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Scale-up of Mycorrhizal-Assisted Phytoremediation System from Technology Readiness Level 6 (Relevant Environment) to 7 (Operational Environment): Cost-benefits within a Circular Economy Context

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Abstract

This chapter analyzes the costs-benefits of a particular phytomining methodology named mycorrhizal-assisted phytoremediation (MAP). This MAP system is responsible for phytostabilization and/or phytoextraction of secondary and critical raw materials from contaminated soil or mining wastes. To this aim, we evaluated the application of MAP in a modified constructed wetland, the vegetable depuration module (VDM), which permits the calibration of physical-chemical-biological variables in a contaminated substrate, as well as the partition of chemical elements within the liquid phase due to leaching and solid phases (biomass and soil). This successful methodology allows to scale-up from a Technology Readiness Level (TRL) 6 (demonstration in a relevant environment) toward TRL 7 (demonstration in an operational environment), which implies the transfer to the territory.

Keywords: phytoremediation, phytomining, circular economy, critical raw materials, heavy metal(loid)s

1. Introduction

Human activities over time have left a legacy of contaminated soils around the world. The intense exploitation of soil and the inadequate disposal of hazardous wastes by urban expansion, industrial and transport activities, mining, military activities and armed conflicts, and even unsustainable agricultural practices are the main sources of soil pollution. These anthropogenic activities release various

chemicals into the environment that are often found to form a complex mixture of numerous contaminants. The different contaminants produce adverse effects on the health of ecosystems and all living beings that inhabit there. Moreover, the frequency and severity of extreme climatic events (droughts, floods, dust storms, and wildfires incidents) caused by climate change exacerbate soil contamination. Anthropogenic activities contribute to changes in the moisture and temperature regimes of soils and groundwater and can increase rates of movement of contaminants *via* soil erosion (wind or water), soil runoff, leaching, and volatilization [1]. In this sense, a detail of natural and anthropic sources of some elements can be seen in **Table 1**. For example, dust storms, volcanic eruptions, geothermal/hydrothermal activity, forest fires increase the level of As and Hg in the environment. Climate change exacerbates these phenomena increasing the natural contribution of metal(loids).

The insufficient registration of contaminated areas in many regions of the world and the lack of regulations for their remediation accentuate this environmental conflict. About 3.5 million sites in the European Union (EU) were estimated to be potentially contaminated, with 0.5 million sites being highly contaminated and needing urgent remediation. There are 400,000 polluted sites in European countries, including Germany, England, Denmark, Spain, Italy, Netherlands, and Finland. Sweden, France, Hungary, Slovakia, and Austria count up to 200,000 contaminated sites. Greece and Poland reported 10,000 contaminated land areas, while Ireland and Portugal reported less than 10,000 contaminated sites. In America, approximately 600,000-ha brownfield sites are polluted with heavy metals [2]. Identification and assessment of potentially polluted sites are the essential first step in the management of soil pollution.

Among the persistent and potentially (eco)toxic heavy metal(loids)s (HMs) ubiquitous around polluted soils are arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), manganese (Mn), nickel (Ni), zinc (Zn), and radionuclides. Many of them are considered trace elements. The concentration of these HMs in soil has increased drastically over the last three decades, thus posing a risk to the environment and human health. A detailed description of the above-mentioned trace elements, including the natural and anthropogenic sources and uses, is given in **Table 1**. Identifying the sources of trace elements in the environment is of key importance to understanding the pollution patterns and natural global cycles, in addition to making decisions concerning soil pollution remediation.

The remediation methods are generally based on physical, chemical, and biological approaches, which may be used in combination with one another to clean-up HMs to an acceptable and safe level [3–5]. The physical and chemical conventional methods are usually expensive and can irreversibly affect the properties of soil, water and the living beings that inhabit them [6]. **Figure 1** resumes the soil remediation techniques based on chemical, physical, and biological processes developed during the last two decades [4, 7–9].

1.1 Physical methods

These methods consist of removing or reducing contaminants by physical methods such as dilution, heating, and solidification of contaminated soil. Some of the technologies involve the soil replacement or isolation, the thermal analytical method, vitrification, and the electric repair technique, which does not change the chemical properties of the pollutants.

—*Soil replacement and isolation method (1 y 2):* The soil replacement method reduces the concentration of contaminants by replacing the original contaminated

