Phytoremediation Using Microbial Communities: II

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15.1 Introduction

Phytoremediation is a novel green technology that uses specialized plants and associated soil microbes to remove, destroy, sequester or reduce the concentrations or toxic effects of contaminant in polluted environment especially soil and water. It refers to a group of plant-based technologies that use either naturally occurring or genetically engineered plants to clean contaminated environments. This technology depends on the ability of both the plant and associated microorganisms to adapt to or survive in high-metal environments. Polluted soil poses a severe problem to both ecosystem health and land development. Soil pollution threatens the health of human, plant and animal. Soil pollution can spread to other parts of the natural environment because soil is at the confl uence of many natural systems. For instance, groundwater that percolates through a polluted soil can carry soil contaminants into streams, rivers, wells and drinking water. Plants growing on polluted soil may contain harmful levels of pollutants that can be passed on to the animals and people that eat them. Dust blown from polluted soil can be inhaled directly by passers-by. Additionally, polluted soil renders valuable open land unusable for parks, recreation or commercial development. The fact that both soil minerals and soil pollutants carry small electric charges that cause each to bond with each other makes polluted soil very hard to clean. A range of technologies such as fi xation, leaching, soil excavation, chemical treatment, vitrifi cation, electrokinetics and landfi ll of the top contaminated soil, bioventing, thermal desorption, soil vapour extraction, biopiles, etc., have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution (Haque et al. 2008). Excavation of p olluted soil for off-site treatment or disposal is labour intensive, consumes a lot of time and requires the use of heavy machinery hence very expensive (Danh et al. 2009). Therefore, cheaper on-site, or in situ, remediation techniques have recently become the focus of research. One of the most interesting and promising of these in situ techniques is phytoremediation. Using plants to remediate soil pollution comprised of two components, one by the root-colonizing microbes and the other by plants themselves which absorb, accumulate, translocate, sequester and detoxify toxic compounds to non-toxic metabolites. Plants frequently lack metabolic capacity for the degradation of many pollutants hence the need to utilize degradation ability of soil organisms. Metal tolerance of plants is generally increased by symbiotic, root-colonizing, arbuscular mycorrhizal fungi (AMF), through metal sequestration in the AMF

hyphae. More also excretion of the glycoprotein glomalin by AMF hyphae can form complex metals in the soil. Exposure of plants to microorganisms within the rhizoplane protects the plants from the toxic effect of the contaminants and also takes part in phytoremediation. Resistant plants can thrive on sites that are too toxic for other plants to grow. They in turn give the microbial processes the boost they need to remove organic pollution more quickly from the soil.

The mechanism responsible for the phytoremediation of contaminated soil has been proved to be as a result of increase in microbial activity. Organic toxins, those that contain carbon such as the hydrocarbons found in gasoline and other fuels, can be broken down by microbial processes. Soil fungi, for example, improve phytoremediation ability of plants by increasing the absorptive area of the roots of plants. The effi ciency of *Tithonia diversifolia* and *Helianthus annuus* in remediating soils contaminated with zinc and lead nitrates could be improved by introducing mycorrhizal fungi in order to increase the absorptive area of the roots of these plants (Adesodun et al. 2010). Plants on the other hand play a key role in determining the size and health of soil microbial populations. All plant roots secrete organic materials that can be used as food for microbes, and this creates a healthier, larger, more diverse and active

