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Phytoremediation of Metal and Metalloid Pollutants from Farmland: An *In-Situ* Soil Conservation

Selvanayaki Krishnasamy, Ramkumar Lakshmanan and Mythili Ravichandran

Abstract

Phytoremediation is an effective technology for *in-situ* remediation of high level polluted soils. Phytoremediation is a plant-mediated approach, which involves the use of plants to absorb and remove elemental pollutants or lower their concentration or bioavailability to soil. Plants have efficacy to absorb compounds in the soil even at low concentration through their root system. Plant root system has geotropism which helps them to extend into the soil matrix and hyper accumulate heavy metals to increase their bioavailability considerably and thereby the polluted soil is domesticated and the soil fertility is enhanced. The heavy-metal-resistant endophytes give the promising effect on plant growth, by decreasing metal phytotoxicity and affecting metal translocation and accumulation in plants. It is an eye opening for researches to implement the phytoremediation of organic contaminants through endophytes that produce various enzymes to metabolize organic contaminants and reduce both the phytotoxicity and evapotranspiration of volatile contaminants. Here, we focus on the most widely used phytoremediation strategies, phytostabilization, phytoextraction, phytovolatilization, and phytofiltration in the remediation of heavy metal-polluted soil.

Keywords: phytoremediation, endophytes, phytostabilization, phytoextraction, phytovolatilization, phytofiltration

1. Introduction

Urbanization and industrialization lead to pollution that make water, air, and soil contaminated with high levels of heavy metals, organic and inorganic materials. These cause bioaccumulation and biomagnification in the ecosystem which in turn reflect in many health issues like colon cancer, heart diseases, liver, and kidney malfunction. These pollutions are solved by various methods such as removal, isolation, incineration, solidification –stabilization, vitrification, thermal treatment, solvent extraction, chemical oxidation, etc. To implement these methods several sophisticated techniques with skilled manpower are needed. They involve the transport of contaminated materials to treatment sites thus, adding risks of secondary

contamination. *In situ* techniques that are eco-friendly and more economical can be used to minimize the problem. In this scenario, biotechnology offers a technique for phytoremediation [1].

Toxic substances that are released from various industrial effluents are loaded in the water bodies. When they enter into the surrounding agricultural fields during irrigation with heavy metals (Pb, Zn, Cd, Cu, Ni, Hg), metalloids (As, Sb), inorganic compounds, radioactive chemical elements (U, Cs, Sr), petroleum hydrocarbons (BTEX), pesticides and herbicides (atrazine, bentazone, chlorinated and nitroaromatic compounds), explosives (TNT, DNT), chlorinated solvents (TCE, PCE) and industrial organic wastes (PCPs, PAHs) pollute the land [2]. Phytoremediation can be considered as one of the effective phenomena in regenerating soil fertility.

Phytoremediation is an efficient phenomenon in which the plant (trees, shrubs, grasses, and aquatic plants) and their associated microorganisms undergo metabolic pathway to remove, degrade or isolate toxic substances from the environment using effective enzymes including both intra and extracellular enzymes [2, 3]. The word “phytoremediation” is coined from the Greek word ‘phyton’, meaning ‘plant’, and Latin ‘remedium’, which means ‘to remedy’ or ‘to correct’. As the meaning indicates heavy metals and the unusual compounds that are transported to cultivated land by the polluted water bodies are converted into nontoxic through phytoremediation. When they are bio-accumulated they are metabolized by the heavy-metal-resistant endophytes. Endophytes play a key role in the reduction and in the decrease of metal phytotoxicity and affect metal translocation which is accumulated in plants. The plant role in phytoremediation and the removal of accumulated toxicity in soil is as follows: modifying the physical and chemical properties of contaminated soils, releasing root exudates and thereby increasing organic carbon, improving aeration by releasing oxygen directly to the root zone, as well as increasing the porosity of the upper soil zones, intercepting and retarding the movement of chemicals, effecting co-metabolic microbial and plant enzymatic transformations of recalcitrant chemicals and decreasing vertical and lateral migration of pollutants to groundwater by extracting available water and reversing the hydraulic gradient [4, 5]. Strategies of phytoremediation and the efficacy of endophytes will enhance the understanding level paving way for further study.

2. Phytoremediation-based strategies

2.1 Phytodegradation (phytotransformation)

A number of plant and microbial enzymes play a major role in degrading (metabolized) or mineralizing the contaminants which are hyper accumulated inside the plant cells. Phytoremediation mostly mediated by the group of enzymes are well documented. It is understood from Nitroreductases degradation of nitroaromatic compounds and glycosyltransferase that bioactivity of plant hormones are altered by glycosylation. This has been reviewed for plant hormones such as auxins, cytokinins, gibberellins and abscisic acid [6] and glutathione transferases (GSTs) that controls the internal cell pressure due to chemical-induced toxicity. It protects cell and provides tolerance by catalyzing S-conjugation between the thiol group of GSH and electrophilic moiety in the hydrophobic and toxic substrate [2].

