Review

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

Jennifer L. Wood 1, Wuxing Liu 2, Caixian Tang 3, 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 (ethylenbis[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 (nitritoltriacetic 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 Noccaea caerulescens [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 (N_2) 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] |
| N₂ fixation                    | Fixation of atmospheric N₂ by *Rhizobia* spp. in root nodules and non-symbiotic diazotrophs | Acquisition of C from plants when excess fixed N₂ 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], |
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 thiweralensis 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]        | Microbacterium arabinogalactanolyticum AY509225 | Ni           |
|             | Microbacterium oxydans AY509223                |              |
| [81]        | Bacillus pumilus S28                            | Cr, Cu       |
|             | Bacillus subtilis S3                            |              |
|             | Brevibacterium halotolerans S29                 |              |
|             | Pseudomonas pseudoalcaligenes S22               |              |
| [82]        | Agrobacterium radiobacter                       | Cd, Pb       |
|             | Arthrobacter mysorens 7                         |              |
|             | Azospirillum lipoferum 137                       |              |
|             | Flavobacterium sp. L30                          |              |
| [50]        | Mycobacterium sp. ACC14                         | Cd           |
|             | Pseudomonas fluorescens ACC9                    |              |
|             | Pseudomonas tolaasii ACC23                      |              |
| Reference | Organism Description |
|------------|----------------------|
| [83] | *Paecilomyces lilacinus* NH1 | Cd |
| [84] | *Bacillus* sp. RJ16 | Cd, Pb |
| [85] | *Pseudomonas* sp. RJ10 | 
| [85] | *Pseudomonas* sp. P1III2, *Delftia* sp. P2III5 | As |
| [86] | *Burkholderia cepacia* | Cd, Zn |
| [87] | *Cedecea daviseae* LCR1 | Cd |
| [88] | *Rhodococcus erythropolis* NSX2 | 
| [88] | *Bacillus cereus* SRA10 | Ni |
| [88] | *Psychrobacter* sp. SRA1 | 
| [88] | *Psychrobacter* sp. SRA2 | 
| [89] | *Achromobacter xylosidans* Ax10 | Ni |
| [90] | *Pseudomonas* sp. A3R3 | Ni |
| [91] | *Phyllobacterium myrsinacearum* RC6b | Cd, Zn |
| [94] | *Klebsiella* sp. BAM1 | Cd |
| [94] | *Pseudomonas jessenii* PjM15 | Ni, Cd, Zn |
| [95] | *Bacillus megaterium* SR28C | Ni |
| [96] | *Phylo bacterium putida* 710, *Comamonas aquatic* 710B | Cd |
| [96] | *Bacillus* sp. J119 | Cd |
| [98] | *Comamonas* sp.TS37 | 
| [98] | *Delftia* sp. TS33 | As |
| [98] | *Delftia* sp.T S41 | 
| [98] | *Rhodococcus* sp. TS1 | 
| [98] | *Streptomyces lividans* PSQ22 | 
| [99] | *Bacillus subtilis* SJ-101 | Ni |
| [100] | *Fusarium oxysporum* | 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 critic 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 genre 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.

Acknowledgments

A. E. Franks and J.L. Wood received funding from the Defense Science Institute Synthetic Biology Initiative. A. E. Franks receives funding from Australian Research Council Linkage Project (LP140100459), Office of Naval Research Global (Award No N626909-13-1-N259) and Asian Office of Aerospace Research & Development, (Award Number FA2386-14-1-4032). C. Tang receives funding from an Australian Research Council Linkage Project (LP100100800).

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.
References

1. Lum A F, Ngwa E S A, Chikoye D, et al. (2014) Phytoremediation Potential of Weeds in Heavy Metal Contaminated Soils of the Bassa Industrial Zone of Douala, Cameroon. *Int J Phytoremediation* 16: 302–319.

2. Rajkumar M, Prasad MNV, Freitas H, et al. (2009) Biotechnological applications of serpentine soil bacteria for phytoremediation of trace metals. *Crit Rev Biotechnol* 29: 120–130.

