



Bio- and phytoremediation: plants and microbes to the rescue of heavy metal polluted soils

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Abstract

Bio- and phytoremediation, being encouraging terms implying the use of biological systems for cleansing purposes, have risen a worthy venture toward environmental restoration in discouraging scenarios, such as the augmentation of indestructible heavy metals. Hyperaccumulating plants and heavy metal resistant microbes own mechanisms embedded in their metabolism, proteins, and genes that confer them with “super characteristics” allowing them to assimilate heavy metals in order to amend polluted soils, and when combined in a symbiotic system, these super features could complement each other and be enhanced to overpower the exposure to toxic environments. Though xenobiotic pollution has been an object of concern for decades and physicochemical procedures are commonly carried out to offset this purpose, a “live” remediation is rather chosen and looked upon for promising results. A variety of benefits have been registered from symbiotic relationships, including plants teaming up with microbes to cope down with non-biodegradable elements such as heavy metals; but a carefully maneuvered interaction might signify a greater insight toward the application of bioremediation systems. These manipulations could consist of genetic engineering and/or additional supplementation of molecules and microbes. In the present study, a contemporary connection between plants and microbes involving their controlled management is summarized in a visionary display.

Keywords Bioremediation · Phytoremediation · Heavy metals · PGPMs · GMOs

1 Introduction

The unbridled extension and careless management of industrial, agricultural, and anthropogenic activities have derived into an overshoot of xenobiotic substances present in former healthy environments. Overage of contemporary pollutants leads to concerning implications covering an extensive invasion of said substances, triggering long-term implications on human and environmental

health; a subject of matter requiring immediate remedy, as well as new clean-up technologies.

Whereas some polluting agents can be easily treated, some elements cannot be degraded, which is the case with heavy metals. Their environmental accumulation favors their dispersion through water and soil, compromising the life of the organisms. In this sense, human health is mainly threatened by lead, cadmium, mercury, and arsenic exposure [30]. These metals are comprised

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in a hazardous category and are visualized as primary threats due to their toxicity to both humans and plants [13]. The gravity of this type of contamination signifies tissue bioaccumulation in living organisms that brings about a biomagnification in the trophic levels, not mentioning the transcendental diseases that heavy metals provoke and their outlet into water consume featuring their involvement in water cycles [32].

Outdated methods against heavy metal pollution consist of landfilling, which is the construction of cells usually built of cement or steel, to confine the contaminants with barriers [18]. Besides being an uneconomical choice, the "solution" means a temporary cover up. Furthermore, the risks implied in secondary pollution and workers integrity have lessened the attractiveness on maintaining the execution of these processes. Other efficiency-questioned methods include physicochemical separation, membrane separation, solvent extraction, ion exchange, and other physicochemical and electrochemical methods [9]. Wetlands are also commonly employed for heavy metal removal through reactions of sedimentation, flocculation, absorption, co-precipitations, cation and anion exchange, precipitation, and oxidation/reduction; along with microbiological activity and plant uptake [47]. According to Brar et al. [9], bioremediation was already the most adopted method of decontamination with a 32% of preference next to chemical treatments (14%), in situ thermal treatment (6%), soil vapor extraction (6%), thermal desorption (5%), solidification/stabilization (5%), permeable reactive barriers (4%), flushing (3%), off gas treatment (3%), soil washing (3%), phytoremediation (3%), multi-phase extraction (2%), pump and treat (2%), vitrification (2%), and others (10%).

Compared to traditional methods of remediation implying a physicochemical mix of procedures, the use of "living" remediation is rather chosen. Though heavy metals cannot be fully removed, particular organisms contribute to their neutralization in order to reduce their harmful effect on the environment [64].

Bioremediation is therefore defined as the elimination of contaminants using biological systems by their break down, transformation or degradation. Modalities of the process are split into in situ and ex situ remediation. Ex situ remediation consists of the transportation of contaminated matter to another site for further treatment, while in situ remediation implies the procedures to be performed in the place of origin. [32].

Budding from this biotechnological subject, phytoremediation and more elaborated cleansing techniques also take place to this purpose. The upgrowth of bioremediation techniques utilizing plants with microbes is the outcome of the pursuit for alternative methods with clean-up

purposes that has lead to the development of bioaugmentation or rhizoremediation [35].

Microorganisms (comprehending bacteria, yeast, fungi, and even archaeon) and plants are the imperative tools required to remediate industrial wastes such as heavy metals, pesticides, toxic chemical fertilizers, among others [33, 86], due to their resemblance as biological catalysts in a bioremediation system [84] arranged by suitable components to fix contaminated environments, meaning microorganisms that are able to take up and transform heavy metals, as well as suited plants to remove and restrain metals from the ground [33].

Though heavy metals appear to be complex pollutants to handle, bioremediation is an attractive method to mend soils contaminated by them, and furthermore, the combination of both microorganisms and plants is an approach to ensure a more efficient clean-up [13].

Consequently, the handling of plants and their associated microorganisms to restore heavy metal polluted settings is a field of expansion that provides beneficial aspects corresponding to cost-effectiveness, sustainability, and environmental implications [1]. The information provided in the present review has a bioremediation approach in soils.