Oxidases (Metal-modifying enzymes) which is involved in the assimilation of heavy metals into organic molecules (e.g., selenate is metabolized to dimethyl selenide), or in changing the oxidation state of metals e.g., toxic Cr (VI) is reduced to nontoxic Cr (III) [3]. Phosphatases, nitrilases and dehalogenases play a vital role in

the transformation and conjugation of explosives and dehalogenases degradation. These enzymes are involved in the transformation of toxic xenobiotic compounds such as explosives, pesticides, nerve gases, and halogenated organic compounds. Nitro reductases are involved in the degradation of nitroaromatic compounds, chlorinated solvents and pesticides. Many diverse organophosphates detoxify other contaminants by reducing either halogen groups or organically bound phosphate [7].

Many endophytes are resistant to heavy metals and are capable of degrading organic contaminants. The endophyte-assisted phytoremediation has been documented in formulating biofertilizers which are providing promising result for *in situ* remediation of contaminated soils accompanied by phosphate solubilizing, biosurfactant activity in degradation of oil-contaminated soil, siderophore production, and antimicrobial activity. In addition, plants and many microorganisms contain abundance of oxidases such as laccases (degradation of anilines) and peroxidases. These enzymes are involved in forming a defense layer in many plant processes. *Populus* species and *Myriophyllum spicatum* are examples of plants that have these enzymatic systems [8]. Phytoremediation essentially comprises of six different strategies, though more than one may be used by the plant simultaneously. They are as shown in **Figure 1** and **Table 1**.

2.2 Phytostabilization

Metals are precipitated as insoluble forms by the direct action of roots which secrete phenolic and low molecular weight organic exudates subsequently trapped in the soil matrix as contaminants. Later when get accumulated organic or inorganic

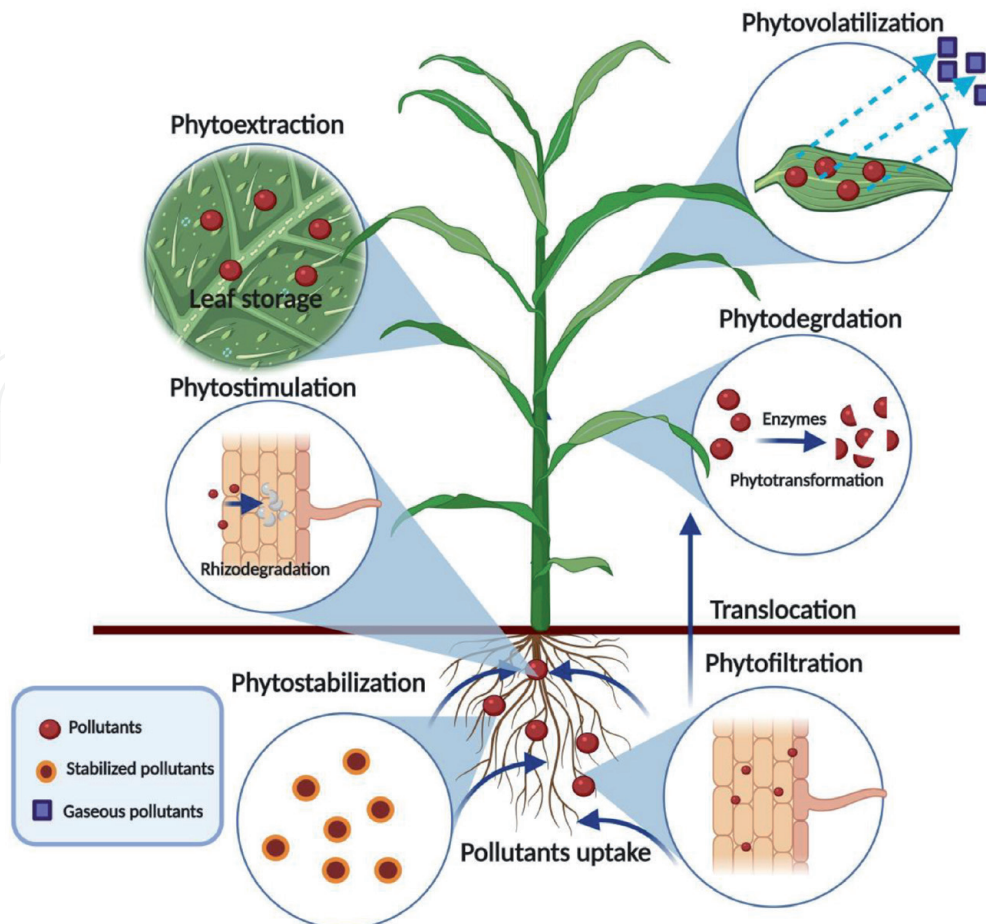


Figure 1.
Schematic representation of phytoremediation.