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microbial population, which in turn causes a faster breakdown of pollutants. Phytoremediation reduces contaminant levels through microbial degradation in the rhizosphere. Phytoremediation systems increase the catabolic potential of rhizosphere soil by altering the functional composition of the microbial community (Siciliano et al. 2003). Plants, through their "rhizosphere effects", support hydrocarbondegrading microbes that assist in phytoremediation in the root zone (Nie et al. 2009). For example, root activities in perennial ryegrass and alfalfa increase the number of rhizobacteria capable of petroleum degradation in the soil (Kirk et al. 2005). In turn, healthy microbial communities enhance soil nutrient availability to the plants (Wenzel 2009). Phytoremediation process can also be enhanced by the addition of specifi c inocula of microorganism to contaminated soils (bioaugmentation). Also, plants that are relatively tolerant to various environmental contaminants are often stunted in the presence of the contaminant. Therefore, plant growth-promoting microorganisms can be added to the roots of plants to remedy this situation. The best bioaugmentation performance can be achieved by the use of microorganisms that are already present in the soil, since indigenous microorganisms are well adjusted to their own environment. Inoculating plants with genetically engineered strains of bacteria that degrade a specifi c contaminant has shown promising results. Biostimulation, a process which involves manipulating the nutrient and pH levels of the soil to increase microbial populations, can also be used to amplify the population of soil organism responsible for biodegradation. Hence, fertilizers can be used together with bioaugmentation to facilitate degradation of pollutants.

15.2 Advantages of Phytoremediation Using Microbes

1. Low-cost: It is less expensive than alternative engineering- based solutions such as soil excavation, incineration or land fi lling of the contaminated materials.
2. Aesthetically pleasing and appealing to the public. Trees

and smaller plants used in phytoremediation make a site more attractive, reduce noise and improve surrounding air quality.

3. Site use and remediation can occur simultaneously.

4. In situ approach: It treats the contamination in place so that large quantities of soil, sediment or water do not have to be dug up or pumped out of the ground for treatment.

5. Environmentally friendly: Poses no health risk to neither plant, human nor animal.

6. Enhance soil nutrient availability to the plants.

7. It takes advantage of natural plant processes and requires less equipment and labour than other methods since plants do most of the work.

8. Saves energy since the site can be cleaned up without digging up and hauling soil or pumping groundwater.

9. Trees and smaller plants used in phytoremediation help control soil erosion.

10. Creates a more fertile soil as soil organic matter is increased as a result of root secretions and falling stems and leaves.

11. Phytoremediation does not degrade the physical or chemical health of the soil as compared to soil excavation method that removes the organic-matter-rich topsoil and, because of the use of heavy machinery, compacts the soil that is left behind.

12. Its by-product can fi nd a range of other uses. Some of the plants used for phytoremediation produce metabolites or phenolic compounds that are of commercial value in the pharmaceutical industry.

13. The roots of plants used create pores through which water and oxygen can fl ow.

15.3 Limitations of Phytoremediation Using Microbes

1. A long time period is required for remediation. It is a slow process that may take many growing seasons before an adequate reduction of pollution is achieved, whereas soil excavation and treatment clean up the site quickly. Multiple metal-contaminated soils require specifi c metal accumulator species and therefore require a wide range of research prior to the application. The cadmium/zinc model hyperaccumulator *Thlaspi caerulescens* , for example, is sensitive towards copper (Cu) toxicity, which is a problem in remediation of Cd/Zn from soils in the presence of Cu by application of this species.

2. Scientifi c understanding of mechanisms is still limited; this is because the technique is still in its infancy state.

3. Hyperaccumulators can be a pollution hazard themselves. For instance, animals can eat the hyperaccumulators and cause the toxins to enter the food chain. If the concentration of contaminant in the plants is high enough to cause toxicity, there must be a way to segregate the plants from humans and wildlife, which may not be an easy task.

15.4 Environmental Contaminants

The following compounds have been reported as contaminants in soil and water:

Pesticides; explosives; oil; heavy metal such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), uranium (U), vanadium (V) and wolfram (W); polychlorinated biphenyls; polycyclic aromatic hydrocarbons (PAHs); chlorinated solvents; xenobiotics;

munitions; semi-coke solid wastes (which contain several

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organic and inorganic compounds such as oil products, asphaltenes, phenols, PAHs, sulphuric compounds); oil shale; and organic synthetic compounds.

15.5 Factors that Affect Phytoremediation

Certain factors affect the uptake, distribution and transformation of contaminants. Some of these factors include the following:

1. Level of contamination: They work best where contaminant levels are low because high concentrations may limit plant growth and take too long to clean up.