Element	Essential	Natural sources	Anthropogenic sourses	Uses
Arseni	No	Dust storms Volcanic eruptions Geothermal/hydrothermal activity Forest fires Arsenic-rich minerals	Metal mining and smelting. Coal mining and burning of arsenic-rich coals. Pesticide. Timber industry. Pyrotechnics.	Wood preservatives. Additive to veterinarian drugs (poultry). Doping agent in semiconductors.
Cadmium	No	Zinc and lead minerals. Phosphates rocks.	Electroplating. Metal industry (non-ferrous metals and steel). Automobile exhaust. Phosphate mineral fertilizer.	Pigments in paints, ceramics, plastics, etc. Cd impurities in Zn coatings used on metal structures.
Chromium	Yes	Chromium minerals	Metal industry Electroplating. Industrial sewage.	Electroplating. Metal alloys. Anticorrosive products. Pesticides, detergents.
Copper	Yes	Sulfides, oxides, carbonates	Domestic and industrial waste, mining waste, animal manure (pig and poultry). Car breaks. Metal industry. Copper-based fungicides.	Electric supplies, electric conductor. Electroplating. Fungicides. Plant residues treated with fungicides are used as soil amendments. Timber treatment chemicals. Copper piping and guttering. Vehicle brake linings.
Lead	No	Lead minerals	Battery manufacturing facilities. Private and industrial waste. Rifle ranges and military facilities. Leaded paints and leaded fuel addition. Insecticides.	Batteries. Alloys, bullets and other munitions.
Mercury	No	Mercury sulfide ores. Volcanoes. Forest fires. Ocean emissions.	Artisanal and small-scale gold mining. Chemical industry. Fossil fuels (coal and petroleum) combustion. Nonferrous metals production.	Catalysts, electrical switches. Batteries, fluorescent lights, felt production, thermometers and barometers. Alloys for dental fillings. Bright-red paint pigments.
Nickel	Yes	Nickel minerals	Metal works, battery plants, electronics. Industrial waste.	Metal alloys, batteries, electronics.

Element	Essential	Natural sources	Anthropogenic sourses	Uses
Zinc	Yes	Minerals	Battery plants. Metal industry. Phosphate fertilizers.	Batteries. Alloys. Construction anticorrosive planting. Tire rubber. Additives in veterinary drugs and pesticides.

Table 1.
Natural and anthropic sources of some elements and their industrial use (Source [1]).
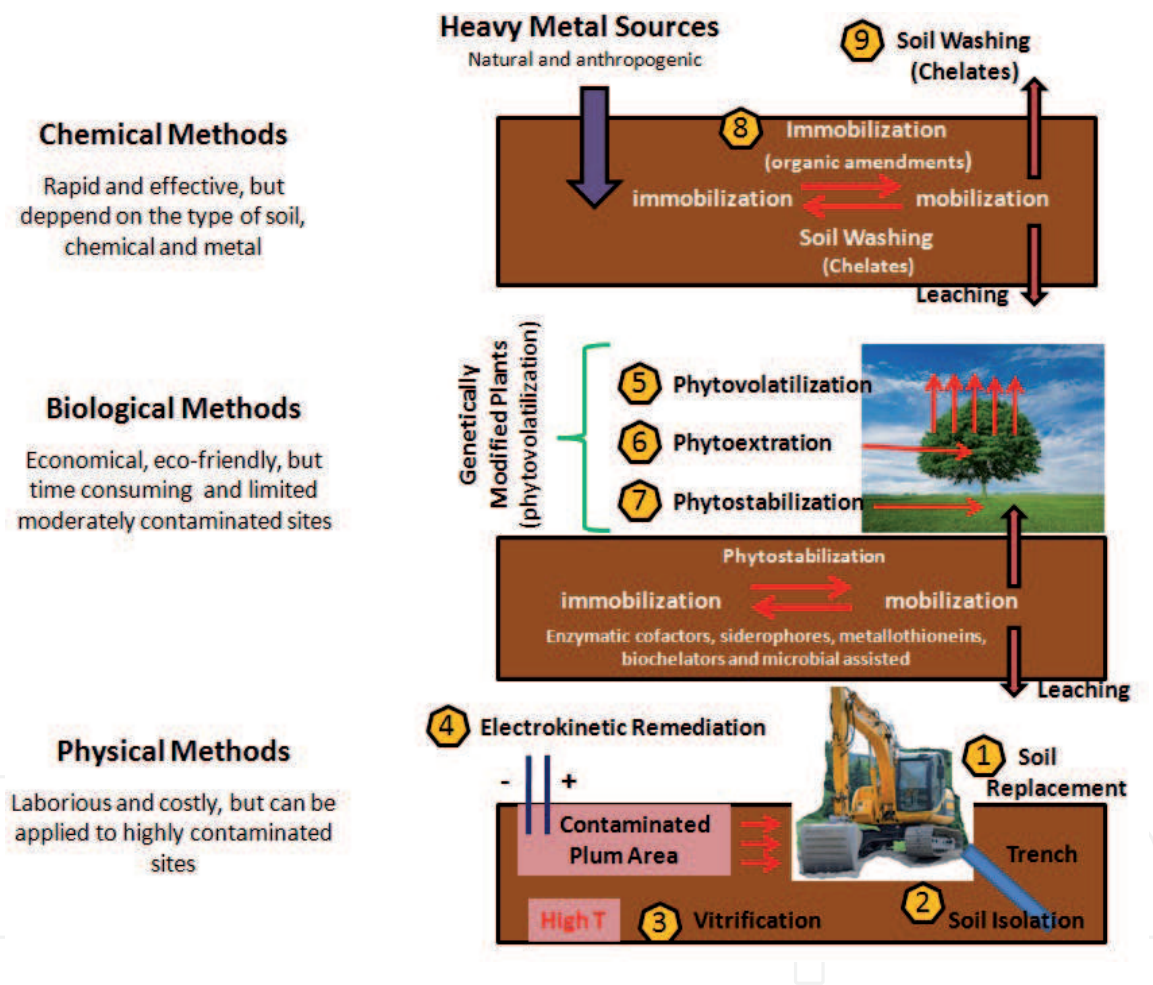

