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RESEARCH ARTICLE

# Phytoremediation- A Green Approach for Soil Decontamination: Concept, Types, Mechanism and Advantages

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## Abstract

The agricultural soil has extremely become contaminated with various pollutants. These pollutants arise from divergent sources including agricultural and industrial drainage, erosion and weathering of rocks, and various human activities, especially after the great development in the industrial sector through the latest decades. Such contaminants negatively affect humankind due to their direct or indirect access to the food chain. Hence, their remediation should be highly considered. A number of techniques have been exploited in contaminant elimination including thermal, chemical or combination of both methods, but these methods are expensive and more complicated. Some plants possess a high capability to eliminate pollutants safely and without the need for a lot of expenses, these plants are called hyperaccumulators. Hyperaccumulators can utilize different techniques to decontaminate soils such as phytoextraction, phytostabilization, phytovolatalization, rhizofitration, and phytodegradation. All the techniques used in soil decontamination are called “phytoremediation”, which is essentially affected by soil and metal properties and plant species. Hyperaccumulators are equipped with various mechanisms to counteract toxic metals safely with no toxicity symptoms. The principle buddies in this process are the phytochelatins and metallothioneins which chelate metals by forming complex with them, which is followed by freeing the sensitive sites from polluting metals or vacuolar sequestration of ligand-metal complex. Phytoremediation is a promising approach that can be utilized for contaminant remediation from impacted sites with some limitations. Therefore, attention has been given to develop modern biological and genetic engineering strategies to increase plant detoxification capabilities

**Keywords:** Phytoremediation; hyper-accumulators; metal toxicity; phytochelatins; metallothioneins; microbes

## Introduction

Heavy metal is a term referring to an element with metallic properties and a proton number higher than 20. The most widespread metal pollutants comprise Hg, Pb, Cd, Cr, Cu, and Zn. These metals could be categorized into micronutrients required in too small quantities like Cr, Co, Cu, Fe, Mn, Ni, and Zn, functioning in (a) redox-processes, (b) molecular stabilization through electrostatic interactions, (c) enzymatic activators, and (d) osmoregulators (Bruins et al., 2000).

Others are non-essential without specific action, like Hg, Pb, Cd, and Pu (Gaur &Adholeya, 2004; Kumar et al., 2013). Soil and water pollution occur naturally and from anthropogenic sources (Khalid et al., 2017). The major natural sources include weathering, erosion and volcanic activity, whereas of anthropogenic sources comprise metal-rich mine tailing, smelting, use of agricultural pesticides and fertilizers, sludge wastes, factories effluents, and atmospheric precipitation (Wuana&Okieimen, 2011). It gets entrance to the body through food, water, air, and touch with the skin causing serious ailments to all life forms (Saad-Allah &Elhaak, 2017). Arsenate is considered as a principle source of drinking water contamination. It causes cancer of skin, lung, urinary bladder and prostate (Martinez et al., 2011). Also, lead is of great concern that causes tissue and organ injuries in children and nerve damage in adults (Sanders et al., 2009). In addition, long-term exposure to cadmium can cause liver, kidney and bone damage (Zhang et al., 2008), inhibition of progesterone and estradiol (Massanyi et al., 2007), and act as estrogen in breast cancer (Brama et al., 2007). Also, mercury can cause dyspnea, fever, tremble, weariness, motor neuropathy, gum disorders, illusions and delirium (Guzzi& La Porta, 2008).

Numerous techniques are certainty used to expurgate the environment from these pollutants, however nearly all of them are expensive and irrelevant from optimal performance. The chemical technologies are costly and discharge great amount of wastes (Rakhshaee et al., 2009). Also, combining chemical and thermal techniques is technically complicated, expensive, and can result in impairing of some soil components (Hinchman et al., 1996). Conventionally, soil remediation from metal pollution could be managed onsite or through excavation after disposal to a dump site (Tangahu et al., 2011). However, this elimination manner has the risk of transporting waste and contaminants from the dump sites to clean soil and the possibility of polluted soil transfer to another non-polluted site. Soil washing is an alternative way for excavation of contaminated soils. However, this technique requires a lot of expenses and can produce heavy metal-rich residues, that need further management. Moreover, remediation techniques prevent soil utilization in plant growth, due to the cessation of all biological processes (Gaur &Adholeya, 2004), along with groundwater contamination.