3. Dalcorso G, Manara A, Furini A (2013) An overview of heavy metal challenge in plants: From roots to shoots. *Metallomics* 5: 1117–1132.

4. Liu H, Probst A, Liao B (2005) Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China). *Sci Total Environ* 339: 153–166.

5. Wasi S, Tabrez S, Ahmad M (2013) Toxicological effects of major environmental pollutants: An overview. *Environ Monit Assess* 185: 2585–2593.

6. Nagajyoti P C, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: A review. *Environ Chem Lett* 8: 199–216.

7. Lebeau T, Braud A, Jézéquel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: A review. *Environ Pollut* 153: 497–522.

8. Agency for Toxic Substances and Disease Registry (2015) Priority List of Hazardous Substances. Atlanta: Agency for Toxic Substances and Disease Registry.

9. Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* 88: 1707–1719.

10. Järup L (2003) Hazards of heavy metal contamination. *Br Med Bull* 68: 167–182.

11. Tongesayi T, Fedick P, Lechner L, et al. (2013) Daily bioaccessible levels of selected essential but toxic heavy metals from the consumption of non-dietary food sources. *Food Chem Toxicol* 62: 142–147.

12. Khan S, Shahnaz M, Jehan N, et al. (2013) Drinking water quality and human health risk in Charsadda district, Pakistan. *J Clean Prod* 60: 93–101.

13. Martin R, Dowling K (2013) Trace metal contamination of mineral spring water in an historical mining area in regional Victoria, Australia. *J Asian Earth Sc* 77: 262–267.

14. Salt D E, Smith RD, Raskin I (1998) Phytoremediation. *Annu Rev Plant Physiol Plant Mol Biol* 49: 643–668.

15. Rasio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Sci* 180: 169–181.

16. Anderson CWN, Brooks RR, Chiarucci A, et al. (1999) Phytomining for nickel, thallium and gold. *J Geochem Explor* 67: 407–415.

17. Robinson BH, Brooks RR, Gregg PEH, et al. (1999) The nickel phytoextraction potential of some ultramafic soils as determined by sequential extraction. *Geoderma* 87: 293–304.

18. Sheoran V, Sheoran AS, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: A review. *Crit Rev Environ Sci Technol* 41: 168–214.