Figure 1.
Comparison of physical, chemical and biological methods of remediation for polluted soils or contaminated substrate. Physical remediation methods include (1) soil replacement, (2) soil isolation, (3) vitrification, and (4) electrokinetic; biological methods generally include (5) phytovolatilization, (6) phytoextraction and (7) phytostabilization; chemical methods contain (8) immobilization and (9) soil washing. Biological and chemical methods can be applied jointly depending on the type of contaminant, soil, plant and chemical reagent. Moreover, the effectiveness of different phytoremediation techniques can be enhanced by microbial-, chelate- and genetic-assisted remediation. Modified from [3].

soil with fresh soil and transferring the contaminated soil to the surrounding environment. The method is simple and reduces the concentration of pollutants in a short time. It does not change the mobility and bioavailability of pollutants in the soil, so it is often required in engineering construction as prevention and control barriers to prevent secondary pollution to the environment.

—*Vitrification method (3):* The soil vitrification consists of treating the contaminated soil with high temperature and pressure for a period, and then cooling it to form a vitreous substance. The result is a stable material where the contaminants are fixed.

—*Electrokinetic techniques (4):* Electrodes are placed into the soil and a direct electrical current is applied, which induces movements of contaminants to the cathode or anode through the electro-osmosis, electrophoresis, and electromigration [10, 11]. This technique has a short cycle and high efficiency but high energy consumption. It can be applied on-site, off-site, and *in situ* depending on the soil conditions.

1.2 Biological method

Phytoremediation technology:

Phytoremediation involves the use of plants to extract and remove chemical pollutants or to decrease their bioavailability in soil [12, 13]. In general, plants used to carry out phytoremediation are known as metallophytes. The main benefits reported for phytoremediation include less secondary waste generation and minimal-associated environmental disturbance *in situ*. However, the main constraint is the long period of remediation due to the growth cycles of plants. This technology can be improved with the inclusion of microorganisms such as filamentous fungi and bacteria with saprophytic or symbiotic nature. The mechanisms of phytoremediation used in the removal of HMs are phytovolatilization, phytoextraction, and phytostabilization.

—*Phytovolatilization (5)* plant roots absorb contaminants from soil and transport them through the xylem. Plants convert the contaminants into less toxic and volatile forms and release them into the atmosphere. Phytovolatilization has been widely used to remove metals such as mercury and selenium, as these metals have high volatility [14].

—*Phytoextraction (6)* is when plant roots absorb the contaminants from soil or water, and transport, and accumulate them in the aboveground biomass such as shoots and leaves. The hyperaccumulator species are the desirable plants to be used for phytoextraction as they have a high ability to accumulate different elements [15]. Plant biomass is comparatively very easy to recycle, dispose of, treat, or oxidize compared to contaminated soil. Phytoextraction guarantees a permanent removal of HMs from the contaminated sites. However, phytoextraction is suitable for those sites with low-moderate levels of HMs, because most plant species are not able to survive in heavily polluted habitats [9, 16]. However, some authors have mentioned the potential use of native hyperaccumulating plants with remarkable tolerance strategies facing polluted conditions [17]. Essential pre-requisites for successful phytoextraction include the following: a high uptake and translocation of HMs to aerial parts, an enhanced loading of HMs into the xylem, and an efficient detoxification in the plant [18, 19]. Physiological studies revealed that enhancement xylem loading of HMs and their transfer to the aerial plant parts are mediated by carrier proteins, generally found in the intracellular or plasma membranes (cation diffusion facilitator, CDF; zinc-regulated transporter proteins, ZRTP; iron-regulated transporter proteins, IRTP; heavy metal(loid) ATPase, HMA; natural resistance and macrophage protein, Nramp) [7, 20, 21]. Other natural chelators such as metallothioneins, phytochelatins, glutathione, thiol compounds, and organic acids are also involved in the improvement of HMs accumulation and translocation to the xylem, besides tolerance to stressful conditions. Secondly, there is also a need to pursue the role of plant growth regulators (indolebutyric acid, cytokinins,

gibberellic acid, naphthylacetic acid, and indole-3-acetic acid) to increase the potential biomass production of hyperaccumulating plants.

