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Enhancing phytoremediation of soils polluted with heavy metals

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Environmental pollution with heavy metals continues to affect soil quality and crops yields. Among remediation solutions, biotechnology offers a number of environmentally friendly options, one of which is phytoremediation. The use of plants as hyperaccumulators for heavy metal ions is beneficial in terms of feasibility, costs, but has the disadvantage that plants may be affected by heavy metals toxicity. Also, heavy metals are often found in soil in less bioavailable forms to be extracted by plant roots. To overcome these shortcomings, various techniques have been proposed to intensify and accelerate the phytoremediation. They are analyzed and concisely described in this paper, emphasizing how these techniques can act to increase plant tolerance to the toxicity of heavy metal ions and can change the conditions in the rhizosphere area to favor heavy metals extraction and the transport in the roots and their translocation towards the aerial parts of the plant.

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Introduction

The presence of heavy metals in the environment is mainly associated with industrial development, agricultural practices and other anthropogenic activities considered as significant sources of heavy metals. The severe toxic effects induced in the environment and aquatic and terrestrial organisms can also manifest by significant changes in the properties of the soil and its biological activity [1••,2••].

Despite intensive laboratory and field research over the past 20–30 years, environmental contamination with heavy metals continues to be a challenge, and today there is still not a perfect technology for remedying and restoring soils contaminated with heavy metals [3]. Remediation strategies include physical, chemical and biological methods. Typically, physical and chemical remedial strategies for removing heavy metals are fairly widespread. Although these techniques are seen as suitable for removing heavy metals from the environment, they are difficult to apply and expensive, and can significantly alter soil properties [2••,4]. In this context, biotechnological solutions, such as phytoremediation, demonstrate increasing opportunities for removal and/or degradation of inorganic and organic contaminants from soils, as they are cheap and feasible alternatives.

Phytoremediation strategies

Phytoremediation is currently considered an emerging, environmentally friendly technique to remove heavy metals from the environment, based on plants capacity of extracting heavy metals from water and soil and bioaccumulating them in various parts (root, stem, leaves, flowers, fruit), without adverse effects on soil structure, fertility and biological activity. In addition, phytoremediation has the advantage to be driven by solar energy and also the potential to remove pollutants from the contaminated sites almost completely [1••,5]. Plants that grow on contaminated soils must have a certain degree of tolerance to various heavy metals (one or more), a high growth rate and can produce large amounts of biomass, thus ensuring a good efficiency of phytoremediation. Also, plants with high capacity to bioaccumulate heavy metals, named hyperaccumulators, when grown on metal rich soils, can accumulate in the roots over 100 mg Cd/kg dry biomass; or more than 1000 mg/kg dry biomass Ni, Cu or Pb; or more than 10,000 mg/kg of Zn or Mn in their shoots [5,6]. Some plant families such as *Brassicaceae*, *Euphorbiaceae*, *Asteraceae*, *Fabaceae*, *Lamiaceae* and *Scrophulariaceae* are recognized as good hyperaccumulators, of which over 500 plant species can be used to accumulate significant amounts of heavy metals [1••].

Phytoremediation is beneficial as it can be applied on large areas of land, requires minimal monitoring and the accumulated metals in biomass can be recovered by physical-chemical, thermal or biological processes, especially in the case of critical metals. Moreover, the properties of the soils are improved, the microflora from the rhizosphere area is restored and the nutrients transfer is intensified. Phytoremediation is based on an autotrophic system, which often involves large amounts of biomass, for which the nutrient intake is relatively reasonable, easy to manage. Also, the process is socially friendly, due to its aesthetic appearance and environmental sustainability [7].

Molecular mechanisms of phytoremediation

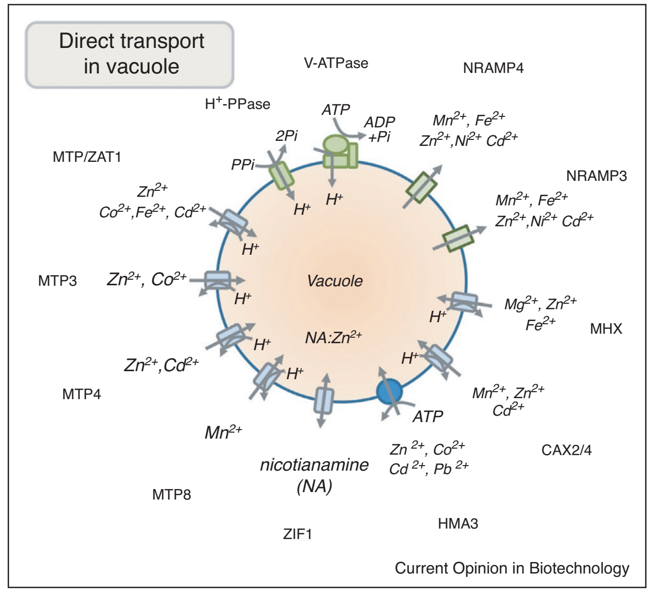
The absorption of heavy metals by plants occurs through the root system, which has a very large-specific surface area and where the mechanisms of tolerance and counteracting the stress induced by heavy metals toxicity also develop. Studies and research conducted in recent years have revealed the complicated mechanisms involved in the bioaccumulation of heavy metals in plants, how plants manage to respond the stress generated by toxicity and high concentrations of heavy metals in the soil [1••,2••,8,9,10].

A deep understanding of the mechanisms underlying the accumulation and tolerance to heavy metals in plants is a condition for the application of techniques for enhancing phytoremediation [11]. Plants can develop strategies and mechanisms at cellular and molecular levels involved in the removal of metals from the environment. The mechanisms are dependent on the structure and composition of cell wall and plasma membrane, root system, involvement of certain enzymes, presence of complexing ligands, vacuolar structure.

Vacuolar compartmentation is dependent on two vacuolar pumps, V-ATPase and V-PPase, respectively, and tonoplast transporters, driven by primary pumps that depend on ATP and all this is essential for heavy metal homeostasis. While plants with low bioaccumulation capacity of heavy metals retain them primarily in root vacuoles, hyperaccumulating plants have the ability to translocate heavy metals through vacuolar transporters and immobilize them in leaf vacuoles. This is due to enhanced root uptake together with controlled compartmentalization of heavy metals into the root cell vacuoles [12,13••]. During this process, metal ions are temporarily retained by weak chelation in the cytoplasm, followed by translocation from root to stem by efficient loading-unloading mechanisms of the xylem and then by compartmentalization of ions in the leaves, respectively in the vacuoles and apoplast of their cells. Figures 1 and 2 concisely describe key vacuolar membrane transporters for homeostasis and detoxification of heavy metal ions [13••].