19. Alford ÉR, Pilon-Smits EAH, Paschke MW (2010) Metallophytes—a view from the rhizosphere. *Plant Soil* 337: 33–50.
20. Finneran KT, Anderson RT, Nevin KP, et al. (2002) Potential for Bioremediation of Uranium-Contaminated Aquifers with Microbial U(VI) Reduction. Soil Sediment Contam 11: 339–357.
21. Gadd GM (2009) Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84: 13–28.
22. Aracic S, Manna S, Petrovski S, et al. (2015) Innovative biological approaches for monitoring and improving water quality. Front Microbiol 6: 826.
23. Das N, Vimala R, Karthika P (2008) Biosorption of heavy metals - An overview. Indian J Biotechnol 7: 159–169.
24. Vijayaraghavan K, Yun YS (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26: 266–291.
25. Ahluwalia SS, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98: 2243–2257.
26. Aryal M, Liakopoulou-Kyriakides M (2015) Bioremoval of heavy metals by bacterial biomass. Environ Monit Assess 187:
27. Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122: 109–119.
28. Lovley DR, Holmes DE, Nevin KP (2004) Dissimilatory Fe(III) and Mn(IV) reduction. Adv Microb Physiol 49: 219–286.
29. Bolan N, Kunhikrishnan A, Gibbs J (2013) Rhizoreduction of arsenate and chromate in Australian native grass, shrub and tree vegetation. Plant Soil 367: 615–625.
30. Kashefi K, Lovley DR (2000) Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by Pyrobaculum islandicum. Appl Environ Microbiol 66: 1050–1056.
31. Williams KH, N'Guessan A, Druhan J, et al. (2010) Electric voltages accompanying stimulated bioremediation of a uranium-contaminated aquifer. J Geophys Res (G Biogeosci) 115.
32. Zhuang K, Ma E, Lovley DR, et al. (2012) The design of long-term effective uranium bioremediation strategy using a community metabolic model. Biotechnol Bioeng 109: 2475–2483.
33. Anderson RT, Vrionis HA, Ortiz-Bernad I, et al. (2003) Stimulating the In Situ Activity of Geobacter Species to Remove Uranium from the Groundwater of a Uranium-Contaminated Aquifer. Appl Environ Microbiol 69: 5884–5891.
34. Gadd GM (2010) Metals, minerals and microbes: Geomicrobiology and bioremediation. Microbiology 156: 609–643.
35. N'Guessan AL, Vrionis HA, Resch CT, et al. (2008) Sustained removal of uranium from contaminated groundwater following stimulation of dissimilatory metal reduction. Environ Sci Technol 42: 2999–3004.
36. Finneran KT, Housewright ME, Lovley DR (2002) Multiple influences of nitrate on uranium solubility during bioremediation of uranium-contaminated subsurface sediments. Environ Microbiol 4: 510–516.
37. Sheoran V, Sheoran AS, Poonia P (2016) Factors Affecting Phytoextraction: A Review. Pedosphere 26: 148–166.
38. Ernst WHO (1996) Bioavailability of heavy metals and decontamination of soils by plants. *Appl Geochem* 11: 163–167.
39. Blaylock MJ, Salt DE, Dushenko S, et al. (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ Sci Technol* 31: 860–865.
40. Cooper EM, Sims JT, Cunningham SD, et al. (1999) Chelate-assisted phytoextraction of lead from contaminated soils. *J Environ Qual* 28: 1709–1719.
41. Tandy S, Bossart K, Mueller R, et al. (2004) Extraction of Heavy Metals from Soils Using Biodegradable Chelating Agents. *Environ Sci Technol* 38: 937–944.
42. Mulligan CN (2005) Environmental applications for biosurfactants. *Environ Pollut* 133: 183–198.
43. Freitas EV, Nascimento C (2016) Degradability of natural and synthetic chelating agents applied to a lead-contaminated soil. *J Soils Sed* 1–7.
44. Liu D, Islam E, Li T, et al. (2008) Comparison of synthetic chelators and low molecular weight organic acids in enhancing phytoextraction of heavy metals by two ecotypes of Sedum alfredii Hance. *J Hazard Mater* 153: 114–122.
45. Karimzadeh L, Heilmeier H, Merkel BJ (2012) Effect of microbial siderophore DFO-B on Cd accumulation by Thlaspi caerulescens hyperaccumulator in the presence of zeolite. *Chemosphere* 88: 683–687.
46. Gunawardana B, Singhal N, Johnson A (2011) Effects of amendments on copper, cadmium, and lead phytoextraction by Lolium perenne from multiple-metal contaminated solution. *Int J Phytoremediation* 13: 215–232.
47. Angle JS, Linacre NA (2005) Metal phytoextraction - A survey of potential risks. *Int J Phytoremediation* 7: 241–254.