Recently, different technologies concerning presence and mobility of inorganic pollutants in the soil, agricultural water and wastewater have been developed (Shtangeeva et al., 2004). Phytoremediation is a proficient, reasonable and practical choice for removal of contaminants from polluted soil. In the current review, we will discuss some fundamental topics in phytoremediation including various mechanisms, types, influencing factors, uptake, translocation and tolerance mechanisms. Modern biological and genetic engineering approaches are also highlighted.

## Phytoremediation concept

Phytoremediation is the utilization of plants and rhizospheric microorganisms, agronomic strategies, and soil amendments in eradication or detoxifying harmful environmental contaminants (Ouyang, 2002). The concept of phytoremediation was proposed by IlyaRaskin in 1994, which comes from two suffixes, the Greek phyto (meaning plant) and the Latin remedium (meaning able to cure or restore) (Vamerali et al., 2010). The most useful target of phytoremediation is its application in removing many classes of soil pollutants including heavy metals, radionuclide, petroleum hydrocarbons, chlorinated organic compounds, pesticides, explosives, etc (Yang, 2008). Plants to be used in phytoremediation, should fulfill four main requirements: a) fast-growing and large biomass producing, b) ability to develop deep roots, c) easily harvestable d) high ability to accumulate metals in their shoots (Schnoor, 1997). The idea of utilizing green plants for heavy metal remediation gains the public acceptance because green remediation of hazardous metals and metalloids is the best alternative for physical and chemical remediation strategies (Ali et al., 2013). Additionally, phytoremediation is considered as an inexpensive prospect for recovering soils from heavy-metals pollution, principally if the produced biomass is used for economic purposes like biodiesel production. Both advantages and disadvantages of phytoremediation are presented in Table 1.

**Table 1.** Advantages and disadvantages of phytoremediation approach

| Advantages of phytoremediation | Disadvantages of phytoremediation |
|---|---|
| 1. Inexpensive and aesthetically attractive. | 1. The prolonged time required for remediation process. |
| 2. Exhibit an ability to reduce soil erosion and metals leaching into the soil. | 2. Limited growth rate and low biomass production of hyperaccumulator used in phytoextraction process. |
| 3. The yielded biomass can be used in regeneration of remediated metals. | 3. Accumulation capacity is much affected by environmental and biological factors. |
| 4. This green remediation technology has a wide range of applicability in terms of toxic heavy metals. | 4. Indigenous flora diversity is potentially affected by hyperaccumulator invasion. |
| 5. Eco-friendly technique. | 5. More handling techniques concerning the accumulation of pollutants into food chain are required (Mahar et al., 2016). |
| 6. Has an ability to eliminate secondary toxic materials from water, soil, and air. | |
| 7. Enhanced public and regulatory authority (Glass, 1999; Hosh & Singh, 2005). | |

There are approximately 45 plant families (comprising about 500 plant species) that have been validated as metal hyperaccumulators. The most significant families comprise Brassicaceae, Euphorbiaceae, Compositae, Leguminosae, Labiatae, and Scrophulariaceae (Hosh& Singh, 2005). Hyperaccumulation potential is the function of plant species and metal type. In this concern, Thlaspicaerulescens is the most operative plant species for zinc, cadmium, and nickel (Mitch, 2002). In phytoremediation, bio-concentration and bio-translocation are the main two factors in identifying hyperaccumulator species. Bio-concentration is the metal ion concentration in plant tissue/soil ratioand bio-translocation factor is heavy metal ions in the shoot/the root ratio. About 0.2% of plants were classified as heavy metal accumulators (Sarma, 2011). However, hyperaccumulators with low biomass production face limitation in their use for bioremediation (Pence et al., 2000). Recently, various approaches have been directed to increase hyperaccumulators growth and biomass production using gene engineering tools. Also, various microbes such as plant growth promoting bacteria (PGPR) and arbuscularmycorrhizal fungi (AMF) can be used as potential biological decontaminants of heavy metals.

