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Use of Bio-Resources for Remediation of Soil Pollution

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ABSTRACT

In recent years, economic boom in fast developing countries has been witnessed with spectacular progress in industrialization and concurrent progress in modern agriculture. Such development is however not without any socio-political and environmental side effects. A major concern has been the environmental pollution. If the current unabated disposal of various forms of wastes to agricultural lands is continued, the inherent capacity of soil to support agricultural production and sustain other ecosystem services will be in peril. Heavy metals with soil residence times of thousands of years present numerous health hazards to higher organisms. They are also known to decrease plant growth, ground cover and have a negative impact on soil biodiversity. Inorganic and organic contaminants typically found in urban areas are heavy metals and petroleum derived products. The presence of both types of contaminants on the same site presents technical and economic challenges for decontamination strategies. In this article we have reviewed the developments to ameliorate the contaminated soils, with special emphasis on biological approaches, which have shown potential to low-cost remediation of soil pollution. Also the limitations of such approaches and direction of further research have been highlighted.

1. Introduction

Intense industrial activity in the 20th century, especially in developing countries, has led to serious environmental pollution, resulting in a large number and variety of contaminated sites which became a threat to the local ecosystems. In India, the application of industrial and city effluents to land has become popular in recent years as an alternative means of treatment and disposal [1-3]. Heavy metals, with soil residence times of thousands of years, present numerous health dangers to higher organisms [4]. They are also known to decrease plant growth, ground cover and have a negative impact on soil microflora [5]. There is increasing and widespread interest in the maintenance of soil quality and remediation strategies for management of soils contaminated with trace metals, metalloids or organic pollutants. Heavy metals are deposited in soils by atmospheric input and the use of mineral fertilizers or compost, and sewage sludge disposal. Conventional remediation methods usually involve excavation and removal of contaminated soil layer, physical stabilization (mixing of soil with cement, lime, apa-

tite etc.), and washing of contaminated soils with strong acids or HM chelators [6]. However, if no remediation action is undertaken, the availability of arable land for cultivation will decrease, because of stricter environmental laws limiting food production on contaminated lands. Inorganic and organic contaminants typically found in urban areas are heavy metals and petroleum derived products. The presence of both types of contaminants on the same site presents technical and economic challenges for decontamination strategies.

Bioremediation, *i.e.* the use of living organisms to manage or remediate polluted soils, is an emerging technology. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes. Initially, bioremediation employed microorganisms to degrade organic pollutants [7]. Microorganisms have been used since 600 B.C. by the Romans and others to treat the wastewater. The first commercial use of a bioremediation system was in 1972 to clean up a Sun Oil pipeline spill in Ambler, Pennsylvania [8]. Since 1972, bioremediation has be-

come a well-developed way of cleaning up different contaminants. But ever since the use of green plants was proposed for *in situ* soil remediation phytoremediation has become an attractive topic of research and development [9]. As concluded by Anderson *et al.* [10], "understanding the mechanisms and critical factors influencing the plant–microbe–toxicant interaction in soils will permit more rapid realisation" of bioremediation of polluted soils. This review aims to contribute towards this goal by examining the current concepts and published data on the biological processes and major controls that may be used for their management in phytoremediation of inorganic and organic soil pollutants.

2. Sources of Soil Pollution

Trace metal contamination of soils can occur naturally from geological sources, for example Cu and Ni contamination of basaltic soils from the basalt parent material [11], or as a result of a wide ranges of industrial and agricultural activities. Therefore, trace metal pollution may harm human food safety and health, and there is much interest in the protection of unpolluted sites and the effective management of contaminated sites. Zinc is an essential plant nutrient and is one of the most ubiquitous trace metals in soils and it is often regarded as a potentially toxic element when present in excessive concentrations [12]. As chromium is widely used in many Industries of which leather industries are the biggest consumers, wastes from tanneries pose a serious threat to the environment. Metals and metalloids enter soils and wa-

ters due to many processes including atmospheric deposition from industrial activities or power generation; disposal of wastes such as sewage sludge, animal manures, ash, domestic and industrial wastes or byproducts; irrigation and flood or seepage waters and the utilization of fertilizers, lime, or agrochemicals. Radionuclides are building up in some areas due to deliberate or accidental releases related to their use of energy production or for military purposes. It has been found that sewage sludge contents maximum amount of metals among different sources.

3. Microbial Remediation of Soil Pollutants

Microbes can reduced the activity of different types of metals or it can convert active forms of toxic metals to inactive forms by the processes as shown in **Figure 1**.