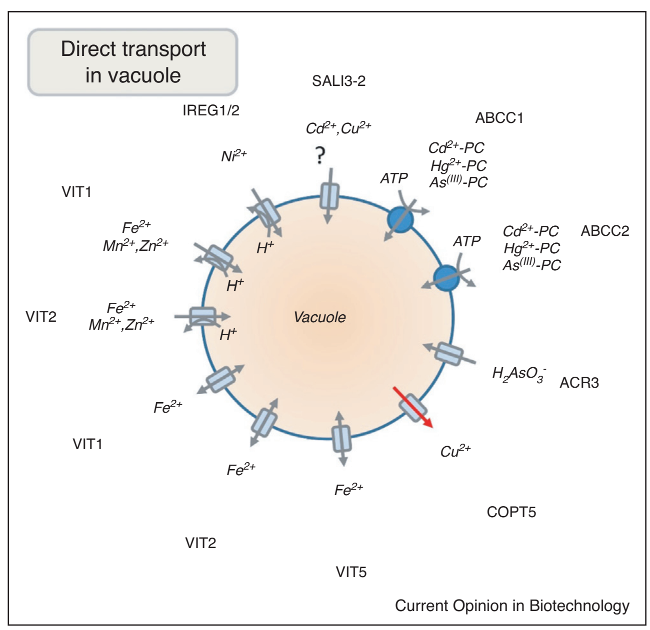
The mechanisms by which this detoxification is achieved can be: phytostabilization or phytoimmobilization, phytovolatilization, phytoextraction (applied mostly to soil, sediment and sludge), rhizofiltration, hydraulic barriers, hydraulic control, vegetative covers, constructed wetlands (applied mainly to polluted water), phytorestoration [14]. They have been studied and explained in recent decades for different combinations plant-heavy metal-environmental compartment. The most common phytoremediation techniques for removing heavy metals are

Figure 1



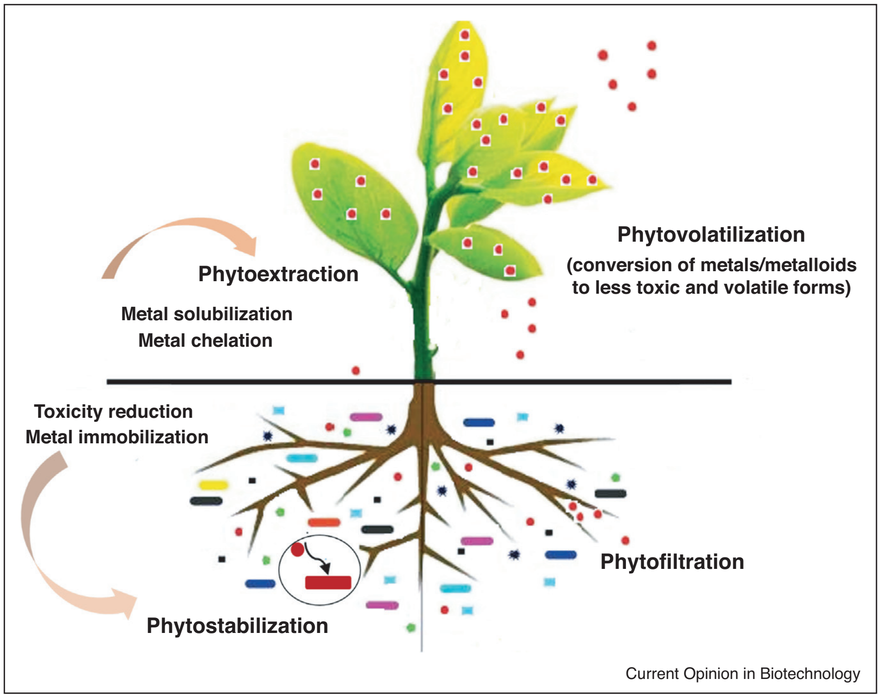
Tonoplast transporters of the MTP, CAX, MHX and NRAMP type that catalyse uptake or release of divalent HMs, for example, Zn^{2+}. Also indicated is the HMA3-primary pump, which carries divalent HMs including Zn^{2+} and Cd^{2+}, and the H^+-pumping V-ATPase and pyrophosphatase (PPase). ZIF1 transports nicotianamine into the vacuoles and eases Zn accumulation by complexation. (Sharma *et al.* [13••], Wiley License 5146690765407 of 12 September, 2021).

Figure 2



Tonoplast transporters of the VIT, VTL, COPT, IREG, SALI and ACR type, which preferentially carry Fe, Cu^{2+}, Ni^{2+} and arsenite. In addition, ABC pumps are shown, which translocate phytochelatin-metal complexes into the vacuolar lumen. (Sharma *et al.* [13••], Wiley License 5146690765407 of 12 September, 2021).

Figure 3



The most common phytoremediation techniques for removing heavy metals.

phytoextraction, *phytostabilization*, *phytovolatilization*, *rhizofiltration* (Figure 3).

Phytoextraction is a process for heavy metals removal by growing plants, when the metal is concentrated from the soil into the harvestable parts of roots and/or aboveground parts of the plant. The plants used for phytoextraction should tolerate the metal as well as possible, have the ability to accumulate as much metal (hyperaccumulators), grow quickly and produce as much biomass as possible [5]. However, among the hyperaccumulative plant species, not all can develop abundant biomass and for this reason high biomass hyperaccumulators are selected for application to a large scale, thus ensuring the efficiency of the phytoextraction process [15]. Phytoextraction has been investigated considerably and has shown that metals can be detoxified in certain zones of the plant, such as the epidermis, cuticle, trichomes. Sequestration of metals in plants is controlled by gene expression, which encodes proteins capable of excluding metals from the cytoplasm (e.g. proteins that transport metals to the tonoplast). However, not all elements that would explain the mechanisms of phytoextraction and bioaccumulation of plants in plants have been elucidated [16].

Vacuoles are considered to be the main place where metals accumulate, but metals can accumulate in cell walls and mucilaginous vesicles, as well, most often in the form of oxides. In addition, the mechanisms involving the chelation of metals with organic ligands (organic acids, amino acids, polypeptides, peptides) is considered responsible for the protection of active metabolic sites in the cytosol of plant cells from the toxic action of metals. In the absence of these mechanisms, plant exposure to the toxic effects of metals leads to the formation of reactive oxygen species and free radicals that affect cells, either by oxidizing amino acids, proteins, membrane fluids, DNA. All this prevents the normal development of plants. A remedy for this situation is the presence of glutathione which has the ability to lower the level of reactive species preventing the consequences of metal presence, but also to be a precursor of phytochelatin biosynthesis, which can bind metals [15].

Phytostabilization means the reduction of heavy metals mobility by plants, usually in the vadose zone, by fixing in the rhizosphere and/or accumulating them in the roots through various mechanisms, thus reducing soil contamination [17]. The immobilization of metals can be achieved by adsorption on the root surface, complexation (by organic acids produced by roots), precipitation in slightly soluble forms (metal complexes, carbonates, sulfides), accumulation in the root tissues. Some metals can be reduced to less toxic forms by redox enzymes (e.g. the toxic hexavalent chromium to trivalent chromium, less

Table 1

Different mechanisms involved in phytoremediation of environment polluted with toxic heavy metals

Plant	Environmental compartment polluted with heavy metals	Heavy metal	Phytoremediation mechanism	Reference
Garlic (*Allium sativum)*	Hydroponic solution	Cd	Phytoextraction	[22•]
Duckweed (*Lemna minor*)	Water		Rhizofiltration	[23]
Rice (*Oryza sativa*)	Soil		Phytoextraction	[24]
Vetiver grass (*Chrysopogon zizanioides*)	Soil		Phytostabilization	[25•]
Mustard greens (*Brassica juncea*)	Soil	Cr	Phytoextraction	[26]
	Water		Rhizofiltration	[27]
White horehound (*Marrubium vulgare*)	Soil	Hg	Phytoextraction	[28]
Water lettuce (*Pistia stratiotes*)	Water		Rhizofiltration	[29]
Stone herb (*Alyssum lesbiacum*)	Soil	Ni	Phytoextraction	[30]
Tall Wheatgrass (*Agropyron elongatum*)	Water		Rhizofiltration	[31]

toxic). Some plants, termed metal exclusion plants, have the ability to grow in soils contaminated with metals due to phytostabilization and maintain low concentrations in the above-ground parts. To exclude metals from tissues, plants develop strategies that involve plasma membranes, mycorrhizae, cell walls. These plants are able to protect metabolic sites like shoots through mechanisms that restrict the movement of toxic metals outside the rhizosphere or roots, but these mechanisms need to be monitored to ensure that there are no unwanted interferences.