—*Phytostabilization (7)* is performed by plants that reduce the mobility and migration of HMs in soil by confining them in the vadose zone (the unsaturated strata above the water table) through the absorption and adsorption of these contaminants on the roots or the precipitation of toxic elements within the rhizosphere [22]. In this process, the plant that is being used to carry out phytostabilization induces changes in their rhizosphere, which has discrete physical-chemical, and biological conditions [23–25]. Metal excluder plants accumulate high levels of HMs from the soil into their roots with the limited transport to their aerial parts [20]. These plants have little potential for HMs extraction to be considered in a phytoremediation process, but are highly efficient for phytostabilization purposes. Phytostabilization can also be used in combination with other remediation approaches, such as the use of soil microorganisms and organic amendments to enhance HMs immobilization in soil. Soil microorganisms are reported to increase root metal contents *via* an increase in plant growth as well as the HMs immobilization in soil [26]. Besides soil organic matter comprises a wide range of organic molecules in different states of mineralization and complexation within the soil matrix, which will behave differently when interacting with contaminants. These organic macromolecules contain many functional groups (carboxylic acids, alcohols and phenols, or amines), dependent on pH- and redox potential, that play a major role in the adsorption of ionizable organic contaminants as well ionic forms of trace elements through covalent and hydrogen bonding, thus reducing accessibility to microbial interactions. Small organic compounds such as amino acids, sugar acids, short-chain aliphatic acids, and phenols can form stable chelates with trace elements, and contaminants can also be complex with Al and Fe oxides. Some substances excreted from microorganisms may contribute to the acidification of soil and increase the mobility of some contaminants. The buffering capacity of soils neutralizes excess anions in exchange for mobilizing cations (e.g., Mg^{2+}, Ca^{2+}, Na^+, K^+) from the surface of soil particles, which results in cations leaching. But this capacity is limited, and if acid deposition exceeds the natural neutralizing capacity of the soil, other cations, such as Al^{3+} or Fe^{2+}, can be mobilized from clay structures and organo-mineral complexes, entering the soil solution [1]. Once the sites are phytostabilized continuous monitoring is required to make sure that the stabilizing condition is maintained. Soil amendment used to reduce HMs mobility in soil may need to be occasionally reapplied to retain immobilizing conditions [22].

1.3 Chemical methods

These methods include soil washing and immobilization technologies. Different chemicals or solvents (metallic oxides, clays, or biomaterials) are added into the soils to stabilize the pollutants and convert them into less toxic forms to living organisms, thus reducing their bioavailability, adsorption, or transformation [27]. The remediation chemical methods are faster than biological ones and could also be applied *in situ*. However, the harmful effects of the use of chemical methods should also be considered before its implementation.

—*Immobilization (8):* It is a sequence of precipitation-adsorption, ion exchange, humification, and other oxidation-reduction reactions by adding a fixative to the contaminated soil and changing the existing form. This process reduces the metal bioavailability in soil and its toxicity. The fixed repair technology has a short cycle and quick effect. Sometimes, this method does not completely remove the metal/oids, only changes its occurrence state, and can cause secondary pollution.

—*Soil washing (9):* Contaminated soil is repaired using injection wells to infiltrate the water or chemical auxiliary eluent under the action of gravity or external force, so the HMs present in the contaminated soil are fully combined with it and desorbed by the eluent. This method uses liquids that contain chelation agents, freshwater, and other solvents to wash the contaminated soil with mechanical processes [28, 29].

2. Phytomining

As it was previously mentioned, the bioavailability and mobility of HMs in soil substrate are greatly influenced by the soil physicochemical properties (pH, Eh, electrical conductivity, cation exchange capacity, and soil mineralogy), the biological conditions, and the presence of soil inorganic and organic ligands. Careful risk assessments should be undertaken to select the appropriate hyperaccumulating plant species and determine safe and acceptable use of the aboveground plant biomass. As this aerial plant biomass gradually accumulates trace elements and other contaminants and its toxicity is likely to increase, it is important to select those hyperaccumulating species that are unlikely to enter the food chain or implement a protection system to avoid this important issue. There are several post-harvest management options for crops including energy generation, biofuel production, gasification, composting, recovery of critical and secondary raw material recovery, and phytomining.

Phytomining or agromining refers to the full agronomic process using hyperaccumulator plants as "metal crops." The process involves the farming of "metal crops" on subeconomic deposits or industrial or mineral wastes to obtain valuable element(s) from their harvested biomass *via* the production of a "bio-ore." However, defined considerations after implementing this management option should be given to ultimate the fate of chemical elements that have been concentrated in plant biomass along the phytomining process [1].

Microbial-assisted phytomining of HMs also represents a promising method for the remediation of contaminated soil [30]. Microbial-assisted phytomining of HMs involves several mechanisms such as biosorption, intracellular accumulation, enzyme-catalyzed transformation, bioleaching and biomineralization, and redox reactions [31]. In many cases, plant-microbe associations are highly efficient in absorbing, accumulating, translocating, and tolerating HMs because of their capacity to produce various substances that participate in stimulating growth and HMs accumulation (monocyclopropane-1-carboxylate deaminase, siderophores, indole acetic acid) [30]. In microbial-assisted phytomining, the exudates of mycorrhizal roots play a significant role in the efficiency of phytoextraction of the elements in the soil. For instance, concentrations of amino acids (glutamine, glutamic acid, valine, and methionine) and organic acids (citric acid, malic acid, and oxalic acid) in the root exudate of *Andropogon virginicus* were increased under P-deficient conditions [32], and the extraradical hyphae of AM fungi could exude diverse metabolites that are influenced by P levels and diverse AM fungal species [33]. In previous reports, we observed an increase in translocation for Mn, Fe, As, Zn, Ti, Cr, Cu, Rb, Sr., Al, Ba, K, and Ca when the MAP system based on the arbuscular mycorrhizal (AM) symbiosis established between the sunflower *Helianthus annuus* and the AM fungal species *Rhizophagus intraradices* (*GA5* strain, https://bgiv.com.ar/strains/Rhizophagus-intraradices/ga5). The MAP system was applied for the recovery of critical and secondary raw material in sunflower plant biomass, and bioremediation of contaminated mining substrate [34, 35].