The choice of micro organisms to be used for bioremediation depends on availability of energy and carbon source, environmental conditions like temperatures, oxygen, moisture and the presence of hazardous contaminants. The aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium* (**Table 1**). These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy. The contact between the bacteria and contaminant is a precondition for degradation. This is not easily achieved, as neither the microbes nor contaminants are uniformly spread in the
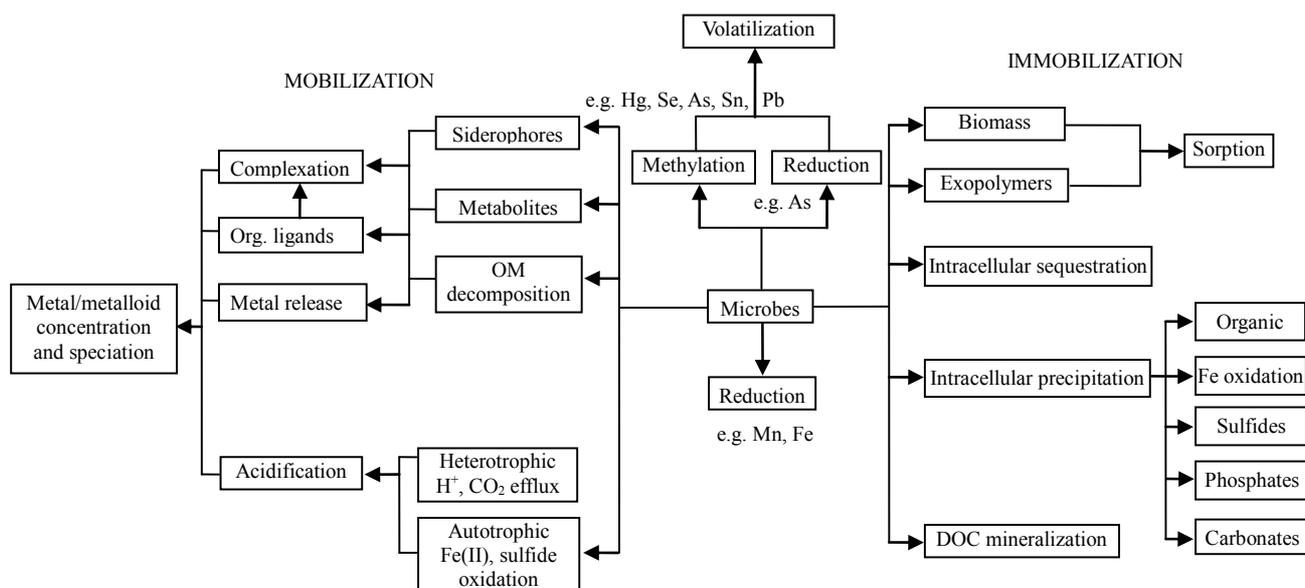

Figure 1. Processes involved in microbial mobilization and immobilization of metals and metalloids in soil (modified from Wengel [9]).

soil. Some bacteria are mobile and exhibit a chemotactic response, sensing the contaminant and moving toward it. Other microbes such as fungi grow in a filamentous form toward the contaminant. It is possible to enhance the mobilization of the contaminant utilizing some surfactants such as sodium dodecyl sulphate. Substrates can be used to facilitate the contact between contaminants and microbes by enhancing the mobilization of contaminants (http://www.clu-in.org).

There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls in river sediments, dechlorination of the solvent trichloro-ethylene, and chloroform. Ligninolytic fungi, such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs. Methylotrophs are the aerobic bacteria that grow by utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1,2-dichloroethane.

4. Bioremediation Strategies

Different bioremediation techniques are employed depending on the degree of saturation and aeration of an area. *In situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance, whereas *ex situ* techniques are applied to at the site which has been removed via excavation (soil) or pumping (water) (**Table 2**).

4.1. *In Situ* Bioremediation

These techniques are generally the most desirable options due to lower cost and fewer disturbances since they provide the treatment in place avoiding excavation and transport of contaminants (http://www.epa.gov/tio). *In situ* treatment is limited by the depth of the soil that can be effectively treated. In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases. The most important in situ land treatments are *bioventing*, *in situ biodegradation*, biosparging *and bioaugmentation*.

Table 1. Miroorganisms capable of degrading heavy metals.

Heavy metal	Microorganisms	References
Cr	• *Pseudomonas fluorescens* • *Pseudomonas aeruginosa* • *Pseudomonas mendocina* • *Enterobacter cloacae*.	[25] [26] [27] [112]
Ni	• *Pseudomonas* • *Methylobacterium*, *Rhodococcus* and *Okibacterium*	[29] [14]
Se	• *Stenotrophomonas sp*	[31]
U	• *Glomus intraradices* took	[35]
Zn, Cd and Mn	• *Arbuscular mycorrhizae*	[40-47]

Table 2. Summary of bioremediation strategies.

Technology	Examples	Benefits	Limitations
In situ	*In situ* bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties Chemical solubility, biodegradability and distribution of pollutants Biodegradative ability of indigenous microbes
Ex situ	Landfarming Composting Biopiles	Cost efficient Can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitations
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetics Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relatively high operating cost

Bioventing is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.

In situ biodegradation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment.

Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater.

Bioaugmentation involves the addition of microorganisms indigenous or exogenous to the contaminated sites to enhance the degradation of the contaminants.

4.2. *Ex Situ* Bioremediation

These techniques involve the excavation or removal of contaminated soil from ground. The most important ex-situ treatments are *landfarming, composting, biopiles and bioreactors.*

Landfarming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate the aerobic degradation of contaminants. The practice is limited to the treatment of superficial 10–35 cm of soil. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up abilities, it has received much attention as a disposal alternative.

Is a technique that involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristics of composting.

Biopiles are a hybrid of landfarming and composting, typically used for treatment of surface contamination with petroleum hydrocarbons [13]. They are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Bio-

piles provide a favorable environment for indigenous aerobic and anaerobic microorganisms.