Phytovolatilization involves the transformation of some metals absorbed by plants and translocated to the shoot, into less toxic and volatile forms, which can be released into the atmosphere by transpiration through the stomatal leaves [18]. In fact, the mechanism of this process consists in the assimilation of metals (a select group of heavy metals and metalloids: As, Hg, Se) into organic compounds with a certain volatility (amino acids, cysteine, methionine), achievable after a biomethylation reaction, resulting in biomolecules that reach the atmosphere [19]. The disadvantage of this mechanism is that it can generate toxic products for the atmosphere, although some research has considered that it is not necessary to assess the potential risk because these compounds are dispersed and diluted in the atmosphere.

Rhizofiltration involves a mechanism specific to some aquatic and land plants, capable of taking a large amount of water from the soil, which favors the precipitation of heavy metals (cadmium, chromium, copper, lead, nickel, zinc) on the surface of the roots or the absorption by the roots of soluble pollutants existing in solution surrounding the root zone (rhizosphere). For an efficient process, the surface area of the roots should be large enough [20,21].

Table 1 shows examples taken from the literature published in the last two decades on the phytoremediation of some of the most toxic heavy metals.

Therefore, the accumulation of heavy metals in plants is the consequence of processes that involve, as cited by Yan *et al.* [1••]: ‘heavy metal mobilization, root uptake, xylem loading, root-to-shoot transport, cellular compartmentation, and sequestration’. By releasing root exudates that can change the pH in the rhizosphere, plants are able to develop mechanisms to increase bioavailability of metals, which exist in the soil predominantly in insoluble forms, and this mechanisms have to be intensified. The capture and translocation of heavy metals in the plant are mediated by specialized transporters (protein channel), a variety of molecules located in the plasma membrane of the root cells, and also complexing agents.

Plants counteract heavy metals stress through morphological and physiological adaptations, which are transmitted by well-coordinated molecular mechanisms [12]. Plants react to the toxic action of heavy metals by developing adaptive and constitutive tolerance mechanisms, which should be accelerated for enhancing phytoremediation. The adaptive mechanism can include [8]: induction of stress proteins, synthesis of specific heavy metal transporters, immobilization, chelation and sequestration of heavy metals by specific ligands, and so on. Phytochelatins (PC) and metallothioneins (MT) are two classes of peptides that play important roles as metal chelators. Recent studies have reported the role of PCs and MTs in detoxifying heavy metals and developing plant tolerance to metals [6,32,33]. For example, cadmium toxicity induces rapid synthesis of PC that forms complexes with Cd and decreases its activity in the cytosol [8]. Metallothionein (MT) is another important peptide, with a low molecular weight and rich in cysteine, which plants use to combat the stress generated by the presence of heavy metals. The expressions of many MTs in plants is induced by metal stress, ensuring the increase of cell resistance to the toxicity of heavy metals. In the presence of heavy metal-induced toxicity, the metallothionein genes are transcriptionally activated, which protects cells from damage [9]. MT is involved in the homeostasis of essential heavy metals and protection against oxidative stress, and a number of recent researches highlight the important role played by this peptide in increasing plant tolerance to heavy metals [9,34].

Enhancing phytoremediation

The applicability of phytoremediation is unfortunately limited by the biological cycle of plants, remediation occurrence predominantly in the rhizosphere at shallow depths, and the concentration levels of heavy metals in the soil that cannot be too high due to their toxicity and limited bioavailability [2••,3,11]. The need to find feasible alternatives for enhancing phytoremediation stems from some limitations of traditional phytoremediation, related to long remediation time, variations in soil pH that influence the concentration and bioavailability of metal ions, insufficient knowledge about plants behavior and potential in phytoremediation, difficulties in the management plant crops.

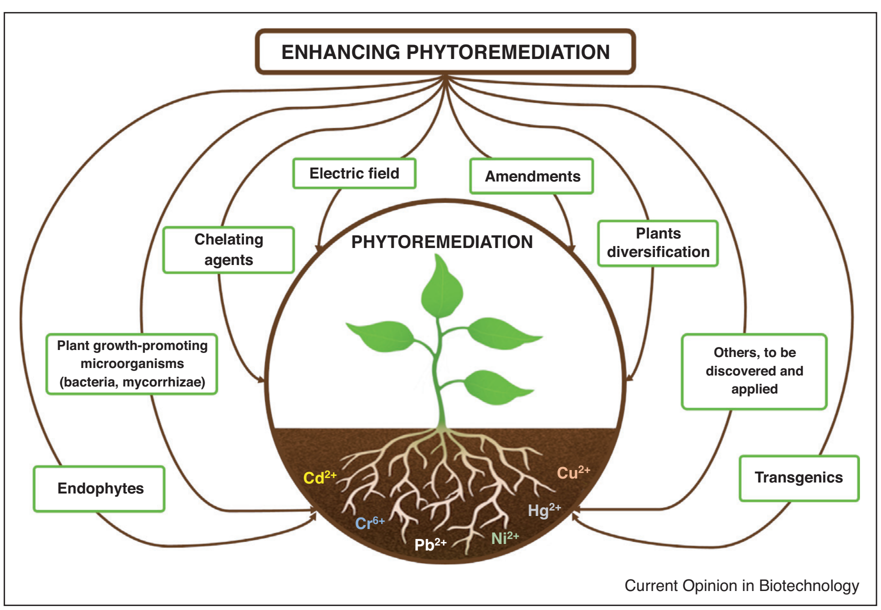
Recent research highlighted the need to intensify the phytoremediation efficiency, by applying strategies that favor and accelerate the process, such as: synergic growth of plants and plant-growth-promoting microorganisms (bacteria, mycorrhizae); addition of chelating agents; application of an electric field; addition of amendments; spatial planting patterns of different plant species; use of transgenic plants and so on (Figure 4). Hence, adaptation and combination of alternatives and eco-friendly strategies is greatly required. The purpose of this paper is to review some techniques for enhancing phytoremediation, discussed in the literature mostly in the last five years.

Enhancing phytoremediation with plant-grow-promoting microorganisms

Studies and experiments have shown that the toxic action of heavy metals, especially when found at high concentrations in water or soil, can affect the normal growth of the plant, the amount of biomass formed, and also can alter plant functions, including those that can allow to extract and accumulate metals. Because phytoremediation is generally a longer-term *in situ* process, achieving a synergism between plants and microorganisms (bacteria, fungi) can help to accelerate the heavy metals phytoremediation [11,35–37]. Microorganisms are contained and/or can be added in the soil adjacent to the plants root system thus building an important ecosystem which can significantly contribute to reducing the influence of factors such as soil chemistry, metal solubility and level of contamination oh phytoremediation efficiency.