2 Heavy metals

Heavy metals belong to a group of elements characterized by a high atomic weight and density, corresponding to above 5 g/cm^3 [52]. From 90 elements that occur naturally, 21 are non-metals, 16 are light-metals, and 53 are designated as heavy metals [89]. These elements can be categorized in two main groups: the ones that could be required in minimal quantities by some organisms (including Co, Cu, Fe, Mn, Mo, Ni, V, and Zn) and those that are considered as entirely menacing (Pb, Cd, Hg, and As) [13].

Natural liberation of these elements occurs by volcanic activity, comets, erosion, and the weathering of minerals. Natural states of heavy metals are not ready for plant uptake or available for living organisms; but anthropogenic activities involving battery production, mining, explosive manufacturing, use of pesticides, phosphate fertilizers, sewage irrigation, steel and electroplating industries, textiles and wood preservation, among others; lead to the excessive liberation of these elements and contribute to their accumulation and dispersion. These activities put in risk all life forms when exceeding normal levels, leading to threatening consequences in their composition [5].

These elements are endured in different ways, depending on the organism they face. Beginning with human vulnerability, heavy metals are conferred with an

intriguing toxicity according to their bioavailability and their lipid solubility. Molecular affections resulting from heavy metal exposure consist of their attachment to the binding sites of proteins; removing other molecules and causing cellular malfunction [32]. The incomplete d orbitals of most heavy metals allow them to form complex compounds in the cells, leading to toxic effects [52]. Although the intracellular concentration of metal ions can sometimes be controlled by protein families in the cells, such as the ABC, P-type ATPases or RND proteins; their effect on living organisms can be counterproductive. Major affections are caused by dermal contact, ingestion or inhalation, wherein absorption of heavy metals induces the transport of these elements and distributes them through body tissues, persisting in organs such as bones, liver, or kidneys [32].

Highlighting the overriding elements of concern and their fatalities, Arsenic is a carcinogenic, cardiovascular and neurobehavioral disorder trigger; besides, intoxication by this element is surprisingly ordinary. As for Cadmium, exposure to this metal derives into lung and stomach cancer, osteoporosis, chromosomal aberrations and damage, as well as liver, kidney, lungs, and heart dysfunction. Lead, however, causes anemia and conditions related to kidney and liver illness, as well as to the reproductive, gastrointestinal, and central nervous system maladies. Least but not less important, Mercury, a remarkable toxic topic, is known to develop neurotoxic effects and is absorbed by the gastrointestinal tract to reach the brain, kidneys, liver, and nervous tissue [32], a threatening element to adipose tissue.

In a vegetative point of view, metal ions are known to damage cell membranes, inhibit enzymatic and photosynthetic activities, and drive the generation of reactive oxygen species in plants. These stress effects make plants more susceptible to climate change, as well as plant productivity [65].

As for microbes, bacteria and fungi can take up elements that might not be vital for microbial metabolism from the soil and store them, such as Cd, Ni, or Cu [33]. Cd & Hg are not required for biological functions [67], yet they can be accumulated, as well as Au, Cs, and U [64]. But on the other hand, some other metals could be required in their metabolic processes as essential micronutrients in trace concentrations, like Co, Cr, Ni, Fe, Mn, and Zn [53].

Since they cannot be destroyed or decomposed, bioremediation principally involves processes such as reducing the solubility of these metals through pH and redox reactions modulation, mobilization, and immobilization, as well as the confinement of the contaminants from polluted environments [28], among other mechanisms furtherly described.

Though the profile that is built around heavy metals seems discouraging, the literature proposes a transformation ratio, by microorganisms alone, of 27% for Cr, 20% for Co, 31% for Cd, 22% for Pb, 7% for Ni, 5% for Zinc, and about 18% for As & Cd [64]. If an additional setting is built where these microorganisms team up with the proper plants, a powerful combination is set on board, but understanding their individual capacities first is in order.

3 Phytoremediation and hyperaccumulating plants

The plants that are utilized for heavy metal removal in phytoremediation usually possess a rapid growth rate, high biomass production, extensive root system, and high heavy metal tolerance [46]. These plants are defined as hyperaccumulators. In order to point out a hyperaccumulator, it needs to meet a higher concentration than 0.1% for Al, As, Co, Cr, Cu, Ni, and Se; higher than 0.01% for Cd, and higher than 1.0% for Zinc, in their shoot [6]. They can transport and accumulate from 100 to 1000 times higher metal concentrations than non-accumulating ones without suffering apparent phytotoxic effects.

Some mechanisms involved in metal detoxification in plants consist of plant cell wall binding, active transport of ions into cell vacuoles, intracellular complexation with peptide ligands such as phytochelatin (PCs) and metallothioneins (MTs), and sequestration of metal-siderophore complexes in root apoplasm or soil [40]. Others consist of exudate production, one of the most critical strategies in plants to tolerate high metal concentrations [40], especially low molecular weight organic acids (LMWOAs) like citric, oxalic, malic, and succinic acid [48], to detoxify As, Cd & Pb [49]. Naturally involved proteins and molecules in plant metabolism when in heavy metal exposure are summarized in Table 1.