Bioreactors. Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor is a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soilbound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable.
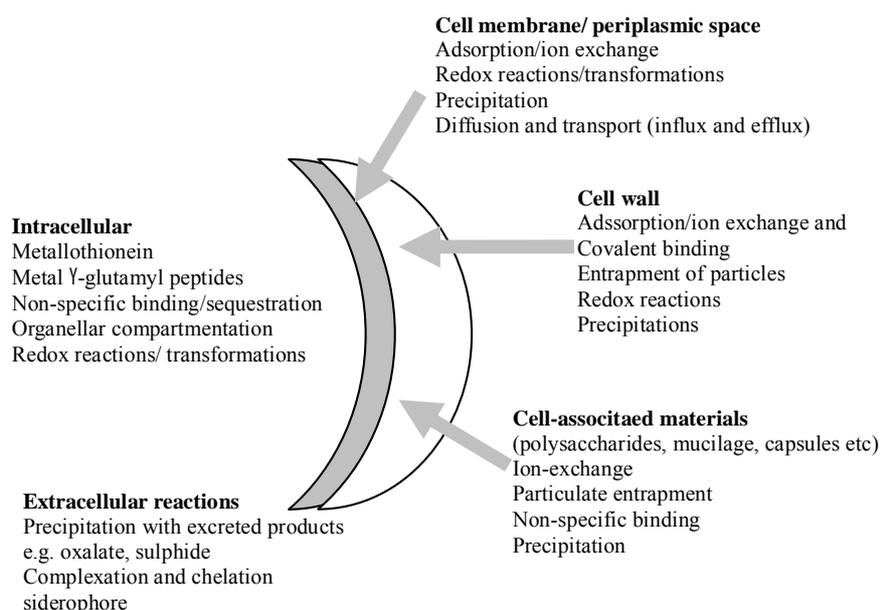
5. Role of Bacteria in Bioremediation

5.1. Rhizosphere Bacteria Affect Plant Growth and Metal Uptake

There is increasing evidence that rhizosphere bacteria contribute to the metal extraction process (**Table 3**), but the mechanisms of this plant–microbe interaction are yet to be fully understood. The rhizosphere of heavy metal accumulating plants provides a niche for adapted metal resistant microorganisms [14,15] and the mobility of heavy metals is higher in the rhizosphere of metal accumulators than in bulk soil, due to active mobilization by roots and microorganisms [16]. Bacterial IAA, ACC deaminase, siderophores, organic acids or specific ligands have been associated with enhanced growth and accumulation and mobilization of heavy metals under heavy metal exposure [17-19].

The rhizosphere bacteria of Salix caprea were reported to influence metal mobilization and uptake. Experimental results indicated that plant growth promotion might be an important parameter besides the enhancement of metal uptake. Zinc resistances of the Salix caprea rhizosphere bacteria ranged between 2 and 11 mM and were much higher than those of bacteria associated with Zn hyperaccumulating Thlaspi. This suggests a high bioavailability of Zn in the rhizosphere of Salix caprea and a specific adaptation of the associated bacteria. Salix caprea trees growing at the contaminated site in Arnoldstein were found to accumulate higher amount of Zn and Cd, but less Pb which indicates the relatively low Pb tolerance of the bacteria [15]. The possible mechanisms of microbial uptake and detoxification of toxic metals in soil matrix is presented in **Figure 2**.

Table 3. Overview of phytoremediation applications.

Technique	Plant mechanism	Surface reacting medium
Phytoextraction	Concentration of metal into the plant tissue via direct uptake with subsequent removal of the plant	Soils
Phytotransformation	Plant uptake and degradation of organic compounds	Surface water, ground water
Phytostabilization	Root exudates cause metals to precipitate and become less available	Soils, ground water, mine tailing
Phytodegradation	Enhances microbial degradation in rhizosphere	Soils, groundwater within rhizosphere
Rhizo filtration	Uptake of metals into plant roots	Soils, Surface water
Phytovolatilization	Plants evaporate selenium, mercury and volatile hydrocarbons	Soils, ground water

Cell membrane/ periplasmic space
Adsorption/ion exchange
Redox reactions/transformations
Precipitation
Diffusion and transport (influx and efflux)

Intracellular
Metallothionein
Metal Υ-glutamyl peptides
Non-specific binding/sequestration
Organellar compartmentation
Redox reactions/ transformations

Cell wall
Adssorption/ion exchange and
Covalent binding
Entrapment of particles
Redox reactions
Precipitations

Cell-associated materials
(polysaccharides, mucilage, capsules etc)
Ion-exchange
Particulate entrapment
Non-specific binding
Precipitation

Extracellular reactions
Precipitation with excreted products
e.g. oxalate, sulphide
Complexation and chelation
siderophore

Figure 2. The microbial uptake and detoxification of toxic metals.

5.2. Chromium in the Environment: Factors Affecting Biological Remediation

The detoxification of chromium in soil is based on the fact that Cr(VI) is readily reduced to Cr(III) and immobilized in organic matter rich soils. The use of Cr(VI)-contaminated groundwater to irrigate organic matter rich soil based on the mechanism of reduction and precipitation of Cr in the soil as Cr(III) [20,21]. In a study to examine the processes responsible for Cr(VI) reduction in soil, it was reported that organic matter content, bioactivity, and oxygen status were among the important factors [21]. Under aerobic, field-moist conditions, organic matter rich soil (amended with 50 tons ha^{-1} organic matter) was reported to reduce 96% of added Cr(VI), whereas sterile soils receiving similar amendments reduced only 75% of the original Cr(VI), demonstrating the impor-

tance of the presence of soil microorganisms in conjunction with a readily available carbon source. These studies assert that soil organic matters play a key role in reduction of Cr(VI) to Cr(III). Organic matter enhances the reduction of chromate in soil by increasing microbial activities, acting as electron donors, and by lowering the O2 level of the soil, thus creating reducing conditions.

