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REVIEW PAPER

Assessing the applicability of phytoremediation of soils with mixed organic and heavy metal contaminants

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Abstract Soil pollution is a major environmental problem and many contaminated sites are tainted with a mixture of organic and heavy metal contaminants. Compared to other remedial strategies, phytoremediation is a low cost, environmentally-friendly, sustainable means of remediating the contamination. This review first provides an overview of phytoremediation studies where the soil is contaminated with just one type of pollutant (heavy metals or organics) and then critically evaluates the applicability of phytotechnologies for the remediation of contaminated sites where the soil is polluted by a mixture of organic and heavy metal contaminants. In most of the earlier research studies, mixed contamination was held to be detrimental to plant growth, yet there were instances where plant growth was more successful in soil with mixed contamination than in the soil with only individual contaminants. New effective phytoremediation strategies can be designed for remediation of co-contaminated sites using: (a) plants species especially adapted to grow in the contaminated site (hyperaccumulators, local plants, transgenic plants); (b) endophytic bacteria to enhance the degradation in the rizhosphere; (c) soil amendments to increase the contaminants bioavailability [chelating agents and (bio)surfactants]; (d) soil fertilization to enhance the plant growth and microbial activity in the soil; and (e) coupling phytoremediation with other remediation technologies such as electrokinetic remediation or enhanced biodegradation in the rhizosphere.

Keywords Mixed contamination · Soil · Phytoremediation · Heavy metals · Organic contaminants · Chelates · Electrokinetic remediation

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1 Introduction

The primary consequence of increased industrialization and overpopulation is the contamination of soil and groundwater, which presents health risks to humans and the environment. Removal of these toxins is essential to ensure the safety of the public and permit continued use and development of urban and rural lands. Both organic and inorganic contaminants, such as heavy metals, petroleum-based hydrocarbon compounds, solvents and agricultural pesticides, can affect the quality of soil (EGWRTAC 1997; USEPA 1997; Khan et al. 2004).

Heavy metals are a major concern for the environment and public health due to their toxicity (Singh et al. 2011). Metals can originate from many sources (USEPA 1995; Reddy et al. 2003). For example, high lead (Pb) levels in the soil can be a result of lead paints, pipes and automobile emissions (USEPA 1996). Cadmium (Cd) can originate from commercial fertilizers, batteries and automobile emissions (Lu et al. 2007). The presence of arsenic (As) in soil can be attributed to pesticide use, burning coal and smelting processes (Garelick et al. 2008). Different methods were developed and applied with different success to the remediation of heavy metals, including soil washing, stabilization and solidification, electrokinetic remediation, vitrification, phytoremediation, pump and treat, in situ flushing, permeable reactive barriers, and monitored natural attenuation (EGWR-TAC 1997; USEPA 2006; Wuana and Okieimen 2011).

Many organic pollutants found at contaminated sites also cause great concern for public safety and health as the increased production of synthetic organic chemicals over the last few decades has led to the release of large quantities of them into the environment. Organic contaminants of special concern in soils and groundwater include hydrocarbons (Kamath et al. 2004; Banks and Schultz 2005), organic solvents, volatile organic compounds (VOCs), halogenated organics (pesticides, PCBs), and polycyclic aromatic hydrocarbons (PAHs) (Schwarzenbach et al. 1993; Pignatello et al. 2010). Most of these compounds cause acute toxicity to living organisms and the exposure to these compounds, even at low concentrations, results in accumulation in tissues and can lead to toxic concentrations. VOC contamination is especially problematic due to the transference from soil and water to air with inhalation risks for the public (Lee et al. 2002).

Most of the more problematic organic contaminants have a very low solubility in water, forming the group of the so-called hydrophobic organic compounds (HOCs). In addition to their hydrophobic nature, HOCs show low reactivity with other chemicals, and have relatively high stability (Sawyer et al. 1978; Gillette et al. 1999). Given these unique characteristics, HOCs remain concentrated in the soil and are neither diluted nor transported readily by flowing water (Saichek and Reddy 2005). Both, their hydrophobic and persistent nature creates great challenges for their removal from the environment (USEPA 1997; Luthy et al. 1994; Loehr and Webster 1996). Nonetheless, while insoluble in water, HOCs still tend to leach into groundwater or surface water slowly, which results in contamination of the subsurface that may persist for as long as 100 years (National Research Council 1997). The groundwater with low concentration of HOCs represents a major threat for the environment due to the high toxicity of many of HOCs, and that groundwater requires remediation to reach safe concentrations. A special case of interest is the NAPLs (non-aqueous phase liquids). Once these compounds are spilled in the soil, they may move down by gravity being accumulated when they reach the water table. The LNAPLs (light non-aqueous phase liquids) accumulate floating in the groundwater and the DNAPLs (dense non-aqueous phase liquids) sink in the saturated zone of soil until being accumulated over the impermeable bedrock. NAPLs accumulated in the soils will slowly leach into groundwater as a continuous source of contaminants for the surrounding environment (Essaid et al. 2015; Schubert 2015).

Sites contaminated with organic pollutants can be remediated with technologies such as soil vapor extraction, soil washing, stabilization and solidification, electrokinetic remediation, thermal desorption, bioremediation, in situ chemical oxidation, phytoremediation, pump and treat, in situ flushing, permeable reactive barriers, in situ air sparging, and monitored natural attenuation (Sharma and Reddy 2004). However, some of these methods are only applicable to specific organic contaminants.

The applicability of remediation techniques depends on factors including contaminant type and site-specific conditions, such as soil type and depth of groundwater table from the surface, cost, and end use of the land. The technology and the specific operating conditions successfully applied at one contaminated site cannot be extrapolated to other contaminated sites (Hyman and Dupont 2001). Given these complications, even the remediation of sites with only one class of contaminants is challenging. Unfortunately, many of the polluted sites contain a variety of mixed contaminants. The U.S. Environmental Protection Agency (USEPA) National Priority List (NPL) indicates that 40 % of the hazardous waste sites are co-contaminated with organic and metal pollutants (USGAO 2010). In such cases, the remedial strategies must consider the above factors for the remediation of sites contaminated with a single type of contaminant plus the possible synergistic effects that occur when more than one type of contaminant is present.

2 Complexities with mixed contamination

Sites with mixed contamination pose technical challenges associated with each class of contaminant (e.g. hydrophobic organics and heavy metals) plus the new problems and challenges that arise due to the present of two (or various) classes of contaminants with different physico–chemical properties, because they will respond in a different way to the remediation technologies. Additionally, the physicochemical interactions among the contaminants might create new and unexpected problems that could limit the efficiency of the remediation technology. As a result, the fate and transport of contaminants in the subsurface at mixed contamination sites can be complex and unpredictable (Reddy 2011).

Several reports in literature demonstrate interactions between organic and inorganic contaminants that increase the complexity of their remediation and the implementation of physical and chemical treatments to remove or permanently immobilize those metals in the soil (Galvez-Cloutier and Dube 2002). As an example, volatile organic compounds (e.g. benzene) can impair the solidification/stabilization of the soil mass, while organic substances with high viscosity reduce the effectiveness of soil washing techniques. These considerations were highlighted in batch experiments performed by Poly and Sreedeep (2011), who demonstrated that sorption isotherms of individual contaminants differ across multi-contaminated soils.

Other difficulties are related to the inherent toxicity of heavy metals that can inhibit the biodegradation of organic contaminants by the microorganisms in soil (Said and Lewis 1991; Sandrin and Maier 2003) and can hamper efforts at bioremediation, phytoremediation and monitored natural attenuation. A possible solution to heavy metal toxicity is found in a two-stage approach. In the first stage, heavy metals are removed with a physicochemical technology, followed by a second stage in which the organic contaminants are extracted or degraded by a biological remediation technology that assures its efficacy once the metal toxicity can no longer hinder the biological activity (Dermont et al. 2008). However, the first stage with the physico-chemical technology can seriously damage the native bacteria and fungi in soil, so that the second stage of bioremediation may require bioaugmentation to start the biological degradation and be effective in a reasonable period of time (Tyagi et al. 2011; Megharaj et al. 2011).

The presence of organic contaminants may positively or negatively affect the transport and removal of heavy metals in soils. Thus, Dubé et al. (2002) fount that residual LNAPL in carbonaceous soil contaminated with Cd, Cu and Pb, enhanced heavy metal mobility and decreased metal retention by the soil. The enhanced mobility was attributed to soil hydrodynamic changes induced by residual LNAPL rather than those caused by chemical interactions between the metals and LNAPLs. Galvez-Cloutier and Dube (2002) found that the presence of NAPL in saturated soil causes water to bypass regions it occupies forming preferential pathways. It is evident that no remediation of heavy metals occurs in the dead-zones. Furthermore, NAPL may also block small pores and mask active surface sites. It results in an altered mobility of heavy metals with the subsequent implications in both contamination assessment and remediation perspectives. Overall, the mobility of heavy metals is altered by the presence of NAPL and the result of that alteration can be only determined by considering the contaminants and site characteristics of each specific case.

3 Phytoremediation and phytotechnologies for contaminated soils

Several technologies for the remediation of contaminated soils have been developed over the past three decades. Their applicability is often limited to a particular kind of contaminant or specific site conditions. In the case of contaminated sites with mixed contamination, few technologies have proven to be efficient, but they also have important limitations. Some of those technologies require the use of chemicals (e.g. soil washing, stabilization and solidification and in situ flushing); others are so intense that they change the texture and physicochemical properties (i.e. pH or organic content) of the soil mass (e.g. stabilization and solidification, vitrification, and electrokinetic remediation). Certain methods do not destroy or remove the contaminants, but instead leave them in the soil in a stabilized form (e.g. stabilization and solidification, bioremediation of heavy metals and vitrification) so, the risk of future contaminant re-mobilization remains. Additionally, most of the methods mentioned above require long treatment time and high amounts of energy, so the application at field scale results very expensive. In this context, phytoremediation arises as a benign, cost effective alternative for the treatment of contaminated sites with mixed contamination (Cameselle et al. 2013).