Plants provide numerous ways of opposing contaminants, as shown in Table 2.

Hyperaccumulating plants of toxic heavy metals are distributed in about 45 plant families, including *Scrophulariaceae*, *Lamiaceae*, *Fabaceae*, *Asteraceae*, *Euphorbiaceae*, and *Brassicaceae*, but about 500 plant species have been used for this purpose [4]. Examples of hyperaccumulating plants (and their remarkable accumulation capacities in mg/kg^{-1}) include *Corrigiola cheiradenia* (2110 of As), *Euphorbia cheiradenia* (1138 of Pb), and *Azolla pinnata* (740 of Cd) [83]. However, more commonly known hyperaccumulators include the sunflower (*Helianthus annuus*), brown mustard (*Brassica juncea*), or duckweed (*Lemna minor*); in addition with a growing protagonism of legumes for the task [36].

Table 1 Proteins involved in heavy metal tolerance and resistance in plants

Type of Proteins	Function	Elements involved	Reference
NRAMP OPT MATE ZIP ZTR/IRT HMA4	Transporters	Se, As, Cd, Ni, Zn, Mn, Co, Fe	[60]
PC MT GSH PAL oxML LMWC	Detoxification		

NRAMP, Natural resistance-associated macrophage protein, *OPT*, oligopeptide transporters, *MATE*, multidrug and toxic compound extrusion, *HMA4*, heavy metal ATPase 4, *PC*, phytochelatin, *MT*, metallothionein, *GSH*, glutathione, *PAL*, phenylalanine ammonia-lyase, *oxML*, oxy monolignol, *LMWC*, low molecular weight compounds

Ricinus communis L. & *Helianthus annuus*, for instance, have been used for Cd removal, as well as Zn. Multimetal removal can also be performed, an example is *Suaeda salsa* with Cu, Zn, As, & Cr (Zhang 2019) and *Pennisetum giganteum* with Cu, Pb, and Ni [76].

4 Heavy metal bioremediation mechanisms in microorganisms

Microbes may own biochemical or molecular mechanisms to bear up against contaminants. In a biochemical appraisal, microorganisms are able to carry out

processes of acidification, precipitation, chelation, and complexation. Resistance activity can also be described in mechanisms as cell surface biosorption, cell transporting systems via active efflux pumping of metals out of the cell, sequestration of metals in intracellular compartments, exclusion of metal chelates into the extracellular space, and enzymatic redox reactions to convert a metal ion into a non-toxic or less toxic state [40]. Due to the multiple oxidation states that inorganic compounds display, redox reactions occur to reach the least hazardous stage, where commonly, the higher oxidation state, the less soluble [64], therefore, more dangerous. However, their ability to degrade or restrain and transform pollutants (according to their organic or inorganic nature depends on the suitability of environmental conditions for their growth and metabolism, which include conditions such as temperature, pH, moisture [86], as well as density and type of charge in soil colloids [46], soil aeration, mineral composition and microbial activity [44].

Potential microorganisms with remarkable transforming capacities include *Cupriavidus metallidurans*, *Pseudomonas aeruginosa*, *Pseudomonas putida* (referring to bacteria), *Aspergillus versicolor*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Penicillium chrysogenum* (referring to fungi), *Candida utilis*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae* (referring to yeast), and Phylum *Crenarchaeota* sp. & *Phanerochaete chrysosporium* (referring to archaea) [64].

Regarding a molecular perspective, some microorganisms own heavy metal resistance genes and/or proteins that provide them of unique characteristics to endure these elements. Examples are provided in Tables 3 and 4.

Table 2 Bioremediation mechanisms in plants

Mechanism	Description	References
Phytoaccumulation or Phytoextraction	Pollutant take up in plant biomass, taken from the soil through the roots into upper plant components	[46, 71, 2, 13, 40, 56, 24]
Phytofiltration	Represents three types of filtration in plants: Rhizofiltration (use of roots), blastofiltration (use of seedlings), and caulofiltration (use of excised plant shoots)	
Rhizofiltration	Elimination of toxic substances from groundwater through root filtration by terrestrial and aquatic plants	
Phytostimulation	Use of exudates from plant roots to stimulate microbial activity and enhance it	
Phytostabilization	Immobilization of metal by plants, reducing their bioavailability; turning them into less harmful and preventing their spread in the environment	
Phytovolatilization	Pollutants taken from the soil are transformed into volatile forms and transpired into the atmosphere, mainly Hg & Se	
Phytodegradation	Breakdown of organic contaminants into non-hazardous forms by plant enzymes	
Detoxification	Involves processes such as adsorption, chelation, transformation, and inactivation of metals	

Table 3 Protein superfamilies that contribute to heavy metal resistance in microorganisms