Realizing the potential importance of soil microorganisms in reducing Cr(VI) in contaminated soils, several groups attempted to identify and isolate microorganisms that can mediate the reduction of Cr(VI) to Cr(III) in soils [22,23]). These microorganisms thought to be Cr resistant as well since the reduction rate is slower than the uptake rate [24]. Some of the bacterial strains found to be resistant to high levels of Cr(VI) include *Pseudomonas fluorescens* [25], *P. aeruginosa* [26], and *Pseu-*

domonas mendocina [27]. Bacterial populations resistant to as much as 500 mg L^{-1} Cr(VI) and fungal populations resistant to 1000 mg L^{-1} Cr(VI) were directly isolated from soils [28].

5.3. Nickel Tolerance and Accumulation by Bacteria from Rhizosphere of Nickel Hyperaccumulators

The bacteria in the rhizosphere of Ni-hyperaccumulators are capable of tolerating high concentration of Ni and also possess nickel uptake potential. The Ni-hyperaccumulators in combination with these Ni-resistant bacteria could be an ideal tool for nickel bioremediation. This high concentration of bioaccessible nickel in the rhizosphere of Ni-hyperaccumulators in turn provides a niche for nickel-resistant microflora.

The predominant Ni-resistant bacteria belonged to *Pseudomonas* in the rhizosphere of Ni-hyperaccumulator, *Alyssum bertolonii* [29], while the same under *Thlaspi goesingense* were identified as *Methylobacterium, Rhodococcus* and *Okibacterium* [14]. The rhizosphere of *Rinorea bengalensis* and *Dichapetalum gelonioides* ssp. *andamanicum*, the endemic Ni-hyperaccumulators from serpentines of Andaman harbor Ni-resistant bacteria, which are capable of accumulating nickel and could tolerate >8 mM Ni and viable cells were capable of accumulating Ni from aqueous solution. This may be attributed to the presence of Ni-binding sites on the cell surface of metallophiles [30].

5.4. Improvement of Selenite and Selenate Abatement in Selenium Contaminated Soils through Rhizospheric Bacterial Population

Brassica juncea cultivation on the soil has been found to result in a higher volatilisation rate when compared with non-vegetated soil. However, the presence of *B. juncea* in soil amended with selenium as selenite or selenate has revealed to promote a significant Se precipitation by eliciting rhizobacteria (*Stenotrophomonas sp*) capable of reducing the metalloid oxyanions to SeO. In fact, the capacity of certain rhizobacteria to precipitate Se oxyanions, reducing their toxicity in contaminated matrices, could be seen as an alternative option to Se phytoextraction or phytovolatilisation, so far emphasized for the removal of the toxic metalloid from soil. *B. juncea* efficiently accumulates as well as volatilizes selenium [31]). Thus, the rhizosphere of *B. juncea* resulted to be an effective source and carrier of microorganisms capable to in vitro reduction and precipitation of toxic Se(IV) and Se(VI) to non toxic elemental Selenium volatilisation by *B. juncea* has been confirmed as a relevant mechanism of Se detoxification in contaminated soil.

6. Role of Arbuscular Mycorrhiza in Bioremediation

6.1. Effects of the Mycorrhizal Fungus *Glomus intraradices* on Uranium Uptake and Accumulation in Uranium-Contaminated Soil

Arbuscular mycorrhizae (AM) are ubiquitous symbiotic associations between higher plants and soil fungi [32] and their extraradical mycelium form bridges between plant roots and soil, and mediate the transfer of various elements into plants. There is also a growing body of evidence that arbuscular mycorrhizal fungi can exert protective effects on host plants under conditions of soil metal contamination. Binding of metals in mycorrhizal structures and immobilization of metals in the mycorrhizosphere may contribute to the direct effects. Indirect effects may include the mycorrhizal contribution to balanced plant mineral nutrition, especially P nutrition, leading to increased plant growth and enhanced metal tolerance. It has been widely reported that ectomycorrhizal and ericoid mycorrhizal fungi can increase the tolerance of their host plants to heavy metals when the metals are present at toxic levels. The underlying mechanism is thought to be the binding capacity of fungal hyphae to metals in the roots or in the rhizosphere which immobilizes the metals in or near the roots and thus depresses their translocation to the shoots [22,33]. Being strongly adsorbed and bound by mycorrhizal structures, metals in soils may be retained within a certain volume of soil, with minimization of leaching processes and restriction of the zone of contamination, and plants may be protected from metal toxicity and environmental stress.

Arbuscular mycorrhizae may play an important role in plant adaptation to U contaminated soils by not only improving plant P acquisition ability, but also enhancing U immobilization by roots, thus reducing U partitioning into plant shoots and environmental risks. As U behaviour in soil is similar to heavy metals such as Pb, it can be deduced that mycorrhizal fungi, may have significant effects on U mobilization and uptake [34,35]. It has been found that extraradical AM fungal mycelium of *Glomus intraradices* took up and translocated U towards root in *in vitro* culture system [35], and hyphae were more efficient in U translocation compared with roots [36]. Soluble uranyl cations or uranyl-sulphate species that are stable under acidic conditions were translocated to a higher extent to roots through fungal tissues, while phosphate and hydroxyl species dominating under acidic to near neutral conditions or carbonate species dominating under alkaline conditions were rather immobilized by hyphal structures. It is documented that plant uptake of U is affected by various factors, such as soil properties [37] and

uranium–phosphorus interactions [38,39].