S.No	Type of contaminants	Medium/mode of remediation	Plant source	References
1.	1,2,4,-trichlorobenzene, Aniline, Benzene ethyl benzene, <i>m</i> -xylene, Nitrobenzene, Pentachlorophenol, Phenol, Trichloroethylene (TCE), Toluene, Methyl Tertiary Butyl Ether (MTBE), Perchloroethylene	Atmosphere/ Phytovolatilization through leaves, trunk, soil	Poplar, Russian olive, Eucalyptus, Pine and Willow trees	[9– 12]
2.	Herbicides, Trichloroethylene and Methyl tert-butyl ether	Plant/ Phytodegradation through root enzymes	Cannas/ Microorganisms	[13]
3.	DDT, Polybrominated diphenyl ethers (PBDEs)	Soil/Rhizofiltration	Sunflower, Tobacco, Spinach, Rye and Indian Mustard	[14]
4.	Dichlorodiphenyl trichloroethane (DDT), Polybrominated diphenyl ethers (PBDEs) and Dichlorodiphenyl dichloroethylene	Soil/ Rhizodegradation	Rhizospheric bacterial population associated with plants	[15]
5.	Pesticides, Hydrocarbons and Animal manure	Soil/ Phytostabilization	Birch, Black locust, Oak, Scots pine and Douglas fir	[16]

Table 1.
Concise view of various phytoremediation strategies.

pollutants are incorporated into the lignin of the cell wall of cells or in humus. The main intention is to cultivate plants like *Haumaniastrum*, *Eragrostis*, *Ascolepis*, and *Gladiolus* in polluted agricultural fields to limit the mobilization and diffusion of contaminants in the soil. [16–18].

The plants are involved in absorbing many toxic elements from rock, soil, and polluted water by the root system. Plant exudates aggregate metals in the soil. Soil microbes which are symbionts can decrease the toxic effects of contaminants in the soil. For example, exudate peptides from the bacterium *Pseudomonas putida* and Arbuscular Mycorrhizal Fungi (AMF) have great potential in phytostabilization and in removing metal contaminants in the soil can decrease Cd toxicity in plants. Plants can also convert contaminants into less toxic forms as well as decrease their bioavailability [19].

Siderophores, organic acids, and phenolics secreted by the microbes associated with the roots of certain plants are natural chelating compounds that form complexes with metals in the rhizosphere. In addition, plants, and their associated soil microbes play a major role in releasing chemicals that act as biosurfactants in the soil that increase the uptake of hydrocarbon toxic pollutants. These contaminants are stabilized in natural and constructed wetlands through a process called phytofiltration. It includes rhizofiltration where metals are precipitated within the rhizosphere zone and in the root membrane. Metal uptake by plants that is generally active diffusion takes place by specific protein transporters (channel proteins) or H⁺ coupled carrier proteins located along the cell membrane of the root. For example,

the Fe regulated transporter (IRT1) allows the uptake of Fe. Uptake of other metals also occurs via IRT1 transporters, especially even in very low concentrations of Fe exist in the soil. By expelling the proton gradient, more ions are concentrated near the root zone. Inadvertent uptake of non-essential metals also takes place via other cell membrane transporters.

2.3 Phytovolatilization

Plants can absorb a high level organic, inorganic and heavy metals through their root system which later is metabolized and converted to nontoxic and also as volatile compounds that are released to the atmosphere by evapotranspiration. Removal of the water-soluble compounds like aldehydes takes up easily than ketones [20]. Distinctively Hg, Se, and As taken up by the roots are converted into non-toxic forms, and then released into the atmosphere during transpiration. The plant species like *Astragalus bisulcatus* and *Stanleya pinnata* for Se or transgenic plants (with bacterial genes) of *Arabidopsis thaliana*, *Nicotiana tabacum*, *Liriodendron tulipifera*, or *Brassica napus* for Hg can be mentioned as examples [17, 21].

