



Review

Microorganisms in heavy metal bioremediation: strategies for applying microbial-community engineering to remediate soils

Jennifer L. Wood¹, Wuxing Liu², Caixian Tang³, and Ashley E. Franks^{1,*}

¹ Department of Microbiology, La Trobe University, Melbourne Campus, Victoria, 3086, Australia

² Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

³ Department of Animal, Plant and Soil Sciences, Center for AgriBiosciences, La Trobe University, Melbourne Campus, Victoria, 3086, Australia

* **Correspondence:** Email: A.Franks@latrobe.edu.au; Tel: +61-3-94792206; Fax: +61-9479-1222.

Abstract: The remediation of heavy-metal-contaminated soils is essential as heavy metals persist and do not degrade in the environment. Remediating heavy-metal-contaminated soils requires metals to be mobilized for extraction whilst, at the same time, employing strategies to avoid mobilized metals leaching into ground-water or aquatic systems. Phytoextraction is a bioremediation strategy that extracts heavy metals from soils by sequestration in plant tissues and is currently the predominant bioremediation strategy investigated for remediating heavy-metal-contaminated soils. Although the efficiency of phytoextraction remains a limiting feature of the technology, there are numerous reports that soil microorganisms can improve rates of heavy metal extraction.

This review highlights the unique challenges faced when remediating heavy-metal-contaminated soils as compared to static aquatic systems and suggests new strategies for using microorganisms to improve phytoextraction. We compare how microorganisms are used in soil bioremediation (i.e. phytoextraction) and water bioremediation processes, discussing how the engineering of microbial communities, used in water remediation, could be applied to phytoextraction. We briefly outline possible approaches for the engineering of soil communities to improve phytoextraction either by mobilizing metals in the rhizosphere of the plant or by promoting plant growth to increase the root-surface area available for uptake of heavy metals. We highlight the technological advances that make this research direction possible and how these technologies could

be employed in future research.

Keywords: heavy metal; soil; remediation; community engineering; phytoextraction

1. Introduction: The Bioremediation of Heavy Metals

Heavy metal contaminants entering our soils and water are a major concern due to both toxicity and persistence within the environment, necessitating physical extraction [1,2]. For the purpose of this review, the term “heavy metals” is extended beyond its strict definition to include metalloids such as arsenic (As) and selenium (Se), which also constitute environmental contaminants and require analogous remediation strategies [3]. Heavy metals can be introduced into the environment via an array of anthropological activities, prominent amongst which are mining (mine tailings, the transport of ores, smelting etc.) and agriculture (the application of fertilizers, pesticides or herbicides containing heavy metal impurities)[4–6]. The accumulation of heavy metals in aquatic and terrestrial ecosystems can have severe health and environmental consequences. The United States Agency for Toxic Substances and Disease Registry (ATSDR) has listed As, lead (Pb), mercury (Hg) and cadmium (Cd) amongst the top ten hazardous substances that pose the greatest threat to human health [7,8].

Heavy metals persisting in the soil can be absorbed by plant tissues, enter the biosphere and accumulate up the trophic levels of the food web [4,9]. In humans, heavy metal poisoning can cause irreparable nerve and organ damage, tumors and intra-uterine retardation. Symptoms of toxicity are variable and often only emerge following several years of exposure [5,10]. Despite the known dangers, the rate of heavy metal deposition into soils continues to increase in many parts of the world, as does human exposure to them [11–13].

Phytoextraction is the predominant bioremediation strategy investigated for the remediation of heavy-metal-contaminated soils. Phytoextraction utilizes plants to move heavy metals from soils into the aerial tissues of the plant where they are sequestered [14]. Upon harvest of the plant material, the metals are permanently removed from the contaminated site. Much research within the field has focused on specialized plants called heavy-metal hyperaccumulators (HMHs) which can obtain concentrations of heavy metals in their above-ground tissues 100-1000 fold higher than what is typically found in non-HMHs [15]. Phytoextraction can also facilitate the recovery of commodity heavy metals through the harvesting of plant biomass, negating the need for excavation in a process termed “phytomining” [16–19].

Bioremediation strategies for heavy-metal-contaminated water include: biosorption, microbial-driven *in situ* precipitation of heavy metals by dissimilatory reducing bacteria, and the incorporation of HMHs in artificial wetlands [14,20–22]. All three of these techniques use microorganisms to facilitate the remediation process either on their own or in conjunction with plants and carry with them their own strengths and weaknesses.

