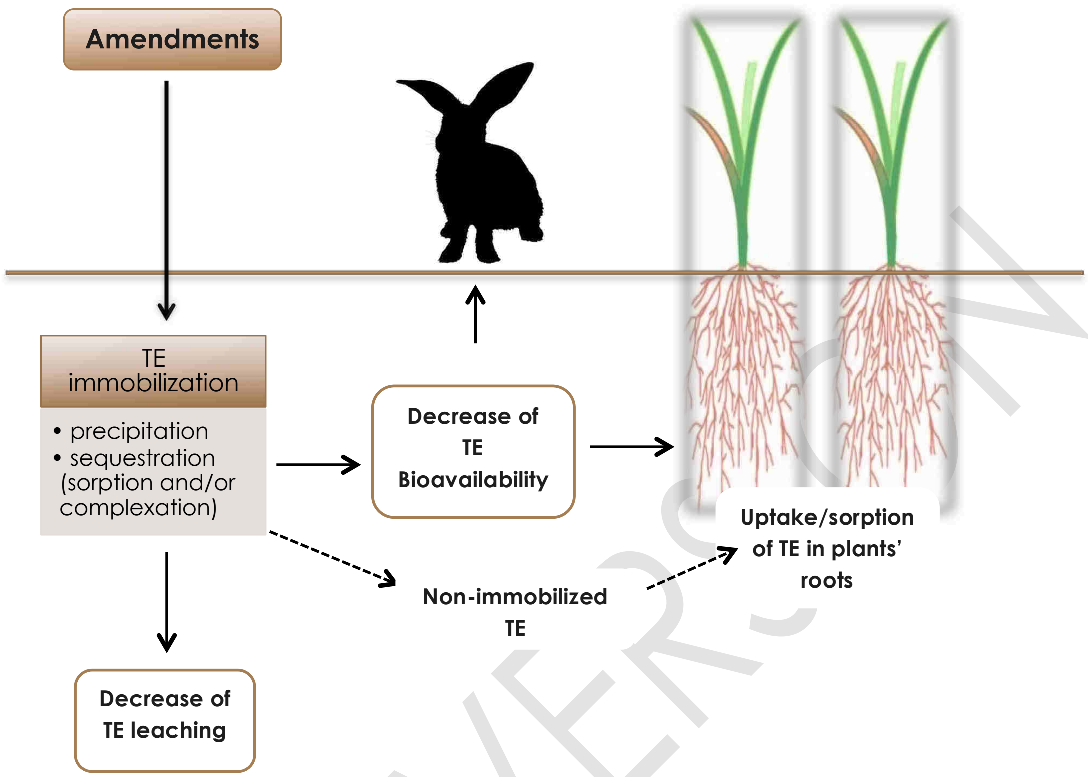

Figure 28: General Impacts of amendments in TE-affected soils.

Among soil amendments, the organic ones are particular interesting because they are easily accessible, low-cost products which, apart from potentially immobilizing TEs, can present several benefits when applied to contaminated soils:

i) Provide essential macro- and micronutrients, such as N and P (major limiting factors for plants' growth in many contaminated areas such as mine tailings);
ii) Improve soil structure and aeration;
iii) Enhance soil moisture and water-holding capacity;
iv) Increase soil organic matter content and stabilization;
v) Increase microbial communities' activities and mesofauna as well;
vi) Increase soil C storage;
vii) Reduce levels of greenhouse gas emission (e.g. less fossil energy used to produce inorganic fertilizers).

The N present in organic amendments is typically of slow release, which limits its runoff and hence the potential degradation of water bodies' quality. Additionally, the high organic carbon content of organic amendments provides an energy and carbon source, which increases soil microbial activity. Organic amendments may also indirectly stimulate microbial growth by promoting plant growth and, hence, the amount of root exudates. The consequent increase in soil organic matter content also improves the soil physical conditions (Varennes, 2003).