2.4 Phytoextraction associated with endophytes

Phytoextraction is either a continuous process by cultivating metal hyper-accumulating plants as well as fast-growing plants or an induced process by using chemicals to increase the bioavailability of metals in the contaminated soil. This phenomenon uses the ability of plants to accumulate contaminants in the above ground. It is applied to heavy metals contaminants like Cd, Ni, Cu, Zn, Pb, Se, and As from industrial effluent mainly from the leather industry, paper and textile industries and organic compounds. Phytosequestration and phytoaccumulation are the techniques that preferentially use hyper-accumulator plants. They can store high concentrations of specific metals in their aerial parts at the rate of 0.01–1% dry weight depending on the metal. Plants such as *Elsholtzia splendens*, *Alyssum bertolonii*, *Thlaspi caerulescens*, and *Pteris vittata* are preferred for hyperaccumulator for Cu, Ni, Zn/Cd [22–24]. This process involves systematic harvesting and renewal of the biomass to lower the concentration of contaminants in the soil. Phytoextraction is a process that takes place in certain plants which undergo the accumulation of contaminants gradually (mainly metals) into their biomass. Certain plants can hyper accumulate metals without any toxic effects. These plants are adapted to naturally occurring metalliferous soils. More than 400 plant species can hyper accumulate various metals. However, most plants have the capability to hyper accumulate at least one specific metal [19].

Physiological, biochemical and molecular approaches are employed to identify the underlying mechanisms such as heavy metal accumulation and tolerance and adaptive mechanisms to cope up with heavy metal stress. Some adaptive mechanisms evolved by tolerant plants with the association of endophytes are the reason behind their gene encoded proteins and enzymes that involve in phytoremediation. This is organized by various factors including immobilization, plasma membrane exclusion, restriction of uptake and transport, synthesis of specific heavy metal transporters, chelation and sequestration of heavy metals by particular ligands, induction of mechanisms contrasting the effects of ROS and MG (such as upregulation of antioxidant and glyoxalase system), induction of stress proteins, the biosynthesis of polyamines and signaling molecules such as salicylic acid and nitric oxide [25–28].

Endophytes are ubiquitous and have been residing in all species of plants. In general, bacterial endophytes colonize the internal tissues of the plant that are

nonpathogenic for their host [29]. Endophytes could produce different plant hormones like IAA, Cytokinin and gibberellic acid to enhance the growth of the host plants. Endophytes have better adaptations against intrinsic and extrinsic stress factors, which lead to enhanced plant growth [30]. Many endophytes are the common rhizospheric bacteria which include *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Actinobacteria*, *Sphingomonas* etc. that are found to be more predominant. They produce various secondary metabolites, volatile compounds and antibiotics to counteract the detrimental effect of pathogens through mechanisms parallel to that of PGPR [31]. Endophytic bacteria are developed as biocontrol agent against the fungal and bacterial phytopathogens [32]. For the phytoremediation of organic contaminants, endophytes have different enzymology to metabolize various organic contaminants and they reduce both the phytotoxicity and evapotranspiration of volatile contaminants.

Although heavy metals are toxic to plants, it has been proved that many plants are metal tolerant and some of them are metal hyperaccumulators [33]. The hyperaccumulator-associated endophytes are metal resistant, due to long-term adaptation to the high concentration of metals accumulated in the plants [34]. Hyperaccumulator associated endophytes and many metal-resistant endophytes were isolated from hyperaccumulating plants, such as *Alyssum bertolonii*, *Alnus firma*, *Brassica napus*, *Nicotiana tabacum*, *Thlaspi caerulescens*, *T. goesingense*, and *Solanum nigrum*. The reported metal-resistant endophytes belong to a wide range of taxa; in bacteria, these include *Arthrobacter*, *Bacillus*, *Clostridium*, *Curtobacterium*, *Enterobacter*, *Leifsonia*, *Microbacterium*, *Paenibacillus*, *Pseudomonas*, *Staphylococcus*, *Stenotrophomonas* and *Sanguibacter* and in fungi *Microsphaeropsis*, *Mucor*, *Phoma*, *Alternaria*, *Peyronellaea*, *Steganosporium* and *Aspergillus* [35].

Case studies emphasize the role of endophytic microbes that involve in the production of IAA. It helps in the plant growth promotion and the production of siderophore which means 'iron carrier' in Greek. They are small, high-affinity iron-chelating compounds that are secreted by microorganisms such as bacteria and fungi and serve primarily to transport iron across cell membranes. Biosurfactant activity of endophytic microbes enhances the emulsification of hydrocarbons and thus they have the potential to solubilize hydrocarbon contaminants and increase their availability for microbial degradation activity in oil-contaminated soil. Antimicrobial activity of endophytes gives promising effect against a broad spectrum of phytopathogen. Inoculation of plant growth-promoting bacteria (PGPR) and AMF can increase plant biomass. The AMF-plant symbionts usually reduce the accumulation of metals in the above ground tissue biomass of plants.