Phytoremediation utilizes a passive, low cost, in situ approach that can decontaminate and restore the site while it sustains the existing biological activity and physical structure and fertility of the soil (Marmiroli et al. 2006; Ouvrard et al. 2011). Because plants utilize solar power for growth and cause contaminant uptake and/or degradation, phytoremediation is considered more sustainable than the typical physico-chemical remediation approaches, which are generally impractical for large sites with shallow contamination. The inherently aesthetic nature of a planted site also makes phytoremediation more attractive than the alternative cleanup methods (Cunningham and Ow 1996; Pradhan et al. 1998).

Phytotechnologies focus on the restoration of contaminated sites as well as surface and subsurface water (Arthur et al. 2005). The main phytotechnologies include phytoaccumulation, rhizofiltration, phytostabilization, phytodegradation, rhizodegradation, and phytovolatilization.

Phytoaccumulation, also called phytoextraction, is based on the uptake of metals and other inorganic contaminants in the soil by the plant roots, and their subsequent accumulation in plant tissues. It is preferable to use plants that translocate the contaminants from the roots to their shoots (the part of the plant above ground), so at the end of the treatment, the contaminated plant biomass can be harvested, treated and disposed of properly. Phytoaccumulation uses hyperaccumulators or plant species that can absorb larger amounts of contaminants than other plant species (Bedmar et al. 2009; Mehmood et al. 2013). The plants have to be carefully selected for each application because they have to be resistant to the toxicity of heavy metals under the specific conditions of the contaminated site (Singh et al. 2013).

Rhizofiltration is the adsorption or precipitation on plant roots or absorption into the roots of contaminants that are in solution surrounding the root zone. Rhizofiltration effectively removes heavy metals (Dushenkov et al. 1995) and radionuclides, such as uranium (Lee and Yang 2010) from contaminated groundwater and aqueous solutions.

Phytostabilization refers to the immobilization of contaminants such as heavy metals in the soil and groundwater through the adsorption by roots or precipitation in the root zone. This process reduces the mobility and bioavailability of the contaminants and is appropriate for the restoration of soil in mines, mine dumps or other areas where the natural vegetation is missing. Usually, metal-tolerant species are used to restore the vegetation as they reduce the mobility of the contaminants and erosion (Gomes et al. 2014; Wójcik et al. 2014).

Phytodegradation, also called phytotransformation, is the breakdown of organic contaminants absorbed by the plant due to its metabolic activity. External enzymes excreted by the plant can also carry out the degradation of contaminants (Lee 2013).

Rhizodegradation is plant-assisted biodegradation or bioremediation in the rhizosphere (the soil around the roots of a plant). Root exudates and enzymes released by the roots of the plants can stimulate bacterial and fungal activity in the rhizosphere that aid in the degradation of organic contaminants (Qiu et al. 2004; Weyens et al. 2009). This process is also known as phytostimulation.

Phytovolatilization involves uptake of volatile organics contained in soil and groundwater by plants and the subsequent release of the gaseous form of the contaminant through stomata in the leaves (Batty and Dolan 2013). In phytovolatilization, plants or trees act as an organic pump that takes up the contaminants from soil and groundwater and disperse them into the atmosphere. So, phytovolatilization does not accumulate or degrade the contaminants, instead they are dispersed in the atmosphere where they can be further degraded or stay as air contaminants. The subsequent risks to the dispersion of the contaminants should be evaluated for each application so that the phytovolatilization become acceptable for the local regulations (Lee 2013).

Phytotechnologies bring about additional benefits associated with the increase of vegetation during the remediation. Among these, plant growth will increase the organic carbon content of the soil and stimulate root zone microbial activity. Plant roots also confer structural stability to the soil that helps to reduce erosion and the generation of windblown dust. The benefits of vegetation can minimize human exposure to soil contaminants via ingestion and inhalation. Plants can also mitigate groundwater contamination by reducing the downward migration of chemicals by absorption and transpiration of groundwater (Schnoor et al. 1995; Arthur et al. 2005).

Effective phytoremediation and the associated benefits for soil and environment can only be achieved if the plant can grow and develop appropriately in the contaminated soil and groundwater. Normal plant growth is dependent on multiple factors that include soil structure and composition, temperature, sunlight, rain, and nutrient availability. At contaminated sites, new factors that counter plant growth are introduced into the environment and must be assessed for their relative impact on growth in order to construct an effective and often site-specific phytoremedial system. The new factors are specifically related to the types and concentrations of the contaminants on-site. The diversity of contamination and its varied effects are not easily classified. In all, their complex interactions make it difficult to predict phytoremediation results with accuracy (McGrath et al. 2001).

Many studies have explored the phytoremediation potential of various plants on both organic and inorganic contaminants, the plant species that are best able to remediate certain contaminant classes, and effective methods to enhance phytoremediation. Contaminants that can be targeted for phytoremediation include heavy metals (Robinson et al. 1998; Bolan et al. 2003; Pulford and Watson 2003), radioactive nuclides (Lee and Yang 2010; Singh et al. 2008), other inorganic contaminants such as arsenic, selenium, antimony and tellurium (Greger et al. 2014; Ogra et al. 2015), explosives (Medina and McCutcheon 1996; Bhadra et al. 2001; Rylott and Bruce 2009; Van Aken 2009), crude oil and oil products (Nedunuri et al. 2000; Pichtel and Liskanen 2001; White et al. 2005; Merkl et al. 2005; Memarian and Ramamurthy 2012), pesticides (Schnoor et al.,1995; Chaudhry et al. 2002), PAHs (White et al. 2005; Huesemann et al. 2009), and PCBs (Sharma et al. 2015). A few select studies on phytoremediation of heavy metals or organic contaminants are reviewed below to create an understanding of the mechanisms of the phytoremediation of these contaminants when each type of contaminants exists individually. This information will be essential in the assessment of strategies for the phytoremediation of contaminated soils and groundwater with mixed contaminants (co-existence of both heavy metals and organic contaminants).

3.1 Phytoremediation of heavy metals

The phytoremediation of heavy metals in soils is based on the use of plant species that are capable of the uptake and accumulation of contaminants in the plant tissues, not only in the roots, but chiefly in the aerial part or shoots. In order to enhance the remediation process, it is important to use plants species that can accumulate high concentrations of heavy metals with minor effects on their growth and development or hyperaccumulators. In general, hyperaccumulators are plant species that accumulate heavy metal concentrations in their shoots at rates 100 times higher than non-hyperaccumulator plants with no significant negative effect on their growth and development (Barceló and Poschenrieder 2003). However, there are three definitions of hyperaccumulator species presently found in the literature, which are based on accumulation capability, bioaccumulation factor, and translocation factor of the metals in the plant. In the case of accumulation capacity, hyperaccumulator plants are those species that can accumulate more than 10,000 mg/kg (dry wt.) for Zn and Mn, 1000 mg/kg for Co, Cu, Ni, As and Se; and 100 mg/kg for Cd in their shoots (Baker et al. 2000). With regard to the bioaccumulation factor, hyperaccumulators are those whose ratio of metal concentration in tissue plant to that in soil is >1.0, and can reach values as high as 50–100 (Brooks 1998). Considering the translocation factor, hyperaccumulators are those species in which the metal concentration in the shoots is greater than that found in its roots (Wei and Zhou 2006).

During the phytoremediation of contaminated soils, hyperaccumulators are capable of accumulating large amount of heavy metals because they have strongly expressed metal sequestration mechanisms and, sometimes, greater internal requirements for specific metals (Shen et al. 1997). Some species may be able to mobilize and solubilize metals from less-soluble forms than the non-hyperaccumulating species (Rascio and Navari-Izzo 2011). However, their effectiveness also depends on the type of heavy metal. For example, different heavy metals have varied patterns of behavior and mobility within tree tissues: Cd, Ni and Zn are more easily translocated to the aerial tissues, while Pb, Cr and Cu tend to be immobilized and held primarily in the roots (Pulford and Watson 2003). After entering the plant, metals commonly bind to cell wall components (free –COOH or –OH groups), sulfur ligands in cytosol (phytochelatins, thiols) or are stored in vacuoles where they are bound to organic acids (Callahan et al. 2006). It is also possible, although less common, to form precipitates with phosphate, sulfate or carbonate and occupy intracellular or extracellular spaces (Marques et al. 2009). An ideal plant for the successful phytoaccumulation of heavy metals should possess high metal tolerance, an ability to grow on low quality soils, high bioaccumulation into aerial tissues (root-to-shoot metal translocation), and the capacity for high yield of biomass (Kärenlampi et al. 2000; Pilon-Smits 2005).

Metals accumulated in plant tissues are not degraded or transformed and plant tissues may require harvesting and proper disposal. The harvested biomass can be incinerated and the ash deposited in a landfill. The volume of ash with heavy metals is much less than that of the plant biomass or contaminated soil, moreover the cost of the process is much less than the excavation and disposal of the contaminated soil in a landfill (USEPA 1999). According to Pulford and Watson (2003), willow plants can be used in phytoextraction of heavy metals and the harvested wood can be burned to produce renewable bioenergy. The biorecovery of the metals from the harvested plant is another possible benefit of phytoremediation to remove heavy metals (Baker et al. 1994; Kikuchi and Tanaka 2012).

When dealing with a site that is contaminated with heavy metals, a phytoremediation study is necessary to determine the ability of the plant to remediate the soil under the specific site conditions before any large scale implementation occurs. This is because a plant that readily uptakes one or more metals at a specific site may not perform equally well at another. In some cases, even though the plant can accumulate a particular metal, the rate may be so slow that remediation is not possible within an economically feasible time frame (Robinson et al. 2000). A hyperaccumulator plant propagated in different soils may hyperaccumulate different metals (Knight et al. 1997). So, the efficiency of a particular species needs to be tested in the targeted soil type and under similar contaminant concentrations before it can be implemented on a field-scale basis (McGrath and Zhao 2003). The phytoremediation potential of different plant species for heavy metal contaminants are summarized in Table 1.

Phytostabilization is an alternative phytotechnology for heavy metal contaminated soil that is based on chemical changes in the rhizosphere that cause the precipitation and immobilization of heavy metals and make them less bioavailable. Chaney et al. (1997) suggested that Cr and Pb may be immobilized by a vegetative cover. Plants achieve Cr immobilization by promoting the reduction of Cr(VI) to Cr(III), which is much less soluble and, therefore, less bioavailable. Pavel et al. (2014) proposed the heavy metal phytostabilization (Zn, Cd and Pb) with *M. sinensis*×. *giganteus* growing in heavily contaminated sites. The addition of red mud, a waste from the alumina industry, favored the inmobilization and stabilization of heavy metals in soil. Phytostabilization is especially attractive for the immobilization of heavy metals in former mining areas. Several species such as *Erica andevalensis* and *Erica australis* plants naturally grow in heavily contaminated sites and suitable for phytostabilization of metal(loid)-polluted sites in abandoned mining areas (Pérez-López et al. 2014).