This review highlights the unique challenges faced when remediating heavy-metal-contaminated soils as compared to static aquatic systems. We discuss the ways in which microorganisms are used in

heavy metal bioremediation, with the aim of highlighting how microbial community-engineering strategies, used in water remediation, could be applied to phytoextraction to remediate soils. We conclude by outlining possible approaches for the engineering of soil communities to improve phytoextraction and highlight the technologies that make this approach possible.

2. The Role of Heavy Metal Mobility in Soil and Water Remediation

A key difference between the remediation of heavy metals in soils compared to water is the desired outcome for metal mobility. In water remediation, a desirable outcome is to precipitate heavy metals out of the water column, precluding their spread in the environment (Figure 1A). Biosorption is a metabolically passive process whereby heavy metals in solution are sorbed onto a solid phase made from living or attenuated biological material, which can then be removed and replaced [23]. Bacterial, fungal, algal and plant biomass have all been proposed as suitable materials for biosorption [24,25]. Biosorption has been shown to effectively remove a variety of heavy metals from aqueous solutions including highly toxic metal ions of Cd, Cr, Pb and Hg [26]. The success of biosorption relies on the diversity of cell wall structures (e.g. Eukaryote, Gram positive and Gram negative cell walls) and charged sites on cell wall components (polysaccharides, chitin, nucleic acids and proteins), which provide chemically diverse binding partners for different heavy metal ions and compounds [25]. Currently, biosorption investigations are still transitioning from fundamental research to industrial-scale studies which need to take into consideration: optimal operating conditions (pH, temperature, kinetics), methods for biomass application (packed columns or immobilized biomass), regeneration of the biosorbent, and the complexities that come with the presence of multiple heavy metals and other contaminants in solution [26].

Whereas biosorption typically uses metabolically inert biological mass, *in situ* bioremediation utilizes the metabolic activity of dissimilatory reducing microorganisms to precipitate heavy metals [27]. It is well documented that a range of metals, including Mn(IV) and Fe(III), can be reduced by dissimilatory metal-reducing bacteria using organic compounds as electron donors [27,28]. By stimulating the activity of Fe(III)-reducing bacteria, the reduction of other metal ions, including toxic metal ions such as U(VI), Cr(VI), Co(III) and Tc(VII), can also occur. The effect of microbial redox reactions on heavy metal mobility depends the oxidation state and the heavy metal in question [29]. For example, where the reduction of Fe(III) generates mobile Fe(II), the reduction of U(VI) to U(IV), Tc(VII) to Tc(IV) or Tc(V), Co(III) to Co(II) and Cr(VI) to Cr(III) generate less toxic, insoluble ions [30].

The activity of indigenous dissimilatory reducing bacteria can be stimulated *in situ* via the addition of appropriate electron donors to drive bioremediation processes. The activity of *Geobacter* species, which reduces Fe(III), but also soluble U(VI) to insoluble U(IV), can be stimulated by the addition of a soluble electron donor, such as acetate, or insoluble electron donors, such as electrodes, which can be added to the environment as part of a microbial electric systems (MES) [31–33]. As well as heavy metals, MES technology can assist the remediation of organic and inorganic contaminants from soils, ground water and waste waters [22].