The role of AMF in regulating metal uptake by plants appears to vary depending on numerous factors like AMF population, plant species, nutrient availability and metal content in the soil. Even the application of specific soil fungicides, the AMF activity has resulted in increased metal accumulation in plants. Endophytes excel in the metabolism of unusual compound degradation including *Achromobacter violaceum*, *Pseudomonas*, *Bacillus*, *Acinetobacter*, etc. They induce phytoextraction which in turn promotes the use of fast-growing crops and chemical manipulation of the soil. The bioavailability of metals in less concentration in the soil is a limiting factor in phytoextraction. The bioavailability of metals can be increased by the use of natural chelators of low molecular weight organic acids or synthetic chelates like ethylenediaminetetraacetic acid (EDTA) or acidifying chemicals like NH_4SO_4 as well as by the microbial activity like phosphate solubilizers, nitrogen fixers and complex organic contaminants degradation microbes.

The use of chelators increases the absorption of metals by the roots and helps in the translocation of metals from the roots to the foliage. The timing of chelate and its efficacy are directly proportional to the biomass production. To chelate Pb from contaminated soil, using EDTA is found to be a promising option and it can be applied to growing corn (*Zea mays*) in Pb-contaminated soil treated with

10 mmol kg⁻¹ EDTA. This in turn will result in the accumulation of Pb at higher rate and facilitate the translocation of Pb from the roots to the parts of the plants. One of the limitations of using synthetic chelates enhances solubility of the metals within the soil and this increases the risk of metal migration into the soil profile and enters the groundwater. This can be avoided by treating the contaminated soil *ex-situ* in a confined site with an impermeable surface. The periodic application of low doses of synthetic chelates reduces the risk of metal migration [19].

2.5 Phytofiltration

Plants absorb soil ionic compounds by their root system through capillary action even at low concentrations. Phytofiltration is a phenomenon where plant roots (rhizofiltration), shoots (caulofiltration), or seedlings (blastofiltration) absorb water along with minerals and pollutants from contaminated or wastewaters [36].

Plants broaden their root system in search of water which deepens in the soil profile. It can establish a network in the root ecosystem. As it mounts up contaminants it aids in regaining the polluted soil and stabilizing soil fertility through the plant exudates. Root exudates often involve in altering the pH of rhizosphere, precipitating ions of heavy metals on plant root and minimizing the movement of heavy metals to underground water. When the roots become saturated, they are harvested and disposed of which minimize the soil contamination but can be dumped from one form to another form. Ideally, plants used for rhizofiltration should have a dense root system, high biomass production, and be tolerant to heavy metal.

In general the terrestrial and aquatic plants can be used for rhizofiltration. Plants cultivated in specific condition achieve biomass with effective root system while other potential submerged organs concentrate on the contaminants, especially heavy metals, radioactive elements and organic pollutants from contaminated water bodies. The plants kept in a hydroponic system absorb the concentrated contaminants when the effluents are passed and 'filtered' by the roots (Rhizofiltration) [17, 37]. Plants with high root biomass or high absorption surface with more accumulation capacity (aquatic hyperaccumulators) and tolerance to contaminants will achieve the best results. Promising examples include *Helianthus annuus*, *Brassica juncea*, *Phragmites australis*, *Fontinalis antipyretica* and several species of *Salix*, *Populus*, *Lemna*, and *Callitriche* [38, 39].

Some terrestrial plants such as Indian mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*) have longer, deeper and hairy root systems with good capacities to accumulate heavy metals during rhizofiltration [40, 41]. Unpredictably woody species make easy accumulation of heavy metals in their shoot system above the soil level. Their deep root system, participate effectively in preventing the soil erosion as well as the distribution of the contaminated soil to the surrounding [42]. In this concern bio-filtration, bioaccumulation and biomagnification are threats related to the food chain and food web. Because of their in-edible nature this approach prevents the availability and probability of the heavy metals entering into the food chain through trees [43].

2.6 Rhizodegradation

Microbes that harbor inside plant parts like endophytic bacteria and fungal population that grows in roots are tend to promote the growth of plants and involve in degrading rhizosphere pollutants. They utilize exudates and metabolites of plants as a source of carbon and energy. In addition, plants provide biodegrading enzymes. The application of phytostimulation is targeted to organic contaminants [37]. The microbial community in the rhizosphere is diverged genetically and physiologically. This varies according to the spatial distribution of nutrients irrespective of factors [17].

There are other strategies, which are of rhizodegradation. These include:

2.6.1 Hydraulic barriers

Some large trees like *Populus* sp. have deep roots which have a major role in transpiration of groundwater in large quantities. Plant enzymes play a key role in eliminating contaminants after metabolized and vaporized together with water in plant tissues.

2.6.2 Vegetation covers

Herbs including grasses, shrubs, or trees planted on landfills, pits, trenches, or tailings minimize the infiltration of rainwater and the spread of pollutants. The roots facilitate soil aeration and in turn enhance the biodegradation, evaporation, and transpiration [44, 45]. Organic soil composed of sawdust, plant remains and NPK-fertilizers promote plant growth which helps in phytoremediation. Many field trials are emphasized at the end of a single biological cycle with 76 different plant species including cereals, shrubs, fruit trees, and even large trees like oaks and pines.