3.2 Phytoremediation of organic contaminants

The degradation organic contaminants can be achieved with phytoremediation due to a combination of mechanisms that include plant-promoted microbial degradation, plant uptake and accumulation, phytovolatilization, and phytodegradation (Kang 2014). Organic contaminants are either degraded in the rhizosphere (rhizodegradation) by root exudates, i.e. enzymes that catalyze contaminant degradation to simple organic molecules, or by the action of microbes in the rhizosphere. The microbial activity in the rhizosphere is enhanced by the root exudates, so the combination of the growing plant and the microflora creates an environment in the rhizosphere that is appropriate for the degradation of contaminants (Dzantor 2007). Plants may also uptake the organic contaminants, and then they will be degraded to simpler molecules by enzymatic transformation in the plant tissues (phytodegradation) (Macek et al. 2004).

The efficiency of the remediation of organic contaminated soil is affected by the solubility and bioavailability of the contaminants. In the case of moderately hydrophobic organic chemicals with

Table 1 Potential plant species for phytoremediation of heavy metals

Species	Phytoremediation potential	References
Solanum Nigrum (Black Nightshade)	Cd	Wei et al. (2010)
Linum usitatissimum (Flax)	Cd	Bjelková et al. (2011)
Albizia amara (Bitter Albizia)	Cr	Shanker et al. (2005)
Casuarina equisetifolia (Coast Sheoak)		
Tectona grandis (Teak)		
Leucaena luecocephala (Leucaena)		
Spirodela polyrhiza (Duckweed)	Ni	Appenroth et al. (2010)
Allium fistulosum (Green Onion)	Pb	Cho et al. (2009)
Pteris cretica (Moonlight Fern)	Pb	Cho et al. (2009)
Pinus sylvestris (Pine)	Cd, Pb	Ostrowska et al. (2006)
Ricinus communis (Ricinus)	Cd, Pb	Zhi-Xin et al. (2007)
Grasses	Cd, Zn	Zhang et al. (2010)
Pennisetum americanum (Cattail Millet)		
Paspalum atratum (Atra Paspalum)		
Silphium perfoliatum (Cup Plant)		
Stylosanthes guianensis (Common Stylo)		
Brassica rapa (Field Mustard)	Cd, Cu, Zn	Meers et al. (2005)
Phragmites australis (Common Reed)	Cu, Hg, Pb	Weis and Weis (2004)
Spartina alterniflora (Smooth Cordgrass)		
Amorpha fruticosa (False Indigo-Bush)	Cu, Pb, Zn	Shi et al. (2011)
Vitex trifolia (Simpleleaf Chastetree)		
Glochidion puberum (Needlebush)		
Broussonetia papyrifera (Paper Mulberry)		
Styrax tonkinensis (Benzoin Tree)		
Species from *Brassica* genus (Mustard Plant)	Heavy metals	Palmer et al. (2001)
Vetiveria zizanioides (Vetiver Grass)	Pb, Cu, Zn, Cd, Mn	Andra et al. (2009)
		Chen et al. (2004)
		Roongtanakiat and Chairoj (2001)
Eichhornia crassipes (Water Hyacinth)	Cd, Cu, Ni, Pb, Zn	Liao and Chang (2004)
	Cd, Cr, Cu, Se	Zhu et al. (1999)
Brassica napus (Canola)	Cd, Cr, Cu, Ni, Pb, Zn	Marchiol et al. (2004)
		Saathoff et al. (2011)
Thlaspi caerulescens (Alpine Pennygrass)	Cr, Cd, Co, Cu, Mo, Ni, Pb, Zn, Mn	Robinson et al. (1998)

octanol–water partition coefficients in the range of log $K_{ow} = 0.5–3.0$ in shallow subsurface soils, the direct uptake of organics (e.g. pesticides, PCBs, dioxins) by plants is a proven efficient removal mechanism (Gao et al. 2008a; Dettenmaier et al. 2009; Chang et al. 2013). Thus, most BTEX chemicals, chlorinated solvents and short-chain aliphatic chemicals are considered amenable to phytoaccumulation. Hydrophobic chemicals with log $K_{ow} > 3.0$ are bound so strongly to the surface of roots that they are not easily translocated to aerial tissues. Water soluble chemicals with log $K_{ow} < 0.5$ are not sufficiently sorbed to roots or actively transported through plant membranes. The expected end product of the degradation of organic components is generally nontoxic constituents such as carbon dioxide, nitrate, chloride, and ammonia (Dhankher et al. 2011). Several studies that investigated the phytoremediation of organic contaminants with different plant species are summarized in Table 2.

Table 2 Potential plant species for phytoremediation of organic pollutants

Species	Phytoremediation potential	References
Brachiaria brizantha (Palisade grass)	Petroleum	Merkl et al. (2005)
Cyperus aggregatus (Inflated-scale Flatsedge)		
Gaillardia aristata (Blanket flower)	Petroleum	Liu et al. (2012)
Echinacea purpurea (Eastern Purple Cone Flower)		
Festuca arundinacea schreb (Fawn)		
Combined *F. arundinacea* (Fire Phoenix)		
Pisum sativum (Pea)	Diesel Fuel	Palmroth et al. (2002)
Pinus sylvestris (Pine)		
Trifolium Repens (White Clover)		
S. alterniflora (Smooth Cordgrass)	Fuel oil	Lin et al. (2002)
Glycine max (Soybean)	Motor oil	Dominguez-Rosado and Pichtel (2004)
Zea mays (maize)		
Trifolium pretense (Red Clover)		
Lolium arundinaceum (Fescue)	Anthracene, Naphthalene, Phenanthrene	White et al. (2005)
Cannabis sativa (Industrial Hemp)	Benzo (a) pyrene, Chrysene	Campbell et al. (2002)
Trifolium Repens (White Clover)	Phenanthrene, Pyrene	Gao et al. (2008b)
Festuca arundinacea (Tall Fescue)	Phenanthrene, Pyrene	Cheema et al. (2009)
Brassica napus (Rapeseed)	Phenanthrene, Pyrene	Sheng-Wang et al. (2008)
Phragmites communis (Common Reed)	Pyrene	Wang et al. (2008)
Typha orientalis (Bullrush)		
Vetiveria zizanioides (Vetiver grass)		
Rohdea japonica (Sacred Lily)		
Bolboschoenus planiculmis (Egorova)		
Morus (Mulberry)	PAHs	Olson and Fletcher (1999)
Cucurbita pepo (Zucchini)	p,p'-DDE*	White (2009)

* p,p'-DDE: 1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene

3.3 Limitations of phytoremediation

Phytoremediation has been reported to be successful in the remediation of soils and groundwater contaminated with organic and inorganic contaminants (Schwitzguébel 2016). Despite the satisfactory results, phytoremediation at large field scale application remains rather limited than it should be considering the promising results from literature. This may be due to some limitations that have to be considered in the design of a phytoremediation application. Phytoremediation is only applicable to low or moderate contaminated soils, where the plants can grow producing significant amount of biomass. The toxicity of heavily contaminated soils inhibits the plant metabolism reducing the biochemical process necessary for the degradation/uptake of the contaminants (Ali et al. 2013). Furthermore, phytoremediation is only applicable to root depth, which depends on the plant species used. The roots of herbaceous species may reach up to 1 m, bushes may reach from 1 to 3 m and phytoremediation with tree species may reach up to 10 m, although phytoremediation is more effective in the first 50 cm–1 m (Cameselle et al. 2013).

Other limitations of phytoremediation are related to the contaminant uptake and translocation from the roots to the stem and possible degradation of the organic contaminants. Metal uptake is favored for some elements (e.g. Cd, Ni, Zn, As, Se, and Cu), whereas other metals (e.g. Pb, Hg, and Cr) tend to show lower metal uptake and translocation factors (Ali et al. 2013). The uptake and translocation of organic contaminants is limited to those compounds that are not highly hydrophobic, and the transport to foliage is limited to those compounds that are not especially hydrophilic (Chaudhry et al. 2002). In general, phytoremediation will show little impact with diffuse contamination (e.g. pesticide contamination), but even in soils highly polluted with pesticides or other organic contaminants, the decontamination rate will not exceed certain value even if the environmental conditions are appropriate for plant growth (e.g. nutrients, light, temperature, pH, and moisture). Nevertheless, the contribution of phytoremediation for the removal of diffuse contamination cannot be neglected under long-term conditions (Chaudhry et al. 2002). This is related with another limitation of phytoremediation: the process is rather slow compared with other remediation technologies because the remediation is related to the metabolic activity of the plant, and that metabolic activity is related to climate and seasonal cycles. Often, phytoremediation requires several harvests and it could mean more than 1 year of treatment (Cheng et al. 2015; Shutcha et al. 2015).

