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Review

Metal phytoremediation: General strategies, genetically modified plants and applications in metal nanoparticle contamination

Maria Angélica da Conceição Gomes [a,*], Rachel Ann Hauser-Davis [b,*], Adriane Nunes de Souza [c], Angela Pierre Vitória [a]

[a] Laboratório de Ciências Ambientais (LCA), Centro de Biociências e Biotecnologia (CBB), Universidade Estadual do Norte Fluminense "Darcy Ribeiro" (UENF), Av. Alberto Lamego, 2000, Parque Califórnia, Campos dos Goytacazes, RJ 28013-602, Brazil
[b] Universidade Federal do Estado do Rio de Janeiro – UNIRIO, Av. Pasteur, 458, Urca, CEP: 22290-240, Rio de Janeiro, RJ, Brasil
[c] Universidade Estácio de Sá – Estácio, Av. 28 de Março, 423 - Centro - Campos dos Goytacazes CEP: 28020-740, Rio de Janeiro, RJ, Brasil

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ABSTRACT

The accumulation of metals in different environmental compartments poses a risk to both the environment and biota health. In particular, the continuous increase of these elements in soil ecosystems is a major worldwide concern. Phytoremediation has been gaining more attention in this regard. This approach takes advantage of the unique and selective uptake capabilities of plant root systems, and applies these natural processes alongside the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant and, although it is a relatively recent technology, beginning in the 90's, it is already considered a green alternative solution to the problem of metal pollution, with great potential. This review focuses on phytoremediation of metals from soil, sludge, wastewater and water, the different strategies applied, the biological and physico-chemical processes involved and the advantages and limitations of each strategy. Special note is given to the use of transgenic species and phytoremediation of metallic nanoparticles.

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1. Introduction

The accumulation of metals in different environmental compartments poses a risk to both the environment and biota health,

* Corresponding authors.
E-mail addresses: maria_angelicagomes@yahoo.com.br (M.A.C. Gomes), rachel.hauser.davis@gmail.com (R.A. Hauser-Davis).

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including humans, since these elements bioaccumulate in living organisms and also suffer biomagnification processes, in which contaminants increase in concentration in tissues of organisms at successively higher levels in a food chain (Alia et al., 2013). In particular, the continuous increase of these elements in soil ecosystems is a major worldwide concern (Pandey et al., 2015; Sharma and Pandey, 2014; Wuana and Okieimen, 2011), and, with novel technological advances and applications, novel forms of metal contamination have been noted and are of concern, such as the rising presence of metallic nanoparticles in the environment (Ebbs et al., 2016). These compounds show many positive impacts in several sectors, such as consumer products, cosmetics, pharmaceutics, energy, and agriculture, among others (Baker et al., 2014). However, the risks associated to their use are still unknown, and they may show potential adverse effects in the environment (Ruffini-Castiglione and Cremonini, 2009), making them target compounds for phytoremediation.

Many types of soil clean-up techniques have been applied over the years, categorized into physical, chemical and biological approaches (Hasegawa and Mofizur, 2015; Lim et al., 2014). Traditionally, remediation of metal-contaminated soils involves either on-site management or excavation and subsequent disposal to a landfill site. This, however, only shifts the contamination problem elsewhere and causes additional risk hazards associated with the transportation of contaminated soil and migration of the contaminants to adjacent environmental compartments (Gaur and Adholeya, 2004). An alternative to this process is soil washing, although this method is very costly, produces metal-rich residues which require further treatment, and usually renders the land unusable for plant growth, since it removes all biological activities (Gaur and Adholeya, 2004; Tangahu et al., 2011). Thus, it is recognized that physical and chemical methods suffer from severe limitations (i.e. high cost, intensive labor, irreversible changes in soil properties and disturbance of native soil microflora), while chemical methods are also problematic, since they usually create secondary pollution problems, generate large volumetric sludge and increase costs (Alia et al., 2013; Tangahu et al., 2011).

In this context, novel and better clean-up solutions for metal-contaminated soils are needed, and biological remediation techniques are considered the most adequate, since they are natural, ecological processes that do not impact the environment (Doble and Kumar, 2005). Biological remediation techniques include bioremediation, phytoremediation, bioventing, bioleaching, land forming, bioreactors, composting, bioaugmentation and biostimulation. Among these approaches, phytoremediation is the most useful (Ullah et al., 2015) and has been gaining more attention in this regard.

Phytoremediation comes from the Greek word *phyto*, meaning plant, and the word *remedium*, in Latin, meaning balance or remediation. This approach takes advantage of the unique and selective uptake capabilities of plant root systems, and applies these natural processes alongside the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant (Hinchman et al., 1995). Phytoremediation can thus be applied to the environment to reduce high concentrations of several pollutants, such as organic compounds and metals (Ahmadpour et al., 2012; Pilon-Smits and Freeman, 2006), and, although it is a relatively recent technology, beginning in the 90's, it is already considered a green alternative solution to the problem of metal pollution, with great potential, since over 400 plant species have been identified as potential phytoremediators (Alia et al., 2013; Lone et al., 2008). In addition, genetically modified plants have also been gaining more attention in this regard, since they can be created to increase phytoremediation capabilities (Macek et al., 2008; Novakova et al., 2010) showing advantages against both abiotic stress and the presence of metals in the environment (Ibañez et al., 2015).

Moreover, phytoremediation allows the restoration of polluted environments with low costs and low collateral impacts (Ibañez et al., 2015), shows benefits regarding the increase of vegetation growth and can be applied in many different ecosystems (Pilon-Smits and Freeman, 2006). Some limitations to this technique do, however, exist, mainly regarding remediation time and the problems of what to do with the toxic plant waste left over after phytoremediation. In this context, the aim of the present study is to discuss and compare phytoremediation techniques in both aquatic and terrestrial ecossystems, with special regard to genetically modified plants and the increasing problem of metallic nanoparticles in the environment.

2. Phytoremediation strategies

Plants can be used for phytoremediation via different physiological processes that allow metal tolerance and absorption capacity (Peuke and Rennenberg, 2005; Pilon-Smits and Freeman, 2006). The main metal phytoremediation techniques can be categorized in: phytofiltration, phytostabilization, phytoextraction, phytovolatilization and phytotransformation (Halder and Ghosh, 2014). Fig. 1 displays a diagram of different phytoremediation technologies involving removal and containment of contaminants and the physiological processes that take place in plants during phytoremediation.

2.1. Phytofiltration

Phytofiltration can be categorized as rhizofiltration (use of plant roots), blastofiltration (use of seedlings) or caulofiltration (use of excised plant shoots; Latin *caulis*= shoot) (Sarma, 2011). In this type of process, contaminants are absorbed or adsorbed from contaminated surface waters or wastewaters, restricting their movement to underground waters. This strategy may be conducted *in situ*, where plants are grown directly in the contaminated water body, decreasing costs (Suthersan and McDonough, 1996).

Blastofiltration takes advantage of the sudden increases in surface to volume ratio that happens after germination and the fact that many seedlings are able to adsorb or absorb large amounts of metal, making them uniquely suitable for water remediation (Krishna et al., 2012). In one study reported in the literature, castor, okra, melon and moringa seeds were investigated with regard to their blastofiltration potential. In 72-h experiments with Pb- and Cd-contaminated water with 60 ppm of each, separately, metal content decreased by 96–99%. Okra and castor seeds were the most efficient, while moringa seeds removed 100% of Cd from the contaminated-water. The author in this cases postulates that plant seeds of lesser economic importance could represent the next generation green technology at bioremediation of heavy metal polluted water (Udokop, 2016). Another report, also using aqueous extracts from *Moringa oleifera* seeds reported metal uptake from contaminated water as 95% for copper, 93% for lead, 76% for cadmium and 70% for chromium (Ravikumar and Sheeja, 2013). Papaya seeds have also recently shown promise in metal removal from contaminated waters. These seeds were added to aqueous solutions contaminated with zinc, at different pH values, and results indicated that Zn uptake increased with increasing contact time and agitation rate of the solutions, while indicating the effective pH for maximum Zn uptake was pH 5.0, demonstrating that absorption efficiency is pH-dependent. In addition, decreases in sorbent particle sizes led to increases in Zn sorption due to increases in surface area and, consequently, binding sites (Ong et al., 2012). Mango seed powder has also been applied in this context, for the removal of Cu, Cd and Pb from aqueous solutions,

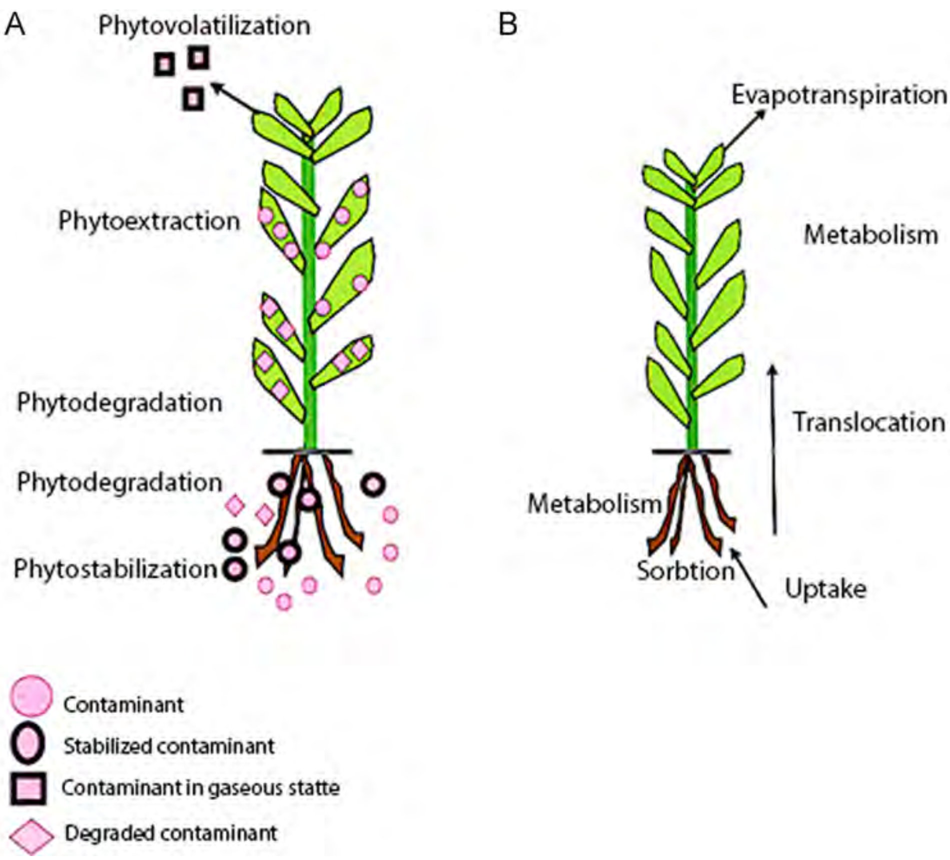

Fig. 1. (A) Diagram of the different phytoremediation strategies and (B) physiological processes that take place in plants during phytoremediation. Adapted from Nature Education (2011).

where removal ranged from 85% to 100% for all three metals (Pareck et al., 2002).

Several advantages exist in using seeds for this phytoremediation process, such as the fact that seedling cultures can be produced in different conditions, such as in light or darkness; cultures are inexpensive, requiring only seeds, water and air (Krishna et al., 2012)l and plant seeds are usually common, easy to obtain, cheap and are, in fact, thrown away as waste, which makes this technology even more sustainable and green (Pareck et al., 2002).