2.6.3 Constructed wetlands

The components of ecosystems comprise of organic soils, microorganisms, algae and vascular aquatic plants. All are involved in the effluent treatment through evaporation, filtration, ion exchange, adsorption and precipitation [46]. Here all the components are interlinked to phytoremediation and the entire system is given a promising effect [47]. The advantages are good cleaning efficacy, less cost of designing along with easy operation and maintenance. It is widely focused in the treatment of domestic, agricultural, and industrial wastewater, and also for treating acid mine drainages [48, 49]. Herbs (grasses, shrubs) or trees planted on landfills or tailings are used to reduce the infiltration of rainwater which is loaded with pollutants from various areas. Since there are difficulties in establishing rooting in tailings some other techniques must be evaluated for future prospective. For example, plants like *Hungarian agronomists* (*Biological Reclamation Process, BRP*) are propagated to utilize residues of organic soil that is composed of sawdust, plant remains and NPK-fertilizers [50].

2.6.4 Phytodesalination

The cultivation of halophytes on salt-rich soil is to improve the productivity of the soil and to remove the excess salt from saline soil [51, 52]. The potential of *Suaeda maritima* and *Sesuvium portulacastrum* is used in removal and accumulation of NaCl from highly saline soil. The plants in saline soil accumulate sodium in shoots and aerial parts which is based on the soil nature and the climatic conditions. The upper horizon of the soil layer is leached by halophytes [53].

3. Recent advancements in phytoremediation

To enhance the rate of phytoremediation, to improve the adaptation to various environmental conditions and to minimize their limitations such as slow-growth several strategies are developed through recombinant DNA technology to create transgenic plants or plant hybridization with fast-growing hyperaccumulators and microbe-associated phytoremediation. The hyperaccumulators can accumulate high levels of contaminants including heavy metals and other pollutants. Electrofusion is used for the fusion of protoplasts between two plants namely *T. caerulea* which has a

high-level Zn accumulator and *Brassica napus* which has a biomass production capability. The resulted somatic hybrids have the properties like hyperaccumulation capability, tolerance derived from *T. caerulescens* and higher biomass production derived from *B. napus* [54]. Moreover in comparison with rhizosphere microorganisms, endophytes have close interaction with their host plants. Genetically modified endophytes can be used as bio-fertilizers that could more efficiently improve phytoremediation [28].

3.1 Genetic engineering

Genetic engineering is a tool for improving strains in industries and clinics. It also enhances the phytoremediation abilities of plants in removing heavy metals. To generate genetically modified plants, a foreign source of the gene of interest which can be obtained from an organism, such as a plant species or even bacteria or animals, is transferred and inserted into the genome of a target plant through a proper vector system. After DNA recombination, the foreign gene gets integrated and inherited that confers specific traits to the plants. Moreover, genetic engineering has tools to transfer desirable characters from hyperaccumulator source plant to sexually incompatible plant species, which is impossible through traditional methods including vegetative propagation [55].

Therefore, creating transgenic plants with the desired gene expression in traits has attracted the researchers in the field of phytoremediation. Genetically modifying traits are fast-growing and high-biomass species with high tolerance against heavy metals. Their accumulation ability is more desirable than hyperaccumulators because sometimes hyperaccumulation may be harmful for biomass. Therefore, the selection of genes for genetic engineering should be based on heavy metal tolerance, construction of metabolic pathways in detoxifying heavy metals and accumulation mechanisms in plants. As heavy metals accumulation may create oxidative stress due to excessive Reactive Oxygen Species (ROS), a defense system provide heavy metal tolerance. To increase heavy metal accumulation through genetic engineering, genes are put under the control of strong promoter. The signal sequences facilitate in the uptake, translocation, and sequestration of heavy metals in elevated levels [56].

As metal chelators act as metal-binding ligands to improve heavy metal bioavailability, they promote heavy metal uptake and root-to-shoot translocation, as well as mediate intracellular sequestration of heavy metal ions in organelles. By over expression of genes encoding natural chelators, heavy metal uptake and translocation can be improved [57]. For example the supply of histidine is a nickel chelating agent and when it is supplied to plants which are originally non-accumulating species for metals greatly increases both its nickel tolerance and nickel transport to the shoot. It indicates the role of histidine in the hyper accumulation of nickel in *Alussum* plants [58].