6.2. Arbuscular Mycorrhiza can Depress Translocation of Zinc to Shoots of Host Plants in Soils Moderately Polluted with Zinc

It has been demonstrated that at high soil heavy metal concentrations, arbuscular mycorrhizal infection reduced the concentrations of Zn, Cd and Mn in plant leaves [40, 41]. Field investigations have indicated that mycorrhizal fungi can colonize plant roots extensively even in metal contaminated sites [42-44], and Zn- and Cd-tolerant fungal strains have been isolated from contaminated sites by several research groups [45,46]. Numerous experimental studies have indicated that under conditions of moderate Zn contamination, arbuscular mycorrhizal plants may exhibit much lower shoot concentrations of Zn and higher plant yields than non-mycorrhizal controls, indicating a protective effect of mycorrhizas on the host plants against potential Zn toxicity [47].

7. Advantages and Disadvantages of Bioremediation

7.1. Advantages

1) Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for the complete destruction of a wide variety of contaminants.

2) The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.

3) Bioremediation can often be carried out on site, often without causing a major disruption of normal activeties. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.

4) Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

7.2. Disadvantages

1) Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.

2) Biological processes are often highly specific. Therefore, the success of the technique requires several site factors like the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.

3) Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment.

4) Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration.

5) Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation and there are no acceptable endpoints for bioremediation treatments.

6) There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.

8. Plant Assisted Bioremediation

Phytoremediation may be defined as use of vegetation to contain, sequester, remove, or degrade organic and inorganic contaminants in soils, sediments, surface water and groundwater. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water [48]. The basic idea that plant can be used for environmental remediation is very old and cannot be traced to any particular source. However, a series of fascinating scientific discoveries combined with an interdisciplinary research approach have allowed the development of this idea into a promising, cost-effective, and environmental friendly technology.

Although the application of microbial biotechnology has been successful with petroleum-based constituents, microbial digestion has met limited success for widespread residual organic and metals pollutants. Vegetation- based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. We can find five types of phytoremediation techniques, classified based on the contaminant fate: phytoextraction, phytotransformation, phytostabilization, phytodegradation, rhizofiltration, even if a combination of these can be found in nature (**Table 3**).

Phytoextraction or *phytoaccumulation*: This is a process used by the plants to accumulate contaminants into the roots and aboveground shoots or leaves. This technique saves tremendous remediation cost by accumulating low levels of contaminants from a widespread area. Unlike the degradation mechanisms, this process produces a mass of plants and contaminants (usually metals) that can be then harvested, incinerated, and the ash related to a confined area or the heavy metals are extracted from it (**Figure 3**, [49]).

Phytostabilization is a technique in which plants reduce the mobility and migration of contaminated soil. Leachable constituents are adsorbed and bound into the plant structure so that they form a stable mass of plant
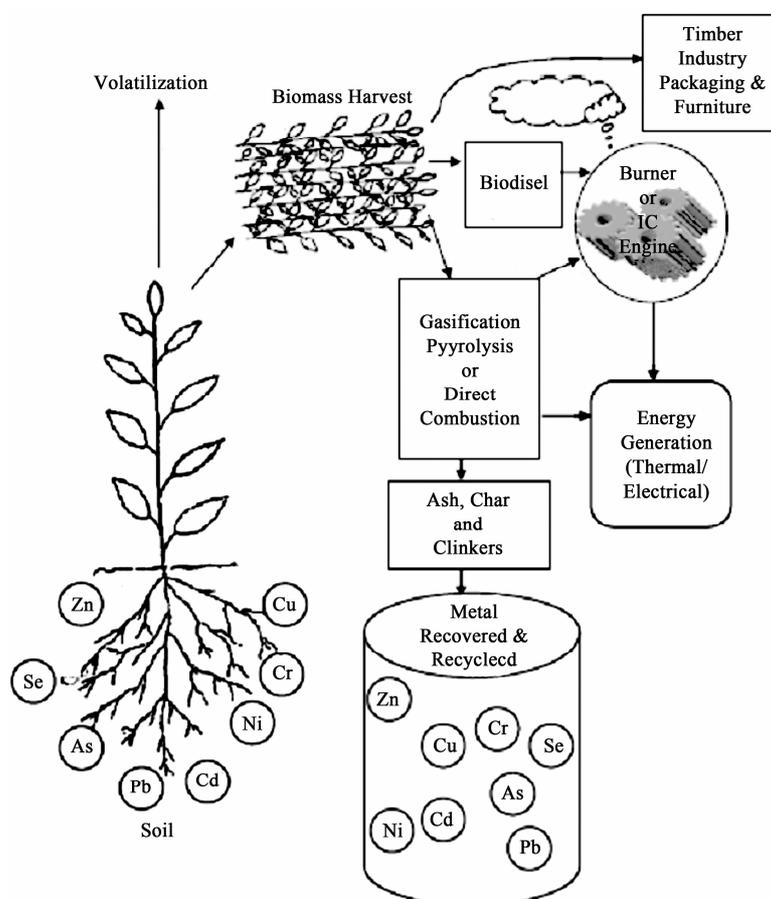
Figure 3. Phytoextraction of metals from soil and their utilization [49].

from which the contaminants will not reenter the environment.