The immobilization of TEs by the use of organic amendments is mainly due to the presence of humic substances (Janos et al., 2010). However, these immobilizations effects depend on the nature of the OM, as well as on the particular soil type and characteristics, and TEs elements concerned (Clemente et al., 2005, 2006; Ruttens et al., 2006a, b; Goecke et al., 2011; Kumpiene et al., 2011; Lagomarsino et al., 2011). Although the most widely described effect of organic matter additions on soil metal(loid) mobility is certainly the abovementioned immobilization, some studies have by contrast shown an enhanced TE solubilization (Almås et al., 1999; Clemente and Bernal, 2006; Tandy et al., 2009). This is due to the fact that, as organic matter decomposes over the time, the release of organic acids increases, and therefore the amendments' initial immobilizing effect may start to revert with a concurrent increase in TE availability and mobility (Lwin et al., 2018). On the other hand, apart from the decrease in soil pH resulting from the release of humic acids derived from the decomposition of the organic C pool provided by the amendment itself, a reduction in soil pH can also occur as a consequence of the nitrification of the ammonium present in the amendment (Antolín et al., 2005). Then, the use of organic amendments with a low mineralization rate within a neutral pH range for avoiding the potential release of TE and thus reduce their uptake and bioaccumulation in plants, has been recommended (Blake and Goulding, 2002). Therefore, the long-term monitoring of TE solubility, mobility and bioavailability is a key element for the sustainability and (self)-maintenance of phytostabilization techniques, since soil reacidification after OM mineralization may result in the re-mobilization of sorbed TEs (Tisch et al., 2000).

In any case, many contaminated sites, where low fertility and poor soils/substrate structure are impeditive of plant establishment, can only be revitalized through the use of soil organic amendments. Moreover, using organic byproducts or wastes as a source of organic matter, can not only improve soil physicochemical and biological properties, but also offer a means of recycling of such wastes.

4.1. Types of organic amendments

There are several categories of organic amendments that can be used in TE contaminated areas. Applying multiple amendments may bring additional positive effects to phytoremediation approaches than applying just one type of amendment (Varennes, 2010). Indeed, organic amendments of different origin (e.g. animal slurry, manure, compost, digestates from the anaerobic treatment of waste, biosolids, sewage sludge, crop residues, etc.) provide essential nutrients to the soil and enhance its organic matter content with concomitant benefits for soil functioning. Then, it is not surprising that organic wastes are most frequently used as amendments in soil remediation initiatives (Epelde et al., 2014; Galende et al., 2014; Gómez-Sagasti et al., 2018). Nonetheless, nutrient availability is affected by the biochemical composition of the amendment: for instance, its carbon-to-nitrogen ratio can limit soil microbial activity and, in consequence, alter OM decomposition and, concomitantly, the pattern of nutrient release (Manzoni et al., 2008).
Organic amendments commonly used in phytoremediation initiatives include biosolids, green composts, manures and biochar (Fig. 29):

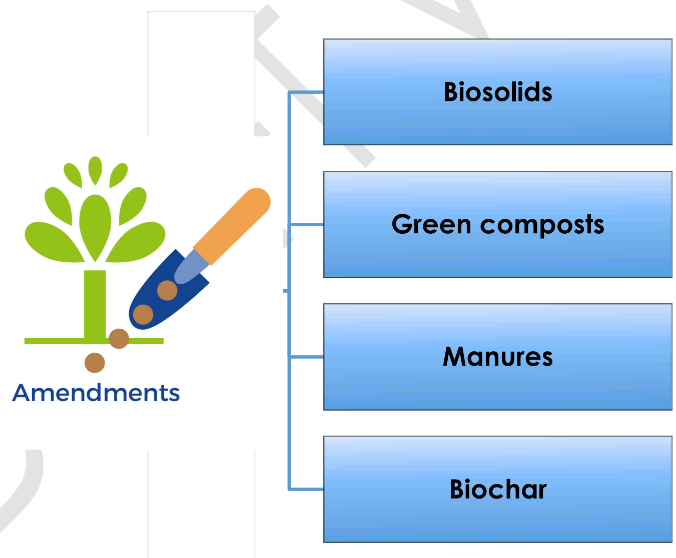

Figure 29: Types of organic amendments used in phytoremediation strategies.