Protein family	Characteristics	Heavy metal resistance	References
RND Transenvelope efflux	Transport proteins constituted by MFP, RND, and OMF	Ni, Co, Zn, Cd, Ag	[11, 29, 39, 52, 53, 70, 54, 46]
P-type ATPases	Bidirectional transport of metal ions	Ion substrates such as H ⁺ , Na ⁺ , K ⁺ , Mg ²⁺ , Ca ²⁺ , Cu ⁺ , Ag ⁺ , Zn ²⁺ , Cd ²⁺	
CDF Efflux proteins	Cation diffusor facilitator driven by chemiosmotic forces	Zn, Cd, Co, Fe	
ABC transporters	Uptake and efflux of metal ions	Mn, Zn, Ni, Fe	

OMF, Outer membrane factors, MFP, membrane fusion protein family, RND, resistance, nodulation, and cell division

Table 4 Examples of genes and proteins that provide heavy metal resistance to microorganisms

Gene	Protein	Type	Microorganism	Heavy metal resistance	References
<i>nccTXH</i>	nccCBA	RND RND	<i>C. metallidurans</i>	Ni, Co	[55]
<i>cnrYXH</i>	czcCBA		<i>C. metallidurans</i>	Co, Zn, Cd and Ni	[54]
<i>cus</i>	CusCFBA proteins	RND	<i>Escherichia coli</i>	Co & Ag	[50]
<i>mer</i>	Mer proteins (MerA, MerB, MerC, MerD, MerE, MerT, MerP)	Mercuric reductase	<i>Proteobacteria, Firmicutes, Actinobacteria</i>	Hg	[3]
<i>CadA & CadC</i>	CadA & CadC	P-Type ATPase	<i>Staphylococcus aureus</i>	Cd, Cu, Pb, Zn	[25]
<i>NikA, NikB, NikC, NikD, NikE</i>	NikA, NikB, NikC, NikD, NikE	ABC Transporters	Archaea & fungi	Ni	[68]
<i>NixA</i> gene	High-affinity nickel-transport protein NixA	Nickel-dependent regulatory protein	<i>Helicobacter pylori</i>	Ni	[23]
<i>HoxN</i> gene	HoxN protein	High-affinity nickel transport protein	<i>Alcaligenes eutrophus, Yersinia pestis, Ralstonia eutropha</i>	Ni/Co resistance	[10]
<i>yohM</i> gene	yohM protein	Ni/Co efflux system	<i>E. coli</i>	Ni/Co resistance	[27]
ars operon	ArsA, ArsB, ArsC	ATPase	<i>P. putida, Bacillus subtilis, P. aeruginosa, E. coli</i>	Arsenic resistance	[7]

5 Plants team up with microbes

Plant-associated microbial diversity, and overall host-microbiome interactions, is a product of evolutionary and ecological events [73, 77]. They are so intimately related that it is almost impossible to separate them as components of the ecosystem, even in modern research, they are nominated as eco-holobionts [77]. Since microbes are ubiquitous, almost any other organism is colonizable, and although this party can become both positive and negative, it is most beneficial and symbiotic [77]. With plants, complex communities are formed in an effort to achieve a healthy state of the environment [34].

Broadly, microbes protect and stimulate plants, while plants provide them with nutrients; but having so much potential belonging to fascinating organisms, this symbiotic interest cannot only happen for survival purposes, but for greater benefits, including heavy-metal bioremediation.

Individual microbial and plant properties that promote heavy metal bioremediation have already been mentioned. In an environmental turn, hyperaccumulating plants alone already imply an approach to remove contaminants, but due to high heavy metal toxicity, the association with specialized microorganisms improves the chances of conquering this target. When these organisms interact with one another, new and enhanced processes occur to arrest heavy metals, leading to multiple cleansing options, where the use of phytoremediation and microbes are a key in providing a multielement remediation option for anthropologically contaminated landscapes [62].

In order to reach this objective and to operate as a directed bioremediation symbiotic system, specialized microbes promote plant activity and survival. They are categorized as Plant Growth-Promoting Microorganisms (PGPMs).

6 Plant growth-promoting microorganisms (PGPMs)

A variety of microbes are considered beneficial for plants, like Plant Growth-Promoting Bacteria (PGPB) and Rhizobacteria (PGPR), Nitrate Fixation Bacteria (NFB), Arbuscular Mycorrhizal Fungi (AMF), and Siderophore Producing Bacteria (SPB), mainly. They are capable of influencing plant activity in terms of root exudate composition modification, growth enhancement [81], and systemic resistance induction to subsequent pathogen attack [15], among other reactions shown in Table 5.

Plant growth promotion is influenced by the plant's rhizosphere, composed of the overall plant roots and its exudates [85] including sugars, amino acids, organic acids, fatty acids, phenols, enzymes, and flavonoids, and lead by PGPR with the production of phytohormones like cytokinins, ethylene, and gibberellic, jasmonic, salicylic or abscisic acid [45, 59, 17]. PGPR have the ability to affect post-embryonic root development too. They can alter cell division and differentiation in the primary root, root hair, and lateral roots; they can also promote shoot growth, but they do not only stimulate root growth and development [85].