## Phytoremediation mechanism

The mechanism of phytoremediation essentially depends on the pollutant type, the bioavailability of the pollutant and soil properties (Cunningham & David, 1994; Laghlimi et al., 2015).

## Plant metal uptake

Metal bioavailability is essential for its uptake by plants. Roots struggle with soil cation/anion exchange sites for ions. Particularlyin clay soils which have high organic matter content, metal bioavailability is low (Ross, 1994). Root activity in the rhizosphere critically affects metal bioavailability to plants (Hinsinger et al., 2005). Additionally, the mechanisms of metal absorption by roots are more complicated. Metal absorption process involves transmittance of metals from the soil solution to the root surface interface, and then diffusion across the cell membrane of root cells. Metal movement through cellular membranes is mainly affected by its charge and valence. Hence, metals and ions passage across cells must be mediated by specific proteins which are known as transporters. Such proteins possess frontiers by which ions connect immediately before their transport, and a transmembrane adherence structure that binds extracellular and intracellular media. The ion uptake can be motivated by the electrochemical gradient across the plasma membrane of the recipient cell, but the transport energetics are not yet fully comprehended (Maser et al., 2001).

## Factors affecting heavy metal bioavailability

There are many factors that affect metal bioavailability like soil reaction (pH), soil organic matter, root exudates, soil redox potential, soil texture, plant species, and properties of the heavy metal. These factors may affect the metal ion release into the soil solution or plant uptake ability in soil (Figure 1).

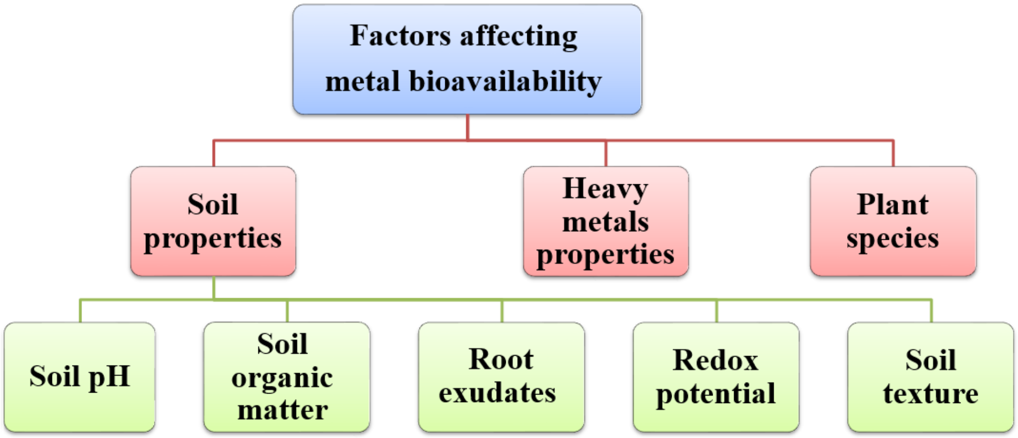

**Figure 1**. Diagram of factors affecting heavy metal bioavailability.

## Soil properties

### Soil pH

Soil pH affects metal solubility and availability to the plant as plants uptake metals in ionic form which is affected by medium pH (Dzantor& Beauchamp, 2002). At certain pH value, heavy metals are divided into two groups: Cd, Ni, and Zn which shows comparatively high mobility, and Cu, Cr, and Pb which exhibits low mobility (Yoon et al., 2015). At low pH, heavy metal adsorption decrease resulting in high metalconcentration in the soil solution. Increasing hydrogen activity at low pH causes heavy metals displacement from exchangeable sites on solid surfaces increasing their concentration. Metal availability for plant uptake is a natural consequence for increased metal concentration. The lower pH values have reported to increase the bioavailability of many cations like cadmium, copper, mercury, lead, and zinc (Sheoran et al., 2016).