4.1.1. Biosolids

Biosolids are solid organic byproducts produced by municipal wastewater treatment processes (generally liming to precipitate the organic matter after anaerobic and/or aerobic digestion). The properties of these low-cost vary according to the incoming sources. In general, biosolids present a high organic matter content, high nutrient availability, and exhibit liming properties. However, the use of fresh biosolids, such as sewages sludges, in phytoremediation scenarios implies some risks due to potential problems associated with high nitrogen and phosphorus content, high electrical conductivity, the presence of toxic metal(loid)s and certain xenobiotics (like antibiotics, other pharmaceuticals, endocrine disruptors, pesticides, etc.), as well as phytotoxic compounds like phenolic acids. Regarding antibiotics, wastewater treatment plants are acknowledged as important reservoirs for antibiotic resistant bacteria and genes (Mao et al., 2015), leading to the introduction and dissemination of these emerging contaminants in sewage sludge-amended soils (Urra et al., 2019b). Relevantly, antibiotic resistance is associated with TE resistance due to co-selection through co-resistance or cross-resistance mechanisms (Urra et al., 2019a) Actually, the presence of TEs in the organic amendments may enhance antibiotic resistance or select for antibiotic resistant bacteria (Bondarczuk et al., 2016).

Furthermore, the introduction of microplastics to soil via the application of sewage sludge is a topic of much concern, due to its potential harmful impact on environmental health. Interestingly, soil contaminants can become adsorbed onto microplastics and then change their availability and ecotoxicity. Regrettably, current data on the effect of microplastics on the functioning of soil ecosystem are still insufficient (Ng et al., 2018). Monitoring and quality control of this type of organic amendments before their application to soil is therefore highly recommended.

Such organic wastes can also be applied after a composting process which produces neutral or alkaline substrates with high richness in organic matter and available essential nutrients (Alburquerque et al., 2006). Composted organic residues present a high proportion of humified organic matter which decreases TE mobility through metal binding to exchange sites, adsorption, and the formation of stable organo-TE complexes (Soler-Rovira et al., 2010). Many studies have reported positive effects of organic waste composts on plant growth and health in TE-contaminated sites, mainly a result of their liming effect, and the reduction of TE mobility and the supply of nutrients (Becerra-Castro et al., 2018). On the other hand, liming has been shown to increase soil microbial activity in acid soils (Mijangos et al., 2010). The amendment of highly acidic Cu mine tailings with composted sewage sludges allowed the establishment of SRC systems and a grassy cover due to the strong reduction in soil acidity, Cu bioavailability and input of organic matter and nutrients (Touceda-González et al., 2017a, b). In addition, soil microbial activity was stimulated and led to the enhancement of vital biogeochemical cycles (Touceda-González et al., 2017a). On the other hand, reports of undesired mobilization of Cu or As in the soil and the leaching of nutrients can also be found (Becerra-Castro et al., 2018). Nonetheless, the application of composted biosolids may still be limited by high nutrient loading (e.g. nitrate and ammonia), and undesirable odors which may bring some problematic issues. Although the levels of metal(loid)s and organic pollutants has decreased in the last years in these types of biosolids due to new biotechnological improvements in wastewater treatment processes, the presence of TE contaminants is still a serious drawback to their use.