Regarding caulofiltration, recent studies have also indicated great potential in this regard for metal removal from contaminated water bodies. For example, a study conducted with *Ipomoea aquatic* exposed to Pb concentrations over 20 mg L^{-1} demonstrated that significant sequestration of excess metal occurred in stem tissue. The ability of the plant to store Pb in its root and lower part of stem coupled with its ability to propagate by fragmentation through production of adventitious roots and lateral branches from nodes raises the possibility of utilizing *Ipomoea aquatica* for Pb phytoremediation from liquid effluents (Chanu and Gupta, 2016). In another study, excised stems from *Stevia rebaudiana* were shown to accumulate significant amounts of As, Cu, Se and Al (Hajara et al., 2014), while excised shoots immersed in concentrated solutions of Cd, Ni, Pb or Zn accumulated significant amounts of these metals in the leaves (Mesjasz-Przybyłowicz et al., 2004). Studies applying this process to metal phytoremediation, however, are not as widespread.

In particular, rhizofiltration has found innumerous applications. This technique is mainly applied using aquatic macrophytes (Dhir et al., 2009; Dushenkov and Kapulnik, 2000; Olguín and Sánchez-Galván, 2012; Rai, 2008), although reports indicate that some terrestrial plants are also able to conduct rhizofiltration, using a root biofilter formed by microorganisms to absorb, concentrate and precipitate metals (Salt et al., 1995). Metal precipitation is caused by root exudates that in turn alter the pH in the rhizosphere (Rai, 2008). The roots of many hydroponically grown terrestrial plants, such as Indian mustard, sunflower and various grasses, have been shown to effectively remove metals (Cu^{2+}, Cd^{2+}, Cr^{6+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} from aqueous solutions (Dushenkov et al., 1995). Roots of Indian mustard, in particular, concentrated these metals ranging from 131 to 563-fold above the initial concentrations, with removal based on tissue absorption and root-mediated Pb precipitation in the form of insoluble inorganic compounds. At high Pb concentrations, precipitation played a progressively more important role in Pb removal than tissue absorption (Dushenkov et al., 1995).

Specifically regarding aquatic macrophytes, several species have shown potential for rhizofiltration. *Eichhornia crassipes*, for instance, has a fast growth rate and high capacity to increase biomass, as well as a well-developed and fibrous root system (Liao and Chang, 2004). It adapts easily to different water conditions and plays an important role in metal extraction and accumulation and, because of this, is considered ideal for use in rhizofiltration (Liao and Chang, 2004). For example, a phytoremediation study by *E. crassipes* in Taiwan found that this species is able to absorb high concentrations of Cu, Zn, Ni, Pb, accumulating these elements mainly in roots, with concentrations 3–15 times higher than in the shoots (Liao and Chang, 2004). In another study, the macrophytes *Salvinia herzogii, Pistia stratiotes, Hydromistia stolonifera* and *E. crassipes*, were highly efficient in the absorption of Cd, with *P. stratiotes* showing higher growth rates in the presence of this element (Maine et al., 2001). The authors suggested that *P. stratiotes* applies various mechanisms for enhanced absorption of Cd, however, without specifying exactly which mechanisms. The increase of Cd concentrations in plant tissues occurred particularly in roots and was linearly related to the amount of Cd added to the experiment, and Cd absorption by the roots was faster than translocation to the shoots and occurred mainly during the first 24 h. More recent studies have also shown the potential of other aquatic species, such as *Azolla pinnata,* to remove metals from aqueous environments through rhizofiltration. In this case, Pb uptake capacity in plants increased with decreasing nutrient concentration in the growth media, and the authors indicate that the efficiency of Pb removal depends on the duration of exposure. After only four days of treatment *A. pinnata* reduced Pb concentrations in the media by 83%, indicating high potential for the removal of Pb from polluted waterways (Thayaparan et al., 2013). Another study regarding Pb uptake by this process was conducted with the wetland plant *Carex pendula*, that also accumulated considerable amounts of this metal by rhizofiltration, further confirming the potential uses for this technique in metal phytoremediation of polluted waterbodies.

Desirable characteristics for efficient rhizofiltration include plant tolerance to metal concentrations, the ability to accumulate high concentrations of these elements, high biomass production and a limited translocation of the contaminants from roots to shoots (Terry and Banuelos, 2000). A plant with high translocation of metals from roots to shoots reduces the advantages of rhizofiltration process, since it increases the number of parts of the plants that are contaminated by metals (Dushenkov and Kapulnik, 2000) and, consequently, the risk of contamination by other organisms through the food chain.

2.2. Phytostabilization

Phytostabilization can involve simple erosion, leaching or runoff prevention or the conversion of pollutants to less bioavailable forms, via precipitation in the plant rhizosphere (Nwoko, 2010). It is usually used for stabilization of metals in contaminated water, soil, sediment or sludge (Ghosh and Singh, 2005; Singh, 2012), preventing their migration to groundwater or their entry into the food chain (Erakhrumen, 2007). This occurs by sorption by roots, precipitation, complexation or metal valence reduction in the rhizosphere, such as the classical case of reduction of Cr^{+6}, the more toxic form of this metal, to Cr^{+3}, a more mobile and less toxic species (Barceló and Poschenrieder, 2003; Ghosh and Singh, 2005; Wu et al., 2010; Fig. 2). Some metals are more prone to phytostabilization than others, due to their chemical characteristics. For example, a research on the comparative performance of metal bioaccumulation by *Typha domingensis* and *Phragmites australis*, both rooted macrophytes, found that these species can be used to phytostabilize Hg and As in sediments (Bonanno, 2013), but are not efficient in phytostabilizing other metals.

Unfortunately, phytostabilization is not a permanent solution to metal contamination, since metals are only inactivated, with their movement in the environmental compartment limited, and still remain in the soil, sediment or plant roots (Vangronsveld et al., 2009). However, phytostabilization does show an advantage over other phytoremediation techniques, since the need to treat the aerial parts is reduced, as the process mostly retains contaminants in the roots, with low translocation to the shoots (Abreu et al., 2012) and is a very effective strategy when rapid immobilization is needed to preserve ground and surface waters (Chhotu et al., 2009).

Appropriate characteristics for plants to be applied in phytostabilization are the ability to develop an extensive root system, show tolerance to metals or other contaminants, the ability to immobilize those elements in the rhizosphere and low translocation to plant aerial parts in general (Kramer, 2005). Phytostabilization studies include many different species in a variety of terrains for a variety of metals, such as Cu in *Bidens pilosa* and *Plantago lanceolata* in contaminated vineyard soils (Andreazza et al., 2015), Cd, Pb, and Zn in forage grasses (*Pennisetum americanum, Pennisetum, Euchlaena mexicana,* and *Sorghum dochna*) (Zhang et al., 2016), and Pb in wetland plants, such as *Juncus effusus* L. (Najeeb et al., 2014).

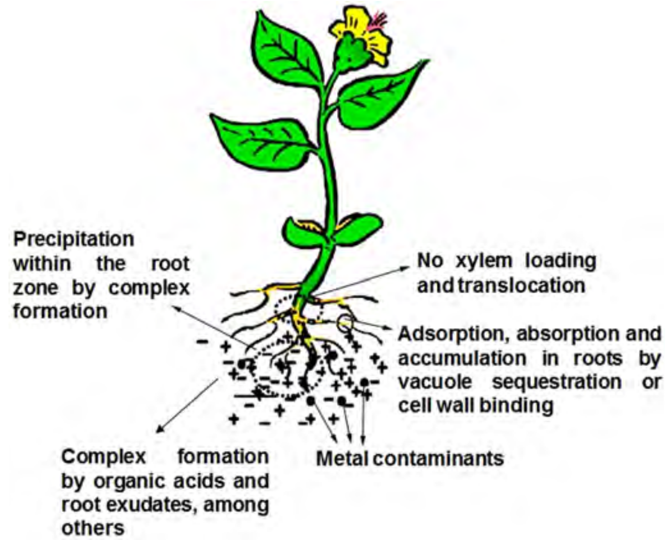

Fig. 2. Phytostabilization mechanism, adapted from Padmavathiamma and Li (2007).

2.3. Phytoextraction

This technique uses the ability that plants have of extracting and accumulating metals into their harvestable tissues (Nwoko, 2010). It is also known as phytoaccumulation, phytoabsorption or phytosequestration, and is composed of metal uptake from soil or water by plant roots and their translocation to and accumulation in the aboveground biomass (Ali et al., 2013; Sekara et al., 2005; Yoon et al., 2006; Fig. 3).

This technique is the main and most useful phytoremediation technique for removal of metals and metalloids from polluted soils, sediments or water, although its efficiency depends on many factors, such as metal bioavailability, soil properties, metal speciation and plant species and, mainly, on shoot metal concentration and biomass (Ali et al., 2013; Li et al., 2010). For example, a study on the phytoextraction of Cd by *Ipomoea aquatica* in a hydroponic solution observed that this plant is promising in this regard, since it has high capacity of translocation of this metal in particular to shoots (Wang et al., 2008). *Polypogon monspeliensis* is considered an appropriate species for phytoremediation of soil and water contaminated by arsenic (As), by this technique (Ruppert et al., 2013). Recent studies include phytoextraction of metals from contaminated soil by many different species, such as Indian mustard, rapeseed, Alpine Penny-cress (Simmons et al., 2015), sunflowers (Shaheen and Rinklebe, 2015) and willow and poplar trees (Kacálková et al., 2015).

According to Ali et al. (2013), two different approaches have been tested for metal phytoextraction: The use of hyperaccumulator plant species (plants that concentrate metals in a minimum percentage which varies according to the metal, for example, more than 1000 mg kg^{-1} dry weight for Ni, Cu, Co, Cr or Pb, or more than 10,000 mg kg^{-1} for Zn of Mn (Baker and Brooks, 1989)), which produce less aboveground biomass but accumulate target metals to a greater extent, and the application of other plant species which accumulate target metals to a lesser extent but produce more aboveground biomass, so that overall accumulation

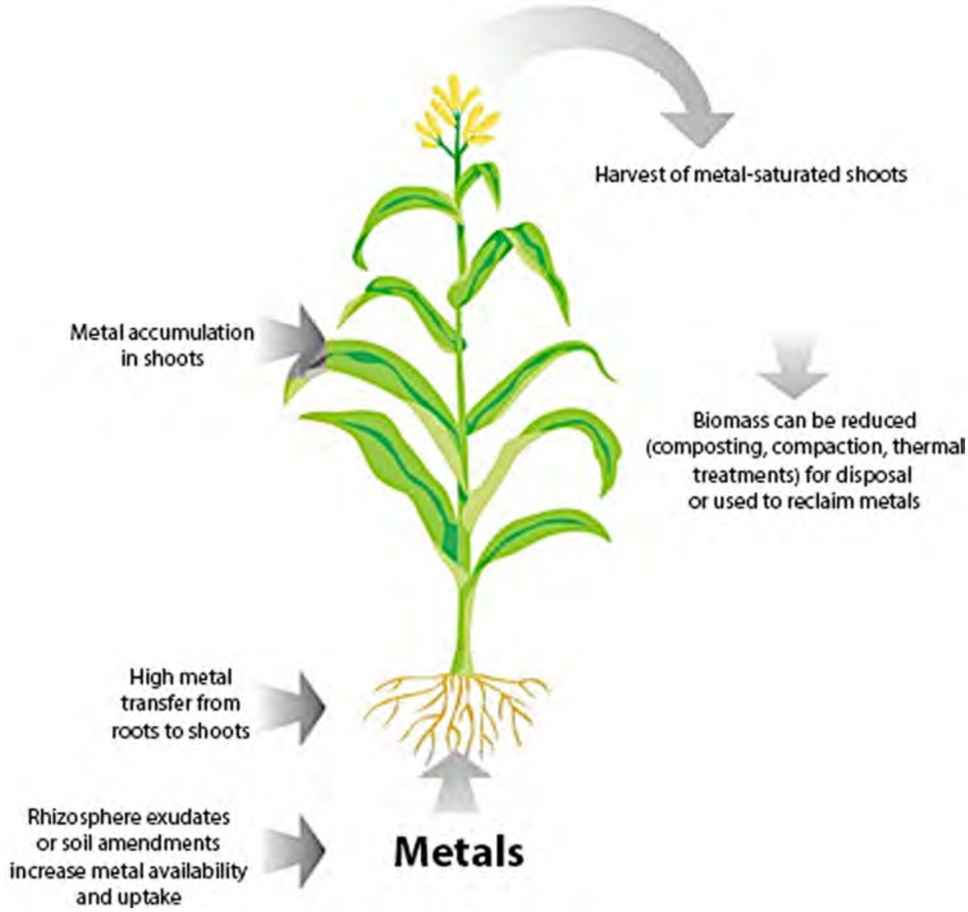

Fig. 3. Phytoextraction diagram.
Adapted from Nascimento et al. (2006)

is comparable to that of hyperaccumulators. However, it has been stated that hyperaccumulation and hypertolerance are more important in phytoremediation than high biomass, and that the use of hyperaccumulators is preferable, since they yield metal-rich, low-volume biomass, which is economical and easy to handle in case of both metal recovery and safe disposal, while, on the other hand, non-accumulators yield metal-poor, large-volume biomass, which will is uneconomical to process for metal recovery and also costly to safely dispose of (Ali et al., 2013; Chaney et al., 1997).