48. Bolan N, Kunhikrishnan A, Thangarajan R, et al. (2014) Remediation of heavy metal(loid)s contaminated soils - To mobilize or to immobilize? *J Hazard Mater* 266: 141–166.
49. Robinson BH, Chiarucci A, Brooks RR, et al. (1997) The nickel hyperaccumulator plant Alyssum bertolonii as a potential agent for phytoremediation and phytomining of nickel. *J Geochem Explor* 59: 75–86.
50. Dell’Amico E, Cavalca L, Andreoni V (2008) Improvement of Brassica napus growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biol Biochem* 40: 74–84.
51. Hernlem BJ, Vane LM, Sayles GD (1996) Stability constants for complexes of the siderophore desferrioxamine b with selected heavy metal cations. *Inorg Chim Acta* 244: 179–184.
52. Neilands JB (1995) Siderophores: Structure and function of microbial iron transport compounds. *J Biol Chem* 270: 26723–26726.
53. Kim JO, Lee YW, Chung J (2013) The role of organic acids in the mobilization of heavy metals from soil. *KSCE J Civ Eng* 17: 1596–1602.
54. Li WC, Ye ZH, Wong MH (2010) Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, Sedum alfredii. *Plant Soil* 326: 453–467.
55. Abhilash PC, Powell JR, Singh HB, et al. (2012) Plant-microbe interactions: Novel applications for exploitation in multipurpose remediation technologies. *Trends Biotechnol* 30: 416–420.
56. Sessitsch A, Kuffner M, Kidd P, et al. (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biol Biochem* 60: 182–194.
57. Glick BR (2010) Using soil bacteria to facilitate phytoremediation. *Biotechnol Adv* 28: 367–374.
58. Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* 63: 541–556.
59. Rajkumar M, Ae N, Prasad MNV, et al. (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol* 28: 142–149.
60. Hayat R, Ali S, Amara U, et al. (2010) Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann Microbiol* 60: 579–598.
61. Badri DV, Weir TL, van der Lelie D, et al. (2009) Rhizosphere chemical dialogues: plant-microbe interactions. *Curr Opin Biotechnol* 20: 642–650.
62. Onofre-Lemus J, Hernández-Lucas I, Girard L, et al. (2009) ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in Burkholderia species, and its growth-promoting effect on tomato plants. *Appl Environ Microbiol* 75: 6581–6590.
63. De Souza MP, Huang CPA, Chee N, et al. (1999) Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants. *Planta* 209: 259–263.
64. Malekzadeh E, Alikhani HA, Savaghebi-Firoozabadi GR, et al. (2012) Bioremediation of cadmium-contaminated soil through cultivation of maize inoculated with plant growth-promoting rhizobacteria. *Bioremediation J* 16: 204–211.
65. Abou-Shanab RA, Angle JS, Delorme TA, et al. (2003) Rhizobacterial effects on nickel extraction from soil and uptake by Alyssum murale. *New Phytol* 158: 219–224.
66. Abou-Shanab RI, Delorme TA, Angle JS, et al. (2003) Phenotypic Characterization of Microbes in the Rhizosphere of Alyssum murale. *Int J Phytoremediation* 5: 367–379.
67. Amprayn KO, Rose MT, Kescskés M, et al. (2012) Plant growth promoting characteristics of soil yeast (Candida tropicalis HY) and its effectiveness for promoting rice growth. *Appl Soil Ecol* 61: 295–299.
68. Whiting SN, De Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by Thlaspi caerulescens. *Environ Sci Technol* 35: 3144–3150.
69. Lodewyckx C, Mergeay M, Vangronsveld J, et al. (2002) Isolation, characterization, and identification of bacteria associated with the zinc hyperaccumulator Thlaspi caerulescens subsp. calamitaria. *Int J Phytoremediation* 4: 101–115.
70. Park JH, Bolan N, Megharaj M, et al. (2011) Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. *J Hazard Mater* 185: 829–836.
71. Zhang YF, He LY, Chen ZJ, et al. (2011) Characterization of ACC deaminase-producing endophytic bacteria isolated from copper-tolerant plants and their potential in promoting the growth and copper accumulation of Brassica napus. *Chemosphere* 83: 57–62.
72. Zhang S, Reddy MS, Kloepper JW (2004) Tobacco growth enhancement and blue mold disease protection by rhizobacteria: relationship between plant growth promotion and systemic disease protection by PGPR strain 90–166. *Plant Soil* 262: 277–288.
73. Shahzad SM, Arif MS, Riaz M, et al. (2013) PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (Zea mays L.) under fertilized conditions. *Eur J Soil Biol* 57: 27–34.