Phytodegradation or *rhizodegradation* is the breakdown of contaminants through the activity existing in the rhizosphere due to the presence of proteins and enzymes produced by the plants or by soil organisms such as bacteria, yeast, and fungi. Rhizodegradation is a symbiotic relationship where the plants provide nutrients necessary for the microbes to thrive, while microbes provide a healthier soil environment.

Rhizofiltration is a water remediation technique that involves the uptake of contaminants by plant roots. Rhizofiltration is used to reduce contamination in natural wetlands and estuary areas. In Table we can see an overview of phytoremediation applications.

8.1. Phytoextraction

Phytoextraction employs metal hyperaccumulator plant species to transport high quantities of metals from soils into the harvestable parts of roots and aboveground shoots [50,51]. Phytoextraction is an innovative, novel and potentially inexpensive technology (**Table 4**) using

higher plants for *in situ* decontamination of metal-polluted soils, sludges and sediments [52-55]. Large biomass production and high rates of metal uptake and translocation into shoots are critical to achieve reasonable metal extraction rates. Effective phytoextraction requires both plant genetic ability and the development of optimal agronomic management practices [1,56]. Hyper accumulators are defined as plants that contain in their tissue more than 1,000 mg kg^{-1} dry weight of Ni, Co, Cu, Cr, Pb, or more than 10,000 mg kg^{-1} dry weight of Zn, or Mn [6]. Apart from metal tolerance, hyper accumulation is thought to benefit the plant by means of allelopathy, defense against herbivores, or general pathogen resistance [57-59].

In-situ phytoextraction of Ni by a native population of *Alyssum murale* on an ultramafic site (Albania) have been reported by Bani *et al.*, [60]. In the case of phytomining, the use of native flora (including local populations of hyperaccumulators) with limited agronomic practices (extensive phytoextraction) could be an alternative to intensively managed crops. Ebbs *et al.* [61] showed that *T. caerulescens* (UK) could achieve approximately

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Table 4. Phytoremediation of heavy metals.

Heavy metal		Plant species	References
Zn and Cd	•	Salix caprea	[15]
Ni	•	*Alyssum bertolonii*	[29]
	•	*Thlaspi goesingense*	[14]
	•	*Rinorea bengalensis* and *Dichapetalum gelonioides* ssp. *andamanicum*	[30]
Se	•	*Brassica juncea*	[31]
Ni	•	*Alyssum murale*	[60]
Cd	•	*T.caerulescens, Brassica spp.*	[61]
Cs	•	*Red root pigweed, Indian mustard , tepary bean*	[62]

10 times higher shoot Cd concentration as compared to *Brassica spp.* Lasat *et al.* [62] conducted a field study to investigate the potential of three plant species for phytoremediation of a ^{137}Cs-contaminated site. Approximately 40-fold more ^{137}Cs was removed from the contaminated soil in shoots of red root pigweed than in those Indian mustard and tepary bean. The greater potential for ^{137}Cs removal from the soil by red root pigweed was associated with both high concentration of ^{137}Cs in shoot and high shoot-biomass production. Among the plants, *Urtica dioica* found to be very effective due to its higher uptake capacity for chromium. *Zea mays* showed high tolerance towards Cr with negligible concentration in leaves. Due to its higher Cr uptake and low biomass production Urtica dioica, commonly known as 'stinging nettle', in German 'Brennnessel', can be considered as the right plant for remediation of Cr contaminated sites. As nettle grows both in tropical and cold climates, therefore its value as 'nature cleaner' is universal.

The lack of success of phytoremediation is largely related to the small biomass of most true hyperaccumulator plants or to metal accumulation by high-biomass (crop) plants being too low. For example while contaminant mixtures appear to be the rule rather than the exception at polluted sites, metal tolerance, as well as efficient metal accumulation by a given plant species is typically restricted to one or few elements. Moreover, high metal/metalloid uptake rates in plants as required for phytoextraction can only be achieved if the metal/metalloid activity in the rhizosphere soil solution is sustained by rapid re-supply from the solid phase [63,64]. The most studied approach is chelant-assisted phytoextraction using EDTA and other artificial chelants. Other researchers have employed acidifying amendments such as elemental sulphur [65,66] and ammonium fertilization along with nitrification inhibitors [67,68] to enhance metal mobility in the rhizosphere of phytoextraction crops. Co-cropping of different plant species has been proposed as a strategy to increase metal bioavailability [69] and to better ex-

plore the soil volume and address the heterogeneous distribution of pollutants in field soils [70], for instance by combining deep rooting metal accumulating willows with small hyperaccumulator species that can efficiently explore the uppermost soil horizons. Co-cropping of metal accumulators with alder trees (*Alnus sp.*) may offer an interesting alternative to chemical mobilisation of metals in phytoextraction crops [71]. Alder species are associated with N_2-fixing actinorhizal symbionts (Frankiae). Nitrogen fixation has been shown to result in substantial acidification in alder rhizospheres [72] because nitrogen uptake relying on N_2-fixation rather than anionic nitrate results in enhanced proton exudation to maintain the cation–anion balance [73]. These indigenous processes could be used to increase metal bioavailability to co-cropped metal accumulators and to improve nitrogen nutrition. Another interesting approach to enhance pollutant tolerance, plant performance and accumulation of metals at root surfaces using recombinant rhizobacterium *Pseudomonas putida* expressing a metal-binding peptide (E20) was recently demonstrated in hydroponic culture by Wu *et al.* [74].