According to Sarma (2011), plants suitable for phytoextraction should ideally present, among other characteristics, high growth rate, production of more above-ground biomass, have a widely distributed and highly branched root system, sometimes with symbiotic mycorrhizal fungi, more accumulation of the target heavy metals from soil, translocation of the accumulated heavy metals from roots to shoots, tolerance to the toxic effects of the target metals, must be of easy cultivation and harvest, and show repulsion to herbivores to avoid food chain contamination. Also, plant species that offer multiple harvests in a single growth period show great potential in phytoextraction (Ali et al., 2013). However, the use of crops for phytoextraction of metals suffers from the disadvantage of contamination of food chain, and the use of field crops for phytoremediation purposes should not consider the use of products for animal feed or direct human consumption (Vamerali et al., 2010).

2.4. Phytovolatilization

Phytovolatilization is a mechanism by which plants convert contaminants into volatile form, with subsequent release into the atmosphere through the stomata, where gas exchange occurs, thereby removing the contaminant from the soil (Terry and Banuelos, 1999; Nwoko, 2010; Sarma, 2011). However, its use is limited by the fact that it does not completely remove the pollutant from the environment, since the contaminant is simply transferred from one environmental compartment (soil) to the other (atmosphere), and is likely to precipitate with rainfall and then return to the ecosystem (Gill, 2014). This makes phytovolatilization the most controversial of phytoremediation technologies (Padmavathiamma and Li, 2007; Sarma, 2011). A diagram of the phytovolatilization process is displayed in Fig. 4.

This technique has been mainly applied for the removal of groups of metals that present high volatility characteristics, such as Hg and Se (Sharma et al., 2015; Wang et al., 2012). Volatilization of Se involves assimilation of inorganic Se into selenoaminoacids selencysteine (SeCys) and selenomethionine (SeMet). Selenomethioine is then methylated to form a volatile, less toxic compound, dimethylselenide (DMSe).

2.5. Phytotransformation

Phytotransformation, or phytodegradation, refers to the capture of contaminants and nutrients from the water, sediment or soil and their chemical modification as a direct result of plant metabolism, often resulting in contaminant inactivation, degradation or immobilization (Pivetz, 2001; Tangahu et al., 2011), and occurs both in the roots (rhizodegradation) and/or shoots (Bulak et al., 2014). Plant-produced enzymes are used to metabolize toxic elements and transform them into less toxic

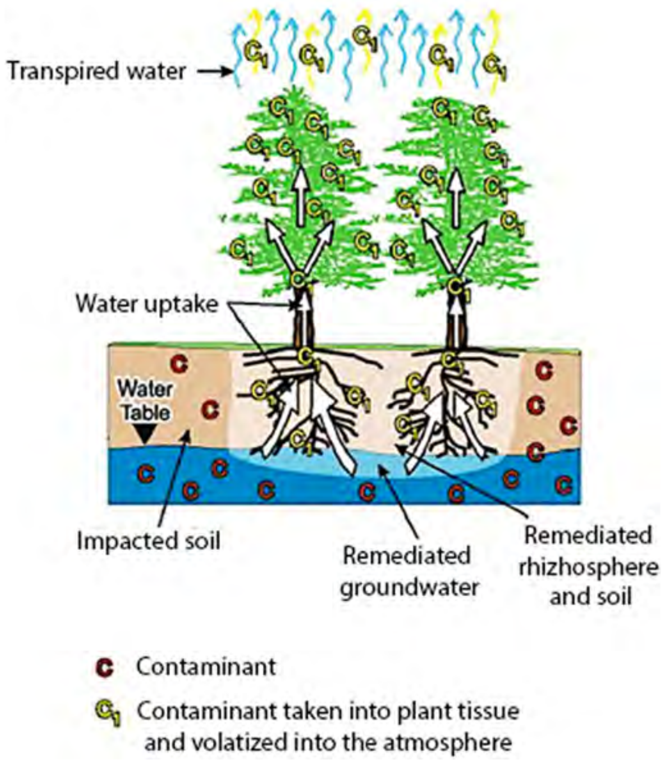

Fig. 4. Diagram of the phytovolatilization process of metals (adapted from Cheung, 2013).

compounds, and microorganisms, such as bacteria, yeasts and fungi also assist in this process (Dobos and Puia, 2009). This process usually occurs for organic compounds, although some cases regarding metals have been observed (Bock et al., 2002). In this case, some plants show the ability to convert metal species into their more stable forms, as in the case of Cr, which is converted from Cr^{6+} to Cr^{3+}, in order to reduce phytotoxicity effects, since Cr^{3+} is less mobile and less toxic than Cr^{6+} (Sen et al., 1987; Shanker et al., 2005).

This technology usually requires more than one growing season to be efficient, and certain soil characteristics must be present, such as less than 3 feet in depth and groundwater within 10 feet of the surface. Soil amendments are also sometimes required, such as the presence of chelating agents to facilitate plant uptake by breaking bonds binding contaminants to soil particles (Miller, 1996).

3. The biology of phytoremediation

According to Nwoko (Nwoko, 2010), the biology of phytoremediation involves several different biological processes, including, but not restricted to, plant-microorganism interactions, among other rhizosphere processes, plant uptake, translocation mechanisms, tolerance mechanisms, such as compartmentation and degradation, and the production or activation of plant chelators involved in storage and transport. However, in order to increase the effectiveness of different forms of phytoremediation, it is important to understand the biological processes involved (Pilon-Smits and Freeman, 2006).

Regarding plant-microorganism interactions, it is widely recognized that plants, through the release of organic materials, nutrients and oxygen, produce a rich microenvironment that can promote the proliferation and microbial activity (Masciandaro et al., 2013). Furthermore, root growth into the ground is a route for air and water access that can alter the carbon dioxide and oxygen concentrations, pH, redox potential, osmotic potential and moisture content in the soil, which provide an environment capable of supporting a high microbial biomass (Lin and Xing, 2008). The interactions between plants and beneficial microorganisms of the rhizosphere can increase plant biomass and tolerance to metals, indicating that microorganisms, such as bacteria, protozoa, fungi and algae, are important components for phytoremediation (Gao et al., 2012; Masciandaro et al., 2013). Thus, many plants and bacteria have their own mechanisms for metal treatment, and the interaction between plants and microorganisms can both increase or decrease metal accumulation in plants, depending on the nature of the plant-microorganism interaction (Sharma et al., 2013). For example, in a study with the aim of evaluating the synergism between metal-resistant microorganisms and chelating agents added to soil contaminated with Cd and Pb, beneficial effects that significantly improved the efficiency of *Solanum nigrum* L. phytoremediation were observed (Gao et al., 2012), while mixtures of arbuscular mycorrhizal fungi have been reported to lead to greater absorption and subsequent accumulation of metals in plant tissues (Leung et al., 2013). In a study on three types of single inoculum mycorrhizae and two types of mixed inoculation in *Pteris vittata* (a hyperaccumulator plant) and *Cynodon dactylon* (a non-hyperaccumulator plant) exposed to 0, 100 and 200 mg kg^{-1} of arsenic, both species showed a significant biomass increase and increased activity of the enzyme arsenate reductase in all arsenic concentration levels (Leung et al., 2013). This also has been shown in the form of positive correlations between the presence of fungi and metal accumulation in leaf sheaths in a survey on the concentration of metals present in the decomposition of *Phragmites australis* in an estuary in the Netherlands (Du Laing et al., 2006).

In addition, some metal-resistant bacteria may be sources of genes used for the improvement of phytoremediation (El-Deeb et al., 2012). For example, in a study on the biotransformation potential of chromium (Cr), the ability of *Pistia stratiots* and *Eichhornia crassipes* in removing Cr^{6+} from a spiked solution is accelerated in the presence of bacterial strains (*Bacillus pumilus, Pseudomonas doudoroffii* and *Exiguobacterium*), due to the fact that plants release exudates that improve the performance of the bacteria strains (Ejaz et al., 2013). Researchers have also used *Bacillus* and *Pseudomonas* for removal of metals in the presence of different hydrophytes, but this area is increasingly under scrutiny, and, for example, chromate removal by *P. stratiotes* and *E. crassipes* in the presence of *Exiguobacterium* has only recently been described for the treatment of wastewater (Ejaz et al., 2013). These findings enable the construction of bioreactors for wastewater treatment of industrial effluents contaminated with Cr (Ejaz et al., 2013).

Additionally, the root system influences the structure and activity of the microbial community, which in turn can affect metal speciation and bioavailability. This has been demonstrated especially with regard to mercury (Hg) (Chattopadhyay et al., 2012; Cosio et al., 2014).

Metal ions can be actively absorbed by root cells via plasmalemma, adsorbed on the walls of the cells by passive diffusion, or ascend through the roots (acropetal transport) of aquatic plants (Choo et al., 2006; Nwoko, 2010). These contaminants can also be absorbed through biological processes involving membrane transport proteins (Pilon-Smits and Freeman, 2006).

The metal translocation process involves proton pumps (ATPases that consume energy and generate electrochemical gradients); cotransporters (proteins using an electrochemical gradient), and proteins that facilitate ion transport within the cell (channels) and each transport mechanism is likely to carry a range of ions (i.e. arsenate is taken up by phosphate transporters, and selenate by sulfate transporters) (Nwoko, 2010; Tangahu et al., 2011). Inside the cells, metals can be sequestered into the vacuole or the cell wall by means of carrier proteins, or may be mineralized or volatilized (Dhankher et al., 2012). Metal ions that are not transported by specialized proteins may complex to low molecular weight ligands, such as mutagenic acid, nicotinamine, organic acids and histidine that ligands may have intracellular roles, including as chelators capturing metal ions in the cytoplasm or in subcellular compartments (Haydon and Cobbett, 2007).

Colageno and Guerinot (2006) emphasize that the uptake and efflux of metals in plant cells is coordinated with the objective of maintaining homeostasis, and that plant genomes encode a large number of metal carriers that vary in their specificity, pattern expression and cellular localization in order to systematize metal translocation. In this context, chelation, one of the most important metal-tolerance mechanisms consists in intracellular complexation of the metal ion to a natural or synthetic chelating agent, either synthesized by the plant or artificially augmented in the environment (Guimarães et al., 2008). Plant roots, aided by these chelating agents are also able to solubilize and use very low micronutrient levels, even from almost insoluble precipitates (Tangahu et al., 2011).

The use of chelating agents has been used to increase metal uptake and translocation, thus opening up a wide range of possibilities for metal phytoextraction (Alkorta et al., 2004). A chelating agent can release a metal from the exchange sites of cations in the soil, forming a metal complex, thus allowing the chelated metals to migrate more easily into the soil. Once the metal has been removed from the cation exchange sites, it can be absorbed by plant roots (Dipu et al., 2012).