3. Cost of remediation of methodologies at field/full scale (TRL 7-9)

Several authors have reported the operating costs related to different remediation technologies, normalized per unit (m^3) of contaminated soil (**Table 2**).

As **Table 2** shows, phytoremediation represents a sustainable and low-cost alternative for the rehabilitation of environments affected by natural and anthropogenic

Applied technology	Cost/ m^3 contaminated soil	Observations	Source
Phytoremediation (phytoextraction)	US$ 37,7	Biological (20 cm soil depth, 2 years), initial capital included in the cost.	[36]
	US$ 50–200	Biological (density 2 tn/m^3)	[37]
	US$ 10–35	Biological	[3]
Plant extraction	US$ 19–78	Physical-biological	[38]
Phytostabilization	US$ 1.3	Biological	[1]
Turnover and attenuation	US$ 4.7–5.6	Physical	[38]
Extraction	US$ 240–290	Physico-chemical	[36]
Solidification	US$ 87–190	Physico-chemical	[36]
Ex situ disposal	US$ 480–813	Physical	[39]
Ex situ high-temperature thermal desorption	US$ 81–252	Physical	[40]
In situ biopile	US$ 130–260	Chemical-Biological	[36]
In situ land farming	US$ 100	Biological, initial capital included in the cost	[41]
Unlined repositories	US$ 9.52	Physical	[42]
Lined repositories	US$ 34.44		
Soil replacement—excavation	US$ 540–920	Physical (*ex situ* disposal, short distance, and soil replacement, density 2 tn/m^3), initial capital included in the cost)	[43]
Excavation and treatment	US$ 145	Physical-chemical, initial capital included in the cost	[1]
Vitrification	US$ 600–1000	Physical, initial capital included in the cost	[37]
Flushing	US$ 150–420	Physical	[37]
In situ bioremediation	US$ 50–150	Biological	[3]
Bioremediation	US$ 50	Biological	[1]
Stabilization/solidification	US$ 240–340	Chemical	[3]
Soil venting	US$ 20–220	Chemical	[3]
Solvent extraction	US$ 360–440	Chemical	[3]
Soil washing	US$ 80–200	Chemical	[3]
Incineration	US$ 200–1500	Chemical	[3]
Phytoextraction+ Chelation	US$ 15	Chemical-biological	[44]

Table 2.
Economical costs of some technologies for remediation of contaminated soils.

pollutants [45, 46]. The differential costs reported among similar methodologies are concerned with the type of chemical elements and their concentration to be extracted, the technical procedures, the type and amount of soil to be remediated, the area to be treated, and the ideal nontoxic concentration value of pollutants to be achieved, among others. For some instances, only high-costing operations are considered, and it is frequently that a plus initial capital has been added for carrying out remediation.

Based on economic implications, the aim of phytoremediation can be three-layered: (1) phytomining (plant-based extraction of metals with a financial benefit, i.e., in the perspective of critical and secondary raw materials recovery from plant biomass [47]); (2) minimization of the risks of bioaugmentation of contaminants in the food chain, for example, by stabilization of Cd in cocoa plantations (https://www.fontagro.org/new/proyectos/bioproceso-cd/en); and (3) sustainable soil management by steadily increases soil fertility allowing for follow-up cultivation of crops with added economic value [48–50]. In concordance, the chelate-assisted and microbial-assisted phytoextraction and use of genetically engineered plants can further reduce the cost of remediation by enhancing metal accumulation and decreasing remediation time. Moreover, the operational costs remain the same as for phytoextraction alone. The implementation of phytoremediation as an effective methodology that guarantees the recovery of elements of interest and the rehabilitation of the soil must contemplate, in the long term, a period greater than 4 months for continuous monitoring, to ensure not having negative impacts due to external and internal variables (e.g., climatic variables, man, animals, changes in pH, Eh) that may affect the efficiency of the process [34, 36, 47].

4. Scaling from TRL 1 to TRL 6: VDM

—Constructed wetland systems:

From the remediation conceptual tests at the laboratory corresponding to technology readiness level (TRL) 1–2 to their applications in the territory (TRL 7), a long way of calibration and adjustments must be executed. Generally, a significant economic loss is given by poor evidence of adaptation and adjustment when technology proceeds from TRL 3 to TRL 7 [51].