### Soil Organic Matter

Trace metals behavior in the soil is found to be affected by organic matter. Organic matter in the soil results in metal-organic complexation leading to decreasing metals phytotoxicity (Gupta &Sinha, 2007). Soil content of organic carbon effectively controls its bioavailability. The high content of organic carbon (>5%) results in low availability of the metal due to strong adsorption, whereas moderate concentrations of organic carbon (1–5%) could limit metal availability (Otten et al., 1997). Thus, soils rich in organic substances help reduce heavy metals absorption by plants, but low concentration of organic carbon in soils make plants more vulnerable to contamination with heavy metals.

### Root exudates

The root zone is of pivotal importance in phytoremediation. Plant root activities result in increasing metal solubility and uptake, and changing heavy metal characteristics through acidification/alkalinization, alteration of the reduction-oxidation potential, secretion of metal chelators and organic ligands (Jones et al., 2004). Consequently, plant root can take up contaminants and accumulate/metabolize it within the target tissue. Another phytoremediation mechanism of pollutants could be achieved by enzymes exuded into the root zone resulting in their degradation (Merkl et al., 2005). Uptake of heavy metals by these proteins is maintained by their conversion and chemical speciation in soils (Mallmann et al., 2014; Laghlimi et al., 2015). Root properties are considerably affected by a wide range of environmental conditions including temperature, drought, precipitation and soil moisture that control growth rate and root length (Mccormack&Guo, 2014). Hence, the root zone properties and composition analysis of enzyme exudates can be the best screening technologies for better identification of hyper-accumulator plant species.

### Redox potential

Redox is an abbreviation for the term “reduction-oxidation reaction”, the processes control the electrons flow from a reducing agent to an oxidizing one. Soil Redox reactions are affected by the aqueous free electron activity, pE, which can also be expressed in terms of Eh, the redox potential (Sposito, 1983). Redox potentials were reported to be high in dry, well-aerated soils, but it has been reported to be low in waterlogged and rich organic matter soils (Evans, 1989). These redox reactions eliminate metal toxicity by decreasing their mobility or through converting them into less toxic or inert forms (Alkorta et al., 2004).

### Soil texture

The texture is a term referring to the particle size, distribution, and content of fine particles like oxides and clay in the soil (Sherene, 2010). Qian et al (1996) studied the impact of soil texture on some metals concentrations like lead and copper and reported that these metals are more concentrated in the clay fraction. Additionally, moderate amounts of available lead and copper were detected in the fine sand fraction, and the clay fraction showed high amounts of available lead. Qian and his coworkers attributed that organic matter, Fe–Mn oxides and sulphides, beside the high surface area and elevated concentration of minerals in the clay, are the main causes of heavy metals accumulation in the clay fraction. They suggested that heavy metals are heavily accumulated in the clay as a result of their adsorption on the clay minerals or their sorption within the clay lattice. The ease with lead to be extracted from the clay was attributed to adsorption of high amounts of lead on the clay particles. Meanwhile, high amount of lead is found to be more extractable from sand fractions as a result of the low binding force with sand particles (Qian et al., 1996).

## Plant species

The capability of the plant to accumulate heavy metals differs significantly among plant species. These differences in metal accumulation capacity among plant species could be attributed to the differences in: a) root architecture; b) water use efficiency; c) rhizosphere chemistry; d) affinity of root surface transporter proteins to metals; e) xylem capacity for metals loading and translocation within the plant (Hamon& McLaughlin, 2003). The recognition of appropriate hyperaccumulator species producing large biomass is the main challenge in phytoextraction technique success (Kumar et al., 2018). In addition, phytoremediation depends upon hyperaccumulator species accumulating contaminants (Lorestani et al., 2011). Plant/cultivar species undergoing hyper-accumulation via utilization of cultivar specific established crop production and management practises, can produce high biomass yields (Rodriguez et al., 2005). Therefore, a zone -specific cropping system exhibiting the highest capacity for heavy metals accumulation should be highlighted.