Many different chelating agents such as EDTA (ethylenediaminetetraacetic acid), citric acid, elemental sulfur, and ammonium sulfate have applied in this context, in order to increase the bioavailability of metals in soil (Ali et al., 2013). However, to obtain positive effects on phytoremediation, a previous evaluation in order to select the appropriate chelating agent is necessary, since a certain agent may provide beneficial effects in the presence of a single metal, but may result in adverse effects in the presence of several metals, or may show differing efficiencies (Gao et al., 2012). For example, in a study on different chelator effects, EDTA and ethylene diamine disuccinic acid (EDDS) were compared with regard to lead phytoextraction through *Cynara cardunculus*. The results indicated that EDTA was more efficient in aiding the uptake of lead by plant roots compared to EDDS (Epelde et al., 2008). The plants treated with EDDS had lower biomass values than those treated with EDTA. However, although EDDS showed a lesser capacity to increase lead phytoextraction, it showed the advantage of increased biodegradability.

In addition, these chemical treatments may also cause secondary pollution problems. For example, synthetic EDTA is non-biodegradable and can leach into ground-water supplies, in itself becoming an environmental hazard (Maine et al., 2001) Furthermore, synthetic chelating agents can also be toxic to plants at high concentrations (Maine et al., 2001). In this context, citric acid shows promise, due to being a natural compound, easily biodegraded in soil and atoxic to plants (Smolinska and Krol, 2012). Uranium concentrations in plant biomass, for example, has been shown to increase significantly after the application of citric acid (Schmidt, 2003).

On the other hand, the right choice of chelating agent can modify metal accumulation patterns, which can be advantageous in phytoremediation efforts. Several studies have been successful in this regard, and are displayed in Table 1.

4. Variables that affect metal phytoremediation processes

Several factors influence phytoremediation processes and efficiency, including plant species, microorganism-plant interactions, as described previously, translocation, tolerance mechanisms and metal and soil characteristics (Pilon-Smits and Freeman, 2006).

Regarding phytoextraction, efficiency is directly influenced by metal bioavailability. Usually, only a small fraction of the metals present in soil is bioavailable for uptake by plants, since metal many times bind strongly to soil particles or precipitation causes many metals to become insoluble (Sheoran et al., 2011, Lasat and Kochian, 2000). To circumvent this, plants have developed biological means for solubilizing heavy metals in soil, such as the secretion of metal-mobilizing compounds in the rhizosphere, called phytosiderophores (Lone et al., 2008). In addition, secretion of H^+ ions by roots can acidify the rhizosphere and increase metal dissolution, since the H^+ ions can displace metal cations adsorbed to soil particles (Alford et al., 2010). Rhizospheric microorganisms may also significantly increase the bioavailability of metals in soil (Vamerali et al., 2010), and interactions of microbial siderophores can increase labile metal pools and uptake by roots (Mench et al., 2009).

In turn, metal mobility and bioavailability are directly influenced by the chemical composition and sorption properties of soil and water (Kłos et al., 2012). Bioavailability can be increased by lowering soil pH, since metal salts are soluble in acidic media rather than in basic media (Ali et al., 2013). The valence of the metals in the water or soil is influenced by several factors, such as pH, oxygen content, water availability temperature and organic matter. For example, in a recent study, *R. communis* was found to be more tolerant to salinity and drought in the presence of Cd and removed more Cd in a given time than Indian mustard (Bauddh and Singh, 2012). This species also produced significantly more biomass than that *B. juncea* when grown in Cd-contaminated soil in the presence of 100 mM NaCl salinity and after a ten-day water withdrawal, indicating the importance of these variables I phytoremdiation processes.

However, some metals are not readily available to the plants because of their insolubility and/or interactions with solid particles (Babula et al., 2008). For example, according to Matagi et al. (1998), the oxygen released through the roots and rhizome, during oxygen translocation by the aerenchyma are responsible for conditions that promote oxidation and precipitation of Fe^{3+} and Mn^{2+}. In research conducted on the oxygen influence on *Elodea canadensis* performance in sediment-water-plant systems, plants were exposed to different levels of oxygen concentrations and high concentrations of dissolved oxygen resulted in less As accumulation in the plants, while average oxygen concentration caused lower release of As from sediment to water and an increase in the accumulation of this metal, demonstrating the importance of knowledge on the chemical characteristics of the environment where the phytoremediation processes (Bergqvist and Greger, 2013).

Particularly in sediment, electrical conductivity and pH can cause changes in the speciation and solubility of metals, which may result in a flow of metals from interstitial water into the water column and/or increased plant uptake (Weis and Weis, 2004). For example, lower soil pH increases metal concentration in solution by promoting their desorption from soil particles (Thangavel and Subbhuraam, 2004). These factors have been reported to directly affect phytoremediation attempts, as occurred in a study with *Lemna gibba,* that observed that this macrophyte has great potential to remove Zn from contaminated waters, particularly at 21 °C and pH between 5 and 6. However, development of this species at temperatures of 17, 25 and 29 °C and pH ranging from 3 to 4 promoted negative effects on the plant and did not favor Zn uptake (Khellaf and Zerdaoui, 2013). In another study, with *Vallisneria natans* (Lour.), a submerged aquatic plant and promising with regard to the removal of arsenic (As), at pH around 7.0, the accumulation of As increased significantly compared to plants exposed to arsenic under pH 5.0 (Chen et al., 2014).

Studies have also reported that higher temperatures and decreased soil pH result in a significant increase of cadmium and

Table 1
Use of different chelating agents in phytoremediation studies.

Species	Experiment	Chelating agent	Effect	Reference
Brassica napus L	Pot experiment, growth in Hoagland's growth solution, exposure to Pb (50 and 100 μmol L^{-1}) for 6 weeks	Citric acid (2.5 mmol L^{-1})	Increased Pb uptake and accumulation in root, stem and leaves at both Pb levels; facilitated Pb translocation from roots to aerial parts	Shakoor et al. (2014)
Brassica napus L	Pot experiment, growth in Hoagland's growth solution, exposure at Cd (10 and 50 μmol L^{-1}) for 8 weeks	Citric acid (2.5 mmol L^{-1})	Increased Cd uptake and accumulation and alleviation of Cd toxicity by reducing oxidative stress (H_2O_2 and MDA accumulations); increased antioxidant capacity, plant biomass and photosynthetic pigments compared to Cd treatment alone, maintenance of gas exchange	Ehsan et al. (2014)
Typha spp.; Pistia sp.; Azolla spp.; Lemna spp.; Salvinia sp.; *Eichhornia sp*	Pot experiment, growth in soil, exposure to As, Cd, Pb and Cd (1 mg L^{-1})	EDTA (1 μg L^{-1})	Increased plant uptake of all metals, especially Pb and Cu However, the pattern of uptake of heavy metals in plants was similar with and without EDTA.	Dipu et al. (2012)
Nasturtium officinale	Pot experiment, growth in soil, exposure to Cr^{3+} (0, 1, 3, and 10 mgL^{-1}) for 15 days	EDTA (10^{-5} and 10^{-4} mol L^{-1})	Increased Cr uptake and accumulation; inhibitory effects on the root and shoot dry biomass. Negative relationship between root Cr^{3+} absorption capability and transportation ability of Cr^{3+} towards the shoots, indicating EDTA stimulates Cr^{3+} root-absorption, but inhibits transport from root to shoot.	Aydin and Coskun (2013)
Salix viminalis L.; Brassica juncea (L.); Zea mays L.; Helianthus annuus L.	Pot experiment, growth in soil, exposure to U (310 mg kg^{-1})	Citric acid (10.5 mg kg^{-1})	Increased U uptake and accumulation 14-fold, from 15 to 200 mg kg^{-1}, optimum U solubilization at pH 4-5, with soluble U concentrations considerably higher than those at pH 6 or 6.8.	(Schmidt, 2003)
Calendula officinalis; Althaea rosea	Pot experiment, growth in soil pH 5.5, exposure to Cd (0, 10, 30, 50, and 100 mg kg^{-1}) for 120 days	EGTA (0.5, 1.0 and 2.0 mg kg^{-1}) and SDS (1.0 mg kg^{-1})	Increased dry biomass and Cd uptake and accumulation in shoots and roots, For *C. officinalis*, maximum total Cd content increased by 72%, For *A. rosea*, maximum total Cd content was 2.5 times higher than controls. For both species Cd removal from soil to shoot was 1.77 and 2.36 times higher than the controls, respectively	Liu et al. (2008)
Helianthus annuus	Pot experiment, growth in soil pH 5.5, exposure to Cd (30 mg kg^{-1}) for 4 weeks	EDTA (0.1 or 0.3 g kg^{-1})	Increased Cd, Cr, and Ni uptake and accumulation, total metal uptake of ~0.73 mg at 0.1 g kg^{-1} compared to ~0.40 mg with 0.3 g kg^{-1} EDTA	Turgut et al. (2004)
Helianthus annuus	Pot experiment, growth in soil pH 5.5, exposure to Cd (50 and 30 mg kg^{-1}) for 4 weeks	EDTA and HEDTA (0.5 g kg^{-1})	Significant increase in Cd and Ni shoot concentrations from 34 and 15-115 and 117 mg kg^{-1}, respectively. Total removal efficiency for EDTA was 59 μg/plant. HEDTA at the same application rate resulted in total metal uptake of 42 μg/plant	Chen and Cutright (2001)
Oilcake	Pot experiment, growth in soil, exposure to Cd (2.5 g kg^{-1}) for 60 days	EDDS (5 mmol kg^{-1})	Increased Cd and Pb accumulation in roots, shoots and flower, Cd accumulation in root, shoot and flower up to 5.46, 4.74 and 1.37 mg kg^{-1} and lead accumulation up to 16.11, 13.44 and 3.17 mg kg^{-1}	Mani et al. (2015)
Wheat	Pot experiment, growth in soil, exposure to Pb (0, 50, and 100 mg kg^{-1}) for 16 weeks	S (0, 150, and 300 mmol kg^{-1})	Improved photosynthetic and transpiration rates, consequent increase in straw and grain yields; enhanced Pb accumulation in roots, translocation from roots to shoot, and accumulation in grain. S and Zn contents of different plant parts were enhanced	Khan et al. (2016)
Brassica juncea; Brassica chinensis	Pot experiment, growth in soil, exposure to U (280 mg kg 1)	Citric acid (0.95 g kg^{-1})	Increased U uptake and accumulation; enhanced soluble U concentration in the soil 35-fold, increase U accumulation in shoots of selected plant species grown in two U-contaminated soils (total soil U, 280 and 750 mg kg − 1) by more than 1000-fold within a few days	Huang et al. (1998)

zinc, while a study on phytoremediation and factors that influence metal absorption in fourteen plant species reported that the addition of lime and lignite (sedimentary rock) added to polluted soil reduced the uptake of cadmium and zinc by plants due to increases in soil pH, with no differences in copper or lead uptakes (Mathé-Gaspar and Anton, 2005). In a study conducted with *Vallisneria natans* (Lour.), a submerged aquatic plant with promise in As removal, a pH near 7.0 caused, increased arsenic accumulation compared to plants exposed to arsenic under pH 5.0, probably due to the fact that arsenic and phosphate transporters have greater affinity for AsO_4^{3-}, which is more electronegative, than for $HAsO_4^{2-}$ and $H_2AsO_4^{-}$ (Chen et al., 2014). Thus, pH soil/water properties should also be taken in account when phytoremediation processes are planned.

The presence of organic matter has also been shown to alter phytoextraction efficiency for certain elements. For example, in a study conducted with *Ricinus communis* L. (castor), the effect of the addition of organic matter (peat) in soil contaminated with metals and boron was evaluated, and it was observed that plants grown with no organic matter showed no accumulation of Cr, Ni, Cd, Cu, Pb and Zn, while B concentrations increased (Abreu et al., 2012).

5. Advantages and limitations of phytoremediation

The idea of phytoremediation is, first and foremost, aesthetically pleasant and has good public acceptance (Ali et al., 2013), and is also popular with the general public as a "green clean" alternative to chemical plants and bulldozers (Pilon-Smits and Freeman, 2006). Also, this technique has low installation and maintenance costs compared to other remediation options, one of its main advantages (Pilon-Smits and Freeman, 2006; Van Aken, 2009).