4 Phytoremediation of mixed contaminants

Phytoremediation of sites with mixed contamination is expected to be more complex than remediation of soils with only one type of contaminant. The different properties of two (or various) kinds of contaminants (heavy metals and organic compounds) and the possible interactions among them as well as with soil and microbiota in the rhizosphere will add complexity to the remediation process. As noted in the previous section, for sites contaminated with only one kind of contaminant, plant selection is critical for an effective remediation, since a plant that is good for the remediation of a particular contaminant may not be effective or even survive in the presence of another contaminant or co-contaminants. Table 3 identifies plants that have been used for multiple heavy metal removal or multiple organic contaminant degradation

Table 3 Potential plant species for phytoremediation of heavy metals and organic contaminants

Species	Phytoremediation potential	References
Avena sativa (Oat)	Zn	Ebbs and Kochian (1998)
	Phenanthrene	Miya and Firestone (2001)
Lolium perenne (Rye Grass)	Cu, Cd, As	O'Connor et al. (2003)
	Cu, Zn	Zhou et al. (2007)
	Petroleum hydrocarbons (Naphthalene, Phenanthrene, Anthracene)	White et al. (2005)
	Organic contaminants (creosote)	Huang et al. (2004)
Medicago sativa (Alfalfa)	Pyrene	Fan et al. (2008)
	Cd, Cr, Ni, Zn	Peralta-Videa et al. (2002)
	Phenanthrene, Pyrene	Sheng-Wang et al. (2008)
	Petroleum contamination	Liu et al. (2012)
Salix spp. (Willow)	Cd, Organics(Oil)	Kuzovkina and Quigley (2005)
	Zn, Cd, Ni, Cr, Pb, Cu	Pulford and Watson (2003)
	Cd	Robinson et al. (2000)
Populus spp. (Poplar Trees)	BTEX	Moore et al. (2006)
	Cd	Robinson et al. (2000)
	BTEX, Nutrient contamination	Schnoor et al. (1995)
Helianthus annuus (Sunflower)	Zn, Cu, Cd	Meers et al. (2005)
	Zn, Pb	Adesodun et al. (2010)
	Motor oil	Dominguez-Rosado and Pichtel (2004)
Brassica juncea (Indian Mustard)	Cd, Cr, Cu, Ni, Pb, U, Zn	Blaylock et al. (1997)
		Liu et al. (2000)
		Lim et al. (2004)
		Singh and Sinha (2005)
	Motor oil	Dominguez-Rosado and Pichtel (2004)

where the soil is contaminated with multiple contaminants only of one kind, either heavy metals or organic pollutants. These plant species are potential candidates for phytoremediation of mixed contaminated soils once their effectiveness for multiple contaminants of each kind of contaminant alone has been proven. However, phytoremediation results may be different from those on Table 3 when multiple organic and inorganic contaminants are present together. There have been few recent studies on phytoremediation of co-contaminated soils with mixed metals and organic contaminants (Chirakkara and Reddy 2014, 2015; Ramamurthy and Memarian 2014). In these studies, the presence of mixed contamination led to very different results when compared with only one kind of contaminants. The differences are related to plant growth and biomass production, metal uptake, organic contaminant degradation, synergistic or antagonistic effects in phyto-toxicity, and physico-chemical interactions among the contaminants that affect their mobility and/or bioavailability. A phytoremediation review by Batty and Dolan (2013) suggests that sustaining a diverse microbial community within the rhizosphere to promote endophyte-plant symbioses is critical for the successful phytoremediation of mixed contaminated soils. This is because soil microorganisms often aid organic contaminant degradation, and may serve to bolster plant health against contaminant toxicity, thus improving the survivability of the plants (Mastretta et al. 2006; Germaine et al. 2013).

When dealing with mixed contaminated sites, it seems that the simplest approach could be to stabilize the heavy metals before inducing organic contaminant degradation. This approach was suggested by Palmroth et al. (2006) who tested the feasibility of phytoremediation in a field scale at a site contaminated with organics (Hydrocarbons) and metals (Cu, Pb and Zn) from bus maintenance activities. Six plant species: *Pinus sylvestris* (pine), *Populus deltoides* x *Wettsteinii* (poplar), *Festuca rubra* (red fescue), *Poa pratensis* (smooth meadow grass), *Lolium perenne* (rye grass) and *Trifolium Repens* (white clover) were tested in the soil with and without amendment (NPK fertilizer or bio-waste compost). Over the 39 month study, a higher hydrocarbon removal rate was observed where the soil had been amended with NPK fertilizer and municipal bio-waste compost than in the amendment-free soil. These results, coupled with observations of greater plant cover in the compost and NPK amended soils relative to the unamended control (which had areas devoid of vegetation), indicate that increased phytodegradation of the hydrocarbons occurred in response to the higher biomass production by the organic amendments. However, plant tissues did not contain the expected high metal content indicative of hyperaccumulation. In this case, the compost addition may have resulted in reduced metal bioavailability, implying that a two-phase approach that targets the organic and inorganic contaminants separately may be the most appropriate process. This suggests that the presence of two kinds of contaminants will result in physico-chemical interactions that will affect the remediation itself, and those interactions must be studied and their effect of phytoremediation evaluated.

The physico-chemical interactions among contaminants and their effect on mobility and bioavailability usually result in a reduction in the effectiveness of the phytoremediation of heavy metals compared to similar tests with only one kind of contaminant. This effect was highlighted in a study by Chen et al. (2004), who investigated the response of Cu and Zn in phytoremediation with *Lolium perenne* (rye grass) of soil contaminated with Cu, Zn and 2,4-dichlorophenol (DCP). The results showed that the water soluble fraction of Cu and Zn was greater in planted soil than in unplanted soil, which indicates increased metal mobility due to phytoremediation. The presence of 2,4-DCP also increased the water soluble fraction of Cu and Zn, which suggested more attention should be paid to the behavior of heavy metals under combined pollution of organic pollutants in the planted soil. The organic contaminant (DCP) did not affect the growth of the plants, but there was an apparent impact on metal uptake, with lower metal accumulation in the plants grown in pots that were treated with 2,4-dichlorophenol. Similar phenomena were observed by Kobyłecka and Skiba (2008), who investigated the effect of herbicides (2,4-dichlorophenoxyacetic acid and 4-chloro-2-methylphenoxyacetic acid) on the uptake of Zn, Cu and Mn by *Triticum Aestivum* (wheat). They found that the heavy metal content of the plants treated with the organic compounds were significantly lower than that of the untreated plants. One possible explanation is the formation of less water soluble heavy metal–organic complexes that reduced the phytoextractability of the metals and limited the metal accumulation by the plant.

The co-occurrence of the two kinds of contaminants has been found to affect the phytotoxicity during phytoremediation and, therefore, the plant growth and remediation efficiency. An increase in phytotoxicity is expected when the plant is exposed to a soil contaminated with heavy metals and organic contaminants, but surprisingly, several researchers have reported a reduction in the phytotoxicity and better remediation results at specific levels of contaminants. For instance, Lin et al. (2006) investigated the dissipation mechanisms for pentachlorophenol (PCP) in copper co-contaminated soil using *Lolium perenne* L (rye grass) and *Raphanus sativus* (radish). PCP removal ranged from 62 to 96 % with ryegrass and 45 to 94 % with radish, depending on the concentration of PCP and Cu, after 12 weeks. These researchers found that the plant growth improved with the increase in Cu concentration at 50 mg/kg of PCP, which suggests that Cu was a limiting nutrient and the better plant growth improves PCP degradation. Conversely, in soils with an initial PCP level of 100 mg/kg, the soil microbial activity and plant growth decreased with the increase in Cu. These contradictory trends illustrate the sensitivity of plants to low levels of certain micronutrients (i.e. Cu) and the resultant variation of plants and microbial responses to mixed contamination due to the differences in both the type and concentrations of the individual contaminants.

The different response of plants to specific levels of mixed contamination was also detected by Batty and Anslow (2008), who studied the effect of pyrene in soil in the remediation of Zn by *Brassica juncea* (Indian mustard) and *Festuca arundinacea* (tall fescue).

These plants grew better in clean soil or pyrene contaminated soil, which confirmed the phytotoxicity of Zn at the concentration tested (8000 mg/kg). Anyway, Zn was effectively removed from the co-contaminated soil by both plant species, although *F. arundinacea* accumulated Zn in the roots and *B. juncea* in the shoots. The translocation of Zn from roots to shoots in *B. juncea* was the reason for its major phytotoxicity of Zn and lower biomass production (especially in co-contaminated soil). Despite its major sensitivity to the mixed contamination in soil *B. juncea* is a better candidate than *F. arundinacea* thanks to its ability to translocate Zn to the shoots.

Zhang et al. (2009) studied the concurrent removal of Cd and pyrene from soil spiked with different concentrations of Cd (0, 2 and 4.5 mg/kg) and pyrene (0, 10, 50, and 100 mg/kg) by *Zea mays* L (maize). Phytoremediation of pyrene was possible since pyrene concentrations were significantly lower in planted than unplanted soil for both pyrene and pyrene-Cd contaminated soils. Minor toxicity in maize was observed in soil with only one contaminant but in the co-contaminated soil the root dry weight decreased, confirming the synergistic toxic effects of pyrene and Cd. These toxics effects were confirmed with the remediation results: residual pyrene in soil tended to become higher as the Cd content increased; and the accumulation of Cd in plant tissues decreased as the pyrene contamination intensified. Overall, these results prove that maize can be an effective remediation tool in soil contaminated with Cd and pyrene, although a decrease in efficiency due to synergistic phytotoxic effects can be expected at higher contaminant level.

Similar phytotoxic effects were obtained by Chigbo et al. (2013) studying the effect of Cu (50 and 100 mg/kg) and/or pyrene (250 and 500 mg/kg) on the growth of *Brassica juncea* and its phytoremediation ability. Planted soil increased the dissipation of pyrene compared to unplanted soil, but the presence of Cu increase the residual pyrene, and when the concentration of Cu was 100 mg/kg the residual pyrene was similar to the unplanted soil. The negative effect of the copper-pyrene co-contamination was also observed in lower shoot and root biomass production and inhibition of Cu uptake. The inhibition of phytoremediation in co-contaminated soil was even more important with *Zea mays* L (Hechmi et al. 2013) cultivated in the presence of pentachlorophenol (0, 50 or 100 mg/kg) and Cd (0, 2 or 6 mg/kg). *Zea mays* L was very effective in the dissipation of PCP alone, but the presence of increasing concentrations of Cd considerably decreased the dissipation of PCP. The Cd toxicity was so negative for the plant that the residual PCP was even higher in planted soil than in unplanted soil test. These researchers also conducted pot culture experiments to evaluate the phytoremediation potential of a wetland plant species *Phragmites australis* in Cd and PCP co-contaminated soil under greenhouse conditions for 70 days (Hechmi et al. 2014). The contaminants used were Cd (0, 5 and 50 mg/kg) and PCP (50 and 250 mg/kg) separately or in combination. Soil dehydrogenase activity (DHA) was measured in the soil because it is considered to be a good indicator of soil microbial activity. The growth of *P. australis* decreased significantly with the addition of either Cd or PCP. Compared to the control, the plant biomass was reduced by 89 and 92 % in the low and high Cd treatments and by 20 and 40 % in the low and high PCP treatments, respectively. However, in the co-contaminated soil with low Cd and PCP, the Cd toxicity was much less than in the test with Cd alone, which resulted in a 144 % plant growth improvement. This result is a clear example of the antagonist effect of phytotoxicity in co-contaminated soil. Unfortunately, the improvement in plant growth in the co-contaminated soil was not useful for soil remediation because the Cd uptake and translocation by *P. australis* were weak. Since a low proportion of Cd was found in the above ground biomass, the researchers concluded that *P. australis* would not be useful for Cd phytoextraction. The removal rate of PCP was very significant (70 %) in planted soil, and they observed significant positive correlations between the DHA and the removal of PCP in planted soils. This confirms that plant root exudates promote the rhizosphere microorganisms and enzyme activity and improve the biodegradation of PCP. Considering the inhibition of plant growth at high contaminant concentrations and the reduced phytoextraction of Cd under co-contamination, they concluded that *P. australis* may not be effective for phytoremediation of soil co-contaminated with Cd and PCP.