## Heavy Metal Properties

The chemical structure of the metal is the main factor affecting its mobility, bioavailability, and toxicity (Fuentes et al., 2004). The major mobile heavy elements comprise cadmium, zinc, and molybedinum, whereas the least mobile are chromium, nickel and lead (Fijakowski et al., 2012). Some heavy metals in the solid phase were found in soils amended with organic matter, these metals can replace primary and secondary minerals in the soil (Pichtel& Anderson, 1997).

## Vacuolar sequestration

As metabolic activities occur in cellular sap and other compartments, these sites have to be freed from the toxic metals. Therefore, the plant cell central vacuole is found to be the most proper compartment for the storage of ions (Marschner, 2011). Through transporter protein family members, principally ZIP (zinc/iron-regulated transporters), heavy metals enter the cytosol and stimulate phytochelatin synthase (PCS) that catalyzes phytochelatins (PCs) synthesis from glutathione. Phytochelatin-heavy metal complexes are driven to the vacuole via tonoplast-localized ATP-binding-cassette (ABC) transporters. Heavy metals are also sequestered in the vacuole by tonoplast-localized cation/proton antiporters which direct exchanges of the HMs with protons. In the vacuole, heavy metals may access the vacuole by means of direct exchange mechanism of different heavy metal-protons exchanger transporters like metal tolerance proteins (MTPs) and natural resistance-associated macrophage-proteins (NRAMPs). These transporter proteins reside in the tonoplast and control metal ions passage to be remobilized or compartmentized (Yang & Chu, 2011).

## Metal translocation to shoots and shoot metabolism

The root to shoot movement of metals is achieved by many types of proteins. Once metals are absorbed via plant roots, they are transported to the aerial parts through xylem in the form of complexes with various chelators (Migeon et al., 2010). Organic acids (e.g., malate and citrate) and different amino acid derivatives (e.g., nicotinamide (NA) and histidine) are chelators of copper, nickel, and iron in the xylem (Marschner, 2011). In shoots, metals overload may cause oxidative stress injuring the exposed cells through the substitution of major ions in many molecules like chlorophyll, proteins and nucleic acids. Photosynthetic machinery is mainly sensitive to heavy metal toxicity. The redox active metals (Cu) and non-redox active metals (Cd and Zn) both can cause oxidative damage. Plants have natural antioxidative defense systems that protect the cells from this damage, these systems are based on reduced metabolites like glutathione (GSH) and antioxidant enzymes like peroxidase and catalase that regulates the redox state. GSH is an essential molecule that is synthesized from Glutamate, Cystine, and Glycine through the activity of glutamyl cysteine synthetase and GSH synthetase. Glutathione is a precursor of phytochelatins. It can bind to metals and metalloids, and scavenge reactive oxygen species (ROS) induced by heavy metals and maintains redox homeostasis for metabolism, signal transduction, and gene expression (Foyer &Noctor, 2005).