Phytoremediation is also effective for treating large areas, where other remediation methods may not be cost effective or practicable (Garbisu and Alkorta, 2003), thus being considered an important tool in ecological engineering. Additionally, the use of plants in synergistic phytoremediation processes results not only in cleaning the environment, but also in restoring ecosystems (Pilon-Smits and Freeman, 2006). This technique also causes fewer disturbances to ecosystems than microbial remediation, where microorganisms are added to the soil or plant to either degrade organic contaminants or to bind heavy metals in more inert and less bioavailable form, or physicochemical interventions (Doran, 2009).

The establishment of vegetation on polluted soils also helps prevent erosion and metal leaching and increases the moisture content of the soil surface (Cameselle et al., 2013; Chaudhry et al., 1998). Furthermore, phytoremediation also provides favorable conditions for microbial colonization of the rhizosphere that assists in symbiotic degradation and detoxification of pollutants (Doran, 2009).

From an economic standpoint, phytoremediation advantages include risk containment, though phytostabilization, important in the case of accidents and spills of toxic metals, phytoextraction of metals with market value such as Ni, Tl and Au, that can be removed and used, sold or recycled after phytoextraction, and durable land management, where this technique may gradually improve soil quality for subsequent cultivation of crops with higher market value (Ali et al., 2013; Chaney et al., 2000; Vangronsveld et al., 2009). Also, depending on the quality of the biomass of the plants after phytoremediation and chemical treatment for decontamination, they can also be used for energy production (biogas or direct combustion), production of ethanol and bricks, and papermaking (Bell et al., 2014; Chaney et al., 2000; Mishima et al., 2008). For example, the use of *Eichhonia crassipes*, an aquatic macrophyte, has been applied in the manufacture of construction bricks, eliminating the problems caused by the presence of toxic elements (Teixeira et al., 2011). The use of dry aquatic plants for metal removal as simple biosorbent material has also shown advantages due to high efficiency in waste treatment, low cost, storage, transportation and handling (Miretzky et al., 2006). In this context, the dried biomass of *Spirodela intermedia*, *Lemna minor* and *Pistia stratiots* have been shown to be effective in removing several metals (Pb^{2+}, Ni^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}), especially Pb and Cd (Miretzky et al., 2006). Macrophytes, in addition to assimilating metals in their tissues, also act as catalysts for purification reactions by increasing the environmental diversity in the rhizosphere, thereby promoting various chemical and biochemical reactions that improve water quality (Jenssen et al., 1993).

However, the use of plants for cleaning the environment often takes longer than other remediation techniques and is best suited for places where the elements are present within the range of plant roots (Doran, 2009; Pilon-Smits and Freeman, 2006). Also, as stated previously, environmental conditions are a great determinant of the efficiency of phytoremediation, and may not always be adequate for most species. Additionally, soil contamination by multiple metals require the use of specific species, well-adapted or tolerant to the environmental conditions and contamination present, and allow a positive synergistic interaction between the plant roots in reaching and tolerating the negative effects caused by metals (Danh et al., 2009; Pilon-Smits and Freeman, 2006). Thus, the application of phytoremediation in these cases also requires a wide range of research prior to the application of the technology (Danh et al., 2009). Metal bioavailability is also an issue; for example, if the metal is tightly bound to the organic portions of the soil, it may not be bioavailable, while, if the metal is water-soluble, it will pass by the root without being accumulated in the plant (Sarma, 2011).

6. Genetically modified plants and phytoremediation

In recent years, many efforts have been made to create genetically modified plants with improved phytoremediation abilities, and many different genes have been used in this regard, involved in several pollutant transport and degradation pathways, isolated from several organisms (bacteria, fungi, animals or plants) and then introduced into candidate plants, as well as techniques involving the overexpression of genes from the same plant species (Ibañez et al., 2015).

The recently coined term "genoremediation"is used in this context (Mani and Kumar, 2014). The reasoning is that metal accumulation and tolerance capacity can be enhanced by over-expressing natural or modified genes encoding several different molecular mechanisms, such as those encoding antioxidant enzymes or those involved and/or phytochelatin biosynthesis for example (Mani and Kumar, 2014). Some biotechnological approaches for genoremediation have been applied, such as in metal homeostasis genes, genes for biotic and abiotic stresses, biodegradative enzymatic genes and risk mitigating genes (Singh et al., 2011). However, in a context of genetically modified plants focusing on metal phytodemediation, the leading biotechnological approach is the enhancement of heavy metal uptake, mainly through genetic manipulation of the expression, activity and location of heavy metal ion transporters, since these proteins directly control the uptake, distribution and accumulation of several metals in plants (Ovecka and Takac, 2014). Genetic modifications in this regard have been, thus, applied for the increased expression of metal chelators, metal transporters, metallothioneins, and phytochelatins (Ibañez et al., 2015). Some of these studies are displayed below in Table 2.

Although the insertion of genes related to metal uptake, translocation and accumulation has been of vital importance,

Table 2
Applications of genetic modifications in phytoremediation.

Original species	Plant species	Experiment	Expression	Effect	Reference
Saccharomyces cerevisae	*Nicotiana tabacum*	Plants grown in soils with low and high Cd and Zn concentrations in a growth chamber for 6 weeks	Metallothionein	Significantly higher Cd accumulation compared to non-. Shoot Cd at higher Cd dosages reached 3.5–4.5-fold higher than that of Cd hyperaccumulation threshold values	Daghan et al. (2013)
Iris lactea var. *chinensis*	*Arabidopsis thaliana*	Treatment with 50 and 100 μM Cu	Metallothionein	Increased Cu concentration and reduced H_2O_2 production in transgenics, as well as longer root length	Gu et al. (2015)
E. coli	*Populus tremula, P. alba*	Plants were cultivated for 80 days in Hoagland solution with 0 or 100 μM Cd^{2+}	g-Glutamylcysteine synthetase	Transgenics accumulated more Cd in their aerial parts, exhibited lower decreases in biomass, higher concentrations of soluble sugars and starch, lower $O_2^{\cdot-}$ and H_2O_2 and higher concentrations of total thiols, GSH and GSSG in the roots and/or leaves, elevated concentrations of soluble phenolics and free proline and greater foliar GR activity compared with wild-type plants	He et al. (2015)
Streptococcus thermophils	*Beta vulgaris* L	Plants were grown for 3 weeks on rooting medium supplemented with 0, 50, 100 or 200 μM Cd^{2+}, Zn^{2+} or Cu^{2+}. For the complex heavy metal stress assays, combinations of two or three ions at 50 μM were added to the medium.	g-Glutamylcysteine synthetase-glutathione synthetase	Transgenics accumulated more Cd, Zn and Cu ions in shoots than non-transgenics, as well as higher GSH and phytochelatin levels under different heavy metal stresses. When multiple heavy metal ions were present at the same time, transgenics resisted two or three of the metal combinations (50 μM Cd-Zn, Cd-Cu, Zn-Cu and Cd-Zn-Cu), with greater absorption in shoots	Liu et al. (2015)
Bacteria	*Nicotiana tabacum*	Tretament with different concentrations (100, 200, and 300 μM) Hg for 15 days. Volatile Hg° was quantitatively trapped in alkaline peroxide liquid traps	Mercury reductase	Transgenics continued to grow well with Hg concentrations in roots up to 2000 μg g^{-1} and accumulated both organic and inorganic Hg at levels surpassing soil concentrations. Organic Hg was absorbed and translocated more efficiently than inorganic Hg, with 100-fold increase in shoots compared to non-transgenics. Transgenics attained a maximum rate of elemental-Hg volatilization in 2 – 3 days, attaining complete volatilization within a week	Hussein et al. (2007)
Staphylococcus aureus	*Populus alba* and *P. tremula* var. *glandulosa*	Growth in 0, 50, or 100 μM HgCl2 and 2 or 5 μM Hg for 4 weeks for mercury tolerance assays, and then placed in closed bottles and cultured for 4 days for gaseous mercury determinations by a mercury vapor analyzer	Mercury reductase	Transgenics grew significantly better than non-transgenics exposed to Hg, and one of strain produced 4.5 – 4.8 times more Hg° than controls when exposed to 50 μM Hg. Mercury content was also lower in the transgenics than in the non-transgenics	Choi et al. (207)
Arabidopsis thaliana	*Brassica juncea*	8 days of Se treatment on agar medium containing 50 and 20 μM selenate	ATP sulphurylase	Dramatic reduction in growth for transgenics and controls, but less for transgenics after exposure, with longer roots and greater biomass. After 50 μM selenate exposure non-transgenics were severely affected and nearly died, whereas transgenics were much less affected, with shoot Se concentrations 2-fold higher and root Se concentrations 26% higher. Similar results were obtained with 20 μM selenate exposure, with 2-fold higher shoot Se concentrations and 1.8-fold higher root Se concentrations compared to non-transgenics.	Pilon-Smits, et al. (1999)
Arabidopsis thaliana	*Brassica juncea*	Exposure to sodium selenate (40 μM) for 2 weeks. Se volatilization was measured from the entire plant over a 24 h period by trapping in an alkaline peroxide solution	Cystathionine-g-synthase	Transgenics showed 2- to 3-fold higher Se volatilization rates, 20 – 40% lower shoot Se levels and 50 – 70% lower root Se levels than wild-types when supplied with selenite. They were also were more tolerant to selenite than the wild type.	Van Huysen et al. (2003)
Astragalus bisulcatus	*Brassica juncea*	Supplementation with selenate. Volatile Se was trapped in alkaline peroxide after 8 days of treatment	Selenocysteine methyltransferase	Production of 2.5 times more volatile Se than the wild type	LeDuc et al. (2004)
Spinacia oleracea	*Nicotiana tabacum*	Heavy metal treatments to Cd, Se, Ni, Pb and Cu for 3 weeks	Cysteine synthase	Transgenics shows tolerance to Cd, Se and Ni, but no significant improvement regarding Pb and Cu	Kawahsima et al. (2004)
E. coli	*Brassica juncea*	Seedling Cd exposure (0, 0.15, 0.20, or 0.25 mm) for 7 days and mature plant exposure to 0.1 mm Cd for 10 days	g-glutamylcysteine synthetase	Significantly higher Cd concentrations in shoots; higher Cd tolerance and growth	Zhu et al. (1999b)
E. coli	*Brassica juncea*	Seedling Cd exposure (0, 0.15, 0.20, or 0.25 mm) for 7 days and mature plant exposure to 0.1 mm Cd for 10 days	Glutathione synthetase	Transgenics accumulated significantly more Cd than wild-types: shoot Cd concentrations were up to 25% higher and total Cd accumulation per shoot was up to 3-fold higher. Moreover, transgenics showed enhanced tolerance to Cd at both the seedling and mature-plant stages.	Zhu et al. (1999a)

other types of genetic modification have also recently arisen as potential techniques for enhanced metal phytoremediation efforts, such as the modification of plant morphology in order to achieve increased metal uptake by obtaining hairy roots. For example, hairy roots were obtained from transgenic *N. tabacum*, which were shown to be very efficient in copper phytoremediation, while hairy roots from transgenic *A. thaliana* expressing the Cu-binding periplasmic protein CopC have also been obtained and were very efficient regarding Cu accumulation (Pérez-Palacios, 2015). However, although hairy roots have shown undeniable potential regarding the use of plants for in vitro phytoremediation studies, in addition to allowing for the study of gene functionality and the role of some key proteins and enzymes involved in plant detoxifying metabolic pathways, research in this regard is still beginning, and some limitations exist, such as the difficulty of hairy roots in adapting to constant environmental fluctuations, metal loads, hydraulic conditions and the presence of undesirable microorganisms (Khandare and Govindwar, 2015).