Sun et al. (2011) investigated the effect of different concentrations of benzo[a]pyrene (0, 2, 5, 10, and 50 mg/kg) on the growth of *Tagetes patula* (marigold) in the presence of three heavy metals: Cd (20 and 50 mg/kg), Cu (100 and 500 mg/kg) or Pb (1000 and 3000 mg/kg). Here, a low concentration of benzo[a]pyrene ($\leq$10 mg/kg) improved the plant growth and resulted in an increase in the plant biomass at the rate of 10.0–49.7 % relative to the control. However, the heavy metals inhibited plant growth and benzo[a]pyrene uptake and accumulation. While only Cd was hyperaccumulated from the co-contaminated soils, the marigold did not absorb Cu and Pb effectively. Consequently, they concluded that marigold can be a good remedial option for benzo[a]pyrene and benzo[a]pyrene-sites contaminated with Cd.

The phytotoxicity effects in co-contaminated soil can be reduced by the addition of hormones that enhance the tolerance mechanisms of the plant. This possibility was tested by Ahammed et al. (2013) when they explored the interactions of Cd and phenanthrene in the phytoremediation of co-contaminated soil with tomato plants. According to their findings, the application of Cd alone was more phytotoxic than the application of phenanthrene alone; but, the combined application of Cd and phenanthrene resulted in improved photosynthetic activity when compared to the single Cd contaminated soil. They suggest that the application of brassinosteroids (a plant hormone related to tolerance mechanisms for a number of abiotic stresses) can reduce phytotoxicity in co-contaminated soil as it stimulates the plant's natural defense mechanisms against cellular stress.

The presence of mixed contaminants often leads to variations in contaminant removal, due to interactions among them. Moreover, complications arise from interspecies variation in metal tolerance, uptake and organic contaminant degradation. This is especially true for poplars in the *Salix* family, which are often favored for groundwater remediation due to high growth and transpiration rates and a demonstrated ability to remove both metal and organic contaminants (Zacchini et al. 2009; Castiglione et al. 2009; Marmiroli et al. 2011). An interesting example of simultaneous removal of metals and organic contaminants is the study of Huang et al. (2011). These researchers investigated the phytoremediation potential of 23 genotypes of *Ricinus communis* (castor oil plant) grown in soil spiked with 2.8 mg/kg Cd and 1.7 mg/kg DDT. There were significant variations among the accumulation of Cd (66.0–155.1 µg per pot) and DDT (83.1–267.8 µg per pot) across the genotypes, but they concluded that the bio-energy crop *R. communis* can be considered as a plant species that accumulates DDTs and Cd.

An additional aspect in phytoremediation of co-contaminated soils is the possible benefits of using several species in the same treatment. Many studies suggested that certain plants can accumulate heavy metals whereas other species can enhance the degradation of organic contaminants. Based on that, intercropping of plant species is a possible option to remediate mixed contaminated soil. Lee et al. (2007) studied four different plant species: *Echinochloa crusgalli* (barnyard grass), *Helianthus annuus* (sunflower), *Abutilon avicennae* (Indian mallow), and *Aeschynomene indica* (Indian jointvetch), in single plant cultures and mixed plant cultures on soil co-contaminated with (mg/kg): Cd (10), Pb (1100), Zn (90), Cu (30) and 2,4,6–trinitrotoluene (50). In the tests with the four plants, seed germination was <20 % for all the four species, but the growth rates of *E.*

crusgalli and *H. annuus* were higher in the four-plant mix. All plant species removed TNT and its metabolites in both single and mixed cultures, whereas the uptake of heavy metals was plant specific. In this study, the results suggested that single plant cultures are better than mixed plant cultures for soil co-contaminated with Cd, Pb and TNT, but other studies, for instance Wu et al. (2012), found a positive effect of intercropping of *Sedum plumbizincicola*, *Elsholtzia splendens, Medicago sativa* and *Houttuynia cordata* for the removal of Cd, Cu and PCB from the soil. Overall, amendments with lime to adjust soil pH to 5.56 combined with intercropping with *S. plumbizincicola* and *M. sativa* produced the best removal rates of Cd, Cu and PCBs.

Recently, Lu and Zhang (2014) also conducted phytoremediation studies by intercropping to two plant species: the hyperaccumulator *Sedum alfredii* and deca-BDE (deca–brominated diphenyl ether) degrader tall fescue (*Festuca arundinaceae*) associated with a BDE degrader microorganism (*Bacillus cereus,* strain JP12). The soil was co-contaminated with BDE and heavy metals (Cd, Pb and Zn). The results showed that the inoculation with JP12 significantly increased the dissipation of BDE and phytoextraction of metals due to the improved plant growth. The authors proposed that intercropping *S. alfredii* with tall fescue combined with the BDE-degrading bacterial strain JP12 is a promising approach for remediation of soil co-contaminated with BDE-209, Cd, Pb, and Zn.

5 Methods for enhancing phytoremediation

The phytoremediation of contaminated soils with heavy metals or organics alone, or a combination of the two types of contaminants (mixed contamination) can be enhanced with several strategies. They include: (a) the increase contaminant mobility and bioavailability (using surfactants or chelating agents); (b) increase overall plant growth (and thus uptake capacity) via nutrient amendments or management strategies (e.g. irrigation); (c) genetic modifications of the plant or the associated rhizosphere or endophytic microorganisms, that increase contaminant tolerance and accumulation or degradation by the plant (Kärenlampi et al. 2000; Kotrba et al. 2009); (d) biomass amendment of the soil (e.g. compost), and (e) electrokinetic enhancement. A combination of these enhancement strategies is also considered.

The amount of metals that a plant is able to accumulate can be improved with procedures that increase their metal-tolerance. In the case of organic contaminants, a reduction in phytovolatilization can be accomplished by genetic modification that will enhance the degradation of organics simultaneously to the contaminant uptake. Inoculation with engineered endophytic bacteria is another alternative to enhance the degradation in the rhizosphere (Weyens et al. 2010, 2011). Further, when coupled with the manipulation of soil conditions via chemical treatments, plant uptake can even be increased in non-hyperaccumulator plant species, which enables the use of high-biomass crops for metal uptake (Sheoran et al. 2012). Some of the significant studies that involve soil amendments are summarized in Table 4.

5.1 Chelate assisted phytoremediation

Chelating agents are soluble chemicals that are able to bind and mobilize other molecules (including both metals and several organic contaminants) into the soil solution, increasing their availability for plant uptake and root-to-shoot translocation (Huang et al. 1997; Evangelou et al. 2007). Both natural and synthetic chelators are available, though their effectiveness varies between plant and soil types. Ethylene diamine tetraacetic acid (EDTA) is a commonly used synthetic chelating agent used to increase plant metal uptake since it forms stable chelates with most of heavy metals (Evangelou et al. 2007; Wu et al. 2007; Huang et al. 1997). Others include hydroxylethylene diamine tetraacetic acid (HEDTA), diethylene triamino pentaacetic acid (DTPA), trans-1,2-cyclohexylene dinitrilo tetraacetic acid (CDTA), ethylene bis[oxyethylenetrinitrilo] tetraacetic acid (EGTA), ethylenediamine-N, N'bis (o-hydroxyphenyl) acetic acid (EDDHA), N-(2-hydroxyethyl)iminodiacetic acid (HEIDA), and N,N'-di(2-hydroybenzyl) ethylene diamine N,N'-diacetic acid (HBED).

Natural chelating agents such as ethylene diamine disuccinate (EDDS), nitrilotriacetic (NTA), citric, oxalic, and malic acids are often preferable to synthetic ones due to their lower toxicity and biodegradability, which reduces their lifetime in soil. The fate of the chelating agent and toxicity to plant and soil microorganisms after its application are highly

Table 4 Soil amendments used for enhanced phytoremediation

Amendment	Contaminants/Plant	Reference(s)	Inference
Chemical amendments			
HEDTA, DTPA, EDTA, EDDHA, EGTA	Pb Pea, Corn, Sunflower	Huang et al. (1997)	After 0.5 g/kg of HEDTA, not much increase in accumulation
EDTA, NTA	Cd Poplar and Willow	Robinson et al. (2000)	Agents caused temporary increase in uptake. EDTA and NTA reduced growth with amendments
EDTA	Cd, Cu, Fe, Mn, Ni, Pb, Zn Barley	Madrid et al. (2003)	EDTA mobilized Cd, Fe, Mn, Ni, Pb, and Zn
EDTA, oxalic, citric and malic acid	Cu, Zn, Pb, Cd Indian Mustard	Wu et al. (2004)	EDTA significantly enhanced the mobility of soil Cu and Pb, but not of Zn and Cd
EDTA and EDDS	Zn, Cu, Cd, Pb, Ni Field mustard, *Cannabis sativa*, Sunflower and *Zea mays*	Meers et al. (2005)	The mobilizing effects induced by EDTA in the soil were found to be too long-lived for application as a soil amendment
Tween 80, Brij35, SDS, CTMAB	Phenanthrene, Pyrene Ryegrass	Gao et al. (2006, 2007)	Tween 80, Brij35 (< 74.0 mg/1) effective at low concentrations. high concentration less effective. SDS, CTMAB are ineffective and phytotoxic
Sorbitan trioleate, salicylic acid and histidine	Ni, phenanthrene, chrysene Candargy	Singer et al. (2007)	Histidine extractable Ni showed high correlation with phytoextractable Ni
Tween 80	Phenanthrene, Pyrene Tall Wheat Grass	Cheng et al. (2008)	Tween 80 increased removal of pyrene but not Phenanthrene. Maximum Pyrene removal at 100 mg/kg tween 80
GA_3 and Tween 80	Cd, benzo[a]pyrene	Sun et al. (2013)	Maximum contamination removal when Soil amended with GA_3 and Tween 80
Tween 80, Triton X-100	Cd, Pb, Engine Oil Indian Mustard	Ramamurthy and Memarian (2012)	Tween 80 effective for improved remediation of mixed metal (Pb, Cr) and petroleum contaminants (engine oil)
Organic amendments			
Chicken Manure, Urea	Cd *Solanum nigrum*	Wei et al. (2010)	Extraction increased due to increased biomass
Cottonwood	Trichloroethylene	Ma and Wang (2010)	Contaminant uptake increased by the addition of amendment
Farmyard Manure Biochar and $FeSO_4$	Hg, Pb *Chromolaena Odorata*	Hamzah et al. (2012)	Amendments reduced pH, and improved C, N, P, K. Better plant growth