## Phytochelatins

Phytochelatins (PCs) are low molecular weight cysteine-rich metal-binding peptides whose synthesis is induced by heavy metals. PCs are synthesized non-translationally from glutathione by the activity of phytochelatin synthase forming (γ-EC) nG molecules (where N=2–11). Many heavy metals are reported to activate PC synthase to produce PCs (Cobbett, 2000). Although PC-synthase activity has been detected many years ago, the gene responsible for its synthesis remained vague till Vatamaniuk and his co-workers identified AtPCS1 gene in Arabidopsis (Vatamaniuk et al., 1999). The activity of AtPCS1 resulted in an increased accumulation of cadmium, suggesting that AtPCS1 plays a role in cadmium chelation and/or sequestration (Vatamaniuk et al., 1999). Phytochelatins (PC) role in the homeostasis of essential metal was reported by (Tennstedt et al., 2009). The cad1-3 (a PC-deficient mutant of Arabidopsis thaliana) and cad1-6 (a newly isolated second strong allele) in context to zinc homeostasis were studied. It was found that zinc accumulation in the root was significantly lowered in the PC-deficient mutants. Expression of AsPCS1 and YCF1 in Arabidopsis resulted in increased tolerance to cadmium and arsenate than corresponding wild-type and individual-gene transgenic lines (Guo et al., 2012). These genes implicated the chelation of heavy metals via thiols and vacuolar compartmentalization. Translocation and access of metal ions into the plant cell are facilitated by overexpression of natural chelators (metallothioneins, and organic acids) (Guo et al., 2012).

## Metallothioneins

Metallothioneins (MTs) are well-known low molecular weight proteins (5–10 kDa) rich in cysteine residue, and common in plants, animals, fungi, and cyanobacteria. In plants, MTs are key players in metal tolerance or homeostasis, detoxification, and distribution via binding metal ions by forming mercaptide bonds (metal-sulfur bond) with various cysteine residues. In addition, scavenging of metal-induced ROS is found to be mediated with the aid of MTs (Hassinen et al., 2011). In addition, metallothionein metal complex can be glutathioned (Brouwer et al., 1993), implying that they can be transported into vacuoles for long-time sequestration.

Expression of plant metallothionein genes using reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization in a detailed study carried on Arabidopsis revealed distinct patterns for two genes, MT1a and MT2a (Garcia-Hernandez et al., 1998). Both genes were expressed in root maturation zones and leaf trichomes. However, the cells of vascular and mesophyll tissues only expressed MT1a. Kumar et al. (1995) correlated Cu tolerance in Arabidopsis ecotypes to the induction of MT2a through Cu treatment. Metallothionein genes have been cloned in many plant species. Cloning of human MT-2 gene into tobacco or oilseed rape plants resulted in Cd tolerance enhancement (Misra et al., 1989). Moreover, MT gene from pea was cloned in Arabidopsis thaliana and resulted in improved Cu accumulation (Evans et al., 1992).

## Phytoremediation types

Five major processes are well recognized in phytoremediation. They comprise phytoextraction, phytostabilization, phytovolatilization, rhizofitration, and phytodegradation (Figure 2). Phytoremediation techniques, their mechanisms of heavy metal removal and selection of plant species are summarised in Table 2.