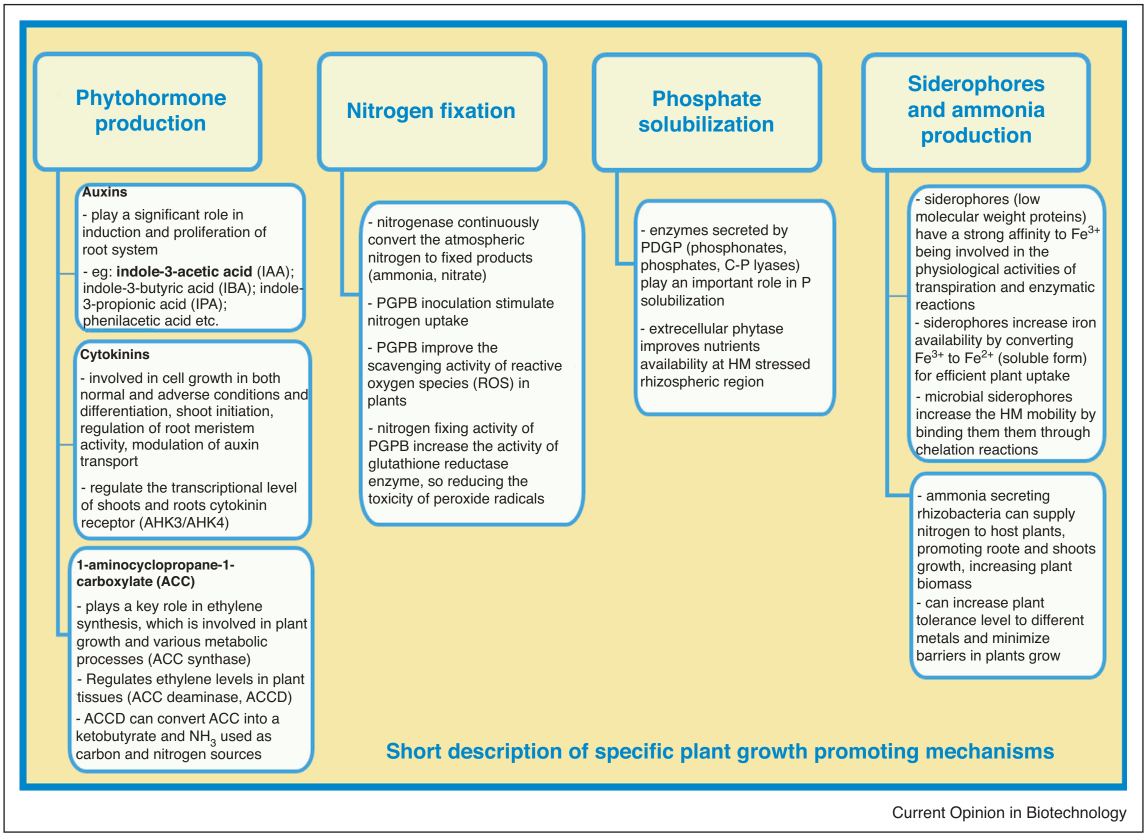
The use of plant-growth-promoting bacteria (PGPB) has attracted the attention, as an important alternative, increasingly applied, to improve biomass production and plant tolerance to heavy metals [38–40]. These bacteria undertake an extraordinary synergism with the plants and support them in their development, alleviating the stress of the plants in polluted environment, by: changing the oxidation state of some metals (e.g. Cr (VI)); phosphate solubilization; production of hydrogen cyanide, auxin (indole-3-acetic acid (IAA)), siderophores, ammonia; nitrogen fixation, and so on [11,40]. Figure 5 concisely describes the main contributions of plant-microorganism synergism to enhancing phytoremediation.

Figure 4



Strategies for enhancing the phytoremediation of soils polluted with heavy metals.

Figure 5



Plant growth promoting substances/metabolites like phytohormones, siderophores and ammonia which can increase the availability of nutrients in soil by phosphate solubilization, nitrogen fixation and organic compounds mineralization, promoting the plant growth.

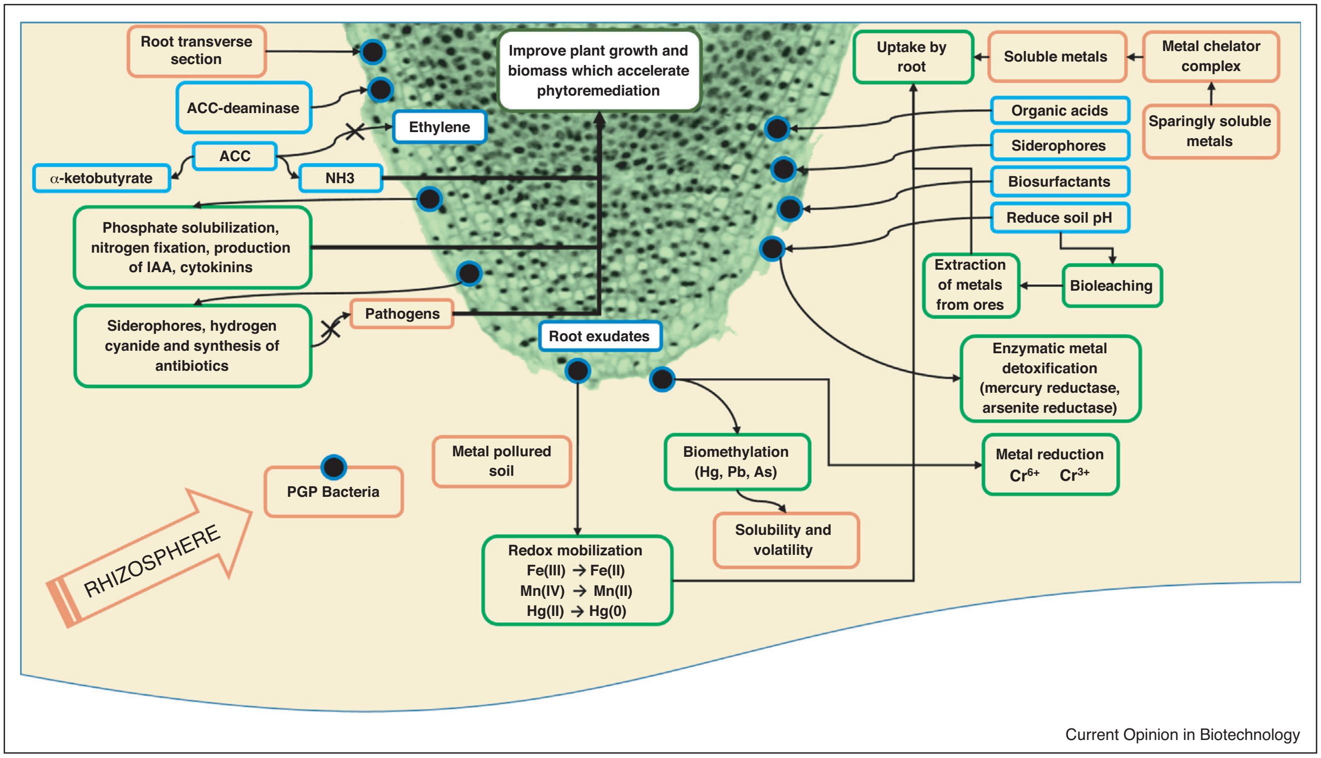
The action of PGPB is achieved either indirectly, by reducing the toxic effects of metals, or directly, by producing phytohormones. For example, PGPB can produce IAA, which supports the enhanced plant growth and better Cu and Zn absorption in *Brassica oxyrrhina* [41]. The ability of PGPB to counteract heavy metals interference with the absorption of nutrients such as phosphorus, by solubilizing phosphates plays a crucial role in improving P uptake by plants. Also, the production of phytohormones by PGPB, such as auxin IAA, even under the stress conditions generated by the presence of heavy metals, plays a key role in plant growth in soils contaminated with heavy metals. On the other hand, the roots of plants in the rhizosphere release phenolic compounds and also 20% of assimilated carbon, which could be used as a direct source of energy for soil microorganisms and metal ion chelating agents [42].