In the study by Scotti et al. [34], a constructed wetland system called vegetable depuration module (VDM) is proposed as a calibrator of variables in MAP tests (**Figure 2A**). The use of VDM allows to determining the balance mass and the metal(loid) partition between soil, fungal structures, mycorrhizal roots, and aboveground plant tissues. The VDM allows the leaching of different HMs under particular conditions of pH-Eh, organic matter and other amendments and co-enzymatic factors (among other elements) taking to account the hydraulic variables such as type of irrigation (vertical, horizontal, continuous, interrupted, laminar, or turbulent), dynamics flow, and constant of hydraulic retention (Kh physical constant dependent on filling). Partitioning among different media usually relies on an equilibrium between the contaminant adsorbed on solid surfaces and the contaminant dissolved in a liquid (or gaseous) phases, controlled by the chemical characteristics of the contaminant (e.g., hydrophobicity, volatility). Several distribution coefficients have been developed over the years (e.g., partitioning coefficient between soil and water: Kd, organic carbon and water: Koc, or octanol-water partition coefficient: Kow) to elucidate processes in nature, but these are usually simple models that do not consider the specificity of sorption sites or competition among molecules and elements [1]. Thermodynamic processes that determine the

Figure 2.
A: The Vegetable Depuration Module (VDM) under construction, B: Vertical flow beds (VFB) under construction in Lima, Peru; C: VFB under construction in Bayawan City, Philippines; D: From left to right: three VFBs (filters) for pre-treatment and two VFBs for secondary treatment in Albondón, Spain (photos by (photos from [57]).

bioavailability of trace elements are complex, and VDM allows to calibrate some of these processes. Once the calibration of these parameters has been obtained, the system can be scaled up to territory by adapting the engineering practices. The description of the VDM [34] shows it as a modified subsurface constructed wetland that allows designing the type and quantity of underground filter, its granulometry, type of substrate, and amendments besides the hydraulic system.

The VDM (located at *Centro de Desarrollo Regional Los Reyunos* of *Universidad Tecnológica Nacional* in Mendoza province, Argentina; 34°35′46″S 68°38′25″W at 702 m elevation) consists of modules with two pools connected to collection chambers through a hydraulic system. Each pool was 2.80 m wide and 5.00 m long, and ranged from 0.6 m (bottom depth) to 0.9 m (top depth), resulting in a difference in height of 0.3 m and a slope of 6%. The collection chambers were 1 m long, 2.8 m wide, and 1 m deep. The VDM is isolated from the external environment by using a waterproofing system and a greenhouse covered with a metal net with a polyethylene film against hail. VDM behaves like a modified subsurface artificial wetland, with vertical/horizontal irrigation flow and regulated inflow and outflow water. Water enters the system through pipes connected to a reserve tank and a water pump that drives vertical/horizontal flow to both pools. The remaining water that is not incorporated into the biosystem is allowed to drain into the collection chambers. When the water enters the chambers, it can eventually be recycled by reintroducing it into the reserve tank or released to the environment if it is sufficiently free of contaminants. The pool is filled as follows: depth layer of 10 cm with large gravel (approximately 10 cm in diameter), covered by 15 cm of gravel of medium size (approximately 5 cm in diameter), and 20 cm of small-size gravel (about 1 cm in diameter). The last 15-cm surface layer consisted of the growth substrate of the bioremediation system.

The VDM is a technological development adaptable to different designs and methodologies with a scale of TRL 6 as a simulated environment. The output of the VDM calibration corresponds to the first engineering cycle of a design to be taken to field scale. Under the experimental conditions in the VDM, the HMs in multi-contaminated soils with high leaching properties pass to the collecting chamber to be recycled and treated in another VDM with different physical-chemical and biological conditions. Consequently, those HMs translocated to plant biomass are considered bio-extracted and the elements retained in the substrate without entering biomass are considered stabilized. Furthermore, the VDM allows calibration of the capacity of phytoextraction or phytostabilization of a given system under certain conditions. The differential behavior between phytoextraction and phytostabilization is mainly given by the soil conditions and the plant-microorganism association. The mycorrhizal plants can retain HMs in soil substrate by physical-chemical fixation, redox reactions, absorption and adsorption in the extra-radical mycelium and spores, and by releasing glomalin, a complex of glycoproteins that acts as a carbon reservoir in soils and is involved in the sequestration of HMs [53]. Recent studies have demonstrated that AM symbiosis performance can fluctuate between phytostabilization and/or phytoextraction depending on certain HMs, the environmental conditions, and the types of plant and fungal partners [54]. As it is known, the bioavailability of HMs is related to the solubility of these elements, which intimately depends on the temperature, pH, and Eh parameters (Pourbaix) [55], among other factors. The VDM allows modifying the retention capacity in the substrate or the leaching rates of HMs by controlling the pH-Eh values according to the soil-plant-microorganisms system applied.