Although the genetic engineering approach is a promising one, a few setbacks are there when the concentration is toxic to cells. On other hand, construction of all desired genes (that involved in mechanisms of detoxification and accumulation of heavy metals) is a time as well as effort consuming process and hence it is not providing a promising effect in the present scenario. Moreover it is difficult to get approval for the cultivation of genetically modified plants in test fields due to its toxicity, allergic levels and risk factor to ecosystem. Therefore, the researchers focus on alternate approaches to improve plants' role in phytoextraction.

3.2 Role of endophytes in phytoremediation

The role of plant-associated microorganisms (rhizospheric microorganisms) can be considered as an alternative approach to improve plant performance for phytoremediation as expressed in **Figure 2** and **Table 2**. The microbial communities

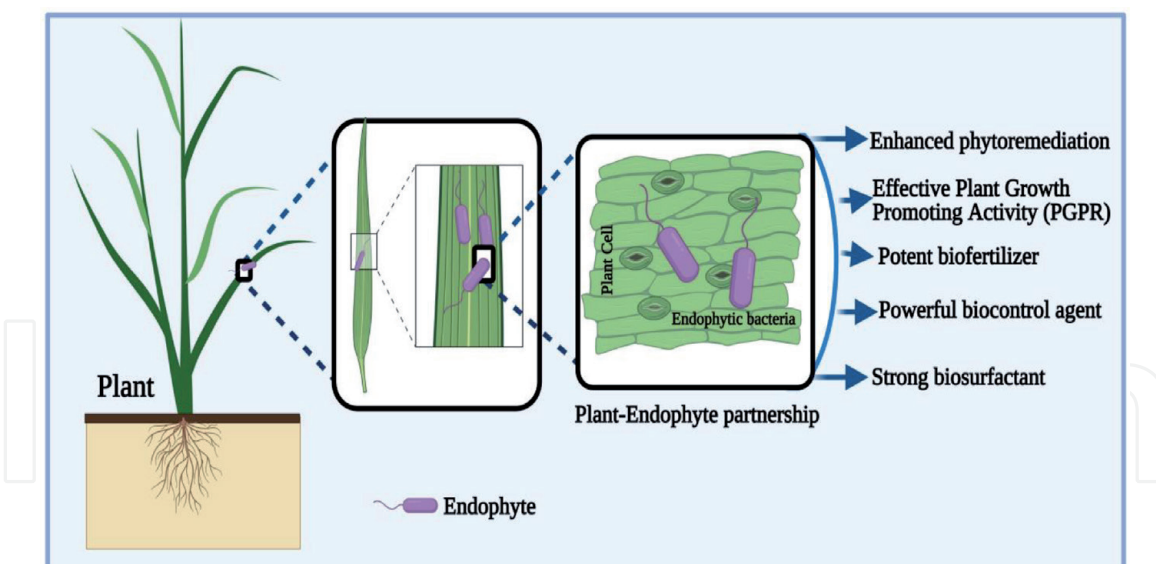


Figure 2.
Role of endophytes in sustainable ecological perspective.

S.No.	Endophytes	Plant Source	Tissue parts used	Resistant metal	References
1.	Bacteria	<i>Prosopis laevigata</i> , <i>Sphaeralcea angustifolia</i>	Root	Pb-Zn	[59]
2.	<i>K. ascorbata</i>	<i>Solanum lycopersicum</i> , <i>Brassica napus</i>	Seed tissue	Ni	[60]
3.	Rhizobacteria	<i>Thalaspia caerulescens</i> , <i>Alyssum bertolonii</i>	Root	Zn and Ni or Ni	[61, 62]
4.	<i>Microbacterium</i> , <i>Bacillus</i> , <i>Arthrobacter</i> , <i>Flavobacterium</i>	<i>Solanum nigrum</i>	Roots	Cd	[63]
5.	Bacteria	<i>Populus alba</i>	Leaves	Cd, Co, Pb	[64]
6.	<i>P. fluorescens</i> , <i>Microbacterium</i> sp., <i>Enterobacter</i> sp., <i>Xanthomonadaceae</i> <i>Pseudomonas</i> sp.,	<i>Brassica napus</i>	Roots	Pb	[65]
7.	<i>Pseudomonas fulva</i> , <i>Stenotrophomonas</i> sp., <i>Clostridium aminovalericum</i> , <i>Sanguibacter</i> sp.	<i>Nicotiana tabacum</i>	Seed	Cd	[66]
8.	Arbuscular mycorrhizal fungi (AMF), <i>Pseudomonas putida</i>	<i>Agrostis tenuis</i> , <i>Festuca rubra</i>	Roots	Cd, Cr(III)	[19]
9.	<i>Kluyvera ascorbata</i> , <i>Pseudomonas tolaasii</i> , <i>P. fluorescens</i> , <i>Variovorax paradoxus</i> , <i>Rhodococcus</i> sp. and <i>Flavobacterium</i> sp.	Graminaceae, and <i>Brassica juncea</i>	Leaves and Roots	Cd, Zn, Cu, Ni, Co, Cr, Pb	[67, 68]
10.	Plant growth promoting bacteria and Arbuscular Mycorrhizal Fungi (AMF)	<i>Liriodendron tulipifera</i>	Roots	methyl-Hg	[19]