The synergistic effect is furthermore the consequence of the ability of bacteria to reduce the level of ethylene stress induced by heavy metals or the possible contribution of endophytes, through their ability to biosorption and bioaccumulation which diminishes the phytotoxic effects of metals. Another mechanism exploited by researchers is the production of siderophores that can stimulate plant growth, both directly, even in conditions of iron limitation, and indirectly by complexing metals into stable chemical forms, thus reducing the pressure of heavy metals on plants [2••,43••].

Figure 6 schematically illustrates the processes and reactions that take place at the plant root in the context of plant-PGPB synergism.

Although a number of PGPB strains are already applied in phytoremediation, there is a growing need to intensify studies and research to find new microbial resources, resistant to the toxic action of heavy metals, which can enhance the efficiency of phytoremediation, especially when the polluted environment contains mixtures of heavy metals. This means increasing concerns for isolating bacterial strains from polluted areas, exposed to toxic

Figure 6



Mechanisms and reactions occurring in rhizosphere of a plant used in phytoremediation in symbiosis with plant-grow-promoting bacteria (Ullah *et al.* [4], Elsevier License 5080380458402 of 1st of June, 2021).

industrial waste, selecting colonies of different morphologies and adapting them to new rhizosphere conditions [43••,44].

Apart from PGPB, various fungi (mycorrhizae) can contribute substantially to intensifying phytoremediation, especially as the network of mycorrhizal hyphae is more extensive than the root system of plants and has the ability to penetrate into relatively small pores of the soil, thus being able to enhance pollution absorption [43••,45]. Phytoremediation could be improved by the synergism between plants and adequate arbuscular mycorrhizae (AMF), ectomycorrhizae (ECM), dark septate endophytes (DSEs) [37]. They greatly improve the absorption of immobile nutrients, such as P by plant roots, improve soil properties and increase subsurface and above-ground biodiversity and resistance to pathogens. Arbuscular mycorrhizal fungi thus promote plant growth and dilute the negative effects of absorbed heavy metals, in a process known as the 'growth dilution effect' [46]. AMF avoids heavy metals transport from roots to stems, so regulating the partitioning of heavy metals. In addition, mycorrhizae can play a key role in increasing plant tolerance to heavy metals, accumulating and transporting them from the roots to the tops of plants.

New phytoremediation technologies assisted by microorganisms are based on genetically modified microorganisms, in which one or more genes are inserted that can, for example, encode biodegradative enzymes, metal absorption regulators, metal homeostasis, and so on [1••,4,38]. Advances in genetic engineering and molecular science can make significant contributions to increasing plant tolerance to heavy metals by modifying the genome using artificial nucleases, thus enhancing the phytoremediation potential.

Enhancing phytoremediation by using transgenics

The role played by genetic engineering in intensifying the capacity of some plants in the phytoremediation process is remarkable. By overexpressing the specific genes involved in the absorption, translocation, sequestration and tolerance of plants to the toxic action of pollutants, the efficiency of phytoremediation in significantly improved. Genetic engineering can provide viable solutions to enhance the potential of plants to detoxify soil and water contaminated with heavy metals since it has identified families of plant genes that may be involved in encoding metal ion transporters in hyperaccumulators. Thus, transgenic plants containing genes from microbes, plants, and animals have a better ability to cope with the phytotoxic effects of pollutants.

The involvement of genes in increasing the ability of plants to tolerate, remove, immobilize heavy metals can be effective in their absorption or detoxification. The mechanisms by which transgenic plants act on the metal are based on overexpressed genes involved in the biosynthesis of proteins and peptides that immobilize the metal. There are numerous studies on the role of single or multiple genes in improving plants ability to struggle metals toxic action so as to enhance their phytoremediation capacity. Singh *et al.* [47] demonstrated that transgenic wheat (*Triticum aestivum*) expressing gene OsNAS2 encoding for nicotianamine synthase 2 from rice (*Oryza sativa*) can achieve dietary significant levels of Fe and Zn in grains. The synergy between two genes introduced into plants was studied in the work of Grispen *et al.* [48], when *Arabidopsis thaliana* genes *AtMT2b* and *AtHMA4* encoding for a second b-class metallothionein and a stellar Zn^{2+} and Cd^{2+} transporting ATPase, respectively, were hosted into *Nicotiana tabacum* plants. The double transformants displayed double improved Cd tolerance and also enhanced Cd/Zn root-to-shoot transport.

Metals have individual molecular mechanisms for absorption, transport and sequestration, and genetic engineering can intervene to increase the number of adsorption sites, attenuation of competition with other ions by changing the specificity of the adsorption sites, increase the number of intracellular binding sites [5].

Enhancing phytoremediation by adding chelating agents

A new approach in enhancing phytoremediation is the integration of sludge treatment from wastewater treatment plants with the use in soil fertilization. Sludges, which have a certain load of heavy metals are converted into artificial soil by cultivating plants capable of extracting heavy metals. The selection of suitable plants for remediation and techniques for artificially improving phytoremediation capacity is the key to rapidly reducing the heavy metal content of artificial soil. Recent research has shown that the fast-growing plants turf grasses (tall fescue — *Festuca arundinacea*, ryegrass — *Lolium perenne* L.) have an extended root system and can develop large biomass. However, their phytoremediation capacity is relatively limited, but acceptable for single HM or heavy metal mixtures in sludge [42,49]. By artificial improvement of the extractive capacity of metals, by adding chelating agents, the remedial efficiency of herbs can be significantly improved. Provided that the rate of application of chelating agents is well controlled, their environmental risks can also be monitored.

Commonly used chelating agents are ethylene diamine disuccinic acid (EDDS), ethylene diamine tetraacetate (EDTA) and nitrilotriacetic acid (NTA) [50,51]. They can increase the solubility of heavy metals and therefore favor the extraction of heavy metals by plants. For example, one of the best known complexing agents, EDTA, has high complexing constants (log k) with heavy metals such as: Cu (18.8), Ni (18.5), Pb (18.0), Zn (16.4), Cd (16.4), and its presence in the soil seriously activates the mobility of heavy metals in the soil [52]. A series of studies analyzed by Li *et al.* [42] demonstrated that the addition of EDTA in concentrations predetermined by laboratory research, significantly increased the solubility of compounds with Cd and Pb in the soil and favored the absorption of Pb, Zn and Cd in rapeseed, corn and wheat.

Enhancing phytoremediation in electric fields

Phytoremediation has some inconveniences, mostly related to the low depth of the soil at which the plant roots can act on pollutants (20 cm, maximum 1 m). The soils polluted in depth with heavy metals cannot be cleaned exclusively by phytoremediation. Research has shown that the coupled technology electrokinetics-phytoremediation can help overcome this limitation of phytoremediation.