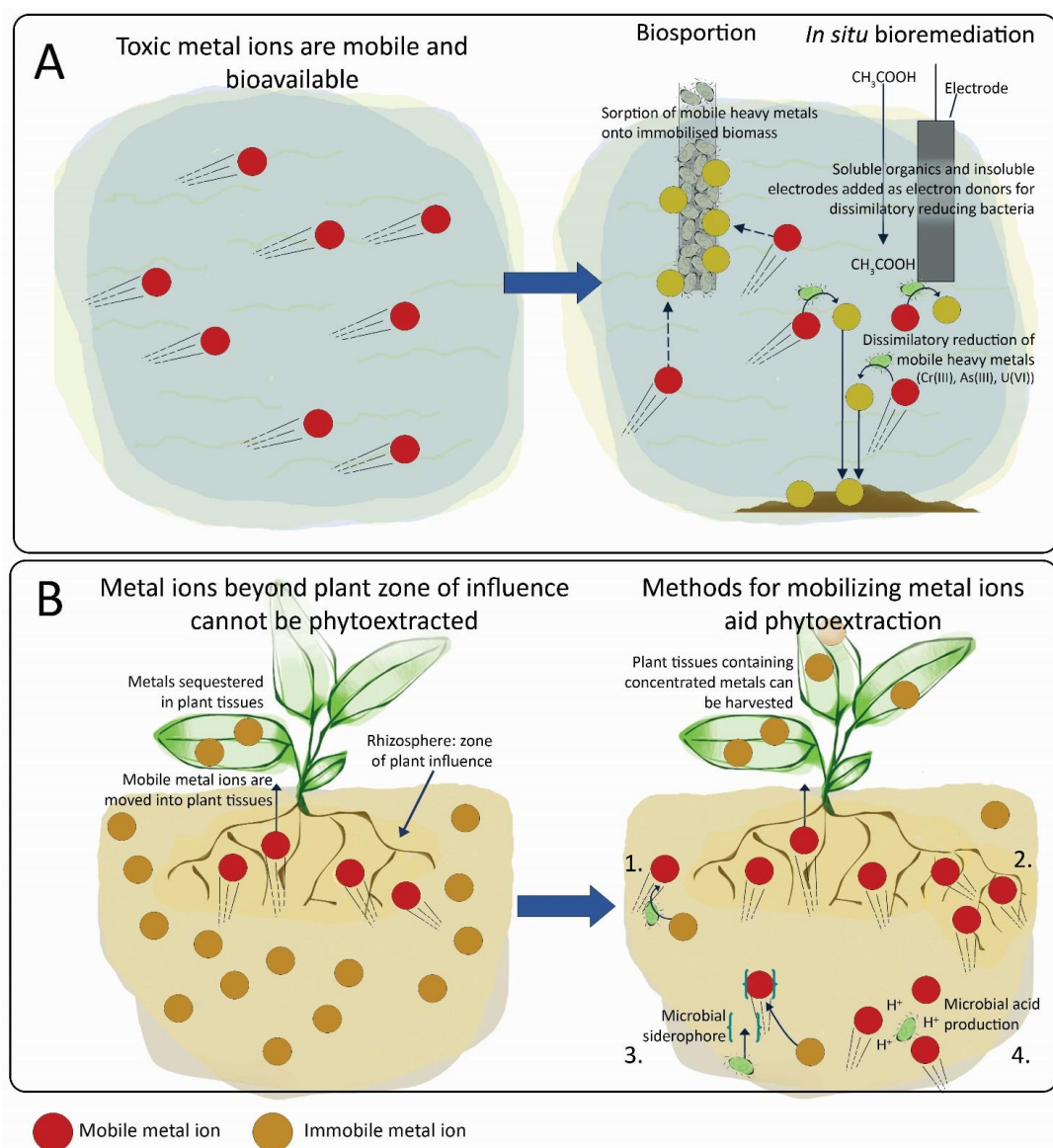


Figure 1. Schematic illustrating heavy metal bioremediation via: immobilization in water and mobilization in soils. **(A)** Immobilization of metal ions is used to remediate contaminated water through: precipitation of metal ions via sorption onto a solid phase made of attenuated microbial biomass (biosorption) and; *in situ* bioremediation via dissimilatory metal reducing bacterial activity stimulated through the addition of soluble (organics) or insoluble (electrodes) electron donors. **(B)** Immobilization of metal ions in soil by binding to the soil matrix limits their bioavailability for phytoextraction. Metals and metalloids in the soil can be mobilized by: **1** dissimilarity metal reducing bacteria activity increasing metal bioavailability; **2** plant-growth-promotion increasing the plant zone of influence; **3** chelation of immobile metals by microbial siderophores; **4** organic acid production by bacteria lowering soil pH which increases metal bioavailability.