74. Wakelin SA, Warren RA, Ryder MH (2004) Effect of soil properties on growth promotion of wheat by Penicillium radicum. *Aust J Soil Res* 42: 897–904.

75. Kamnev AA, Tugarova AV, Antonyuk LP, et al. (2005) Effects of heavy metals on plant-associated rhizobacteria: Comparison of endophytic and non-endophytic strains of *Azospirillum brasilense*. *J Trace Elem Med Bio* 19: 91–95.

76. Latour X, Philippot L, Corberand T, et al. (1999) The establishment of an introduced community of fluorescent pseudomonads in the soil and in the rhizosphere is affected by the soil type. *FEMS Microbiol Ecol* 30: 163–170.

77. Melent’ev AI, Kuz'mina LY, Galimzyanova NF (2000) Effect of temperature and soil moisture content on the colonization of the wheat rhizosphere by antiphytopathogenic bacilli. *Microbiology* 69: 351–356.

78. Gerhardt KE, Huang XD, Glick BR, et al. (2009) Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. *Plant Sci* 176: 20–30.

79. Kang Y, Shen M, Wang H, et al. (2013) A possible mechanism of action of plant growth-promoting rhizobacteria (PGPR) strain Bacillus pumilus WP8 via regulation of soil bacterial community structure. *J Gen Appl Microbiol* 59: 267–277.

80. Abou-Shanab RAI, Angle JS, Chaney RL (2006) Bacterial inoculants affecting nickel uptake by Alyssum murale from low, moderate and high Ni soils. *Soil Biol Biochem* 38: 2882–2889.

81. Abou-Shanab RA, Ghanem K, Ghanem N, et al. (2008) The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. *World J Microbiol Biotechnol* 24: 253–262.

82. Belimov AA, Kunakova AM, Safronova VI, et al. (2004) Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. *Microbiology* 73: 99–106.

83. Gao Y, Miao C, Mao L, et al. (2010) Improvement of phytoextraction and antioxidative defense in Solanum nigrum L. under cadmium stress by application of cadmium-resistant strain and citric acid. *J Hazard Mater* 181: 771–777.

84. He LY, Chen ZJ, Ren GD, et al. (2009) Increased cadmium and lead uptake of a cadmium hyperaccumulator tomato by cadmium-resistant bacteria. *Ecotoxicol Environ Saf* 72: 1343–1348.

85. Lampis S, Santi C, Ciurli A, et al. (2015) Promotion of arsenic phytoextraction efficiency in the fern Pteris vittata by the inoculation of As-resistant bacteria: A soil bioremediation perspective. *Front Plant Sci* 6: 80.

86. Li WC, Ye ZH, Wong MH (2007) Effects of bacteria on enhanced metal uptake of the Cd/Zn-hyperaccumulating plant, Sedum alfredii. *J Exp Bot* 58: 4173–4182.

87. Liu W, Wang Q, Wang B, et al. (2015) Plant growth-promoting rhizobacteria enhance the growth and Cd uptake of Sedum plumbizincicola in a Cd-contaminated soil. *J Soils Sed* 1–9.