The impact on remediation efficiency is significant when applying an electric current (continuous or alternative) of low intensity because, in these conditions the contaminants can be mobilized and transported to the plant roots, increasing the capacity and intensity of phytoremediation. Also, the production of biomass increases either as a result of increased bioavailability of nutrients or the influence of electric current on enzymatic reactions, transport through membranes, water activity [3,53].

However, the application of this combined process requires a series of precautions because it is possible to inhibit the growth or cause death of plants in the immediate vicinity of the electrodes, since water electrolysis can occur, which generates acidic or alkaline pH. Also, the concentration of metals around the plant roots can increase the level of toxicity to plants, with unfavorable consequences on their growth. Unfortunately, the literature provides insufficient information to establish the optimal electric field strength to enhance phytoremediation without causing damage to plants, probably because this parameter depends largely on the properties of plants, contaminants and soil.

Enhancing phytoremediation by applying amendments

An older variant, but which continues to be considered and recommended in phytoremediation, consists in the application of amendments to intensify phytoremediation of soils contaminated with heavy metals, sometimes in the presence of microorganisms that promote plant growth (bacteria and fungi). For this purpose, conventional organic or inorganic materials (compost, ammonium nitrate, citric acid, titanium dioxide nanoparticles

(TiO_2NPs) etc.) with a large-specific area and with very good properties are used, which can produce soil changes, can adsorb and modify the speciation of some heavy metals. Also, these amendments can change the bioavailability of heavy metals either by their adsorption, by complexation with soil humic substances or by precipitation [42,54].

An example of such an amendment is biochar, a coal obtained by pyrolysis of plant biomass in the absence of oxygen, very well known in various applications, but especially in phytoremediation, being a stable solid, rich in carbon and able to remain in the soil for thousands of years. For this reason it is also used to improve the physico-chemical properties of soils and to provide nutrients for plants. Besides the fact that biochar can adsorb and retain metal ions from the water from soil pores due to the negatively charged surface, alkaline nature and other properties (nutrients and water-holding capacity, cation exchange capacity), it can provide favorable conditions in the soil, for beneficial microorganisms and plant roots development. Other potential benefits of biochar in the phyoremediation process refer to coprecipitation with phosphate, carbonates, silicate and chloride, formation of complexes with functional groups on biochar surface, the release of nutrients containing K, N, P, Ca, boosting phytostabilization by enhancing the reductive precipitation of metal ions (e.g. Cr (VI) to Cr (III)), while specific microorganisms can grow based on carbon from biochar [55].

Enhancing phytoremediation by diversification of plants species

The intensification of phytoremediation by applying spatial planting patterns of different plant species is a remediation strategy for heavily polluted soils with mixtures of heavy metals. Usually, in the phytoremediation process, the spatial distribution mode of the plants is insufficiently taken into account, so that the effects of some spatial patterns on the phytoremediation efficiency are not clear enough. There are also few studies on the opportunity to establish planting models for combining various species by intercropping, when plants grow together on the same land and time period (e.g. double intercropping involving the presence of two plant species, triple intercropping with three plant species) [56].

Lu *et al.* [57] established a planting strategy and evaluated the efficiency of phytoremediation for nine planting models (monoculture, double interculture and triple interculture) by multicriteria decision analysis. Three plant species (*Setaria viridis*, *Echinochloa crus-galli* and *Phragmites australis*) were planted and the results showed that combining them can significantly increase the migration rate of heavy metals in the soil and phytoremediation efficiency.

This approach can be also applied as phytoremediation strategy for multiple trace metal elements in soil. However, details on the mechanisms by which the combination of various cultivated plants contributes to metal uptake have not been fully elucidated.

Conclusions

Phytoremediation is an environmentally friendly bioremediation technique, which uses plants as hyperaccumulators plants to extract and accumulate heavy metals ions from soils in roots, shoots, leaves, flowers, fruits. Because of its advantages as an ecological process, but also to the disadvantages related to the long time required for the elimination of heavy metals, the threats that the toxicity of heavy metals can cause on plants integrity and their ability to absorb heavy metals, the need to intensify and accelerate phytoremediation emerged from research and applications. In this context, the paper examines some of the possibilities and strategies for enhancing phytoremediation and creates perspectives for the development of new strategies. The most common way for enhancing phytoremediation is to achieve a synergy between bacteria or fungi that can grow the rhizosphere and plants, which gives them greater resistance and better conditions for metal extraction by increasing their bioavailability and providing substances that facilitate phytoremediation. In addition to this strategy, other strategies can be applied, such as the administration of chelating substances and other amendments in the rhizosphere, the application of an electric potential to modify the mobility of heavy metal ions and soil conditions (electro-kinetic remediation coupled with phytoremediation), the use of multiple plants in various combinations. Also, research in genetic engineering and molecular science can make substantial, innovative contributions to increasing the impact of phytoremediation as a beneficial technique for improving and increasing soil quality, but also as a means for recovering critical metals from heavy metal polluted areas

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Yan A, Wang Y, Tan SN, Mohd Yusof ML, Ghosh S, Chen Z:
•• **Phytoremediation: a promising approach for revegetation of heavy metal-polluted land.** *Front Plant Sci* 2020, **11**:359 http://dx.doi.org/10.3389/fpls.2020.00359
The paper describe in a widespread way the mechanisms of heavy metals uptake, translocated, and detoxified in plants. Also, it focuses on the strategies applied to improve the phytoremediation efficiency, including

the application of genetic engineering, microbe-assisted and chelate-assisted approaches.

2. •• Manoj SR, Karthik C, Kadirvelu K, Arulselvi PI, Shanmugasundaram T, Bruno B, Rajkumar M: **Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: a review**. *J Environ Manage* 2020, **254**:109779 http://dx.doi.org/10.1016/j.jenvman.2019.109779

The paper is a comprehensive review that reveals very well that the applicability of phyto-remediation in contaminated sites is restricted by primary factors. It also provides clear information about the molecular mechanisms exerted by PGPR strains in promoting plant growth and phytoremediation of heavy metals.

3. Cameselle C, Gouveia S, Urrejola S: **Benefits of phytoremediation amended with DC electric field. Application to soils contaminated with heavy metals**. *Chemosphere* 2019, **229**:481-488.

4. Ullah A, Heng S, Munis MFH, Fahad S, Yang X: **Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review**. *Environ Exp Bot* 2015, **117**:28-40.

5. Suman J, Uhlik O, Viktorova J, Macek T: **Phytoextraction of heavy metals: a promising tool for clean-up of polluted environment?** *Front Plant Sci* 2018, **9**:1476 http://dx.doi.org/10.3389/fpls.2018.01476.

6. Rosatto S, Mariotti M, Romeo S, Roccotiello E: **Root and shoot response to nickel in hyperaccumulator and non-hyperaccumulator species**. *Plants* 2021, **10**:508 http://dx.doi.org/10.3390/plants10030508.

7. Mahajan P, Kaushal J: **Role of phytoremediation in reducing cadmium toxicity in soil and water**. *J Toxicol* 2018, **2018**:4864365 http://dx.doi.org/10.1155/2018/4864365.

8. Lal S, Ratna S, Ben Said O, Kumar R: **Biosurfactant and exopolysaccharide-assisted rhizobacterial technique for the remediation of heavy metal contaminated soil: an advancement in metal phytoremediation technology**. *Environ Technol Innov* 2018, **10**:243-263.