Precipitated metal ions in the environment may still require extraction as once the electron donor is removed, and conditions no longer favor microbial dissimilatory metal reduction, the metal ions can

be resolubilized. Spontaneous chemical reactions or microorganisms using the reduced metal ions as an electron source can lead to the resolubilization of the immobilized metal ions [34]. The redox cycling of metals by microbial communities may be a limiting feature of *in situ* ground-water remediation, as without constant inputs to drive microbial reduction over oxidation the remediation work may be undone. For example, upon the cessation of acetate amendments, which stimulate U(VI) reduction, selective pressures on favorable microbial processes (i.e Fe(III) and U(VI) reduction) likewise cease and the immobilized U(IV) is re-oxidized to mobile U(VI) [35]. Thus, although immobilization can prevent the movement of heavy metals in the environment, the reversible nature of immobilization reactions highlights the need to ultimately remove heavy metals from the environment.

Investigations into *in situ* redox cycling have also elucidated strategies for selectively mobilizing metal ions via the addition of specific substrates. For instance, the addition of nitrate facilitates the oxidation of Fe(II) and U(VI) by *Geobacter* spp. using nitrate as an electron acceptor [36]. Whilst metal mobilization may not be desirable in aquatic systems, microbial redox reactions that increase metal availability could be integrated into soil remediation and phytoextraction, as we shall discuss shortly.

Whilst the remediation of heavy-metal-contaminated water requires the precipitation of soluble metal ions, in soils the majority of heavy metals may already be immobile, chemically bound to organic particles or sequestered in insoluble mineral complexes [37]. However, this immobility does not preclude the mobilization and entry of heavy metals into the biosphere. Thus, in contrast to aquatic systems, the immobilization of heavy metal ions is a key limiting feature for soil remediation as the amount of a metal that can be concentrated in plant tissues is directly proportional to the amount of bioavailable metal in the soil [38]. There are a number of strategies that can be employed to enhance heavy-metal-phytoextraction rates to overcome this limitation (Figure 1B).

3. Using Microorganisms to Improve Soil Phytoremediation

3.1. Increasing metal bioavailability in soils for phytoextraction

A clear strategy for increasing heavy metal bioavailability is by increasing metal mobility in the soil. There are a range of chemical chelators that have been shown to increase soil metal-mobility and plant uptake. These include EDTA (ethylenediaminetetraacetic acid), ADA (*N*-2-(acetamido)iminodiacetic acid), DTPA (diethylenetriaminepentaacetic acid), CDTA (*trans*-1,2-cyclohexylenedinitrilotetraacetic acid), EGTA (ethylenebis[oxyethylenenitrilo]tetraacetic acid) and PDA (pyridine-2,6-dicarboxylic acid)[39,40]. Environmental concerns regarding the persistence of chemical chelators in soils has led to research into biodegradable chemical chelators such as; NTA (nitrilotriacetic acid), EDDS ([*S,S*]-ethylenediaminedisuccinic acid), IDSA (iminodisuccinic acid) and MGDA (methylglycine diacetic acid) [41]. There is also considerable interest in biological compounds, such as biosurfactants, siderophores and organic acids as biodegradable alternatives to chemical chelators for mobilizing heavy metals [42]. Compared to synthetic chelators, such as EDTA and NTA, organic acids including citric, oxalic and tartaric acid, have been shown to increase the solubility of heavy metals for short periods of time before being

rapidly degraded in the soil [43,44]. Microbial siderophores, such as desferrioxamine-B (DFO-B), have demonstrably improved Cd hyper-accumulation by 37% and increased root-to-shoot translocation by 27% in *Nocca caerulea* [45]. Similarly, microbial-derived biosurfactants, such as rhamnolipid, have been shown to enhance the accumulation of Cu and Cd in rye grass (*Lolium perenne*) [42,46]. Although chemical and organic chelators do increase metal bioavailability, there is an associated risk that the mobilized metals will be leached into ground-water if the rate of plant uptake is not matched to the rate of chelator application [47,48].

Organic heavy-metal-solubilizing compounds can be added to soils directly, as purified products, or indirectly by adding microbial inoculants capable of their production. Using microorganisms, as opposed to their products, in phytoextraction may reduce the risk of heavy metals leaching into waterways by concentrating metal solubilization near the plant root-surface where microbial activity is greatest and extraction is most likely to take place. Additionally, if the chelate-producing microorganisms persist in the soil environment, there is potential for sustained production of metal-solubilizing compounds that would otherwise be biodegraded.