88. Ma Y, Rajkumar M, Freitas H (2009) Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. *J Hazard Mater* 166: 1154–1161.
89. Ma Y, Rajkumar M, Freitas H (2009) Inoculation of plant growth promoting bacterium Achromobacter xylosoxidans strain Ax10 for the improvement of copper phytoextraction by Brassica juncea. *J Environ Manage* 90: 831–837.
90. Ma Y, Rajkumar M, Luo Y, et al. (2011) Inoculation of endophytic bacteria on host and non-host plants-Effects on plant growth and Ni uptake. *J Hazard Mater* 195: 230–237.
91. Ma Y, Rajkumar M, Luo Y, et al. (2013) Phytoextraction of heavy metal polluted soils using Sedum plumbizincicola inoculated with metal mobilizing Phyllobacterium myrsinacearum RC6b. *Chemosphere* 93: 1386–1392.
92. Plociniczak T, Sinkkonen A, Romantschuk M, et al. (2013) Characterization of Enterobacter intermedius MH8b and its use for the enhancement of heavy metals uptake by Sinapis alba L. *Appl Soil Ecol* 63: 1–7.
93. Prapagdee B, Chanprasert M, Mongkolsuk S (2013) Bioaugmentation with cadmium-resistant plant growth-promoting rhizobacteria to assist cadmium phytoextraction by Helianthus annuus. *Chemosphere* 92: 659–666.
94. Rajkumar M, Freitas H (2008) Influence of metal resistant-plant growth-promoting bacteria on the growth of Ricinus communis in soil contaminated with heavy metals. *Chemosphere* 71: 834–842.
95. Rajkumar M, Ma Y, Freitas H (2013) Improvement of Ni phytostabilization by inoculation of Ni resistant Bacillus megaterium SR28C. *J Environ Manage* 128: 973–980.
96. Rani A, Souche Y, Goel R (2013) Comparative in situ remediation potential of Pseudomonas putida 710A and Commmamonas aquatica 710B using plant (Vigna radiata (L.) wilczek) assay. *Ann Microbiol* 63: 923–928.
97. Sheng X, He L, Wang Q, et al. (2008) Effects of inoculation of biosurfactant-producing Bacillus sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. *J Hazard Mater* 155: 17–22.
98. Yang Q, Tu S, Wang G, et al. (2012) Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by Pteris vittata L. *Int J Phytoremediation* 14: 89–99.
99. Zaidi S, Usmani S, Singh BR, et al. (2006) Significance of Bacillus subtilis strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in Brassica juncea. *Chemosphere* 64: 991–997.
100. Zhang X, Lin L, Chen M, et al. (2012) A nonpathogenic Fusarium oxysporum strain enhances phytoextraction of heavy metals by the hyperaccumulator Sedum alfredii Hance. *J Hazard Mater* 229–230: 361–370.
101. Muehe EM, Weigold P, Adaktylou II, et al. (2015) Rhizosphere microbial community composition affects cadmium and zinc uptake by the metal-hyperaccumulating plant Arabidopsis halleri. *Appl Environ Microbiol* 81: 2173–2181.
102. Muehe EM, Obst M, Hitchcock A, et al. (2013) Fate of Cd during microbial Fe(III) mineral reduction by a novel and Cd-tolerant geobacter species. *Environ Sci Technol* 47: 14099–14109.
103. Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17: 319–339.
104. Ma Y, Prasad MNV, Rajkumar M, et al. (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. *Biotechnol Adv* 29: 248–258.
105. Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol Rev* 31: 425–448.
106. Ryu RJ, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by tyr in Enterobacter cloacae UW5. *J Bacteriol* 190: 7200–7208.
107. Sharma SB, Sayyed RZ, Trivedi MH, et al. (2013) Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2: 87–132.
108. Mattey M (1992) The production of organic acids. *Crit Rev Biotechnol* 12: 341.
109. Siciliano SD, Fortin N, Mihoc A, et al. (2001) Selection of Specific Endophytic Bacterial Genotypes by Plants in Response to Soil Contamination. *Appl Environ Microbiol* 67: 2469–2475.
110. Hamamura N, Olson SH, Ward DM, et al. (2006) Microbial population dynamics associated with crude-oil biodegradation in diverse soils. *Appl Environ Microbiol* 72: 6316–6324.
111. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26: 2460–2461.
112. Caporaso JG, Kuczynski J, Stombaugh J, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7: 335–336.
113. Cole JR, Wang Q, Fish JA, et al. (2014) Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Res* 42: D633–D642.
114. Howe AC, Jansson JK, Malfatti SA, et al. (2014) Tackling soil diversity with the assembly of large, complex metagenomes. *Proc Natl Acad Sci USA* 111: 4904–4909.
115. Langille MGI, Zaneveld J, Caporaso JG, et al. (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 31: 814–821.
116. Abhauer KP, Wemheuer B, Daniel R, et al. (2015) Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* 31: 2882–2884.
117. Tu Q, Yu H, He Z, et al. (2014) GeoChip 4: A functional gene-array-based high-throughput environmental technology for microbial community analysis. *Mol Ecol Resour* 14: 914–928.
118. Andrews SC, Robinson AK, Rodríguez-Quíñones F (2003) Bacterial iron homeostasis. *FEMS Microbiol Rev* 27: 215–237.