9. Liu Y, Kang T, Cheng JS, Yi Y-J, Han J-J, Cheng H-L, Li Q, Tang N, Liang M-x: **Heterologous expression of the metallothionein *PpMT2* gene from *Physcomitrella patens* confers enhanced tolerance to heavy metal stress on transgenic Arabidopsis plants**. *Plant Growth Regul* 2020, **90**:63-72.

10. Dong Q, Xu PX, Wang ZL: **Differential cadmium distribution and translocation in roots and shoots related to hyper-tolerance between tall fescue and Kentucky bluegrass**. *Front Plant Sci* 2017, **8**:113 http://dx.doi.org/10.3389/fpls.2017.00113.

11. Diaconu M, Pavel LV, Hlihor RM, Rosca M, Fertu DI, Lenz M, Corvini PX, Gavrilescu M: **Characterization of heavy metal toxicity in some plants and microorganisms-a preliminary approach for environmental bioremediation**. *New Biotechnol* 2020, **56**:130-139.

12. Kosakivska IV, Babenko LM, Romanenko KO, Korotka IY, Potters G: **Molecular mechanisms of plant adaptive responses to heavy metals stress**. *Cell Biol Int* 2021, **45**:258-272.

13. •• Sharma SS, Dietz K-J, Mimura T: **Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants**. *Plant Cell Environ* 2016, **39**:1112-1126

The paper is a very good synthesis of literature that makes a critical analysis of existing information on vacuolar responses to stress induced in plants by the presence of heavy metals in the environment, covering the molecular and organellar perspective.

14. Li X, Wang X, Chen Y, Yang X, Cui Z: **Optimization of combined phytoremediation for heavy metal contaminated mine tailings by a field-scale orthogonal experiment**. *Ecotoxicol Environ Saf* 2019, **168**:1-8.

15. Sabir M, Waraich EA, Hakeem KR, Ozturk M, Ahmad HR, Shahid M: **Phytoremediation: mechanisms and adaptations**. In *Soil Remediation and Plants. Prospects and Challenges.* Edited by Hakeem KH, Sabir M, Ozturk M, Mermut A. Elsevier; 2015:85-105.

16. Robinson BH, Anderson CWN, Dickinson NM: **Phytoextraction: where's the action?** *J Geochem Explor* 2015, **151**:34-40.

17. Monaci F, Trigueros D, Mingorance MD, Rossini-Oliva S: **Phytostabilization potential of *Erica australis* L. and *Nerium oleander* L.: a comparative study in the Riotinto mining area (SW Spain)**. *Environ Geochem Health* 2019, **42**:2345-2360.

18. Van Oosten MJ, Maggio A: **Functional biology of halophytes in the phytoremediation of heavy metal contaminated soils**. *Environ Exp Bot* 2015, **111**:135-146.

19. Guarino F, Miranda A, Castiglione S, Cicatelli A: **Arsenic phytovolatilization and epigenetic modifications in *Arundo donax* L. assisted by a PGPR consortium**. *Chemosphere* 2020, **251**:126310 http://dx.doi.org/10.1016/j.chemosphere.2020.126310.

20. Oustriere N, Marchand L, Roulet E, Mench M: **Rhizofiltration of a Bordeaux mixture effluent in pilot-scale constructed wetland using *Arundo donax* L. coupled with potential Cu-ecocatalyst production**. *Ecol Eng* 2017, **105**:296-305.

21. Sampaio CJS, de Souza JRB, Damião AO, Bahiense TC, Roque MRA: **Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in a diesel oil-contaminated mangrove by plant growth-promoting rhizobacteria**. *3 Biotech* 2019, **9**:155 http://dx.doi.org/10.1007/s13205-019-1686-8.

22. • Jiang W, Liu D, Hou W: **Hyperaccumulation of cadmium by roots, bulbs and shoots of garlic**. *Bioresour Technol* 2001, **76**:9-13

The authors employed an interesting approach on the uptake and accumulation of Cd^{2+} by garlic roots, bulbs and shoots and found provoking results: Cadmium stimulated root length at lower concentrations, the seedlings exposed to higher (two orders) Cd^{2+} exhibited substantial growth reduction. Concentrations in bulbs and shoots were low, the plants carried only a small amount of CD in these parts of the plant. The study demonstrates the complexity and peculiarities of phytoremediation, the results of which largely depend on the characteristics of the soil, metal and plants.

23. Hou W, Chen X, Song G, Wang Q, Chang CC: **Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*)**. *Plant Physiol Biochem* 2007, **45**:62-69.

24. Murakami M, Ae N, Ishikawa S: **Phytoextraction of cadmium by rice (*Oryza sativa* L.), soybean (*Glycine max* (L.) Merr.), and maize (*Zea mays* L.)**. *Environ Pollut* 2007, **145**:96-103.

25. • Lai H-Y, Chen Z-S: **Effects of EDTA on solubility of cadmium, zinc, and lead and their uptake by rainbow pink and vetiver grass**. *Chemosphere* 2004, **55**:421-430

The authors study the influence of EDTA solution on phytoextraction and phytostabilization in two categories of plants. The results showed that the concentrations of Cd, Zn and Pb in plants increased significantly after the addition of EDTA, but differently for each metal, while the absorption mechanisms is specific to each plant.

26. Zhang X-H, Liu J, Huang H-T, Chen J, Zhu Y-N, Wang D-Q: **Chromium accumulation by the hyperaccumulator plant Leersia hexandra Swartz**. *Chemosphere* 2007, **67**:1138-1143.

27. Diwan H, Ahmad A, Iqbal M: **Genotypic variation in the phytoremediation potential of Indian mustard for chromium**. *J Environ Manage* 2008, **41**:734-741.

28. Moreno-Jimenez E, Gamarra R, Carpena-Ruiz RO, Millan R, Penalosa JM, Esteban E: **Mercury bioaccumulation and phytotoxicity in two wild plant species of Almaden area**. *Chemosphere* 2006, **63**:1969-1973.

29. Skinner K, Wright N, Porter-Goff E: **Mercury uptake and accumulation by four species of aquatic plants**. *Environ Pollut* 2007, **145**:234-237.

30. Singer AC, Bell T, Heywood CA, Smith JAC, Thompson IP: **Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant Alyssum lesbiacum: evidence of histidine as a measure of phytoextractable nickel**. *Environ Pollut* 2007, **147**:74-82.

31. Axtell NR, Sternberg SPK, Claussen K: **Lead and nickel removal using Microspora and Lemna minor**. *Bioresour Technol* 2003, **89**:41-48.

32. Dvorak M, Schnegg R, Niederwanger M, Pedrini-Martha V, Ladurner P, Lindner H, Kremser L, Lackner R, Dallinger R:

Cadmium pathways in snails follow a complementary strategy between metallothionein detoxification and auxiliary inactivation by phytochelatins. *Int J Mol Sci* 2020, **21** http://dx.doi.org/10.3390/ijms21010007.

33. Navarrete A, González A, Gómez M, Contreras RA, Díaz P, Lobos G, Brown MT, Sáez CA, Moenne A: **Copper excess detoxification is mediated by a coordinated and complementary induction of glutathione, phytochelatins and metallothioneins in the green seaweed Ulva compressa**. *Plant Physiol Biochem* 2019, **135**:423-431.