Among root exudate communication with microorganisms, flavonoids play a key component in plant–microbe interactions such as mycorrhiza formation, the establishment of legume-rhizobia symbiosis [80], AMF spore germination, hyphal growth, differentiation, and root colonization in AMF-plant interactions [63].

Other processes, derived from quorum sensing, also occur in plant–microbe interactions. For instance, Acylated Homoserine Lactones (AHLs) control bacterial traits such as symbiosis, virulence, competence, conjugation, mobility, sporulation, biofilm, and antibiotic production [19], and when recognized by plants, modulation of tissue-specific gene expression, plant growth homeostasis, and defense responses are triggered [14].

In a bioremediation aspect, PGPB possess single or multiple traits of heavy metal toxicity alleviation, alteration of metal availability, production of siderophores, biochelation, fixation of nitrogen, and solubilization of mineral nutrients [41]. Most of the bacteria located in the rhizosphere of plants are naturally tolerant to environmental contaminants, which is why they play an essential role in organic and heavy metal removal [56].

Table 5 summarizes PGPM activity.

Commonly used and widely studied bacteria to enhance plant growth and activities include *Pseudomonas*, *Enterobacter*, *Arthrobacter*, *Glucanoacetobacterium*, *Flavobacterium*, *Beijerinckia*, *Klebsiella*, *Erwinia*, *Bacillus*, *Serratia*, among others [4].

7 Plant–microbe interaction for bioremediation purposes

In order to survive in toxic and nutrient-limited environments, many plants interact with rhizospheric and root-associated microbes [1], even from where this interaction's bioremediation purpose originates from. The potential resistant or stimulating microbial rhizosphere communities emerge and develop during plant growth in the same polluted areas [35]. However, the insertion of a microbial strain for xenobiotic degrading activities on plant seeds to further colonize the root and remain on the root system can also be performed [56].

The plant communicates with the microbes during its growth through root exudates and diverse molecules to stimulate the survival and action of bacteria, enhancing pollutant confinement, transformation or degradation [35]. These molecules act as chelating agents and can enhance the phytoavailability of pollutants [1], as well as their mobility [56]. For this reason, such microorganisms' performance is a vital role in phytoremediation systems.

The use of plants for the treatment of contaminated soils with heavy metals or radionuclides like sewage sludge, power plants, or nuclear power reactors wastes [28] is a more conventional approach in bioremediation, and the combination of microorganisms and plants is an approach that ensures more efficient clean-up [13]. Having this in mind, we can present the following definitions:

7.1 Bioaugmentation

The bioaugmentation method improves the degradation and enhances the transformation rate of xenobiotics by the insertion of specific microorganisms [35]. Unlike phytoremediation, which occurs when plant enzymes establish the degradation of pollutants, the attenuation or bioaugmentation occurs when the microbial population performs such degradation. The re-introduction of protective microbes also benefits plant development by creating optimal conditions for crop growth under harsh conditions, hence reducing water, fertilizer, and pesticide use [34].

When a suitable rhizospheric strain is introduced into a suitable plant, the bacteria might settle on the root along with the indigenous population, enhancing the bioremediation process. Then, the efficient root-colonizing pollutant bacteria would profit from the growing root system, spreading through the soil [35]. However, consortia of bacteria often cause the degradation of a pollutant, mainly because several bacterial populations degrade pollutants more efficiently than a single strain [56].