Recently, different modular constructed wetland systems in series were designed with different numbers of vertical flow bed (VFB). In the system designed in **Figure 2B**, the entire surface is used as an inlet area to greywater influents through connected pipes with uniform holes that later are covered with gravel to complete the testing performances. In **Figure 2C**, another example of a VFB is constructed for the treatment of wastewater from a landfill. **Figure 2D** shows modular constructed wetland systems in series without electricity supply as it is built on a slope. It consists of three VFBs for pre-treatment as a filtration step and two VFBs for secondary treatment.

5. Cost of projection of HMs bioextraction at TRL7 by using MAP and VDM

In Scotti et al. [47], we estimated the calibration for an efficient extraction of CRM and SRM per m^3 of mining soil treated in the VDM with the MAP system. Estimated bio-extracting potential (BP) was in the range 2.417 g (K) > BP > 0.14 g (As) per m^3 of contaminated soil, suggesting the eventual subsequent recovery of SRMs and CRMs by hydrometallurgical techniques, with final purification by selective electrodeposition, as a viable and cost-effective option. In this work, the costs of a projection to TRL 7 (real environment) of the BP results reached by using the MAP and the VDM were determined. For that, an economic model used by Wan et al. [36] was followed, separating initial capital costs and operating costs. Also, repositories and constructed wetland costs were considered (**Table 3**). For the operating costs, various models of repositories and constructed wetlands were taken into account depending on the objective to be achieved [34, 52]. The costs of the projection to the territory for the application of MAP using designed models of the VDM are shown in **Table 3**. The costs of the MAP system were divided into initial capital and operational costs. The initial capital includes the following items:

investigation about pollution, selection of remediation strategy, soil preparation, construction of modules repositories, pipes and collector chambers, equipment, temporary store, irrigation system, and incineration equipment. Construction of adequate accesses is required.

Regarding the operational costs, they include the cost of labor and materials, large machines, and other direct or indirect costs. The cost of labor involves seedling, production of AM fungal inocula, transplantation, fertilizer application, insect control, irrigation and recycled, weed control, harvesting, module filling, placement of stones, amendments and chelates, and some other less significant items. The cost of materials includes the purchase of seedling tray, hyperaccumulator seedling production, crop seedlings, farm chemicals, inorganic and organic fertilizer, stones filter, amendments, chelates, and some other less significant items. The cost of using large machines includes rent for machines during harvest, incineration, and disposal of dangerous wastes. The direct cost is the production compensation and rent of land, which are paid to the local farmer; fuel and power cost during the phytoremediation project; construction and environmental supervision, amortization for the initial capital to 10 years. When the land is fiscal (government), the compensation is included in the indirect costs at the level of tax rates. A conversion from ha to m^3 was carried out taking into account 0.2 m of soil depth. The total estimated cost of MAP was US$ 40.775 with initial capital and operational costs accounting for 41.76% and 58.24%, respectively. On the other hand, the operational cost in total was US$ 23.75. It is highlighting that the cost for labor is low compared to total operating costs. This could indicate that the system is simple to be managed, and no extreme technical skills are required to handle it.

In **Table 4**, the commercial value of each chemical element established by the global market was linked to the quantities of each bioextracted element in the VDM corresponding to 1 m^3 of treated soil substrate. Although the commercialization value corresponds to the last prize quote, we observed that there are elements (Mn, Fe, P, Rb Sr., Al, Ba, K, S, and K) that are highly remunerative, and their cost of bioextraction is very low (US$ 40.75/m^3), disregarding the cost of hydrometallurgy to recover metal with high purity. Therefore, an important aspect in applying bioextraction processes is the appropriate selection of the experimental conditions, the combination of chemical elements, the adding of amendments and enzymatic co-factors, and an efficient mycorrhizal hyperaccumulating plant.

Items		Cost (USD)/ha
	Strategy selection	824.8
	Modules, collector chambers, repositories	5770.3
	Equipment	5893.6
	Irrigation system	5986.8
	Possible accesses required	4548.4
	Incineration equipment	7216.5
	Others	3812.4
Initial capital in total		34052.8

Items			Cost (USD)/ha
			Cost (USD/m3)
Operational cost (two years)	Cost of labor	Seedling	0.082
		AM fungal inocula production	0,390
		Transplant	0.103
		Fertilize	0.062
		Insect control	0.062
		Irrigation and recycled	0.062
		Weed control	0.206
		Harvest	0.093
		Module filling, placement of stones, amendments, chelates, others	0.329
		Cost of labor in total	**1.389**
	Cost of materials	Seedling tray	0.041
		Hyperaccumulator seedlings	0.082
		Crops seedlings	1.261
		Farm chemicals	0.021
		Fertilizer	7.446
		Stone filter, amendments, chelates, others	0.461
		Cost of materials in total	**9.312**
	Cost for usage of large machines	Harvest machines	0.148
		Incineration machine	0.161
		Disposal of dangerous wastes	0.103
		Cost for usage of machines in total	**0.412**
	Other direct cost	Production compensation	0.178
		Rent of land	0.155
		Fuel and power cost	0.974
		Construction supervision	0.037
		Environment supervision	2.006
		Regular monitor	1.650
		Other direct cost in total	**5.00**
	Indirect cost	Staff wage	0.495
		Administrative expenses	0.412
		Travel expenses	1.944
		Cost of water and electricity	1.003
		Others (amortizations, taxes)	3.782
		Indirect cost in total	**7.636**

Items	Cost (USD)/ha
Operational cost in total	23.749
Initial capital cost/ m^3 (0.20 m depth)	17.026
Cost in total/ m^3	40.775

Table 3.
Costs of projection to the territory for the application of MAP using designed models of the VDM.