8.2. Phytoremediation of Heavy Metal–Contaminated Soils: Natural Hyperaccumulation versus Chemically Enhanced Phytoextraction

Recent research has shown that chemical amendments, such as synthetic organic chelates, can enhance phytoextraction by increasing HMs bioavailability in soil thus enhancing plant uptake, and translocation of HMs from the roots to the green parts of tested plants [42,75,76]. Of the chelates tested, ethylene diamine tetraacetic acid (EDTA) was often found to be the most effective [77,78]. Huang *et al.* [76] found that among different chelating agent EDTA is more effective in accumulation Pb in corn and pea and also found that on increasing the concentration of EDTA accumulation efficiency of Pb in shoot of

corn and pea was increased Therefore, the potential risks of use of EDTA or other chelators for phytoextraction should be thoroughly evaluated before steps towards further development and commercialization of this remediation technology are attempted. Research (reference) indicated up to 104.6, 2.3 and 3.2-fold increase of Pb, Cd and Zn concentration, respectively, in leaves of Chinese cabbage grown on EDTA (10 mmol kg^{-1}) treated soil. EDTA effectively prevents cell wall retention of HM and influenced not only HM uptake but also enhanced HM translocation in the plant [77]. The use of chelates as soil amendments to increase the bioavailability of HM has raised some concern over the potential increased mobility of the metalchelate complex in the soil. Several authors have emphasized the possibility of HM groundwater contamination or other off site migrations [79]. The adverse effects of HMs on the occurrence of arbuscular mycorrhizal fungi, HM tolerance in these micro-organisms, and their effects on metal uptake and transfer to plants are well documented [80]. There is, however, very little information on the direct effects of EDTA on arbuscular mycorrhiza [81]. The toxicity of EDTA on soil bacteria, actinomycetes and fungi was studied with PLFA and DGFA methods. PLFA and DGFA are relatively new tools in environmental microbiology and enable the insight into the structure of microbial populations in complex substrates, and give an indication of environmental stress inflicted on microbial populations [82]. The results are in accord with phytotoxicity and arbuscular mycorrhize tests. Increasing doses of EDTA increased the cultural stress (DGFA analysis, trans/cis ratio of PLFA methyl esters) of soil microflora. The PLFA results indicated that soil fungi are more sensitive to EDTA or to EDTA mediated increase of HMs bioavailability than are soil bacteria and actinomycetes. This can be partly explained by a very diverse bacterial metabolism which enables bacterial species to adjust to different environmental conditions. Therefore, emphasize the importance of EDTA risk assessment for each specific soil and phytoextraction conditions. New non-toxic chelates, and methods to prevent the leaching of the HMs-chelate complex down the soil profile need to be evaluated.

8.3. Rhizovolatilisation

Rhizovolatilisation of inorganic contaminants differs significantly from other remediation techniques as it releases the contaminants in the atmosphere. In the case of selenium, volatile methylated species are less toxic than inorganic forms [83]. The concern related to volatilisation of contaminants is significant especially for elements such as mercury and arsenic, which are not essential and can form extremely toxic volatile compounds [84]. Elemental mercury is far less toxic than methylmercury and its half-life in the atmosphere is in the order of years which should enable a substantial dilution into the large atmospheric pool [85]. For this reason an approach based on the accumulation of Hg^{2+} in the plant shoot rather than volatilisation of elemental mercury has been pro- posed as an alternative strategy [86]. Studies on arsenic uptake and distribution in higher plants indicate that ar- senic predominantly accumulated in root and only small quantities are transported to shoots. However, plant may enhance the biotransformation of arsenic by rhizospheric bacteria, thus increasing rates of volatilization.

8.4. Rhizodegradation

Field-contaminated soils that have undergone prolonged periods of ageing [87-91] generally appear to be much less responsive to rhizodegradation than fresh soil [92-98]. Characterising root exudation in terms of chemical composition and quantity and investigation of utilization pattern by microbial strains competent to degrade organic pollutants is a prerequisite for this purpose. In long-term field contaminated soil, enhancement of bioavailability appears to be the key of successful biodegradation. Selection and engineering of plants and microbial strains that can modify solubility and transport of organic pollutants through exudation of biosurfactants holds promise [99]. Recent attempts to genetically engineer plant– microbial systems to enhance rhizodegradation include gene cloning of plants containing bacterial enzymes for the degradation of organic pollutants such as PCBs and of recombinant, root-colonising bacteria (e.g. *Pseudomonas fluorescens*) expressing degradative enzymes (e.g. ortho-monooxygenase for toluene degradation) [100].