4.1.2. Green Composts

Green composts are aerobically decomposed organic materials, such as yard trimmings (e.g. grass and leaves from residential, institutional and commercial sources and food wastes. Municipalities offer a wide amount and a variety of these green organic wastes. Green compost composition varies widely, but it tends to have a lower N content than biosolids or animal manures. Crop residues and green composts can also provide protection against erosion, control weeds (Kruidhof et al., 2011), enhance the physicochemical and biological properties of the amended soils and, finally, improve the fertility of the recipient soils (Turmel et al., 2015). These organic amendments are also deployed in contaminated soils to reduce TE availability and provide nutrients and organic matter. For instance, Van Herwijnen et al. (2007) showed that in calcareous contaminated soils the use of green waste compost decreased the uptake of Pb, Cu, and Zn in greek cress (*Lepidium sativum*) by 54, 21, and 16%, respectively. Still, the effects of deploying organic amendments on the availability of TEs strongly depends on the TE, soil type and characteristics, and on the amendments general properties (e.g. pH, electrical conductivity (EC) and humification rate; Bernal et al., 2004). Moreover, some composts present high EC, which can lead to increased soil salinity (Scotti et al., 2015.)

4.1.2. Livestock Manures

Livestock manures are another major source of organic amendments, which can be obtained from chicken, swine, poultry and cattle. They greatly vary in their characteristics, namely in moisture content, nutrient content, stability (degree of OM decomposition), etc., depending on factors such as animal type, type of feeding, storage conditions and so on (Miller et al., 2003). The N content of animal manures is usually readily available to plants and does not persist in the soil for relatively long periods like the N from biosolids. Animal manure-based organic amendments can enhance soil microbial biomass, activity and diversity (Liu et al., 2016; Reardon et al., 2016).

Composting livestock manures is advisable to increase OM stability and, most importantly, to decrease potential human pathogens, such as *Escherichia coli, Salmonella* sp. and *Listeria* sp.

Animal manures have often been used to reduce TE availability in contaminated and phytoremediated areas. For example, the employment of manure was able to decrease the concentration of Cu, Zn, and Pb in *Chenopodium album* L. plants, compared with plants grown in both control soil and compost-amended soil (Walker et al., 2004).

4.1.3. Biochar

Biochar (BC) has been used more recently as soil amendment in contaminated soils (Wang et al., 2020; Lomaglio et al., 2018). This type of amendment is a C-rich product obtained through the pyrolysis of OM (Inyang et al., 2016). The feedstock and pyrolysis settings (e.g. temperature, residence time, pressure) affect the BC's physicochemical properties, namely structure, area and charged surface, which affect its adsorptive capacity of TEs (Cha et al., 2016; Sizmur et al., 2017).

Biochar has been mainly used in phytostabilization processes (Bolan et al., 2011; Nartey and Zhao, 2014; Wu et al., 2019; Shen et al., 2018), due to its' ability to adsorb TE, reducing its extractable forms in soil and consequent bioavailability (Nartey and Zhao, 2014; Wang et al., 2019). Specific functional groups located on BC's surface (e.g. oxygen-containing groups, -OH) can establish strong connections with TE by ion exchange, electrostatic attraction and surface complexation (Li et al., 2017). Biochar can also increase soil pH, thus limiting metal bioavailability and positively impacting soil microbial communities (Oliveira et al., 2017; Zhen et al., 2017). This beneficial relationship between BC and soil microbiota can then be

reflected in a positive effect on plant growth and development owing to the well-known aboveground-belowground links between plants and soil microorganisms, specifically rhizospheric microorganisms (Rajput et al., 2020; Sun et al., 2018).
Different particle-sized BCs were applied to soil vegetated with *S. viminalis* in a highly As and Pb contaminated site (Lebrun et al., 2018). This willow was not able to grow in the site without biochar (BC particle size did not have a significant effect on plant growth). Trace elements mainly accumulated in roots, showing its potential for phytostabilization.

4.2. Choice of Amendments

As addressed before, the choice of the appropriate amendment for applying in TE-contaminated soils depends on several factors, as summarized below:

Figure 30: Selection of the right amendment to TE-contaminated soils.