3.2. Increasing plant uptake and accumulation of heavy metals to enhance phytoextraction

Promoting plant growth is an alternate strategy for enhancing phytoextraction. Processes that stimulate plant growth increase the amount of harvestable, heavy-metal-containing biomass as well as the available below-ground surface area at which reactions, including heavy metal mobilization and uptake, can take place. Increases in plant-root biomass, following fertilizer application, have been associated with the improved accumulation of Ni in *Alyssum bertolonii* by as much as 300 % [49]. Microorganisms can also be used to improve heavy metal phytoextraction by promoting plant growth. For instance, increases in canola biomass, due to *Pseudomonas fluorescens* and *P. tolaasii* inoculations, improved Cd-phytoextraction by 72% and 107%, respectively [50]. The improvements in Cd-phytoextraction were due only to microbial plant-growth-promotion (PGP), as Cd concentrations in the plant tissues remained constant.

Microbial plant-growth-promotion can be achieved via the secretion of PGP compounds such as indole-3-acetic acid (IAA), gibberellins or cytokines, or by improving plant nutrition via the solubilization of inorganic phosphates (P) and ferric iron (Fe(III)) or nitrogen (N₂) fixation. It is worth noting that a number of PGP processes can also mobilize heavy metals in the soil: Microbial siderophores promote plant growth by solubilizing ferric iron and increasing plant Fe(III) acquisition, but can also chelate and mobilize heavy metals [51,52]. Similarly, the production of organic acids by phosphate-solubilizing microorganisms (PSMs) improves plant P acquisition and growth but has also been shown to increase heavy metal mobility through chelation and/or altering soil pH [53,54]. Selected microbial PGP processes proposed to influence phytoextraction are summarized in Table 1; extensive reviews of microbial PGP processes can be found elsewhere [42,55–58].

Table 1. Summary of selected plant-growth-promoting (PGP) mechanisms, the functions that they perform for microbes and how they stimulate plant growth.

PGP attribute	Mechanism of action	Microbial function	PGP effect	Ref.
Siderophore production	Microbes secrete siderophores (low-molecular-weight, chelating complexes) that chelate and mobilize Fe(III).	Fe(III) acquisition	Fe(III) acquisition via plant uptake of microbial siderophores	[59]
Phosphate solubilization	Production and secretion of organic acids including gluconic acid, oxalic acid, tartaric acid, formic acid and acetic acid to lower soil pH and solubilize phosphate	P acquisition	P acquisition	[53]
N ₂ fixation	Fixation of atmospheric N ₂ by <i>Rhizobia</i> spp. in root nodules and non-symbiotic diazotrophs	Acquisition of C from plants when excess fixed N ₂ stimulates plant growth	Additional N	[60]
Plant-growth-hormone production	Microbes synthesize and secrete molecules that regulate plant growth including auxin (IAA), gibberellins and cytokines	Acquisition of C and N, additional root exudate that accompanies plant growth	Hormones induce plant-growth signaling pathways	[61]
ACC-deaminase production	Degradation of 1-aminocyclopropane-1-carboxylic acid (ACC), an intermediate in the ethylene (plant stress) hormone production pathway	Acquisition of N via the degradation of ACC	Inhibition of stress signaling that would otherwise inhibit growth	[62],

3.3. The success and challenges of using microorganisms to improve phytoextraction

To date, multiple studies have provided evidence that rhizosphere-associated microorganisms have a positive impact on phytoextraction efficacy, increasing heavy metal accumulation as much as 4 fold when compared to rates obtained in sterilized soil [63–68]. However, there are a number of limitations yet to be overcome.

A typical strategy for using microorganisms to improve phytoextraction is to “mine” PGP or metal-solubilizing microorganisms from contaminated soils and rhizospheres. Promising isolates are then applied during the phytoextraction process to the rhizosphere of the plant exogenously, either as a single-strain inoculum or as a consortia of microorganisms with PGP and metal-mobilizing attributes [69–71]. The two main techniques for microbial inoculation of the rhizosphere are soil drenching and direct inoculation onto the plant seeds before planting [72]. This strategy has shown promise; some 49 microbial isolates with the potential to improve the phytoextraction of six different heavy metals have been identified from just 24 publications in the last decade, a list that is by no-means exhaustive (Table 2).