This type of technology, however, has been the subject of ethical controversy for the last quarter century (Thompson, 2012). The current debate is a serious conflict between agri-biotech investors and their affiliated scientists, who consider agricultural biotechnology as a solution to food shortage, the scarcity of environmental resources, weeds and pests infestations and methods to remediate the environment, and independent scientists, environmentalists, farmers and consumers who warn that genetically modified plant species introduces new risks to food security, the environment and human health, including as loss of biodiversity; the emergence of superweeds and superpests and increases in antibiotic resistance, among others (Maghari and Ardekani, 2011). Laws and guidelines are now in place, and countries and companies are obliged to obey them for production, handling and consumption of genetically modified materials, for whatever end, as well as risk assessments performed on the three major spheres in this regard, Agriculture (gene flow, reducing biodiversity), Food and Food safety (allergenicity, toxicity), and Environment (including non target organism) (Maghari and Ardekani, 2011). These regulatory issues have been created in order to protect the planet from any adverse effects, although the long-term effects of this technology are yet to be seen, so the implementations of this type of technology must proceed with caution more stringent practices and guidelines being developed and implemented.

7. Nanoparticles and phytoremediation

Nanoparticles (NPs) are atomic or molecular aggregates, usually between 1 and 100 nm, classified as natural (originating from volcanic or lunar dust or mineral composites), incidental (resulting from anthropogenic activities, such as exhaust originated from combustion processes or welding fumes) or engineered (Masarovičová and Kráľová, 2012). Anthropogenic and engineered nanoparticle concentrations in the environment are on the rise, and there are currently extensive discussions on the risks of these compounds to biota and human health. Many NPs are metallic in nature, such as AgNP, AuNP, as well as TiO_2, ZnO and Al_2O_3 (Lin and Xing, 2007). Recent reports indicate that NPs show toxicity to several organisms, however this knowledge is limited only to species used in regulatory testing and freshwater species, and more studies are necessary (Masarovičová and Kráľová, 2012). Some, scarce, reports on higher (vascular) plants are available. In this context, CuNP were shown to be toxic to *Phaseolus radiatus* (mung bean) and *Triticum aestivum* (wheat) (Lee et al., 2008), while AgNP at 500 and 100 mg L^{-1} resulted in 57% and 41% decreases in plant biomass and transpiration in *Cucurbita pepo* (zucchini) (Stampoulis et al., 2009), and also showed toxic effects on *Lemna gibba* exposed to AgNPs over 7 days to 0, 0.01, 0.1, 1, and 10 mg L^{-1}, with growth inhibition, decrease of frond numbers, reduction in plant cellular viability and significant increase of intracellular ROS formation by 1 and 10 mg L^{-1} of AgNP exposure (Oukarroum et al., 2013).

However, many studies have reported low or no significant effects of the presence of NP to higher plants, indicating potential for the phytoremediation of these compounds in the environment, in what could be termed “nanoremediation”. For example, Zhang et al. (2005) demonstrated that TiO_2 even increased plant dry weight, chlorophyll formation, photosynthetic rate and the activity of some enzymes, with no significant toxic effects, while Gao et al. (2006) observed that TiO_2 increased Rubisco carboxylase activity and Doshi et al. (2008) analyzed the uptake and transport of Al NPs in *Phaseolus vulgaris* and *Lolium perenne*, and showed no negative growth effects.

Some authors have recently investigated the potential for metallic NP phytoremediation. Table 3 displays some studies in this regard, showing the main conclusion the authors obtained from the experiments.

In particular, NPs have also been show to enter the leaf surface, originating from atmospheric particulate matter deposition, indicating another phytoremediation option, for removing these compounds from the atmosphere (Da Silva et al., 2006) This type of phytoremediation was also shown to be morphology-dependent, as different types of leaves can show greater or lesser metallic NP accumulation, depending on characteristics such as peltate trichomes and hypodermis (Da Silva et al., 2006).

However, metallic NP phytoremediation, or nanoremediation, is still in its infancy, with scarce reports in the literature, all conducted in the last ten years or so. The results however, are very promising, and nanoremediation is sure to be increasingly applied to metallic NP environmental contamination, although further studies are needed on which type of plant species is more adequate for each metallic NP, since morphology-, species- and NP-dependent results have been reported, and the fact that the metal may be less bioavailable in nanoform should also be taken into account.

8. Disposal of toxic plant waste after phytoremediation

Metal-accumulating plants are classified as hazardous waste and need to be harvested and either recycled or disposed of in compliance with applicable regulations in order to prevent potential risk (USEPA, 2000). Depending on regulations and metal concentrations in the plants, the contaminated biomass may need to be landfilled, or the metals reclaimed through smelting, pyrolysis of biomass, or extraction (USEPA, 2000). If plants are first incinerated, the resulting ash must also be disposed of in a hazardous waste landfill, although the ash volume is approximately less than 10% of the volume that would be created if the contaminated soil itself were dug up for treatment, still being advantegous in ths regard (UNEP, 2016). If plants are contaminated by radioactive compounds, such as in the case where one study applied sunflower plants to extract cesium (Cs) and strontium (Sr) from surface water, they must be disposed of as radioactive waste (Adler, 1996). Care regarding plant disposal is very important in this regard, since some species, for example, *Brassica juneca*, need to be harvested right after the plants mature, since they become dry, crumbly and flaky, and may become a source of secondary emissions of the metals they have ad/absorved, while others, such as cabbage, even after withering, still retain their structure and the dead leaves do not crumble, so the accumulated metals are in no danger of returning the environment (Szczygłowska et al., 2011).

Table 3
Investigation regarding the potential for metallic NP phytoremediation.

Metallic NP	Plant species exposed to NPs	NP particle size	Media	Uptake form	Main conclusion of the study	Reference
Pd	*Hordeum vulgare*	Colloidal NPs, 1 – 12 nm; insoluble Pd NPs, 1 µm	Hoagland medium	Dissolved Pd, not NPs.	Particle-size dependent uptake via roots, smaller particles were more taken-up	Battke et al. (2008)
Fe_3O_4	*Cucurbita maxima; Phaseolus limensis*	20 nm – 2 µm	Growth media	Fe_3O_4NPs	Uptake is species-dependent: *C. maxima* showed significant uptake and accumulation, while *P. limensis* did not absorb or translocate the investigated NPs	Zhou et al. (2008)
Cu	*Halimione portulacoides* and *Phragmites australis*	< 50 nm	Elutriate solution + estuarine water	Both dissolved Cu and CuNPs	Both plants accumulated Cu in roots, but accumulation was lower when the metal was added in NP form; interactions between plants and NPs differ with plant species.	Andreotti et al. (2015)
Ag	*Brassica juncea; Medicago sativa*	Dissolved silver nitrate, not NP	Demineralized water	Both dissolved Ag and AgNPs	Formation of AgNPs after uptake by the plant. AgNP uptake is directly correlated to concentration and exposure time, distributed in the cellular structure	Harris and Bali (2008)
Au	*Arabidopsis thaliana*	Dissolved Au, not NP	Water + sucrose	Both dissolved Au and AuNPs	Formation of AuNPs after uptake in the plant, NPs present only in roots, other parts showed only Au	Taylor et al. (2014)

Thus, for proper disposal of the toxic biomass, knowledge about the life cycle of the species applied in phytoremediation processes is also paramount.

9. Conclusions

Phytoremediation strategies applied to metal contamination are fairly recent, with great potential in removing harmful or excess metals from the environment, since they are considered a "green" approach. However, these techniques are very dependent on many different factors, such as soil pH, temperature, depth of the contamination and metal species, among others. Thus, there is need for a full understanding of plant physiology, biochemistry and uptake of these contaminants, as well as proper evaluation of possible synergistic effects and specific metal species contamination and further research regarding synthetic approaches to metal phytoremediation, such as the addition of chelators or organic matter to the soil or rhizosphere or the use of transgenic species, which has increased in the last few years. In particular, given that the nanotechnology industry is growing at extremely fast rates, nanoparticle contamination is of increasing concern, and NP phytoremediation, or nanoremediation, is being rapidly demonstrated as feasible. Thus, the suite of phytoremediation strategies used for conventional metal contamination can now also include NP phytoremediation. In addition, proper care is paramount at the end of phytoremediation processes, to adequately dispose of the toxic waste, in order to avoid reintroduction of the remediated metals into the environment.

Conflict of interest statement

The authors declare no conflict of interest.

References

Abreu, C.A., et al., 2012. Organic matter and barium absorption by plant species grown in an area polluted with scrap metal residue. Appl. Environ. Soil Sci. 2012, 1–7.

Adler, T., 1996. Botanical cleanup crews. Sci. News 150, 42–43.

Ahmadpour, P., et al., 2012. Phyto-remediation of heavy metals: a green technology. Afr. J. Biotechnol. 11, 14036–14043.

Alford, E.R., et al., 2010. Metallophytes – a view from the rhizosphere. Plant Soil 337, 33–50.

Ali, H., et al., 2013. Phytoremediation of heavy metals—concepts and applications. Chemosphere 91, 869–881.

Alia, H., et al., 2013. Phytoremediation of heavy metals—concepts and applications. Chemosphere 91, 869–881.

Alkorta, I., et al., 2004. Chelate-enhanced phytoremediation of soils polluted with heavy metals. Rev. Environ. Sci. Biotechnol. 3, 55–70.

Andreazza, R., et al., 2015. Evaluation of two Brazilian indigenous plants for phytostabilization and phytoremediation of copper-contaminated soils. Braz. J. Biol. 4, 868–877.

Andreotti, F., et al., 2015. Interactions between salt marsh plants and Cu nanoparticles – effects on metal uptake and phytoremediation processes. Ecotoxicol. Environ. Saf. 120, 303–309.

Aydin, D., Coskun, O.F., 2013. Effects of EDTA on Cr uptake, accumulation, and biomass in *Nasturtium officinale* (Watercress). Ekoloji 22, 16–23.

Babula, P., et al., 2008. Uncommon heavy metals, metalloids and their plant toxicity: a review. Environ. Chem. Lett. 6, 189–213.

Baker, A.J.M., Brooks, R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements – A review of their distribution. Ecol. Phytochem. Biorecovery 1, 81–126.

Baker, T.J., et al., 2014. Impacts of metal and metaloxidenanoparticles on marine organisms. Environ. Pollut. 186, 257–271.

Barceló, J., Poschenrieder, C., 2003. Phytoremediation: principles and perspectives. Contrib. Sci. 2, 333–344.

Battke, F., Ernst, D., Fleischmann, F., Halbach, S., 2008. Phytoreduction and volatilization of mercury by ascorbate in Arabidopsis thaliana, European beech and Norway spruce. Applied Geochemistry 23 (3), 494–502.