Element	Purity (%)	BP g/VDM (m^3)	State	Price USD/g (Market)	Recovery (USD) in VDM (m^3)	Source
Mn	99.7	34.82	scales	0.15	5.223	[56]
Fe	99.99	60.01	powder 450 μ	1.19	71.4119	[57]
Ga	99.99	1.02	spheres	30.97	31.5894	[58]
P	99.5	114.7	red	91.72	10,520	[59]
As	99.99	0.145	lump	40.78	5.91	[60]
Zn	99.5	43.22	Ingot	0.023	0.99	[61]
Ti	99.95	2.2	Ingot	0.16	0.352	[62]
Cr	99.5	0.21	Ingot	1.2	0.252	[63]
Ni	99.99	0.22	spheres	0.14	0.0308	[64]
Cu	99.99	0.55	tabs	0.12	0.066	[65]
Rb	99.99	3.39	tabs	421	1.427	[66]
Sr	99.8	12.14	dendritic pieces	9	109.26	[67]
Al	99.99	23.99	granules	1.8	43.182	[68]
Ba	99	0.65	lump	348.4	226.46	[69]
K	99.97	2,417	lump	8.47	20471.99	[70]
S	99.8	243.9	powder	11.29	2.754	[71]
Ca	99	690	lump	29.76	20.534	[72]

Table 4.
The commercial value of each chemical element established by the global market linked to the quantities of each bioextracted element in the VDM corresponding to 1 m^3 of the treated soil substrate.

6. Standardization of methodologies

Sustainable remediation is now covered by the International Organization for Standardization with the ISO standard 18504:2017 "Soil quality – Sustainable remediation" [73]. In the United States of America, the international American Society for Testing and Materials (ASTM) has developed the "Standard Guide for Greener Clean-ups ASTM E2893 - 16e1" [74]. Australia has developed a National Remediation Framework and technical guidance to support its legislation on polluted sites [75]. In this regard, ISO Technical Committee on Soil Quality has developed a valuable catalog of standard methods for the analyses of soil contaminants, as well as the design and implementation of soil sampling from contaminated sites.

ISO also includes methods to assess the toxicity of contaminated soils to plants, microorganisms, earthworms, insects, and other biota [56]. However, this extensive catalog is only available on a standard purchase basis, making it difficult to access, especially in developing countries. To facilitate universal access to internationally developed and agreed standards, the Global Soil Partnership works in collaboration with experts from around the world to identify, agree, and make harmonized sampling and analytical procedures available worldwide through the global networks of soil laboratories (GLOSOLAN1, http://www.fao.org/global-soil-partnership/glosolan/en/) and soil information institutions (INSII2, http://www.fao.org/global-soil-partnership/insii/en/).

7. Conclusions

Phytoextraction is a safe, least destructive, eco-friendly, and cost-efficient remediation technique that allows soil clean-up over a large scale. The cost of phytoremediation of HMs contaminated soils can be minimized by better understanding the mechanisms and processes involved in bioremediation, and the many options at the different remediation steps.

Phytomining is an incipient methodology for both remediation and recovery of chemical elements of interest. The UE in its 4th list [76] declares 20 critical raw materials due to their availability in nature and the increasing demand in the industry. Many of these elements can be recovered in toxicity-tolerant hyperaccumulators plants.

This methodology can be improved by modulating the physical-chemical and biological variables and their dynamism. For instance, amendments, enzymatic co-factors, and chelators could be incorporated by both artificially and naturally ways to set physical-chemical variables. But results about biological exudates are not constant and reproducible because they depend on an elapsed time, nutrients, and microorganisms present in soils.

Variables on phytomining techniques are currently under study, and many of these have not been elucidated yet, leading to failures when the technology is scaled up. To carry out this calibration, we propose the application of the VDM at a TRL 6 scale (1 to 10 m^3 of soil) before taking it to the territory (TRL 7). Through the controlled experiences in the VDM, it is possible to obtain information on phytoextraction, phytostabilization, and leaching of the elements under study.

In this sense, to successfully transfer this methodology to territory, we can generalize the knowledge about the partition of a certain chemical element: a) root and aerial biomass (translocation factor), b) root and soil biomass (bioconcentration), and c) solid-liquid matrix phases. These partition compartments are dependent on various physical-chemical and biological factors.

Regarding the economic aspect, phytoremediation is a very convenient option compared to other techniques of remediation used. In turn, the possibility of recovering valuable chemical elements for the global market, this methodology becomes even more convenient.

Finally, the social license for phytoremediation, under recovery of commercially important chemical elements and minimization of wastes in the environment, makes this methodology a good option toward a circular economy.

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