Table 5 Role of PGPMs in plant growth and bioremediation

Mechanism	Type	Effect	References
Siderophore production	SPB	Siderophores as metal chelators increase the solubility of nutrients, enhancing metal accumulation in plant tissues and solubilizing unavailable forms of heavy metals by complexation	[16, 74]
Phosphate solubilization	PGPM	Insoluble metal sulfides can precipitate When phosphorus is unavailable for plants: PSMs convert phosphorus to a soluble form for plants PSB inhabit the rhizosphere and supply phosphorus to the plant	[40] [56]
ACC Deaminase production	PGPR	It allows plants to mitigate abiotic stress conditions and stimulates plant growth by breaking ACC (ethylene precursor that inhibits plant growth), developing the plant root system	[56] [22]
IAA production	PGPR	Production and release of auxins such as IAA, stimulate the growth of the root system and protect the plant from abiotic stress	[78]
ROS balancing	PGPR	ROS regulates the transition from proliferation to differentiation independent of auxin and cytokinins, and PGPR may alter ROS balance within the root resulting in growth and developmental changes	[72]
Endogenous mechanisms manipulation	PGPR	Bacteria manipulate the hosts' inner mechanisms to regulate post-embryonic root development to alter root growth	[85]
Production of biochelators	PGPB	Production of organic acid or biosurfactants	[40]
Defense mechanism induction	PGPM	PGPMs stimulate plant growth indirectly via the induction of defense mechanisms against phytopathogens	[40]
Quorum sensing	PGPM	Diketopiperazines (DKPs) and N-Acyl-homoserine lactones (AHLs) compounds are produced by numerous bacterial species involved in quorum sensing	[85]
Metal bioavailability	PGPM	Change in metal bioavailability in the soil through various biochemical and molecular mechanisms	[40]
Metal toxicity immobilization and transformation	AMF	Metal induced toxicity reduction, metal availability change through alteration of soil pH, and metal translocation affection	[12] [65] [40]
Plant activity enhancement	AMF	Boosting plant growth and nutrient uptake	[58] [12]
VOC emission (Acetoin and 2,3-Butanediol)	PGPB	Establish communication with plants, trigger plant defense, and growth promotion mechanisms by enabling host plants to colonize nutrients or soils	[40]
VOC emission	PGPB	Impact to act as bioprotectants (induced systemic resistance, ISR), biopesticides (antibiotic functions), and phytoestrogens (triggering hormonal networks)	[69] [82] [91] [40]
VOC emission	PGPB	Ability to affect mycelium growth and sporulation of different fungal species (Ammonia, Butyrolactones, Hydrogen cyanide, Phenazine-1-carboxylic acid, or Alcohols)	[31] [40]
Signaling molecules	AMF & Rhizobia	Synthesis of Myc (AMF) and Nod (Rhizobia) factor can modulate root system architecture, such as stimulation of lateral root branching, the formation of new organs, and nodule	[57] [40]
Fertility improvement	NFB	Enhance biological Nitrogen and improve the fertility of polluted soils for plant growth by catalyzing the reduction of atmospheric N ₂ to biologically available ammonium	[40] [51]
Production of Biosurfactants	PGPR	Microbial metabolite production that facilitates metal mobilization by complexation of metals in their nonionic forms to remove them from surfaces, acting as soil washing agents	[74]
Microbial EPS Production	PGPR	EPS can sequester metal ions by their functional groups such as carboxyl, phosphoric, amine, and hydroxyl groups that are naturally negatively charged to form organo-metallic complexes	[74]

ACC, 1-Aminocyclopropane-1-carboxylate, IAA, Indole-3-Acetic Acid, PSMs, Phosphate-Solubilizing Microorganisms, PSB, Phosphate-Solubilizing Bacteria, ROS, Reactive Oxygen Species, SPB, siderophore Producing Bacteria, VOC, Volatile Organic Compounds, NFB, Nitrogen Fixating Bacteria, EPS, Extracellular Polysaccharide Substances

7.2 Rhizoremediation

When phytoremediation and bioaugmentation combine, rhizoremediation takes place. During this process, exudates derived from plant roots can improve efficiency of phytoremediation [34] and spread the bacteria through the soil. Substantial degradation of pollutants is due to the microbes living in the rhizosphere, dominated by Gram-negative rods such as *Pseudomonas* spp. [35], but the first studies toward the degradation of compounds in the rhizosphere were mainly focused on herbicides and pesticide removal [35], rather than heavy metal remediation. For instance, it has been proven to work against trichloroethylene (TCE), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) [35], and for PCB rhizoremediation, sugar beet was inoculated with *P. fluorescens* [8]. Providing an example with heavy metals, Cd²⁺ was rhizoremediated using *Astragalus sinicus* and *Mesorhizobium huakuii* [79].

Retaking the importance of the individual mechanisms previously presented, the gathering of these qualities and their potential for environmental engineering purposes will be understood.

Probably the main mechanism of bioremediation by microbes is chelation. Plants and microbes can release organic chelating compounds such as metal-binding compounds, organic acid anions, siderophores, biosurfactants, and metallophores [75], to take metals ions and prevent them to be resorpted [20]. Metallophores are LMWOLs released from microorganisms that can regulate intracellular metal concentrations to avoid toxicity or maintain appropriate concentrations for their growth [40]. Organic acids involved in rhizospheric processes also promote heavy metal detoxification and mobilization or solubilization in soil [65]. Bioaccumulation is characterized by the metabolic activities involving the use of MTs and PCs for intracellular sequestration, extracellular precipitation, metal accumulation, and complex formation [20]; these mechanisms are useful for plant metal toxicity reduction and uptake [41].

As for metal immobilization mechanisms, plants teamed up with microorganisms can promote the precipitation of radionuclides and toxic metals by enzymatic catalysis or reduction processes [61]. In precipitation, bacteria protect the host plant against the inhibitory effect of heavy metals [40]. Besides the use of enzymes, insoluble mineral forms of radionuclides, and metals can be immobilized by bacterial Fe oxidation or interactions between inorganic acids such as hydrogen sulfide, bicarbonate, and phosphate [92]. These last components interact with dissolved metals and form insoluble precipitates. SRB can also influence in this mechanism, where bacteria can solubilize minerals, promote plant growth, and immobilize metals in soil in phytostabilization [92].