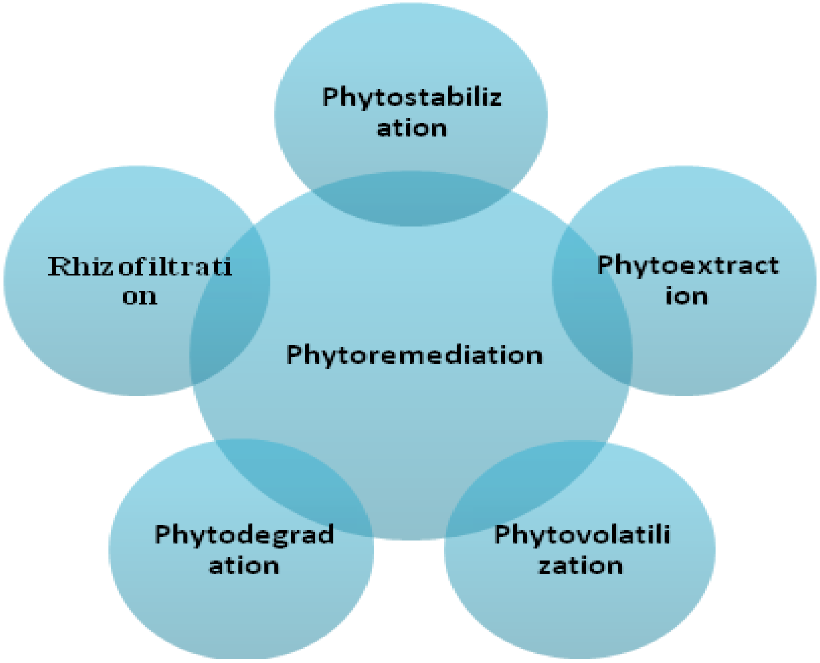

**Figure 2.** Diagram of phytoremediation types.

## Phytoextraction

Phytoextraction, also named phytoaccumulation, phytoabsorption or phytosequestration, is the absorption of contaminants from soil or water via plant roots and their mobility and accumulation in aboveground tissues, that simply burned after harvesting for energy production and possible restoration of the metal from the ash (Rafati et al., 2011). The metal-accumulating plants can be sown or transferred into soils heavily contaminated with these metals and then cultivated with traditional agricultural practices. Salt et al (Salt et al., 1995) reported that the costs of phytoextraction would be ten-folds lower than usual remediation techniques. Phytoextraction had been assured a complete elimination of some contaminants like Pb, Cd, Ni, Cu, Cr, and V, from the soils. Nevertheless, its application is limited to low or moderately metal contaminated sites due to restricted plant growth in heavily contaminated sites (Padmavathiamma& Li, 2007). There are different methods by which efficacious phytoextraction of heavy metals along with their accumulation in plant species have been achieved. These include biosynthesis of metal ligands/transporters, alternations in enzyme kinetics responsible for sulfur metabolism, variations in redox states leading to heavy metal formation, and synthesis of some products of primary metabolism such as intermediate moieties (Fasani et al., 2018). Plants used in phytoextraction should exhibit specific features like (a) fast rate of growth and high biomass content, (b) deep and highly branched root systems, (c) high adaptability to various growth sites, (d) simplicity of cultivation and growing (e) more or less inedible by animals to prevent accumulation of metals in food chain (Seth, 2012).

**Table 2.** Examples of some plants that can be used for phytoremediation of heavy metals

| Metal | Plant | Process | Remarks | References |
|---|---|---|---|---|
| As | *Pterisvittata* | Phytoextraction | *P.vittata* exhibits high capability for phytoextraction of As when treated with water-soluble chitosan. | (Yang et al., 2017a; Yang et al., 2017b) |
| Cd | *Athyriumwardii* | Phytostabilization | *Athyriumwardii* is a promising plant for Cd phytostabilization with a fast growth rate and large amount of biomass. | (Zhang et al., 2012) |
|  | *Pistiastratiotes* L | Rhizofiltration | *Pistiastratiotes* L is effective for removing Cd from surface waters. | (Das & Goswami, 2014) |
| Ni | *Typhadomingensis* | Phytostabilization | T. *domingensis*is capable of surviving under Ni-contaminated sites.<br>T. *domingensis*has high ability to accumulate high levels of Hg. | (Bonanno & Vymazal, 2017) |
|  | *Arundodonax* L | Phytoextraction | *A. donax* L. is considered as a great candidate for Ni phytoextraction. | (Atma et al., 2017) |
| Mn | *Pistiastratiotes* | Rhizofiltration | *P. stratiotes* has high accumulation potential.Therefore, this plant can be used successfully in rhizofiltration process. | (Neuberg, 2012) |
| Pb | *Miscanthussinensis* | Phytostabilization | It is suggested that *M. sinensis*can be used in aided phytostabilization for Pb mine tailings. | (Lee et al., 2014) |
|  | *Plantago major* L | Phytostabilization | *P.major*major is considered a bioaccumulator species for Pb | (Romeh & Khamis, |