Despite the success of this approach, there have been reports of isolates that induce significant improvements to phytoextraction under laboratory conditions, exhibiting variable or poor performance in the field. Soil nutrient and contaminant characteristics may account for variable PGP performance exhibited by *Pseudomonas thivervalensis* STF3 and *Serratia marcescens* STJ5, between laboratory and field experiments [73]. Significant shoot- and root-growth-promotion exhibited in axenic pot trials translated to only root-growth-promotion in field trials. Additionally, root-growth-promotion by the two strains was reduced from 68 to 31% and 82 to 25% respectively, from laboratory to field [73]. The issue of performance variability is a multifaceted one that can be due to both abiotic (soil nutrient characteristics, pH, level and types of contamination present) and biotic (selection by the plant, *in situ* competition with indigenous microorganism) factors [74–77]. Even in instances where initial inoculation results in measurable PGP, competition with native soil microorganisms may reduce the effect of an inoculum over time [57,78]. The PGP effect of *Pseudomonas chlororaphis* RA6 was lost after 60 days when the inoculum was no longer detectable by DGGE in the rhizosphere [79].

Table 2. Summary of microorganisms that demonstrably improved phytoextraction from selected of publications (ranging from 2001–2015). Organisms listed in square parentheses [] were added as a consortia.

Publication	Microorganism	Target metal
[80]	<i>Microbacterium arabinogalactanolyticum</i> AY509225 <i>Microbacterium oxydans</i> AY509223	Ni
[81]	<i>Bacillus pumilus</i> S28 <i>Bacillus subtilis</i> S3 <i>Brevibacterium halotolerans</i> S29 <i>Pseudomonas pseudoalcaligenes</i> S22	Cr, Cu
[82]	<i>Agrobacterium radiobacter</i> <i>Arthrobacter mysorens</i> 7 <i>Azospirillum lipoferum</i> 137 <i>Flavobacterium</i> sp. L30	Cd, Pb
[50]	<i>Mycobacterium</i> sp. ACC14 <i>Pseudomonas fluorescens</i> ACC9 <i>Pseudomonas tolaasii</i> ACC23	Cd

[83]	<i>Paecilomyces lilacinus</i> NH1	Cd
[84]	<i>Bacillus</i> sp. RJ16	Cd, Pb
	<i>Pseudomonas</i> sp. RJ10	
[85]	[<i>Pseudomonas</i> sp. P1III2, <i>Delftia</i> sp. P2III5] [<i>Variovorax</i> sp. P4III4, <i>Pseudoxanthomonas</i> sp. P4V6, <i>Bacillus</i> sp. MPV12]	As
[86]	<i>Burkholderia cepacia</i>	Cd, Zn
[87]	<i>Cedecea davisae</i> LCR1	
	<i>Rhodococcus erythropolis</i> NSX2	Cd
[88]	<i>Bacillus cereus</i> SRA10	Ni
	<i>Psychrobacter</i> sp. SRA1	
	<i>Psychrobacter</i> sp. SRA2	
[89]		
	<i>Achromobacter xylosoxidans</i> Ax10	Ni
[90]	<i>Pseudomonas</i> sp. A3R3	Ni
[91]	<i>Phyllobacterium myrsinacearum</i> RC6b	Cd, Zn
[64]	<i>Bacillus mycoides</i> , <i>Micrococcus roseus</i>	Cd
[92]	<i>Enterobacter intermedius</i> MH8b	Cd, Zn
[93]	<i>Klebsiella</i> sp. BAM1	Cd
[94]	<i>Pseudomonas jessenii</i> PjM15	Ni, Cd, Zn
[95]	<i>Bacillus megaterium</i> SR28C	
[96]	[<i>Pseudomonas putida</i> 710, <i>Comamonas aquatica</i> 710B]	Cd
[97]	<i>Bacillus</i> sp. J119	Cd
[68]	[<i>Microbacterium saperdae</i> BJ1 <i>Pseudomonas monteilii</i> BJ5, <i>Enterobacter cancerogenus</i> BJ10]	Zn
[98]	<i>Comamonas</i> sp. TS37	As
	<i>Delftia</i> sp. TS33	
	<i>Delftia</i> sp. T S41	
	<i>Rhodococcus</i> sp. TS1	
	<i>Streptomyces lividans</i> PSQ22	
[99]	<i>Bacillus subtilis</i> SJ-101	Ni
[100]	<i>Fusarium oxysporum</i>	Cd, Cu, Pb, Zn