2. Plant species used for phytoremediation: Certain plants are better at removing contaminants than others. This may be due to differences in root exudate patterns, differences in root architecture as well as differences in genetic composition of the plant. Tall fescue with fi brous root system, for example, increases the potential of soil microbial community to degrade hydrocarbons, whereas rose clover with a coarse, woody root system decreases it (Siciliano et al. 2003). Plants used for phytoremediation must be able to tolerate the types and concentrations of contaminants present. They also must be able to grow and survive in the local climate. Chemical, physical and microbiological plants with low biomass yield and reduced root systems do not support effi cient phytoremediation and most likely do not prevent the leaching of contaminants into the aquatic system.

3. Depth of contamination: Small plants like ferns and grasses have been used where contamination is shallow. Because tree roots grow deeper, trees such as poplars and willows are used for hydraulic control or to clean up deeper soil contamination and contaminated groundwater.

4. Plant growth and development stage. Phytoremediation is most effective during the vegetative growth stages of plants. Plant vegetative growth stage is the most important phase for phytoremediation (Nie et al. 2011).

5. Type and properties of inoculum used for bioaugmentation.

6. Soil condition: Soil abiotic and biotic factors may determine the survival and activity of the introduced microorganisms (Juhanson et al. 2007). Some of the abiotic factors include temperature, soil pH, soil organic matter, soil moisture, cation exchange capacity, etc.

7. Bioavailability of contaminant to the microbial community is another factor infl uencing biodegradation of pollutants.

8. Age of the contaminants.

9. Physical and chemical properties of the contaminant. Contaminants that are soluble in water may pass by the root system without being accumulated.

10. Climatic factors. Plant survival and growth are adversely affected by extreme climatic factors.

11. Toxicity of soil.

12. Bioavailability of contaminant to plants. Metal that is tightly bound to the organic portions of the soil may not be available to plants.

13. Contaminant source.

15.6 Phytoremediation Strategies

These technologies to be discussed below are based on the plant's ability to absorb, accumulate, sequester and detoxify toxic metals:

1. *Hydraulic control* : In this process of phytoremediation, plants act like a pump, drawing the groundwater up through their roots to keep it from moving. It reduces the movement of contaminated groundwater towards clean areas off-site.

2. *Phytoaccumulation* (*phytoextraction*)： Plants absorb, accumulate and transport pollutants from the soil to aboveground plant parts (shoots). Removing the metals is as simple as pruning or cutting the plant aboveground mass. Plants, and their associated soil microbes, can release chemicals that act as biosurfactants in the soil that increase the uptake of contaminants. The aboveground plant parts rich in accumulated metal can be easily and safely processed by drying, ashing or composting. The plants used in a phytoextraction scheme should ideally have large biomass production and accumulate high concentration of metals in the aboveground portions (Adesodun et al. 2010). Over 500 plant species (101 families) and approximately 0.2 % of angiosperms have been reported to possess metal hyperaccumulation ability (Krämer 2010).

3. *Phytostabilization* involves the use of plants to reduce the mobility and bioavailability of contaminants in soil either through precipitation or adsorption onto roots. Plants adsorb contaminants onto their roots where microorganisms that live in the soil break down the adsorbed contaminants to less harmful chemicals. Mycorrhizal association, for example, is known to inhibit transport of metallic cations into plant roots. Some plant species such as *Combretum* and *Rhus* (Anacardiaceae) have the ability of in situ stabilization of some metals (Regnier et al. 2009 ; Mokgalaka-Matlala et al. 2010).

4. *Phytodegradation* is the breaking down of contaminants into less toxic substances in the soil through the activities of microorganisms in the rhizosphere of plant roots or externally through metabolites produced by plants. For instance, exudates (peptides) from the bacterium *Pseudomonas putida* can decrease cadmium (Cd) toxicity in plants. Natural exudates such as siderophores, organic acids and phenolics released by the roots of certain plants can form complexes (chelates) with metals in the rhizosphere.