Bauddh, K., Singh, R.P., 2012. Growth, tolerance efficiency and phytoremediation potential of *Ricinus communis* (L.) and *Brassica juncea* (L.) in salinity and

drought affected cadmium contaminated soil. Ecotoxicol. Environ. Saf. 85, 13–22.
Bell, T.H., et al., 2014. Increasing phytoremediation efficiency and reliability using novel omics approaches. Trends Biotechnol. 32, 271–280.
Bergqvist, C., Greger, M., 2013. Oxygen influences on arsenic behaviour in sediment-water-plant system of *Elodea canadensis*. Digitala Vetenskapliga Arkivet.
Bock, C., et al., 2002. Advances in phytoremediation: phytotransformation. innovative approaches to the on-site. Assess. Remediat. Contam. Sites, 115–140.
Bonanno, G., 2013. Comparative performance of trace element bioaccumulation and biomonitoring in the plant species Typha domingensis, Phragmites australis and Arundo donax. Ecotoxicology and environmental safety *97*, 124–130.
Bulak, P., et al., 2014. Plant growth regulators-assisted phytoextraction. Biol. Plant. 58, 1–8.
Cameselle, C., et al., 2013. Electrokinetic-enhanced phytoremediation of soils: status and opportunities. Chemosphere 93, 626–636.
Chaney, R. L., et al., 2000. Progress in risk assessment for soil metals, and in-situ remediation and phytoextraction of metals from hazardous contaminates soils. US-EPA conference Phytoremediation, State of the Science, Boston.
Chaney, R.L., et al., 1997. Phytoremediation of soil metals. Curr. Opin. Biotechnol. 8, 279–284.
Chanu, L.B., Gupta, A., 2016. Phytoremediation of lead using Ipomoea aquatica Forsk. in hydroponic solution. Chemosphere 156, 407–411.
Chattopadhyay, S., et al., 2012. Phytoremediation of mercury-and methyl mercury-contaminated sediments by water hyacinth (*Eichhornia crassipes*). Int. J. Phytoremediat. 14, 142–161.
Chaudhry, T.M., et al., 1998. Phytoremediation-focusing on accumulator plants that remediate metal-contaminated soils. Aust. J. Ecotoxicol. 4, 37–51.
Chen, G., et al., 2014. Arsenic species uptake and subcellular distribution in *Vallisneria natans* (Lour.) Hara as influenced by aquatic pH. Bull. Environ. Contam. Toxicol. 92, 478–482.
Chen, H., Cutright, T., 2001. EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere 45, 21–28.
K. H., Cheung, 2013. Bioremediation of Toxic Metals (Doctoral dissertation, The University of Hong Kong Pokfulam, Hong Kong).
Chhotu, D., et al., 2009. Phytoremediation of heavy metals: recent techniques. Afr. J. Biotechnol. 8, 921–928.
Choi, Y. I., et al., 207. Mercury-tolerant transgenic poplars expressing two bacterial mercury-metabolizing genes. J. Plant Biol., vol. 50**,** pp. 658.
Choo, T.P., et al., 2006. Accumulation of chromium (VI) from aqueous solutions using water lilies (*Nymphaea spontanea*). Chemosphere 62, 961–967.
Colangelo, E.P., Guerinot, M.L., 2006. Put the metal to the petal: metal uptake and transport throughout plants. Current opinion in plant biology 9 (3), 322–330.
Cosio, C., et al., 2014. Effects of macrophytes on the fate of mercury in aquatic systems. Environ. Toxicol. Chem. 33, 1225–1237.
Da Silva, L.C., et al., 2006. Response of restinga plant species to pollution from an iron pelletization factory. Water Air Soil Pollut. 175, 241–256.
Daghan, H., et al., 2013. Transformation of tobacco with ScMTII gene-enhanced cadmium and zinc accumulation. Clean. Soil Air Water 41, 503–509.
Danh, L.T., et al., 2009. Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. Int. J. Phytoremediat. 11, 664–691.
Dhankher, O.P., et al., 2012. Biotechnological approaches for phytoremediation. In: Altman, A., Hasegawa, P.M. (Eds.), Plant Biotechnology and Agriculture. Academic Press, San Diego, pp. 309–328.
Dhir, B., et al., 2009. Potential of aquatic macrophytes for removing contaminants from the environment. Crit. Rev. Plant Sci. 39, 754–781.
Dipu, S., et al., 2012. Effect of chelating agents in phytoremediation of heavy metals. Remediat. J. 22, 133–146.
Doble, M., Kumar, A., 2005. Biotreatment of Industrial Effluents. Butterworth-Heinemann, Oxford, UK.
Dobos, L., Puia, C., 2009. The most important methods for depollution of hydrocarbons polluted soils. Bull. UASMV Agric. 66, 67–72.
Doran, P.M., 2009. Application of plant tissue cultures in phytoremediation research: incentives and limitations. Biotechnol. Bioeng. 103, 60–76.
Doshi, R., et al., 2008. Nano-aluminum: transport through sand columns and enviromental effects on plant and soil communication. Environ. Res. 106, 296–303.
Du Laing, G., et al., 2006. Metal accumulation in intertidal litter through decomposing leaf blades, sheaths and stems of *Phragmites australis*. Chemosphere 63, 1815–1823.
Dushenkov, V., Kapulnik, Y., 2000. Phytofiltration of metals. In: Raskin, I., B.D, E. (Eds.), Phytoremediation of Toxic Metals—Using Plants to Clean-Up the Environment. John Wiley and Sons, New York, pp. 89–106.
Dushenkov, V., et al., 1995. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ. Sci. Technol. 29, 1239–1245.
Ebbs, S.D., et al., 2016. Accumulation of zinc, copper, or cerium in carrot (*Daucus carota*) exposed to metal oxide nanoparticles and metal ions. Environmental Science: Nano. Environmental Science: Nano.
Ehsan, S., et al., 2014. Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. Ecotoxicol. Environ. Saf. 106, 164–172.
Ejaz, S., et al., 2013. Biotransformation potential of hexavalent chromium by *Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8 in association with hydrophytes. Int. J. Environ. Sci. Technol. 10, 709–718.
El-Deeb, B., et al., 2012. Molecular characterization of *endo*phytic bacteria from metal hyperaccumulator aquatic plant (*Eichhornia crassipes*) and its role in heavy metal removal. Geomicrobiol. J. 29, 906–915.
Epelde, L., et al., 2008. Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. Sci. Total Environ. 410, 21–28.
Erakhrumen, A.A., 2007. Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Educ. Res. Rev. 2, 151–156.
Gao, F., et al., 2006. Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach. Biol. Trace Elem. Res. 111, 239–253.
Gao, Y., et al., 2012. Metal-resistant microorganisms and metal chelators synergistically enhance the phytoremediation efficiency of *Solanum nigrum* L. in Cd-and Pb-contaminated soil. Environ. Technol. 33, 1383–1389.
Garbisu, C., Alkorta, I., 2003. Basic concepts on heavy metal soil bioremediation. Eur. J. Miner. Process. Environ. Prot. 3, 58–66.
Gaur, A., Adholeya, A., 2004. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr. Sci. 86, 528–534.
Ghosh, M., Singh, S.P., 2005. A review on phytoremediation of heavy metals and utilization of it's by products. Appl. Ecol. Environ. Res. 3, 1–18.
Gill, M., 2014. Phytoremediation: green technology to clean the environment. Int. J. 2, 879–886.
Gu, C.S., et al., 2015. The heterologous expression of the Iris lactea var. chinensis type 2 metallothionein IlMT2b gene enhances copper tolerance in *Arabidopsis thaliana*. Bull. Environ. Contam. Toxicol. 94, 247–253.
Guimarães, M.D.A., et al., 2008. Toxicidade e tolerância ao cádmio em plantas. Rev. Trópica–Ciências Agrar e Biológicas 2, 58–68.
Hajara, E.W.I., et al., 2014. Assessment of heavy metals tolerance in leaves, stems and flowers of *Stevia rebaudiana* plant. Procedia Environ. Sci. 20, 386–393.
Halder, S., Ghosh, S., 2014. Wetland macrophytes in purification of water. Int. J. Environ. Sci. 5, 432–437.
Harris, A.T., Bali, R., 2008. On the formation and extent of uptake of silver nanoparticles by live plants. J. Nanopart. Res. 10, 691–695.
Hasegawa, H., Mofizur, I.M.D., 2015. Environmental Remediation Technologies for Metal-Contaminated Soils.
Haydon, M.J., Cobbett, C.S., 2007. Transporters of ligands for essential metal ions in plants. New Phytol. 174, 499–506.
He, J., et al., 2015. Overexpression of bacterial g-glutamyl cysteine *syn*thetase mediates changes in cadmium influx, allocation and detoxification in poplar. New Phytol. 205, 2240-254.
R. R., Hinchman, et al., 1995. Phytoremediation: using green plants to clean up contaminated soil, groundwater, and wastewater. Argonne National Laboratory Hinchman, Applied Natural Sciences, Inc.
Huang, J.W., et al., 1998. Phytoremediation of uranium contaminated soils: Role of organic acids in trigerring uranium hyperaccumulation in plants. Environ. Sci. Technol. 32, 2004–2008.
Hussein, S.H., et al., 2007. Phytoremediation of mercury and organomercurials in chloroplast transgenic plants: enhanced root uptake, translocation to shoots, and volatilization. Environ. Sci. Technol. 41, 8439–8446.
Ibañez, S., et al., 2015. Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants. New Biotechnol. S1871–6784, 00267–00268.
Jenssen, P., Maehlum, T., Krogstad, T., 1993. Potential use of constructes wetlands for wastewater treatment in northern environments. Water Sci. Technol. 28 (10), 149–157.
Kacálková, L., et al., 2015. Phytoextraction of risk elements by willow and poplar trees. Int. J. Phytoremediat. 17, 414–421.
Kawahsima, C.G., et al., 2004. Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. Biotechnol. Lett. 26, 153–157.
Khan, M.N., 2016. Elemental sulfur improves growth and phytoremediative ability of wheat grown in lead (Pb) contaminated calcareous soil. Int. J. Phytoremediation.
Khandare, R.V., Govindwar, S.P., 2015. Phytoremediation of textile dyes and effluents: current scenario and future prospects. Biotechnol. Adv. 33, 1697–1714.
Khellaf, N., Zerdaoui, M., 2013. Phytoaccumulation of zinc using the duckweed *Lemna gibba* L.: effect of temperature, pH and metal source. Desalination and Water Treatment. 51, 5755–5760.
Kramer, U., 2005. Phytoremediation: novel approaches to cleaning up polluted soils. Curr. Opin. Biotechnol. 16, 133–141.
Krishna, R., et al., 2012. Rhizofiltration: a green technology for remediation of heavy metals. Int. J. Innov. Bio-Sci. 2, 193–199.
Lasat, M.M., Kochian, L.V., 2000. Physiology of Zn hyperaccumulation in. In: Terry, N., Bañuelos, G. (Eds.), Thlaspi caerulescens. Phytoremediation of contaminated soil and waterLewis Publishers, Boca Raton, pp. 159–169.
LeDuc, D.L., et al., 2004. Overexpression of selenocysteine methyltransferase in Arabidopsis and Indian mustard increases selenium tolerance and accumulation. Plant Physiol. 135, 377–383.
Lee, W.-M., et al., 2008. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestrivum*): plant agar test for water-insoluble nanoparticles. Environ. Toxicol. Chem. 27, 1915–1921.
Leung, H.M., et al., 2013. Mixed arbuscular mycorrhizal (AM) fungal application to improve growth and arsenic accumulation of *Pteris vittata* (As hyperaccumulator) grown in As-contaminated soil. Chemosphere 92, 1367–1374.
Li, J.T., et al., 2010. Cadmium tolerance and accumulation in cultivars of a high-biomass tropical tree (*Averrhoa carambola*) and its potential for phytoextraction. J. Environ. Qual. 39, 1262–1268.
Liao, S.W., Chang, W.L., 2004. Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. Photogramm. Eng. Remote Sens. 54, 177–185.