In complexation, extracellular polysaccharide substances (EPS) secreted by plant-associated microbes form a protective barrier against harmful effects through metal biosorption through mechanisms like ion exchange, adsorption, and precipitation [26]. Microbial biosorption consists of the use of living or dead biomass and their components to remove or recover metals and metalloids. Biosorption also contributes to heavy metal speciation, defined as the identification and quantification of metals to determine their bioavailability and therefore, their toxicity in terms of pH, temperature or time; complying information regarding their specie, form or phases in which they occur [5]. EPSs are a key point for biosorption, and most bacteria produce them. These exopolymers are able to create strong bonds with trace elements and form organo-metallic complexes. Also, biofilms (communities of microorganisms) produce EPS and adsorb heavy metals in a better way than the planktonic species. EPS are composed by polysaccharides, mucopolysaccharides, humic substances, and proteins. Quorum sensing defines the production of EPS by two systems, the LasI/LasR and RhII/RhIR. Microorganisms such as *Rhizobium meliloti*, *Pseudomonas* sp., *Cupriavidus paulucus*, *Azotobacter* sp., are reported to produce EPS [74].

When speaking of heavy metal speciation, these may be organized according to their biosorption behaviors in different pH (Table 6).

Table 6 Classes of metal species and their pH biosorption behaviors

Class	pH bonding	Characteristics	Examples	References
I	Bonds strongly in a neutral medium	Taken up from biological substrates at pH < 2	$Al^{3+}, Cu^{2+}, Cd^{2+}, Cr^{3+}, Co^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Zn^{2+}$	[43, 42, 90]
II	Bond strongly at low pH but weakly at pH > 5	Binding due to protonated nitrogen atoms of amine groups	$PtCl_4^{2-}, CrO_4^{2-} \& SeO_4^{2-}$	
III	Binding independent to pH	Biosorption due to covalent bonds between the metals and ligands on cell surface	$Ag^+, Hg^{2+} \& AuCl_4^-$	

Heavy metal speciation improves their biosorption while profiling the conditions at which they can be adsorbed, facilitating the manipulation and enhancement of the process.

Continuing with immobilization processes, alkalization affects stability in soil. For instance, AMF and bacteria can absorb metals through substrate alkalization activity by releasing OH⁻ to activate nitrate uptake by microbes and reduce metal phytoavailability in the rhizosphere by secreting glomalin [21], an insoluble metal-sorbing glycoprotein that reduces metal mobility or sequesters metals [88].

On the other hand, in terms of acidification, pH represents an essential aspect for soil remediation effectivity, as well as the mobility, and solubility of metals [66]. Mobilized metals increase pH, and plant roots secrete hydrogen ions that can displace heavy metal cations adsorbed in soil, which leads to rhizosphere acidification; however, root exudates lower pH [40].

In a briefly summarized picture, Fig. 1 describes the benefits of microbes teaming up with plants.

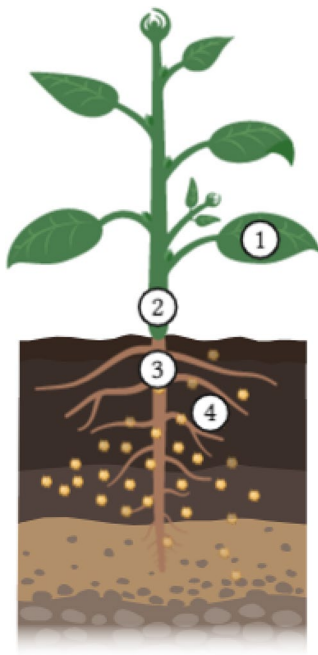


Fig. 1 PGPMs enhance phytoremediation processes in plants by plant biomass expedition (1), bioaccumulation or metal translocation facilitation from soil to root (2), translocation or transport from root to shoot tissues (3), increase in heavy metal availability in soil by phytoextraction or decrease in metal availability in soil in phytostabilization (4)

8 Visionary super powers

Phytoremediation assistance includes PGPMs, heavy metal accelerators, transgenic plants, nanoparticles [37], chelating agents, and even biochar addition [76]. Phytoremediation expands an “old process” that occurs naturally, but in a manipulated and conscious way, manual supplementation and genetic engineering could contribute to the development of a perfect bioremediation system. Once that Bioremediation mechanisms proper of plants and microorganisms are fully understood, their controlled combination can be standardized to be applied in situ or ex situ. Edited plants, microorganisms and enzymes to degrade harmful substances and facilitate remediation processes, as well as creating biomarkers to detect contamination of a particular type, are a field of expansion. All of these aspects, combined, can create a potential weapon against contamination of non-biodegradable elements, as well as organic contaminants.

Also, PGPMs, by displaying numerous plant benefits, suggest that the identification of the responsible genes could be selected from these microbes, which deliver future insights to discover preferential associations between specific genes and phytobeneficial traits. Initially, the attractive characteristics to be taken and exploited with bioremediation efforts consisted on the plant’s growth rate, leading to a focus of identifying the responses of high biomass expedition, but lately, this interest leans more strongly to metal accumulating genotypes.

There are four main approaches related to the development of genetically engineered microorganisms with Bioremediation focus: Bioaffinity bioreporter sensor applications in chemical sensing, end point analysis and toxicity reduction; bioprocess development, monitoring and control; enhancement of affinity and enzyme specificity; and construction and pathway regulation [87].

Table 7 summarizes genetically engineered organisms with heavy metal remediation purposes.