Table 15.1 Phytoremediation strategies of various groups of contaminants

Technology	Action on contaminants	Main type of contaminant	Vegetation
Phytostabilization	Retained in situ	Organics and metals	Cover maintained
Phytodegradation	Attenuated in situ	Organics	Cover maintained
Phytovolatilization	Removed	Organics and metals	Cover maintained
Phytoextraction	Removed	Metals	Harvested repeatedly
Phytofiltration	Retained in situ	Metals	Cover maintained

Metals such as the toxic Cr(lll) can be converted to the much less toxic Cr(Vl) by enzymes found on the roots of wetland plants. During detoxification plants release gluta-thione conjugates into the rhizosphere where they could be metabolized by microbes (Schroder et al. 2007).

5. *Phytovolatization* involves use of plants to take up certain contaminants and then converts them into gaseous forms that vaporize into the atmosphere. This process is driven by the evapotranspiration of plants. Plants that have high evapotranspiration rate are sought after in phytovolatiliza-tion. Organic contaminants, especially volatile organic compounds (VOCs), are passively volatilized by plants. For example, hybrid poplar trees have been used to volatil-ize trichloroethylene (TCE) by converting it to chlorinated acetates and CO_2. Metals such as Se can be volatilized by plants through conversion into dimethylselenide [Se$(CH_3)_2$]. Genetic engineering has been used to allow plants to volatilize specific contaminants. For example, the abil-ity of the tulip tree (*Liriodendron tulipifera*) to volatilize methyl-Hg from the soil into the atmosphere (as HgO) was improved by inserting genes of modified *E. coli* that encode the enzyme mercuric ion reductase.

6. *Phytofiltration*: This involves rhizofiltration where con-taminants such as metals are precipitated within the rhi-zosphere. Metal plaque forms typically on the roots of wetland plants through the release of oxygen via the parenchyma of roots. Iron oxides, for example, can pre-cipitate along with other metals into the metal plaque. Metal plaque on roots acts as a reservoir for active iron (Fe^{2+}), which in turn increases the tolerance of plants to other toxic metals (Table 15.1).

The ability of the plants to degrade or metabolize xenobiotic pollutants can be improved by transferring genes from organ-isms (bacteria, fungi, plant and animals) which have poten-tial for degradation and mineralization of xenobiotic pollutants. That is, catabolic genes essential for the degrada-tion of contaminants are boosted in a plant resulting in enhanced phytoremediation. The plants which received the genes are called transgenic plants. The genes are introduced into the candidate plants using *Agrobacterium*-mediated or direct DNA method of gene transfer. Phytoremediation pro-cess in plant can also be improved by constructing plants with enhanced secretion of enzymes capable of degrading xenobiotics into the rhizosphere (Gerhardt et al. 2009) (Tables 15.2 and 15.3).

Table 15.2 General processes affecting rhizoremediation

Processes	Effects
Root exudates	Microbial growth stimulation
O_2—redox reaction	Microbial growth stimulation
CO_2—soil pH plant chelators and biosurfactants	Contaminant bioavailability
H^+ & OH^-—soil pH acid/base reaction	Contaminant bioavailability
Microbial enzymes	Plant growth
Ion uptake	Plant growth
Microbial chelator—plant nutrient delivery	Plant growth

15.7 Phytoremediators

PHYTOREM database (complied by Environment Canada) estimates that more than 750 plant species worldwide have potential for phytoremediation (Sarma 2011). Some of these include:

Bromus hordeaceus, Festuca arundinacea, Trifolium fra-giferum, Trifolium hirtum, Vulpia microstachys, Bromus carinatus, Elymus glaucus, Festuca rubra, Hordeum califor-nicum, Leymus triticoides, Nassella pulchra, Combretum sp., *Rhus* sp., *Phragmites australis, Alyssum corsicum, Alyssum murale,* mustard greens, *Helianthus annuus, Agrostis tenuis, Thlaspi caerulescens* (alpine pennycress), *Brassica juncea* (Indian mustard), *Liriodendron tulipifera* (yellow poplar) and *Nicotiana glauca* (Table 15.4).