CTMAB cetyltrimethylammonium bromide, *DTPA* diethylenetriamine pentaacetic acid, *EDDHA* ethylenediamine-di(2-hydroxyphenyl)acetic acid, *EDDS* ethylenediaminedisuccinic acid, *EDTA* ethylenediamine tetraacetic acid, *EGTA* ethylene glycol tetraacetic acid, *HEDTA* (N-Hydroxyethylethylenedinitrilo)triacetic acid, *NTA* nitrilotriacetic acid, *SDS* sodium dodecyl sulphate

significant factors in selecting the appropriate chelating agent. It should not persist in the soil system for a long time without degradation because if it does remain, the risk of heavy metal migration in the subsurface with the possible dispersion and contamination of groundwater is high (Evangelou et al. 2007).

Huang et al. (1997) investigated the effectiveness of chelates in Pb contaminated soils to increase Pb accumulation in plants. Chelates were very effective since the concentrations of Pb in the shoots of *Zea mays* (corn) and *Pisum sativum* (pea) increased from <500 mg/kg to more than 10,000 mg/kg after the addition of chelates. The rise in phytoaccumulation was directly caused by the increase in Pb bioavailability in the soil solution due to the addition of chelates. The effectiveness of different chelating agents was EDTA > HEDTA > DTPA > EGTA > EDDHA. Furthermore, the EDTA significantly increased the Pb translocation from roots to shoots. The Pb concentration in the corn xylem sap increased

140-fold and the net Pb translocation from roots to shoots increased 120-fold as compared to the control without EDTA, all within 24 h after the application of the EDTA solution (1.0 g of EDTA/kg of soil) to the contaminated soil. Their results indicate that the use of chelates augmented the Pb desorption from soil to soil solution, facilitated Pb transport into the xylem and increased Pb translocation from roots to shoots, all of which are important factors in phytoaccumulation.

A greenhouse pot-based experiment and a laboratory leaching column experiment were conducted by Wu et al. (2004), who studied the EDTA enhanced phytoextraction of heavy metals by *Brassica juncea* (Indian mustard). They added 3 mmol/kg of EDTA to pots of a paddy soil polluted with Cu (169 mg/kg total Cu) and spiked with Zn (500 mg/kg), Pb (500 mg/kg) and Cd (50 mg/kg). The application of EDTA significantly enhanced the mobility of the Cu and Pb, but not Zn and Cd. The concentrations of Cu and Pb in the shoots of the plants were also boosted by the EDTA, but the uptake rates were too low for an effective remediation of the soil. The researchers estimated at least 200 crops would need to be planted over time to remediate the soil. They also investigated the addition of oxalic, citric and malic acid to the soil at the same rate (3 mmol/kg), but with virtually no effect on uptake of the metals by *B. juncea*. Rainfall was simulated in the column leaching experiments, which showed that the concentrations of Cu, Zn, Pb, and Cd in the leachate increased linearly as the dosage of EDTA increased. Soil macronutrients, including Fe, were lost due to the application of EDTA. Since the shoot uptake of Pb and Cu were low and the chelating agent posed the threat of groundwater pollution, the study concluded that chelate-assisted phytoremediation was unsuitable for the combination of pollutants in the soil used in the study and the Indian mustard, especially during periods of high rainfall.

Chigbo and Batty (2013) evaluated the role of single and combined applications of chelates to single or mixed contaminated soils where the contaminants were Cr and benzo[a]pyrene (B[a]P). After growing *Medicago sativa* in this contaminated soil, the soil was amended with citric acid and/or EDTA. The dissipation of B[a]P was effective even without plants or amendments in the single B[a]P-contaminated soil what suggest that the removal mechanisms is biodegradation by the soil microflora and possible volatilization to the atmosphere. In the co-contaminated soil, the B[a]P dissipation improved with the application of either EDTA or EDTA + citric acid suggesting that these organic acids may enhance the microflora activity in soil. In the Cr contaminated soil, the application of EDTA + citric acid significantly decreased the shoot dry matter of *M. sativa* by 55 %, so this also decreased the Cr removal from the soil even though the soluble Cr concentration was enhanced with the chelates. The chelating agents or any other chemical added to the soil to increase the bioavailability of the contaminants must be carefully selected because their presence in soil may reduce the development of the plant. Chigbo and Batty (2015) reported a biomass reduction in Zea mays cultures of 43 and 44 % due to the addition of EDTA and citric acid, respectively. However, the tests with the combination of the two chemicals (EDTA + citric acid) resulted in a biomass increase of 41 % and this test showed the best remediation results of the two contaminants in soil: pyrene and Cr.

Overall, several studies have demonstrated that the addition of chelating agents increased the potential for metal mobility as well as leaching into the subsurface. The high risk for contaminant remobilization and the persistence of certain synthetic chelating agents in the environment necessitate the careful selection of the proper chelating agent for the particular soil and plant type employed. Natural chelating agents such as NTA, EDDS and citric acid are much less harmful to the environment than synthetic agents (Alkorta et al. 2004). NTA has high biodegradability and good chelating strength (Bolton et al. 1996). Kos and Leštan (2003) indicated that the use of a biodegradable chelating agent like EDDS might allow environmentally safe chelate-induced Pb phytoextraction. However, given the risks of metal remobilization via chelating agents, the addition of these chemicals should be carefully evaluated on a case by case basis before field implementation is pursued.

5.2 Surfactant enhanced phytoremediation

Surfactants are a group of natural and synthetic chemicals that can desorb, solubilize, and/or emulsify poorly soluble substrates (Mulder et al. 1998; Noordman et al. 2002), and can be used to remediate both organic and metal contamination (Miller 1995), but their main application is to raise the solubility and bioavailability of hydrophobic organics. Mass transfer and the rate of plant uptake are the major factors that limit the phytoremediation of HOCs (Gao et al. 2007). In phytoremediation, surfactants can assist the mobilization of contaminants into the soil solution where they are more accessible and easily accumulated by plants. However, there are some possible negative effects of surfactant use, such as surfactant phytotoxicity and preferential biodegradation of the surfactant itself by soil microflora (Volkering et al. 1997). The toxicity of surfactants can be minimized by the use of biosurfactants: surfactants produced by plants, animals and many microorganisms. Their merits over synthetic surfactants include biodegradability, cost effectiveness and the potential for in situ production (Miller 1995).

Differences in improvements to plant uptake and the biodegradation of HOCs among types of surfactants were illustrated by Gao et al. (2007), who showed that the phytoremediation of pyrene contaminated soil was significantly enhanced by the presence of some nonionic surfactants, including polyoxyethylene sorbitan monooleate (Tween 80) and polyoxyethylene(23)dodecanol (Brij35), at relatively low concentrations. However, the anionic surfactants (sodium dodecyl sulfate, SDS) and the cationic surfactants (cetyltrimethylammonium bromide, CTMAB) were phytotoxic, which led to much lower removal rates. They concluded that surfactants can stimulate the microbial biodegradation of the HOCs and promote the plant uptake of HOCs.

Ramamurthy and Memarian (2012) studied the influence of non-ionic surfactants Triton X-100 and Tween 80 on the removal of mixed contaminants (Cd, Pb and used engine oil) from sandy soil by *B. juncea* (Indian mustard) in a greenhouse study. After 30 days of growth, surfactants were applied to the test pots where the soil had been spiked with 50 mg/kg of $CdCl_2$, 500 mg/kg of $PbCl_2$ and 500 mg/kg of used engine oil. Triton X-100 and Tween 80 enhanced the Cd and Pb accumulation in the plant roots. Cd, but not Pb, was translocated to plant shoots. Better results were obtained when the surfactant concentrations were above the critical micelle concentration. When applied at equal concentrations, Tween 80 was more effective than Triton X-100 in facilitating the rhizodegradation of used engine oil. Leaching test results indicated that the enhanced phytoremediation could remove the mixed contaminants safely without causing groundwater contamination. Overall, this demonstrated that the phytoremediation of mixed contaminated soil can be enhanced with the non-ionic surfactant Tween 80. The same surfactant was tested by Gao et al. (2008b) for PAHs (phenanthrene, pyrene) uptake form an aqueous solution using *Trifolium pretense* L (red clover). At the test concentrations, Tween 80 (0–105.6 mg/l) did not cause any apparent phytotoxicity to the plant but the PAHs uptake was only enhanced up to the Tween 80 concentration of 6.6 mg/l. This study concluded that surfactants can effectively enhance phytoremediation of PAH but the results were very sensitive to surfactant concentration.

The combination of surfactants with amendments to boost plant growth was investigated by Sun et al. (2013). They conducted pot experiments to evaluate the effectiveness of GA_3 (a vegetable hormone that can promote plant growth) and Tween 80 as soil amendments for enhanced phytoremediation of soil co-contaminated with Cd (200 mg/kg) and benzo[a]-pyrene (5 mg/kg) using *Tagetes patula* (marigold). The amendments GA_3 and Tween 80 enhanced the plant growth the remediation of soil. These results support the use of combined treatments in co-contaminated soils to bolster plant health and improve both phytoextraction and phytodegradation.