4. The Hidden Potential of Native Soil-Microbial Communities

Whilst complex native soil-microbial communities can hinder the use of microbial inocula in enhancing phytoextraction, they may also present opportunities. The importance of indigenous-microbial communities in metal accumulation was highlighted by microcosm studies that

compared *Arabidopsis halleri* Cd- and Zn-accumulation in the presence of “native” and a strongly disturbed (gamma-irradiated) soil-microbial communities. The disturbance of the soil-microbial community significantly reduced the amount of Zn and Cd accumulated by the plant even though the plant biomass was unaffected [101]. There is also growing evidence that PGP abilities can be gained by indigenous-microbial communities and that this functional change can be stimulated by PGP-microbial inocula, even if the inoculum itself is lost over time. For instance, indigenous-microbial communities were able to promote the growth of successive faba bean plantings even after the initial PGP-inoculum, *B. pumilus* WP8, was lost from the rhizosphere [79]. The ability of the indigenous community to promote plant growth coincided with a change in the structure of the original soil community [79]. The exact changes that the inoculum elicited in the community and how it did so require further investigation. Similarly, recent work has demonstrated that indigenous PGP-rhizosphere bacteria are enriched in rhizosphere communities following the application of a PGP *Rhodococcus erythropolis* NSX2 or *Cedecea davisae* LCR1 inoculum, the latter of which was itself lost from the community after three months [87].

5. Engineering of Communities to Improve Phytoextraction

The potential for using indigenous soil-microbial communities to enhance phytoextraction has yet to be fully explored. Just as the *in situ* bioremediation of uranium-contaminated ground-water utilizes ubiquitous microorganisms and metabolic processes, community engineering could be used to select for indigenous microorganisms that mobilize heavy metals in soils or promote plant growth [20]. The use of *in situ*-microbial communities in soil remediation has two advantages:

- 1) Using *in situ* communities negates the issue of variable inoculum performance under different soil conditions by utilizing the best plant-growth-promoters from each given soil.
- 2) Competition within the community could be used as leverage to increase the numbers of phytoextraction-aiding microorganisms (via the addition of amendments that selectively advantage them) rather than an obstacle impeding the colonization and retention of an inoculum.

As previously mentioned, redox reactions can increase or decrease heavy metal mobility in soils and strategies to promote metal mobilization (such as the addition of nitrate as a soluble electron acceptor) have been identified [36]. Thus, *in situ*-microbial communities could be exploited to improve phytoextraction by stimulating bacteria that mobilize heavy metals to assist plant-uptake. The reversible nature of mobilization/immobilization reactions that can limit *in situ* ground-water remediation is less likely to impede phytoextraction since heavy metals are physically *removed* from the soil. Indeed, the coupling of the successional mobilization and immobilization of Cd by *Geobacter* sp. Cd1, to phytoextraction, has been suggested for this reason [102].

In addition to microbial redox reactions, community engineering to improve phytoextraction could target the promotion of known PGP genre such as the *Bacillus*, *Enterobacter*, *Pseudomonas* and *Rhizobium* [103]. However, the conditions that favor the proliferation of these microorganisms or induce PGP activities in the presence of heavy metals have not been explicitly investigated.

An alternate community engineering approach to stimulate microbial PGP is to target biochemical processes thought to be linked to plant-growth-promotion (Table 1). The microbial production of indole-3-acetic acid (IAA), is repeatedly cited as a microbial process that can improve

phytoextraction [57,85,88,104]. Although there are multiple IAA synthesis pathways, at least five of the known pathways (Indole-3-acetamide pathway, Indole-3-pyruvate pathway, Tryptamine pathway, Tryptophan side-chain oxidase pathway and the Indole-3-acetonitrile pathway) converge on the precursor tryptophan [105]. The addition of tryptophan *in vitro* can stimulate microbial IAA production and could be investigated as a substrate for upregulating IAA pathways *in situ* [106]. Other microbial processes that could be targeted for community engineering include fermentative processes that produce short-chain carboxylic acids such as citric acid, acetic acid, oxalic acid and tartaric acid, which are produced by PSMs [107]. Sugars such as glucose and beet molasses, which have been used for the industrial production of lactic and citric acid respectively, are potential starting points for stimulating the production of organic acids by PSMs [108].