34. Ziller A, Fraissinet-Tachet L: **Metallothionein diversity and distribution in the tree of life: a multifunctional protein**. *Metallomics* 2018, **10**:1549-1559.

35. Kaur P, Singh S, Kumar V, Singh N, Singh J: **Effect of rhizobacteria on arsenic uptake by macrophyte *Eichhornia crassipes* (Mart.) Solms**. *Int J Phytoremediation* 2017, **28**:114-120.

36. Ma Y, Rajkumar M, Moreno A, Zhang C, Freitas H: **Serpentine endophytic bacterium Pseudomonas azotoformans ASS1 accelerates phytoremediation of soil metals under drought stress**. *Chemosphere* 2017, **185**:75-85.

37. Khalid M, Ur-Rahman S, Hassani D, Hayat K, Zhou P, Hui N: **Advances in fungal-assisted phytoremediation of heavy metals: a review**. *Pedosphere* 2021, **31**:475-495.

38. Sobariu DL, Tudorache Fertu DI, Diaconu M, Pavel LV, Hlihor RM, Drăgoi EN, Curteanu S, Lenz M, Corvini PFX, Gavrilescu M: **Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation**. *New Biotechnol* 2017, **39**:125-134.

39. Hassan TUI, Bano A, Naz I: **Alleviation of heavy metals toxicity by the application of plant growth promoting rhizobacteria and effects on wheat grown in saline sodic field**. *Int J Phytoremediation* 2017, **19**:522-529.

40. Montalban B, Thijs S, Lobo C, Weyens N, Ameloot M, Vangronsveld J, Perez-Sanz A: **Cultivar and metal-specific effects of endophytic bacteria in Helianthus tuberosus exposed to Cd and Zn**. *Int J Mol Sci* 2017, **18**:2020 http://dx.doi.org/10.3390/ijms18102026.

41. Ma Y, Rajkumar M, Zhang C, Freitas H: **Beneficial role of bacterial endophytes in heavy metal phytoremediation**. *J Environ Manage* 2016, **174**:14-25.

42. Li F-L, Qiu Y, Xu X, Yang F, Wang Z, Feng J, Wang J: **EDTA-enhanced phytoremediation of heavy metals from sludge soil by Italian ryegrass (*Lolium perenne* L.)**. *Ecotoxicol Environ Saf* 2020, **191**:110185 http://dx.doi.org/10.1016/j.ecoenv.2020.110185.

43. •• Franco-Franklin V, Moreno-Riascos S, Ghneim-Herrera T: **Are endophytic bacteria an option for increasing heavy metal tolerance of plants? A meta-analysis of the effect size**. *Front Environ Sci* 2021, **8**:603668 http://dx.doi.org/10.3389/fenvs.2020.603668

The paper analyzes data published in research papers from the last decade on the extent of the effects of the presence of endophytic bacteria to increase plant tolerance to heavy metal toxicity. The analysis highlights the general positive effects of inoculation with endophytic bacteria on the growth and tolerance to heavy metals in different plant species, highlighting the influence of the type of host plant (hyperaccumulator or nonhyperaccumulator) and heavy metal properties.

44. Sarma H, Islam NF, Prasad R, Prasad MNV, Ma LQ, Rinklebe J: **Enhancing phytoremediation of hazardous metal(loid)s using genome engineering CRISPR–Cas9 technology**. *J Hazard Mater* 2021, **414**:125493 http://dx.doi.org/10.1016/j.jhazmat.2021.125493.

45. Garcia-Sanchez M, Kosnar Z, Mercl F, Aranda E, Tlustos P: **A comparative study to evaluate natural attenuation, mycoaugmentation, phytoremediation, and microbial-assisted phytoremediation strategies for the bioremediation of an aged PAH-polluted soil**. *Ecotoxicol Environ Saf* 2018, **147**:165-174.

46. Pedrosa Gomes M, Zorman Marques R, Cristina Nascentes C, Scotti MR: **Synergistic effects between arbuscular mycorrhizal fungi and rhizobium isolated from As-contaminated soils on the As-phytoremediation capacity of the tropical woody legume *Anadenanthera peregrine***. *Int J Phytoremediation* 2020, **22**:1362-1371 http://dx.doi.org/10.1080/15226514.2020.1775548.

47. Singh SP, Keller B, Gruissem W, Bhullar NK: **Rice Nicotianamine synthase 2 expression improves dietary iron and zinc levels in wheat**. *Theor Appl Genet* 2016, **130**:283-292.

48. Grispen VMJ, Hakvoort HWJ, Bliek T, Verkleij JAC, Schat H: **Combined expression of the Arabidopsis metallothionein MT2b and the heavy metal transporting ATPase HMA4 enhances cadmium tolerance and the root to shoot translocation of cadmium and zinc in tobacco**. *Environ Exp Bot* 2011, **72**:71-76.

49. Zhang J, Yang N, Geng Y, Zhou J, Lei J: **Effects of the combined pollution of cadmium, lead and zinc on the phytoextraction efficiency of ryegrass (*Lolium perenne* L.)**. *RSC Adv* 2019, **9**:20603-20611.

50. Tananonchai A, Sampanpanish P, Chanpiwat P, Tancharakorn S, Sukkha U: **Effect of EDTA and NTA on cadmium distribution and translocation in *Pennisetum purpureum* Schum cv. Mott**. *Environ Sci Pollut Res* 2019, **26**:9851-9860.

51. Jiang M, Liu S, Li Y, Li X, Luo Z, Song H, Chen Q: **EDTA-facilitated toxic tolerance, absorption and translocation and phytoremediation of lead by dwarf bamboos**. *Ecotoxicol Environ Saf* 2019, **170**:502-512.

52. Chauhan G, Pant KK, Nigam KDP: **Chelation technology: a promising green approach for resource management and waste minimization**. *Environ Sci Processes Impacts* 2015, **17**:12-40.

53. Mao X, Han FX, Shao X, Guo K, McComb J, Arslan Z, Zhang Z: **Electro-kinetic remediation coupled with phytoremediation to remove lead, arsenic and cesium from contaminated paddy soil**. *Ecotoxicol Environ Saf* 2016, **125**:16-24.

54. Gul I, Manzoor M, Kallerhoff J, Arshad M: **Enhanced phytoremediation of lead by soil applied organic and inorganic amendments: Pb phytoavailability, accumulation and metal recovery**. *Chemosphere* 2020, **258**:127405 http://dx.doi.org/10.1016/j.chemosphere.2020.127405.

55. Sun W, Zhang S, Su C: **Impact of biochar on the bioremediation and phytoremediation of heavy metal(loid)s in soil**. In *Advances in Bioremediation and Phytoremediation.* Edited by Shiomi N. IntechOpen; 2018:149-168.

56. Bian F, Zhong Z, Wu S, Zhang X, Yang C, Xiong X: **Comparison of heavy metal phytoremediation in monoculture and intercropping systems of Phyllostachys praecox and Sedum plumbizincicola in polluted soil**. *Int J Phytoremediation* 2018, **20**:490-498.

57. Lu J, Lu H, Li J, Liu J, Feng S, Guan Y: **Multi-criteria decision analysis of optimal planting for enhancing phytoremediation of trace heavy metals in mining sites under interval residual contaminant concentrations**. *Environ Pollut* 2019, **255**:113255 http://dx.doi.org/10.1016/j.envpol.2019.113255.