1. Characteristics of the target soil (physico -chemical)
   - should not induce drastic changes in soil properties
2. Intended goals for the target site (TE immobization and/or plant establishment/growth)
   - should inactivate TE rapidly and suplly nutrients to plants
3. Potential off-site runnof of contaminants and nutrients
   - should prevent TE and other contaminants leaching
4. Composition, stability and maturity (existence of phytotoxic organic substances)
   - unstable and/or imature amendments increase OM mineralization rates
5. Availability and costs (including transportation)
   - should be inexpensive and ready available
6. Salt content and pH of amendment
   - should not increase soils salinity; should be able to change pH when necessary
7. Presence of pathogens and weed seeds
   - should not introduce into soil human or phytophatogens, as well as unintended seeds
8. Metal(loid) contents
   - should be in compliance with legislation.

Readily bioavailable organic amendments (e.g. biosolids and livestock manure) are not retained for long time periods in soil, showing a fast but temporary effect on soil properties. More recalcitrant amendments, such as woody biomass, are less labile and show a smaller but longer-lasting effect on soil properties.

4.3. Application Rates

Appropriate application rates of organic amendments are dependent on the specificities of the contaminated site but are usually higher than those applied in agriculture practices. Usually, in phytomanagement initiatives, only one high-loading application is done at site implementation, in an attempt to facilitate plant establishment and growth.

Some approaches can be used to determine the appropriate application rate in TE contaminated soils:

1. Evaluation of OM in a nearby soil

- The amount of OM in a nearby healthy soil can be assessed, and values used as reference. It should be taken into account that a significant portion of the OM applied to the contaminated site will decompose to carbon dioxide and water in a relatively short time, and therefore a higher amount should be added in order to compensate the initial rapid decomposition.

2. Application of rates used in similar sites

Another approach is to look at rates that have been used at similar sites and apply similar ones.

3. Laboratory protocols

Laboratory protocols can define the amount of organic amendments that can be added to a particular soil, taking into account the analysis of soil (CEC, pH) and organic amendment charcateristics.

4. Qualitative analysis

- This is generally the case for amendments used to increase soil organic matter or to rebuild soil. Higher application rates of soil amendments are required when rebuilding soil rather than simply enhancing damaged soil.

5. Regulations

- The use of soil amendments is regulated in some countries, and these regulations are usually formulated to protect against excessive leaching of N to groundwater while assure success of the re-vegetation effort.

Figure 31: How to select the proper application rate of organic amendments.

5. CONCLUSIONS

Soil contamination with hazardous concentrations of TE is a serious environmental problem and prompt responses to mitigate its negative consequences are mandatory and must comprise complementary strategies that encompass both prevention and restoration actions. The latter includes phytomanagement, which can bring not only environmental benefits but also economic profits to stakeholders, by being a source of e.g feedstock for bioenergy production. Furthermore, the use of microorganisms, namely TE-tolerant PGPB and AMF fungi, as well as cropping systems, can further assist selected plants species to better cope with harsh soil conditions and improve its biomass and ecosystems services. Several examples of plants and microbial inoculants targeting TE-contaminated soils were given in this technical guide supporting the trustworthiness of phytomanagement options. Soils restoration is profoundly aligned with the United Nation's vision that that humans and natural ecosystems are indivisible, such that ecosystem services constitute an important source of livelihoods. Therefore, promoting the recovery and long-term sustainable use of natural resources while minimizing environmental risks are the foundations to promote ecosystem's resilience while contributing to public well-being. Hence, phytomanagement strategies directly contribute to UN's 2030 Sustainable Development Goals', particularly goal #15, which aims to "protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification and halt and reverse land degradation and halt biodiversity loss".

Despite the promising results achieved so far, more research is needed to further investigate the plethora of poorly understood links and mechanisms that still undercut the large-scale employment of phytomanagement. Particularly, the potential environmental and economic benefits, field-based implementation and deployment strategies, or the best host plants - microbe combinations. A cornerstone for current development lies in understanding which plants with economic value could be paired with which microbial inoculants to maximize plants' TE extraction and/or stabilization capacity while achieving higher yields and socioeconomic benefits. Moreover, more field trials are of key importance and deeply required to clearly demonstrate the feasibility of phytomanagement.

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