Ideally, processes targeted by community engineering will be most active in the rhizosphere. The assembly of rhizosphere communities from the bulk soil is believed to be a largely deterministic process and there is evidence that microorganisms with advantageous growth strategies for a given soil are further enriched in the plant rhizosphere [109,110]. For instance, in the presence of hydrocarbons microorganisms capable of their degradation are selectively advantaged in soil [111]. However, comparisons of microorganisms encoding hydrocarbon metabolising genes (*ndoB*, *alkB* and *xylE*) have demonstrated that, in the presence of contamination, the enrichment of hydrocarbon-degrading ecotypes is greater in the rhizosphere relative to bulk soils [110]. Thus, it is likely that the engineering of soil communities to promote a specific microbial process related to plant-growth-promotion or metal solubilization will also drive the enrichment of these processes in the rhizosphere where they will have the greatest impact on phytoextraction.

6. The Technological Advancement to Aid Rhizosphere Community Engineering

The technologies available for unraveling soil communities to a point where community-engineering strategies can be developed are becoming increasingly sophisticated and accessible. The increasing ease with which high-throughput amplicon-based sequencing (such as 16S rRNA profiling) can be used to taxonomically characterize a community is partly due to decreasing costs of sequencing materials and partly to software development. In particular, software associated with data QC and analysis have been streamlined by platforms such as QIIME (quantitative insights into microbial ecology), USEARCH and the RDP (ribosomal database project) to enable rapid visualization of the taxonomic structure of microbial communities [112–114]. 16S rRNA profiling can be used to track the response of key genera to soil treatments and identify community-engineering strategies that stimulate their growth. However, identifying which treatments stimulate the growth of desirable microorganisms will remain a challenge.

The use of high-throughput sequencing to investigate biochemical pathways and metabolic processes of interest is more difficult. Shotgun metagenomics enables the reconstruction of entire genomes from multiple organisms in a community. However, the high diversity in soils makes it difficult to generate adequate read-coverage of rarer genomes without massive amounts of sequencing [115]. Thus, typically only the genomes of the most abundant community members are able to be assembled.

Predictive metagenomics software, such as PICRUSt and tax4fun, take advantage of strength of amplicon-based and metagenome sequencing approaches by linking taxonomic information from 16S rRNA sequences to existing databases of assembled genomes to predict the functional potential of communities [116,117]. Predictive metagenomics could be used in community-engineering as a preliminary strategy to test whether changes to taxonomic profiles are translating to changes in the functional potential of communities. Alternatively, microarray technologies such as GeoChip can be used in conjunction with community DNA or mRNA to track changes to community functional potential and functional activity, respectively [118]. GeoChip microarrays capture information on major biogeochemical cycles, including carbon, nitrogen, phosphorus, sulfur and various metals as well as information on antibiotic resistance, organic contaminant degradation, stress responses, and energy production [118]. Used in conjunction with mRNA extracted from communities, GeoChip can be used to measure changes in mRNA expression of environmentally relevant genes to inform community-engineering studies. The addition of microarray probes that target genes in biosynthesis pathways associated with plant-growth-promotion, such as IAA (*ipdC* and *iaaM*) or siderophore (*entABCDEF*) biosynthesis pathways, would further improve the value of GeoChip and other functional microarrays to community-engineering phytoextraction studies [105,106,119].

7. Conclusion

Phytoextraction is the predominant bioremediation strategy available for remediating heavy-metal-contaminated soil. To date, much has been learned regarding the ways in which microorganisms can improve phytoextraction through the use of plant-growth-promoting and metal-mobilizing inocula. The bioremediation of heavy-metal-contaminated water has demonstrated that microbial processes can be stimulated *in situ* to assist with bioremediation. Recent developments in sequencing technologies and software now permit research into the community engineering of *in situ*-microbial communities to augment the phytoextraction of heavy metals in soil. Using *in situ*-microbial communities to assist phytoextraction could further improve the technology by utilizing microorganisms optimally adapted to contaminated soil and rhizosphere conditions. This avenue of research will also contribute greatly to our fundamental understanding of soil-microbial ecology.

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Conflict of Interest

The content of the manuscript has been approved for publication by all the authors, and we have no conflicts of interest to disclose.

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