The toxicity of surfactants in plant growth during phytoremediation has been reported by Liao et al. (2015). These researchers used maize (*Zea mays* L.) in the phytoremediation of a soil contaminated with phenanthrene (50 mg/kg) and pyrene (50 mg/kg) enhanced with Triton X-100, Saponin and Rhamnolipid. The use of surfactant clearly increased the desorption of the PAHs from soil and their removal rate compared to the tests with no surfactants. However, surfactants had no effect on the accumulation of PAHs in maize tissues. It is supposed that PAHs were mainly removed by the biological degradation by the soil microflora which population increased with increasing concentrations of surfactants. Maize biomass production was inhibited with Triton X-100 and enhanced with the biosurfactant Rhamnolipid. These results proved that the application of surfactants in soil should be carefully considered due to their phytotoxic effects.

Surfactant can play an important role in soil remediation due to its unique characteristics for the desorption and mobilization of organic contaminants. Heavy metals can also be mobilized via surfactant-associated complexation and ion exchange process. Furthermore, the presence of surfactants may enhance biological activity in the soil with the subsequent degradation of organic contaminants. There is a significant amount of surfactants in the marketplace at low cost that make attractive their use at large scale applications (Mao et al. 2015). The main drawback during phytoremediation is their toxicity for the growing plants, since a limitation in biomass production results in lower remediation efficiency. However, toxicity of surfactants can be avoided with the use of biosurfactants, which show minor toxic effects. Recently, the use of gemini surfactants has been proposed in soil remediation. Gemini surfactants are a group of surface active compounds possessing more than one hydrophobic and hydrophilic groups. These compounds shows lower CMC (critical micelle concentration) than the corresponding monomeric surfactant (Liu et al. 2014), thus lower dose will result in lower phytotoxic effects.

5.3 Bacterial endophyte enhanced phytoremediation

The enhancement of the rhizodegradation of organics by endophytic bacteria and fungi that exist within the plant root zone is gaining interest as a potential phytoremediation enhancement strategy. If the contaminants are degraded in the rhizosphere, the amount of pollutants taken up by the plant is reduced, avoiding possible phytotoxicity and limited plant growth, as well as reducing the possible volatilization of the chemicals through the plant leaves. Thus, the risks associated to inhalation and remobilization of contaminants upon plant death is minimized.

The enhanced rhizodegradation can be achieved via the stimulation or inoculation of plants with natural or engineered endophytic bacteria that favor the degradation of organic contaminants, as well as aid in the bioavailability of some contaminants by extending the reach of the root zone and secreting organic acids and enzymes that can enhance local mobility. One successful demonstration of endophyte-enhanced rhizodegradation of toluene was conducted by Barac et al. (2004). They introduced engineered endophytic bacteria, which degraded toluene and resulted in a marked decrease in its phytotoxicity as well as a 50–70 % reduction of its evapotranspiration through the leaves. This strategy improves the efficiency of phytoremediation when treating soil for volatile organic contaminants.

Similar improvements were noted with other organic contaminants by Germaine et al. (2006), who studied bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid, which is widely used throughout the world as a herbicide for the control of broad-leaf weeds. This compound is particularly toxic to some other broad-leaved plants, such as poplar and willow, which are often used in phytoremediation projects. Germaine et al. (2006) proposed as a possible solution to this problem the inoculation of *P. sativum* (pea) with a genetically tagged bacterial endophyte that naturally possessed the ability to degrade 2,4-dichlorophenoxyacetic acid. The bacterial strain colonized both the plant and rhizosphere, leading to a significant increase in the plant biomass relative to the control. They observed a higher rate of removal of 2,4-dichlorophenoxyacetic acid in the inoculated plants without any contaminant accumulation in the aerial tissues of inoculated plants. This study demonstrates the effectiveness of bacterial endophytes in enhancing phytoremediation of herbicide contaminated soil and groundwater.

Research efforts were made by Weyens et al. (2010) to reduce the effects of phytotoxicity and evapotranspiration by inoculating *Lupinus luteus* (yellow lupine) plants with engineered endophytic bacteria where the soil was contaminated with Ni and trichloroethylene (TCE) for trichloroethylene degradation and Ni resistance/sequestration system. The root mass of the inoculated plants increased by 30 % indicating decreased phytotoxicity. The inoculated plants showed a decreasing trend for TCE evapotranspiration when compared to un-inoculated controls. The inoculated plants also had 5 times higher Ni uptake than the control plants: the Ni concentrations in the roots were approximately sevenfold higher and the concentrations in the shoots increased fivefold in the inoculated plants than in the control plants. This study confirmed that engineered bacteria can be equipped with mechanisms that will successfully degrade metal tolerance and organic contaminants and yield promising results for application at mixed contaminated sites. A related study from Weyens et al. (2011) found similar improvements in Ni uptake in co-contaminated soil that contained Ni and toluene after the root zone was inoculated with engineered endophytic bacteria, and using *Lupinus luteus*. The interesting result of this study is that the inoculation with some strains of bacteria considerably reduced the evapotranspiration and increased the Ni uptake. Although further research to identify the most effective inoculum for different plant and contaminants is suggested by the researchers, these results suggest that engineered endophytes can help the host plant to deal with co-contamination of toxic metals and organic contaminants.

Pot-based experiments by Zhu et al. (2012) examined the combined effect of phytoremediation with *Sedum alfredii* (a known hyperaccumulator plant) and bacterial inoculum in soil co-contaminated with Cd and DDT. The presence of microbes did not seem to affect Cd removal but microbes clearly decreased the residual DDT concentration in soil by 52 % compared to the un-inoculated test. The study demonstrated that even though the bacterial inoculum did not have any noticeable effect on the Cd extraction, it helped to increase the rhizodegradation of DDT, possibly by conferring protection against Cd toxicity to the plant. Overall, these studies illustrate that the combination of phytoremediation and bacterial inoculation is a promising approach for remediation of co-contaminated soils even containing hydrophobic, toxic and xenobiotic organic contaminants such as: TCE (Weyens et al. 2010), toluene (Weyens et al. 2011), benzo(a)pyrene (Gutiérrez-Ginés et al. 2014), hexachlorocyclohexane (Becerra-Castro et al. 2013).

5.4 Enhancement of phytoremediation by the biomass enhancement

Increasing the biomass of the plant through the use of fertilizers, compost or other amendments is a way of augmenting the efficiency and uptake capacity of phytoremedial systems. Increased plant growth leads to increased rates of uptake and organic contaminant degradation in the soil. Amendments used to increase the biomass include NPK fertilizer (Pichtel and Liskanen 2001), chicken manure, urea (Wei et al. 2010), farmyard manure, and biochar (Hamzah et al. 2012). The application of vegetable (Sun et al. 2013) and plant hormones (Ahammed et al. 2013) to stimulate plant growth has shown promising results. The effects of biomass enhancement in phytoremediation of post-oil spill habitat restoration site soil were evaluated in an early study by Lin and Mendelssohn (1998). Two years after application of the oil, plants were transplanted into oil contaminated and oil-free soil samples, with fertilizer applied 1 and 7 months after transplantation. They observed increased microbial numbers and activities in response to an increase in vegetation spurred by the fertilizer application, resulting in increased oil degradation. Several recent studies have confirmed the effectiveness of organic amendments for improved growth, which is often incorporated with other technologies to improve remediation, e.g. Rentz et al. (2003) uses an 10/5/5 NPK fertilizer combined with the oxygenation of rhizosphere to increase the biomass production of poplar trees by 145 % growing in hydrocarbon-contaminated soil. Willscher et al. (2013) used soil amendments (increasing pH and organic matter, fertilizing) to increase biomass production and plant tolerance to heavy metals when Triticale, *Helianthus annuus* and *Brassica juncea* was growing in a soil contaminated with heavy metals and radionuclides.

The use of amendments in field applications is limited to their availability, price and absence of other contaminants or phytotoxicity. Compost appears to be the most appropriate amendment for general purposes (Alaribe and Agamuthu 2015) although not always results successful (Marchal et al. 2014).

5.5 Combination of phytoremediation with other technologies

Phytoremediation can be combined with certain other remediation technologies to enhance removal efficiency. Most of these strategies employ a coupled technology (e.g. chemical amendments or electrokinetic remediation) to increase the availability of contaminants to the plants used for phytoremediation. The combination of electrokinetic remediation with phytoremediation has shown significant promise for promoting heavy metal mobility and plant uptake (Cameselle et al. 2013), although its application for mixed contamination has been limited so far. Phytoremediation was commonly combined with bioremediation in the rhizosphere for the degradation of organics and removal/immobilization of heavy metals. The biological activity in the rhizosphere is a natural process since many microorganisms growth in a symbiotic relation with the plants. However, several efforts have been done to enhance biological activity in the rhizosphere oriented to improve the removal of contaminants. Thus, the combination of phytoremediation with microbial biodegradation using omic tools and new bioinformatics approaches will allow understanding integrated activity patterns between plants and microbes, with the final objective of understand how the organisms can be modified to maximize growth, appropriate assembly of microbial communities, and, ultimately, phytoremediation activity (Bell et al. 2014).

Phytoremediation can also be used to clean up residual contaminants after the primary remedial treatment has been applied as a final 'polishing' step. Along with residual contaminant clean up, phytoremediation can help recover the soil structure and texture after physical or chemical treatment by providing organic nutrients and encouraging the growth of endophytic microorganisms in the rhizosphere.

5.6 Electrokinetic-enhanced phytoremediation

Electrokinetic (EK) remediation has received recent attention as a means of increasing plant uptake of both inorganic and organic compounds, though the majority of studies coupling electrokinetics with phytoremediation have focused on enhancing plant uptake of heavy metals (Gomes et al. 2012). Evidence for increased plant growth and contaminant mobility in the presence of an electric field (e.g. Lemström 1904) indicate a possible applicability for use with mixed contaminants (Cameselle et al. 2013). When an electric field is applied to the soil, the movement of the pore fluid (electro-osmosis), ions (electromigration) and colloids (electrophoresis) can be induced, which allows greater metal accumulation in the interstitial fluid in the rhizosphere and uptake by the plant as noted by Hodko et al. (2000). They suggested that the effectiveness of phytoremediation can also be improved if the soil is prevented from becoming strongly acidic or basic through the manipulation of the electric field. Acidic or alkaline environments in soil seriously affect the plant metabolism and biomass production. Keeping the electric current intensity in low values will limit the extension of the electrolysis of water, and therefore avoid rapid changes in the pH in the area close to the electrodes. The periodic inversion of polarity (with DC current) and the use of AC current are two interesting approaches to avoid pH changes in the soil (Aboughalma et al. 2008). Apart from the pH, the main effect of an electric field in phytoremediation is the increased exposure of plant to heavy metals, which may increase the stress on the plants. As such, researchers have found that only plants that are able to tolerate high metal concentrations, (i.e. hyperaccumulator plants with rapid growth periods) are suitable for use in electrokinetic-enhanced phytoremediation (Bedmar et al. 2009).