Soils and sediments polluted with crude oil hydrocarbons are of major environmental concern on various contaminated sites. Hydrocarbon-degrading microorganisms are ubiquitously distributed in soils and constitute less than 1% of the total microbial communities but may increase to 10% in the presence of crude oil [101]. Degradation of HC further requires a balanced nutrient supply in soil which can be achieved by fertilisation (biostimulation). Mainly nitrogen and, to a lesser extent, phosphorus are reported to be limiting factors of HC degradation processes in oxic soil environments [102-104]. Microorganisms are able to use HC as a carbon and energy source [103] preferentially in the absence of a readily available carbon source like labile natural organic matter. Read e*t al.* [105] observed increased phosphorus mobilisation due to exudation of biosurfactants by lupine (*Lupinus angustifolius* L. cv. Merrit). The identified biosurfactants consisted of phospholipids which could provide an additional phosphorus source to microorganisms. Polycyclic aromatic hydrocarbons (PAHs) are contaminants generated from many sources such as the

combustion of coal and fossil fuels for energy production and are potential carcinogens that can induce mutations. As lipophilic compounds, they present a significant health risk if they enter the food chain [106]. These compounds can be used by soil microorganisms as an energy and carbon source, although four-, five-, and six-ring PAHs are more resistant to biodegradation. Bacteria initiate PAH degradation via dioxygenase attack, increasing PAH chemical reactivity and solubility [107].

8.5. Phytostabilisation/Phytoimmobilisation

Inoculation with metal-resistant PGPR can support the establishment and improve vitality of the phytostabilisation crops, and detoxification mechanisms in the rhizosphere may be enhanced by inoculation with microbial associates. Some plants and microorganisms are able to precipitate metal compounds in the rhizosphere. This was shown for lead pyromorphite [108,109], and may provide an effective means to reduce metal toxicity as well as metal mobility (phytoimmobilisation) [110]. The design of phytostabilisation systems relates to combining different approaches to ameliorate multiple constraints (*i.e.*, nutrient and water deficiency, toxicity due to mixed contamination) and to control their efficiency in field conditions. The combined use of alders, frankiae and mycorrhizae for the remediation of contaminated ecosystems is thought to improve plant nutrition through both the actinorhizal symbiosis (*Frankiae*) and the mycorrhizal symbioses, and to protect the plant from toxicity through the mechanisms discussed above for metals and organic pollutants [71].

8.6. Advantages and Disadvantages of Phytoremediation

Phytoremediation is well suited for use at very large field sites where other methods of remediation are not cost effective or practicable; at sites with a low concentration of contaminants where only polish treatment is required over long periods of time and in conjunction with other technologies where vegetation is used as a final cap and closure of the site. There are some limitations to the technology which include long duration of time for remediation, potential contamination of the vegetation and food chain and difficulty in establishing and maintaining vegetation at some sites with high toxic levels.

8.7. Integrated Approaches

The complexity and heterogeneity of sites often polluted with multiple metals, metalloids and organic compounds requires the design of integrated phytoremediation systems that combine different processes and approaches. Co-cropping different species may enhance the overall capabilities of a phytoremediation system to explore the contaminated soil volume, address different pollutants, and support differential microbial consortia in their rhizospheres. Shared rhizospheres may be designed to optimise the nutritional status, e.g. by combining plants that support N_2-fixing and P-solubilising microorganisms. Co-cropping could be also used to modify the bioavailability of pollutants, e.g. by combining Alnus sp. with metal-accumulating willows [71] or to combine metal phytoextraction crops (e.g. willows) with plants that support rhizodegradation of organic pollutants (e.g. grasses). Engineering rhizobacteria capable of heavy metal accumulation and enhanced degradation of organic pollutants such as trichloroethylene offers further opportunities to address multiple contaminated sites [111]. While some phyto-/rhizoremediation technologies are being used commercially, it is obvious that the complexity of interactions in the plant–microbe–soil pollutant system requires substantial further research efforts to improve our understanding of the rhizosphere processes involved.

9. Conclusion and Future Research Needs

It can be concluded that bioremediation is indeed an environmentally friendly, gentle management option for polluted soil as it uses solar-driven biological processes to treat the pollutant. The success of a phytoextraction technique is largely dependent on the continuous availability of the metal of interest to the phytoextracting plants. It appears attractive because in contrast to most other remediation technologies, it is not invasive and, in principle, delivers intact, biologically active soil. There is a need to enhance research efforts on this emerging and environmentally friendly "green" technology. Research should focus on identifying remediating plants that are adapted to the local climate and soil conditions.

As phytoremediation is a slow process, biotechnological as well as classical hybridization techniques should be used to develop more efficient metal hyperaccumulator plant species having increasing pollutant tolerance, root and shoot biomass, root architecture and morphology, pollutant uptake properties, and degradation capabilities for organic pollutants etc. [9].

Other approaches to be the management of microbial consortia: the selection and engineering of microorganisms with capabilities for pollutant degradation, beneficial effects on the phytoremediation crops, or modifying effects on pollutant bioavailability. Selection and engineering of plants and microbial strains that can modify solubility and transport of organic pollutants through exudation of biosurfactants holds a great promise [99].

Additional strategies should include proper management of the soil, e.g. via fertilisation or chelant addition to increase pollutant bioavailability, and of the phytore-

mediation crops, e.g. via optimisation of coppicing, harvest cycles, development of mixed cropping systems etc. In long-term field contaminated soil, enhancement of bioavailability appears to be the key of successful biodegradation. Phytoremediation is still a new field which holds great potential and in order to develop this potential requires a multidisciplinary approach, spanning field as diverse as plant biology, agricultural engineering, agronomy, soil science, microbiology and genetic engineering. Finally, the applicability of these bio-approaches for rehabilitation of contaminated soils needs to be fully demonstrated in the fields for wider acceptability.

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