Table 2.
Documented records of role endophytes- based phytoremediation.

have symbiotic association with rhizosphere stimulating root proliferation and thus, promoting plant growth. They have increased heavy metal tolerance and plant fitness among the flora in local biosphere. [69]. Plant growth-promoting rhizobacteria (PGPR) have a key role in phytoremediation. PGPR can promote plant growth via IAA production, antimicrobial activity, increase plant tolerance against heavy metals and improved nutrient uptake through diffusion as well as uptake of heavy metal from contaminated soil, translocation etc., [22]. This is achieved by producing various compounds such as organic acids for organic pollutant degradation, iron chelating siderophores, antibiotics, various enzymes involved in phytoremediation and growth promoting phytohormones [22]. PGPR can degrade the ethylene precursor ACC by synthesizing the 1-aminocyclopropane-1-carboxylate (ACC) deaminase. PGPR minimizes ethylene production and thus in turn promotes plant growth [70, 71].

Plants inoculated with PGPR containing extensive root and shoot densities result in enhanced uptake of heavy metals by the influence of ACC deaminase which promote phytoremediation efficiency [70, 72]. PGPR induces the formation of lateral root and root hair development, thus promoting plant growth and improving phytoremediation with bacterial indole acetic acid (IAA) [73]. Arbuscular mycorrhizal fungi (AMF) are the vast group of fungi, an important microbial community are predominant in soil profile that support plants for phytoremediation. AMF in rhizospheres increases the surface area for root absorption with an extensive hyphal network. They improve the uptake of water, nutrients and heavy metal bioavailability [74]. AMF can also produce phytohormones to promote plant growth and biosurfactant aids in phytoremediation [75].

A plant employs various strategies to enhance heavy metal bioavailability for better absorption. Root exudates promote desorption of heavy metals by making insoluble complexes of contaminants to free ions, by decreasing soil pH, which thus facilitate the accumulation of heavy metals in the soil for easy absorption near the roots [76]. Plants secrete metal-mobilizing compounds such as phytosiderophores, carboxylates, and organic acids in rhizosphere. According to the bioavailability heavy metals/metalloids in the soil are classified as high, moderate and low bioavailable heavy metals/metalloids. The high bioavailable are Cd, Ni, Zn, As, Se, Cu, moderately bioavailable heavy metals are Co, Mn, Fe, and least bioavailable are Pb, Cr [77].

4. Conclusion

Heavy metal pollution is a major issue which invade even in the breast milk of mother. Their toxic effect leads to multi organ failure and several cancers. They readily enter into the agricultural products and food. Health deteriorates due to the toxic effects and rapid accumulation in the environment through irrigation of contaminated water bodies. To prevent or mitigate heavy metal contamination and renovate the contaminated soil, a variety of techniques have been developed including phytoremediation. It has been proved to be a promising technique to remediate heavy metal-polluted soil.

Hyper-accumulation is the most straightforward approach for phytoremediation, and hundreds of hyper-accumulator plants have been identified so far. Phytoremediation has a few limitations including time-consuming process of plants in clearing the contaminants due to their slow growth in altered soil. But the genetic engineering is a powerful tool to modify the plants with resistant traits like fast growth even in polluted soil, high biomass production, heavy metal tolerance by designing their metabolic pathways and good adaption for surviving in various climatic and geological conditions.

Hence, a better understanding of the plant mechanisms for phytoremediation is more essential which comprises of absorption, translocation, and detoxification of pollutant in plants. These are mediated by different biomolecules and metabolic pathways. Their limitations can be overcome by genetic engineering of plants and endophytes to promote more effective way to create sustainable ecosystem. These engineered microbial consortiums can be used to improve soil health and further promote plant growth and fitness. Practically, a single approach will never be effective to the revival of heavy metal-polluted soil. So the combination of different new approaches such as genetic engineering, microbe-assisted bio fertilizer for plant growth promotion as well as detoxification of pollutants, and chelate-assisted approaches to concentrate the pollution near to rhizosphere are vital for highly effective and extensive phytoremediation in future.

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Author details

Selvanayaki Krishnasamy*, Ramkumar Lakshmanan and Mythili Ravichandran
Department of Microbiology, K.S. Rangasamy College of Arts and Science,
Tiruchengode, Tamil Nadu, India

*Address all correspondence to: selbiofilms@gmail.com

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