Though Genetic Engineering sounds like a lovely bet to modify and increase organism’s bioremediative characteristics, there are limiting factors for their development. For instance, revising microbes, such microorganisms are suggested to include suicidal genes to avoid horizontal gene transfer with native microbes in order to prevent the native microbial population displacement. Also, modified microbes cannot be liberated into polluted areas, and one of the reasons includes the hostile environment that this signifies for them [87]. In the case of bioaugmented plants, these must coexist with its natural rhizobacteria. However, the perfection and continuous research about these mechanisms is still necessary for new proposed solutions.

Table 7 Examples of genetically engineered organisms with heavy metal remediation characteristics in modern times

Organism	Gene/Protein	Bioremediation Trait	References
<i>E. coli</i>	ArsR Manganese transporter MT MerR Phytochelatin Synthase (SpPCS) Cd Transporter (MntA) Lead-binding peptide	As(III), As(V), Cd ²⁺ , Pb ²⁺ , Hg ²⁺ Synthesis of PC and MT	[90, 87, 60]
<i>Saccharomyces cerevisiae</i>	MT tandem repeats <i>Arabidopsis thaliana</i> Phytochelatin Synthase (AtPCS) Arsenic transporters Fps1p and Hxt7p Pb transporting P1-type ATPases	Cd ²⁺ , As, Pb ²⁺ PC MT	
<i>P. putida</i>	Monkey MT α tandem repeats	MT Cd ²⁺	
<i>Mesorhizobium huakuii</i> subsp. <i>Rengei</i>	<i>Arabidopsis thaliana</i> PCS; PCSAt	PC	
<i>Rhodospseudomonas palustris</i>	pEBZ141 from <i>Alcaligenes eutrophus</i> Mercury transport system and MT	Cr, Hg transport, MT, Hg ²⁺	
<i>Pseudomonas fluorescens</i> O58	MerR CadC ZntR Pmer PcadA PzntA	Cd, Pb, Hg, Zn	
<i>Deinococcus radiodurans</i>	merA	Hg(II)	
<i>Arabidopsis thaliana</i>	MerC AdPCs1–2–3 ZmUBP15–16–19 PCs1 CsCCoAOMT YSL SsMT2	Hg, Cd, Na	

As a closure, what comes after phytoremediation activities is as important as the remediation itself. This last procedure also needs to encourage more research to achieve optimal remediation processes. Harvesting of plants used in phytoremediation of heavy metals creates heavy contaminated bio-waste [37], but processes to treat them are proposed, including heat treatment, incineration, pyrolysis, gasification, or carbonization [37]. In these processes, fly ash is released, containing heavy metals and discharging them. The higher the temperature, the higher vaporization of the HM, where the remaining ashes are bioleached [37].

During pyrolysis, biomass is decomposed at elevated temperatures, resulting with biochar, bio-oil and pyrolysis gases. However, heavy metals enter biochar and the distribution is similar to incineration. In this process, the temperature is a key factor to decrease risk potential. An additional use of the resulting biochar could include the plant-stimulation and biochemical improvement of soil during phytoremediation of heavy metals.

As it can be perceived, part of an efficient plant–microbe team up involves multiple variables toward a futuristic insight of environmental amendment.

9 Conclusion

Modern bioremediation does not longer include plants and/or microbes solely, but proposes a mixture of both organisms along with biochemical, ecological and genetic engineering aspects, along with nanotechnology. The use of biotechnology for the restoration of polluted sites is a modern alternative to contamination cleansing. Urges related to pollution and its consequences lead to new options and technological adaptations, where the combination of microorganisms and plants is an exciting update to this task. In addition, microbial and/or plant efficiency can be improved with genetic engineering assistance. Deeper research still needs to be conducted to clarify doubts about the safety of GMO release; in the meantime, the gathering of scientific

disciplines makes the building of new tools an intriguing step toward this goal. Whereas phytoremediation and bioremediation using microorganisms provide numerous possibilities for contamination removal, the combination of both is translated into an enhanced process. Plant–microbe interaction has been present in nature in different ways, especially in a rhizospheric aspect, and the forms in which these interactions favor each other are quite impressive. A close-up to understanding these interactions has opened doors to further opportunities where the use of PGPMs and hyperaccumulating plants manipulated in a specific way could mean the primary tool for the success of a combined cleansing system. It provides a high number of perspectives and applications to develop more robust bioremediation activity toward heavy metal contamination, and of course, other sources of contamination. Since modern approaches keep on surging, genetically engineered plants, microbes, or enzymes could be right around the corner for huge phytoremediation applications. Advances of this nature imply the manipulation of traits such as chelating properties or new technological derivatives like nano-phytoremediation and biochar-assisted phytoremediation. The understanding and discovery of the presented mechanisms can lead us toward a restoration future, where the recipe of the perfect system involving hyperaccumulating plants, microbial genes and enzymes, hormone and chelating agents supplementation among multiple mechanisms used as ingredients, could be cooked.

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Declarations

Conflict of interest The authors declare that there are no conflict of interests.

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