The use of a combination of electrokinetic remediation and phytoremediation to decontaminate two metal-polluted soils (Cu, Cd and As) was demonstrated in laboratory-scale reactors by O'Connor et al. (2003). *Lolium perenne* (perennial ryegrass) was sown in the chambers and 30 V was continuously applied across the soil. Tests of the metal content in the soil and leaves revealed a significant redistribution of the metals from the anode to cathode after the electrokinetic treatment. Though no clear patterns in Cd uptake were noted, an upsurge in Cu uptake was observed in the cathode region. Significant soil acidification near the anode due to the EK treatment negatively affected plant growth, although the growth and soil pH were apparently unaffected elsewhere within the reactor. Further, no visual signs of metal toxicity were noted in either polluted soil in response to the combined treatment, which indicates the feasibility of this approach for enhanced metal uptake with minimal oxidative stress on the plants.

The addition of an electric field around *B. juncea* (Indian mustard) plants in conjunction with a chelating agent (EDTA) to increase the uptake of lead was studied by Lim et al. (2004). The studies were conducted at different ranges of the parameters such as operating current/voltage, concentration, application time of EDTA, and electric potential. The optimal results were obtained with 5 mmol EDTA/kg, 30–40 V and an electric field application time of 1 h per day where the plants were harvested 9 days after the EDTA application. This shows that the combined procedure resulted in better remediation. Zhou et al. (2007) studied the effect of direct current (DC) on metal uptake by *Lolium perenne* (rye grass) in greenhouse experiments. They combined phytoremediation with electrokinetic remediation and the application of EDTA or EDDS to strengthen the uptake of Cu and Zn from the contaminated soil. The results showed that the combination of a chelating agent and the electric field had better phytoremediation results than the control or individual treatments. EDTA/EDDS application significantly increased the uptake of Cu and Zn as it increased the aqueous concentrations of each metal even though the increase in uptake was greater for Cu than for Zn. The redistribution of Cu and Zn concentrations were due to the vertical electric field. The study also showed that better control over the leaching of Cu and Zn was achieved with the application of an electric field.

A set of laboratory-scale greenhouse experiments by Aboughalma et al. (2008) combined electrokinetic remediation and phytoremediation to decontaminate soil polluted with Zn, Pb, Cu, and Cd. *Solanum tuberosum* (potato tubers) were planted in plastic containers filled with contaminated soil. The study compared the application of alternating current (AC) versus direct current (DC). The application of the DC current provoked a pH gradient along the soil sample from pH 3 at the anode to pH 8 at the cathode. The pH gradient was the responsible metal precipitation in the zone where the soil pH was 5. Furthermore, the DC current tests showed 27 % lower biomass production that the control test with no current. The AC treated samples showed no significant metal redistribution or pH variation between the anode and cathode regions, and the biomass production of the plants was 72 % higher than the control. The higher biomass production and the absence of pH gradient in the AC tests resulted in higher metal accumulation in biomass, especially in the shoots, than in the DC tests. Bi et al. (2011) also tested the capability of AC and DC current to enhance phytoremediation using *Brassica napus* (rapeseed) and *Nicotiana tabacum* (tobacco). The three soils used for the experiments included contaminant-free soil from a forest area (S1), soil artificially contaminated with 15 mg/kg Cd (S2) and co-contaminated soil (Cd, Zn and Pb) from an industrial area (S3). The plants grown in containers with contaminated soil were subjected to three treatment conditions: AC electric field (1 V/cm), DC electric field (1 V/cm), or no electric field (control). The polarity of the DC electric field was switched every 3 h to eliminate the known pH variation from the anode to cathode region. The plants had different responses to the electric field. The rapeseed biomass was not affected significantly by the DC electric field, but under the AC treatment, its biomass increased. In the case of tobacco plant, its biomass was decreased under DC electric field, but did not evidence enhancement in the plants subjected to the AC electric field. In general, the metal uptake of rapeseed was higher in the AC treated samples, a point attributed to the increased biomass. Better performance of electrokinetic assisted phytoremediation was found in the Cd contaminated soil where there were no other contaminants present than when multiple metals were present in the soil.

Cang et al. (2012) conducted laboratory experiments on the impact of electrokinetic-assisted phytoremediation of heavy metal contaminated soil on its physicochemical properties and enzymatic and microbial activities. *B. juncea* (Indian mustard) was grown in the presence of 0, 1, 2, or 4 V/cm DC electric field for 8 h per day. Soil respiration and key enzyme activities (i.e. urease, invertase and neutral phosphatase) were negatively affected by the electric field, especially in the tests at 4 V. Plant biomass production was maximized at 2 V but the plant growth was inhibited at 4 V. Plant growth did help to minimize any drop in the enzymatic activity relative to the activity in the control plants. Thus, depending on the electric field strength, electrokinetic remediation may negatively impact soil microbial health and plant growth, affecting the phytoremediation capacity.

Chirakkara et al. (2015) has proven the capability of phytoremediation enhanced with AC electric current (25 AC V per 3 h/day). These researchers used a soil contaminated with 50 mg/kg naphthalene and 100 mg/kg phenanthrene and heavy metals (500 mg/kg Pb, 200 mg/kg Cr and 50 mg/kg Cd) as a model soil to represent complex mixed contamination commonly found in former industrial areas. Oat plant and sunflower were the plants selected in this study because these species can grow in the contaminated soil (Chirakkara and Reddy 2015). The results confirm a significant reduction of heavy metals and organic contaminants in soil. However, there was no noticeable improvement of heavy metal phytoextraction or PAH degradation due to the application of electric field despite the increase in biomass production by the plants subjected to the electric current. The electric potential application time and frequency were suggested to be increased to have noticeable effects in heavy metal uptake and PAHs degradation.

Overall, the enhancement of phytoremediation with electric fields (AC, DC or DC with polarity inversion) shows interesting perspectives for the remediation of soil contaminated with heavy metals or organic contaminants or mixed contamination that deserve to be explored. It is considered of special interest to understand the effect of the electric field in the growing and development of the plants, as well as the modifications induced by the electric field in the geochemical interactions among the soil, plant, microflora and contaminants.

6 Conclusions

Phytoremediation has been studied extensively for the remediation of contaminated soils with one contaminant or one kind of contaminants (e.g. heavy metals, metalloids, other inorganic elements, and specific groups of organic contaminants). However, there is still limited information in literature about the effectiveness of phytoremediation for mixed contaminated soils. The published literature suggests that phytoremediation has great potential for the remediation of contaminated soils with moderate concentrations of mixed contaminants. Based on the literature review, the following conclusions can be drawn:

- Plants that have proved their effectiveness in the phytoremediation of heavy metals or organic contaminants alone are the best candidates for the remediation of co–contaminated sites. However, the remediation results with mixed contamination may differ from those with only one kind of contaminants.
- It is expected that mixed contaminated soils exert more phytotoxic effects on plants than that of the same contaminants when exist individually. This is mainly true at high concentrations, but with low/moderate concentrations of contaminants, the presence of heavy metals may enhance the degradation/removal of organic contaminants. As an example, pyrene was effectively removed in the presence of low concentrations of Cu (50 mg/kg). Phytotoxicity appears at 100 mg/kg of Cu. This phenomenon can be related with the role of Cu as a necessary nutrient at low concentrations. The same behavior was found with PCP-Cu and pyrene-Cd, but latter this case, there is not known metabolic function for Cd.
- The limitations of phytoremediation due to phytotoxicity can be avoided in part with the selection of: (a) hyperaccumulator plants and fast-growing plants; (b) local plants that naturally grow in the contaminated site (they are naturally adapted to the site contaminants); (c) transgenic plants specially designed to withstand high levels of contaminants (i.e. engineered transgenic alfalfa plants); and (d) the use of engineered endophytic bacteria to promote better degradation in the rhizosphere.
- Phytoremediation can be enhanced with the addition of amendments/chemicals that favor the biomass production (e.g. use of fertilizers and compost) or increase the bioavailability of contaminants (e.g. chelating agents, organic acids, and surfactants).
- Soil fertilization usually enhances remediation by increasing biomass production and microbial activity in the rhizosphere. The use of biosolids or compost as a source of nutrients may show a negative effect upon remediation by adsorption/ immobilization of heavy metals, reducing plant metal uptake, whereas the degradation of organic contaminants is favored.
- Chelating agents and surfactants are in general effective amendments for the enhanced removal of heavy metals and organic contaminants. The main drawback is potential for phytotoxic effect on plant and soil microflora. Thus, biosurfactants are preferred over other chemical surfactants available in the market. Despite this, Tween 80 has shown good results and low toxicity when used at moderate concentration (100 mg/l). Gemini surfactants (surface active compounds possessing more than one hydrophobic and hydrophilic groups) are a new group of surfactants that should be tested for phytoremediation because of their lower CMC that results in minor phytotoxic effects.
- The use of organic acids (e.g. citric acid) with very low toxicity is a better option than using other common complexing agents (e.g. EDTA) that shows more toxic effects on plants. The combination of several complexing agents at low concentration is another option to avoid phytotoxicity and enhance metal bioavailability at the same time.
- Coupling phytoremediation with other remediation technologies (i.e. electrokinetic remediation or enhanced biodegradation in the rhizosphere) can improve plant survival and growth rates, and may be used to improve the remediation process for recalcitrant contaminants in complex subsurface conditions.

Finally, the system composed of plant, soil microflora, mixed contaminants, and soil will result in complex interactions that will impact the remedial results. As a result, it is common to find significant differences in phytoremediation efficiency from site to site, even if similar conditions (e.g. plant and amendments) are applied. Given the sensitivity of plant growth and contaminant uptake/degradation to soil geochemistry and contaminant interactions, contaminant-specific and site-specific investigations may be necessary to successfully implement phytoremediation at a particular mixed contaminated site.

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