15.8 Phytoremediation Traits

Plant adaptation and responses to contaminated environ-ments depend on many physiological, molecular, genetic and ecological traits. Indicators of a plant's phytoremediation potential include the following:

1. Tolerance to high pH and salinity.
2. Tolerance to extreme drought and waterlogged conditions.
3. Good rooting system and adequate depth of root zone. Fibrous rooting system provides large surface area for root-soil contact.
4. High level of tolerance with respect to the contaminant known to exist at the site.
5. High growth rate and biomass yield.

Table 15.3 Some examples of microorganisms used for phytoremediation

Plants	Microbes	Contaminants	References
Sugar beets	*Pseudomonas* sp.	PCBs	Villacieros et al. 2005
Thlaspi goesingense	*Methylobacterium* sp.	Nickel	Idris et al. 2004
Rock cress	*Pseudomonas* sp.	PCBs	Narasimhan et al. 2003
Alfalfa	*Pseudomonas* sp.	PCBs	Brazil et al. 1995
Wheat	*Pseudomonas* sp.	TCE	Yee et al. 1998
Thlaspi goesingense	*Sphingomonas* sp.	Nickel	Idris et al. 2004
Wild rye	*Pseudomonas* sp.	Chlorobenzoic acid	Siciliano and Germida 1998
Pea	*Pseudomonas* sp.	24-D	Germaine et al. 2006
Popular	*Pseudomonas* sp.	MTBE, TCE, BTEX	Germaine et al. 2004; Moore et al. 2006
Pea	*Pseudomonas* sp.	Naphthalene	Germaine et al. 2009
Barmultra grass	*Pseudomonas* sp.	Naphthalene	Kuiper et al. 2004
Barley	*Pseudomonas* sp.	Phenanthrene	Anokhina et al. 2004
Common reed	*Sinorhizobium meliloti* P221	Phenanthrene	Golubev et al. 2009
Switch grass	Indigenous degraders	PCBs	Chekol et al. 2004
Red clover, ryegrass	Indigenous degraders	24-D	Shaw and Burns 2004
Ryegrass	Indigenous degraders	PCPs	He et al. 2005
White mustard	Indigenous degraders	Petroleum hydrocarbon	Liste and Prutz 2006
Hybrid poplar	Indigenous degraders	BTEX, toluene	Barac et al. 2009
English oak, common ash	Indigenous degraders	TCE, toluene	Weyens et al. 2009
Birch	Indigenous degraders	PAHs	Sipilä et al. 2008
Altai wild rye, tall wheat grass	Indigenous degraders	Petroleum hydrocarbon	Phillips et al. 2009
Corn	*Gordonia* sp. S2Rp-17	Diesel	Hong et al. 2011
Yellow lupine	*Burkholderia cepacia*	Toluene	Barac et al. 2009
Poplar	*Burkholderia cepacia*	Toluene	Taghavi et al. 2005
Barley	*Burkholderia cepacia*	24-D	Jacobsen, 1997
Wheat	*Azospirillum lipoferum* spp.	Crude oil	Muratova et al. 2005; Shaw and Burns 2004
Tall fescue grass	*Azospirillum brasilense* Cd	PAHs	Huang et al. 2004
Tall fescue grass	*Enterobacter cloacae* CAL2	PAHs	Huang et al. 2004
Poplar	*Methylobacterium populi* BJ001	TNT, RDX, HMX	Van Aken et al. 2004a; Van Aken et al. 2004b

PAH polyaromatic hydrocarbon; *TCE* trichloroethylene; *PCB* polychlorinated biphenyl; *MTBE* methyl tert-butyl ether; *BTEX* benzene, toluene, ethylbenzene and xylenes; *24-D* 2,4-dichlorophenoxyacetic acid; *PCP* pentachlorophenol; *TNT* 2,4,6-trinitrotoluene; *RDX* hexahydro-1,3, 5-trinitro-1,3,5-triazine; *HMX* octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine

Table 15.4 Some examples of phytoremediators and contaminants

Plants	Metals	References
Arabis gemmifera	Cd and Zn	Kubota and Takenaka 2003
Crotalaria dactylon	Ni and Cr	Saraswat and Rai 2009
Thlaspi caerulescens	Cd and Zn	Kupper and Kochian 2010
Pelargonium sp.	Cd	Dan et al. 2002
Arabidopsis halleri	Cd	Kupper et al. 2000
Crotalaria juncea	Ni and Cr	Saraswat and Rai 2009
Thlaspi caerulescens	Cd, Pb and Zn	Banasova and Horak 2008
Brassica napus	Cd	Selvam and Wong 2009
Arabidopsis thaliana	Zn and Cd	Saraswat and Rai 2009
Thlaspi caerulescens	Zn, Cd and Ni	Assuncao and Schat 2003
Pistia stratiotes	Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn	Odjegba and Fasidi 2004
Chengiopanax sciodophylloides	Mn	Mizuno et al. 2008
Pteris vittata	As	Dong 2005
Sedum alfredii	Pb and Zn	Sun et al. 2005
Tamarix smyrnensis	Cd	Manousaki et al. 2008
Potentilla griffithii	Zn and Cd	Hu et al. 2009
Rorippa globosa	Cd	Sun et al. 2010

6. High level of tolerance to waterlogging and extreme drought condition.
7. High level of accumulation, translocation and uptake potential of contaminant.
8. Habitat preference of plant, e.g. terrestrial aquatic or semiaquatic.

15.9 Effects of the Metals on the Phytoremediators

Plants that have been successfully used as phytoremediators were able to tolerate, accumulate or translocate the metals by reasons of the following effects of the metals on the plants:

1. The plant physiology: Metals affect the physiology of plants either by promoting or inhibiting the growth of the plant. Some develop metal tolerance characteristics through apoplastic or symplastic detoxifi cation mechanisms (Pilon-Smits et al. 2009). Some are absorbed from soil solution through passive transport. Hg, for example, may preferentially bind with sulphur- and nitrogen-rich ligands (amino acids) and enter inside the cells. Cd can induce changes in lipid profi le (Ouariti et al. 1997) and can also affect the enzymatic activities associated with membranes such as the H $_+$ ATPase (Fodor et al. 1995).

2. Biomass production of the plants that have been successfully used as phytoremediators.

15.10 Responses of Microbial Communities to Phytoremediation

Different plant species have different effects on microorganisms in the soil. For instance, *Alyssum corsicum* , *Alyssum murale* and *Brassica juncea* (Ni hyperaccumulators) have been reported to increase both the population and biomass of soil microorganisms. By absorbing nickel from the soil and excreting root exudates, the plants reduced nickel toxicity and improved the living environment of the microbes (Cai et al. 2007). Phytoremediation increased the number of phenol- degrading bacteria as well as metabolic diversity of microbial community in semi-coke polluted soil (Truu et al. 2003). Perennial ryegrass supports a general increase in microbial activity and numbers in the rhizosphere, some of which have catabolic activity towards petroleum hydrocarbons in petroleum-contaminated soil. Alfalfa, on the other hand, seems to specifi cally increase the number of microorganisms capable of degrading more complex hydrocarbons (Kirk et al. 2005). Plant-dependent changes in microbial functionality are the result of some form of communication between the associated microorganisms and the plant. For example, bacterial products, such as lumichrome, stimulate root respiration and thereby increase the availability of root exudates for bacteria (Phillips et al. 2009).

15.11 Sources of Environmental Pollution

1. Increased toxic waste from increased population
2. Anthropogenic activities such as agriculture
3. Metal purifi cation procedure, which includes mining, smelting and the tailings from industries

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