Lim, K.T., et al., 2014. Physical, chemical, and biological methods for the removal of arsenic compounds. BioMed. Res. Int. 2014, 1–9.
Lin, D., Xing, B., 2007. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ. Pollut. 150, 243–250.
Lin, D., Xing, B., 2008. Root uptake and phytoxoxicity of ZnO nanoparticles. Sci. Technol. 42, 5580–5585.
Liu, J.H., Inoue, H., Moriguchi, T., 2008. Salt stress-mediated changes in free polyamine titers and expression of genes responsible for polyamine biosynthesis of apple in vitro shoots. Environmental and Experimental Botany *62* (1), 28–35.
Liu, D., et al., 2015. Enhanced heavy metal tolerance and accumulation by transgenic sugar beets expressing *Streptococcus thermophilus* STGCS-GS in the presence of Cd, Zn and Cu alone or in combination. PLoS One 10, 1–15.
Lone, M.I., et al., 2008. Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. J. Zhejiang Univ. Sci. B 9, 210–220.
Macek, T., et al., 2008. Novel roles for genetically modified plants in environmental protection. Trends Biotechnol. 26, 146–152.
Maghari, B.M., Ardekani, A.M., 2011. Genetically modified foods and social concerns. Avicenna J. Med. Biotechnol. 3, 109–117.
Maine, M.A., et al., 2001. Cadmium uptake by floating macrophytes. Water Res. 35, 2629–2634.
Mani, D., Kumar, C., 2014. Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. Int. J. Environ. Sci. Technol. 11, 843–872.
Mani, D., et al., 2015. Hyperaccumulator oilcake manure as an alternative for chelate-induced phytoremediation of heavy metals contaminated alluvial soils. Int. J. Phytoremediat. 17, 256–263.
Masarovičova, E., Kráľová, K., 2012. Plant-heavy metal interaction: phytoremediation, biofortification and nanoparticles. In: Montanaro, G. (Ed.), Advances in Selected Plant Physiology Aspects. InTech, Rijeka, Croatia, pp. 75–102.
Masciandaro, G., et al., 2013. Organic matter–microorganism–plant in soil bioremediation: a synergic approach. Rev. Environ. Sci. Bio/Technol. 12, 399–419.
Matagi, S.V., et al., 1998. A review of heavy metal removal mechanisms in wetlands. Afr. J. Trop. Hydrobiol. Fish. 8, 23–25.
Mathé-Gaspar, G., Anton, A., 2005. Phytoremediation study: Factors influencing heavy metal uptake of plants. In: Proceedings of the 8th Hungarian Congress on Plant Physiology, Vol. 49(1–2), pp. 69–70, 2005 and the 6th Hungarian Conference on Photosynthesis. 49**,** pp. 69–70.
Mench, M., et al., 2009. Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. Environ. Sci. Pollut. Res. 16, 876–900.
Mesjasz-Przybyłowicz, J., et al., 2004. Uptake of cadmium, lead, nickel and zinc from soil and water solutions by the nickel hyperaccumulator *Berkheya coddii*. Acta Biol. Crac. 46, 75–85.
Miller, R., 1996. Phytoremediation, Technology Overview Report, Ground-Water Remediation Technologies Analysis Center, Series Os. 3.
Miretzky, P., Saralegui, P.A., Fernandez Cirelli, A., 2006. Simultaneous heavy metal removal mechanism by dried macrophytes. Chemosphere 62, 247–254.
Mishima, D., et al., 2008. Ethanol production from candidate energy crops: water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes* L.). Bioresour. Technol. 99, 2495–2500.
Najeeb, U., et al., 2014. Enhancing the lead phytostabilization in wetland plant *Juncus effusus* L. through somaclonal manipulation and EDTA enrichment. Arabian J. Chem.
Nascimento, C.W.A.D., Xing, B., 2006. Phytoextraction: a review on enhanced metal availability and plant accumulation. Scientia agricola *63* (3), 299–311.
Novakova, M., et al., 2010. Cloning the bacterial bphC gene into *Nicotiana tabacum* to improve the efficiency of phytoremediation of polychlorinated biphenyls. Biotechnol. Bioeng. 1, 419–423.
Nwoko, C.O., 2010. Trends in phytoremediation of toxic elemental and organic pollutants. Afr. J. Biotechnol. 9, 6010–6016.
Olguín, E.J., Sánchez-Galván, G., 2012. Heavy metal removal in phytofiltration and phycoremediation: the need to differentiate between bioadsorption and bioaccumulation. New Biotechnol. 30, 3–8.
Ong, S.-T., et al., 2012. Papaya (*Carica papaya*) seed as a low-cost sorbent for zinc removal. Afr. J. Agric. Res. 7, 810–819.
Oukarroum, A., et al., 2013. Silver nanoparticle toxicity effect on growth and cellular viability of the aquatic plant *Lemna gibba*. Environ. Toxicol. Chem. 32, 902–907.
Ovecka, M., Takac, T., 2014. Managing heavy metal toxicity stress in plants: biological and biotechnological tools. Biotechnol. Adv. 32, 73–86.
Padmavathiamma, P.K., Li, L.Y., 2007. Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Pollut. 184, 105–126.
Pandey, V.C., et al., 2015. Sustainable phytoremediation based on naturally colonizing and economically valuable plants. J. Clean. Prod. 86, 37–39.
Pareck, D.C., et al., 2002. Removal os trace metals with mango seed powder. Indian J. Chem. Technol. 9, 540–542.
Pérez-Palacios, P., 2015. Environmental Biotechnology, Development of Biotechnological Strategies for the Bioremediation of Heavy Metals. Vol. Doctoral Thesis, Spain.
Peuke, A.D., Rennenberg, H., 2005. Phytoremediation: molecular biology, requirements for application, environmental protection, public attention and feasibility. EMBO Rep. 6, 497–501.
Pilon-Smits, E.A., Freeman, J.L., 2006. Environmental cleanup using plants: biotechnological advances and ecological considerations. Front. Ecol. Environ. 4, 203–210.
Pilon-Smits, E.A.H., et al., 1999. Overexpression of ATP sulfurylase in Indian mustard leads to increased Selenate uptake, reduction, and tolerance. Plant Physiol. 119, 123–132.
Pivetz, B.E., 2001. Ground Water Issue: Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites. EPA, Washington, D.C., United States, pp. 1–36.
Rai, P.K., 2008. Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: an ecosustainable approach. Int. J. Phytoremediat. 10, 133–160.
Ravikumar, K., Sheeja, A.K., 2013. Heavy metal removal from water using *Moringa oleifera* seed coagulant and double filtration. Int. J. Sci. Eng. Res., 4.
Ruffini-Castiglione, M., Cremonini, R., 2009. Nanoparticles and higher plants. Caryologia 62, 161–165.
Ruppert, L., Lin, Z.Q., Dixon, R.P., Johnson, K.A., 2013. Assessment of solid phase microfiber extraction fibers for the monitoring of volatile organoarsinicals emitted from a plant–soil system. Journal of hazardous materials *262*, 1230–1236.
Salt, D.E., et al., 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat. Biotechnol. 13, 468–474.
Sarma, H., 2011. Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J. Environ. Sci. Technol. 4, 118–138.
Schmidt, U., 2003. Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. J. Environ. Qual. 32, 1939–1954.
Sekara, A., et al., 2005. Cadmium and lead accumulation and distribution in the organs of nine crops: implications for phytoremediation. Pol. J. Environ. Stud. 14, 509–516.
Sen, A.K., et al., 1987. Studies of uptake and toxic effects of Cr (VI) on *Pisita stratioites*. Water Sci. Technol. 19, 119–127.
Shaheen, S.M., Rinklebe, J., 2015. Phytoextraction of potentially toxic elements by Indian mustard, rapeseed, and sunflower from a contaminated riparian soil. Environ. Geochem. Health 37, 953–967.
Shakoor, M.B., et al., 2014. Citric acid improves lead (Pb) phytoextraction in *Brassica napus* L. by mitigating Pb-induced morphological and biochemical damages. Ecotoxicol. Environ. Saf. 109, 38–47.
Shanker, A.K., et al., 2005. Chromium toxicity in plants. Environ. Int. 31, 739–753.
Sharma, P., Pandey, S., 2014. Status of Phytoremediation in World Scenario. Int. J. Environ. Bioremediation Biodegrad. 2, 178–191.
Sharma, R., et al., 2013. Rhizosphere biology of aquatic microbes in order to access their bioremediation potential along with different aquatic macrophytes. Recent Res. Sci. Technol. 5, 29–32.
Sharma, S., et al., 2015. Phytoremediation: role of terrestrial plants and aquatic macrophytes in the remediation of radionuclides and heavy metal contaminated soil and water. Environ. Sci. Pollut. Res. 22, 946–962.
Simmons, R.W., et al., 2015. Towards practical cadmium phytoextraction with *Noccaea caerulescens*. Int. J. Phytoremediat. 17, 191–199.
Singh, J.S., et al., 2011. Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480, 1–9.
Singh, S., 2012. Phytoremediation: a sustainable alternative for environmental challenges. Int. J. Green. Herb. Chem. 1, 133–139.
Smolinska, B., Krol, K., 2012. Leaching of mercury during phytoextraction assisted by EDTA, KI and citric acid. J. Chem. Technol. Biotechnol. 87, 1360–1365.
Stampoulis, D., et al., 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ. Sci. Technol. 43, 9473–9479.
Suthersan, S.S., McDonough, J., 1996. Remediation Engineering: Design Concepts. CRC Press, Boca Raton, Florida.
Szczygłowska, M., et al., 2011. Use of brassica plants in the phytoremediation and biofumigation processes. Int. J. Mol. Sci. 12, 7760–7771.
Tangahu, B.V., et al., 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through Phytoremediation. Int. J. Chem. Eng. 2011, 1–31.
Taylor, A.F., et al., 2014. Investigating the toxicity, uptake, nanoparticle formation and genetic response of plants to gold. PLoS One 9, e93793.
Teixeira, S.R., et al., 2011. The effect of incorporation of a Brazilian water treatment plant sludge on the properties of ceramic materials. Appl. Clay Sci. 53, 561–565.
Terry, N., Banuelos, G.S. (Eds.), 1999. Phytoremediation of contaminated soil and water. CRC Press, p. 408.
Terry, N., Banuelos, G., 2000. Phytoremediation of Contaminated Soil and Water. CRC Press, Boca Raton, Florida.
Thangavel, P., Subbhuraam, C., 2004. Phytoextraction: role of hyperaccumulators in metal contaminated soils. In: Proceedings of the Indian Natural Sciences Academy Part B, vol. 70**.** pp. 109–130.
Thayaparan, M., et al., 2013. Rhizofiltration of Pb by *Azolla pinnata*. Int. J. Environ. Sci. 3 (6), 3.
Thompson, P.B., 2012. Genetically modified food: Ethical issues.
Turgut, C., et al., 2004. The effect of EDTA and citric acid on phytoremediation of Cd, Cr, and Ni from soil using *Helianthus annuus*. Environ. Pollut. 131, 147–154.
Udokop, U., 2016. Studies on the blastofiltration potentials of selected plant seeds for the treatment of lead and cadmium contaminated water samples. Vol. Chemistry, Africa. pp. 37.
Ullah, A., et al., 2015. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ. Exp. Bot. 117, 28–40.
UNEP, 2016. Phytoremediation: An environmentally sound technology for pollution prevention, control and redmediation - An introductory guide to decision-makers. Available at: ⟨http://www.unep.or.jp/Ietc/Publications/Freshwater/FMS2/2.asp⟩.
USEPA, 2000. Introduction to Phytoremediation. EPA 600/R-99/107. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.
Vamerali, T., et al., 2010. Field crops for phytoremediation of metal-contaminated

land. Environ. Chem. Lett. 8, 1–17.
Van Aken, B., 2009. Transgenic plants for enhanced phytoremediation of toxic explosives. Curr. Opin. Biotechnol. 20, 231–236.
Van Huysen, T., et al., 2003. Overexpression of cystathionine-gamma-synthase enhances selenium volatilization in *Brassica juncea*. Planta 218, 71–78.
Vangronsveld, J., et al., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ. Sci. Pollut. Res. 16, 765–794.
Wang, J., et al., 2012. Remediation of mercury contaminated sites–a review. J. Hazard. Mater. 221, 1–18.
Weis, J.S., Weis, P., 2004. Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ. Int. 30, 685–700.
Wu, G. s, et al., 2010. A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J. Hazard. Mater. 174, 1–8.
Wuana, R.A., Okieimen, F.E., 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. Int. Sch. Res. Not. Ecol. 2011, 1–20.
Yoon, J., et al., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci. Total Environ. 368, 456–464.
Zhang, L., et al., 2005. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of Spinach. Biol. Trace Elem. Res. 105, 83–91.
Zhang, Z., et al., 2016. Phytostabilization of acidic soils with heavy metal contamination using three forage grasses in combination with organic and inorganic amendments. Soil Sediment Contam.: Int. J. 25, 1–10.
Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G., 2008. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta *606* (2), 135–150. http://dx.doi.org/10.1016/j.aca.2007.11.018.
Zhu, Y.L., et al., 1999a. Overexpression of glutathione *syn*thetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiol. 119, 73–79.
Zhu, Y.L., et al., 1999b. Cadmium tolerance and accumulation in indian mustard is enhanced by overexpressing gamma-glutamylcysteine synthetase. Plant Physiol. 121, 1169–1178.