| | | | | |
|---|---|---|---|---|
| | | | and can be used as a bioindicator of pollution with lead. | 2016) |
| | *Plectranthusamboinicus* | Rhizofiltration | *P. amboinicus*is a promising plant for the clean-up of Pb-contaminated waste water in combination with safe biomass disposal alternatives. | (Ignatius et al., 2014) |
| Zn | *Anthyllis vulnerary* | Phytoextraction | *A.vulnerary* is used efficiently for phytoextraction of Zn after inoculation with *R. metallidurans*, a natural symbiont. | (Grison et al., 2015) |
| | *Cyperusalternifolius* | Phytostabilization | *C. alternifolius* has the potential for use in phytoremediation of Zn-contaminated wetlands. | (Yang et al., 2017a; Yang et al., 2017b) |
| Cu | *Oenotheraglazioviana* | Phytostabilization | *O. glazioviana* has great potential for the phytostabilization of copper-contaminated soils and a high commercial value without risk to human health. | (Guo et al., 2014) |
| Cr | *Tradescantiapallida* | Phytoextraction | *T. pallida* is considered as an excellent candidate for continuous removal of Cr(VI) from contaminated water. | (Sinha et al., 2016) |
| Co | *Helianthus annuus* | Phytoextraction | Sunflower roots show high potential for Co phytoextraction | (Lotfy & Mostafa, 2018) |
| Al | *Solanumnigrum* | phytoextraction | *S. nigrum* can accumulate Al in its organs to an extent higher than that of the polluted soil. | (Saad-Allah &Elhaak, 2017) |
| Hg | *Lepidiumsativum* L. | Phytoextraction | *Lepidiumsativum*L. is used as Hg extractant. | (Smolinska & Rowe, 2015) |
| | *Phragmitessustralis* | Rhizofiltration | *P. australis* is a promising species used to remediate Hg-contaminated wetlands. | (Bonanno & Vymazal, 2017) |

hytoextraction could be linked with income making processes like forestry and bioenergy production (Van Ginneken et al., 2007). For example, castor bean is reported to accumulate Cd (Melo et al., 2009) and Pb (Romeiro et al., 2016). Phytoextraction of cadmium by carambola tree is an ideal solution for soils decontamination from Cd as it has proven that 50% of Cd could be extracted throughout 13 years in a slightly contaminated soil with Cd (Li et al., 2009). In this respect, jatropha tree used in energy production must be explored for its possible use in phytoextraction process.

## Phytostabilization

Phytostabilization, also recognized as phytoimmobilization, is the use of specific plants in stabilizing heavy metals in polluted soils (Singh, 2012). This technique could be applied in regulating the transformation of metal-pollutants into a stable form, either organic or inorganic, lowering their risks to the environment. The main goals of this process are to (a) decrease the leaked water amount into the soil, which could form harmful leachate, (b) form barrier preventing direct contact with the polluted soil and (c) avoid soil erosion and dispersion of the heavy metals to other sites (Ensley, 2000). Plants used in phytostabilization are characterized by slow translocation rate of the contaminants from the root to the aerial parts, fast growing, having developed root systems and large shoot system, and must be tolerant to environmental and biological stresses (Ismail, 2012). In this context, willow species showed high capacity towards phytostabilization of soils contaminated with heavy metals (Tack et al., 2005). Some metals like cadmium and zinc, are reported to be accumulated mainly in roots. However, their occurrence in the shoots increased following the increase of their concentration (Soudek et al., 2012). In addition, Agrostis species and Festuca species are considered as the most popular plants utilized for the phytostabilization of soils contaminated with Cu, Zn and Pb in European countries (Mahar et al., 2016). Bare sites as a result of high metal contamination levels could be reclaimed using phytostabilization. Once a community of tolerant plant has been established, the soil becomes stable and coherent and resists the spread of the pollutant metals, and the leakage of the contaminants into the soil. Phytostabilization is a convenient approach because throwing away of contaminated biomass is not required, and it is very useful in preserving ground and surface waters (Wuana&Okieimen, 2011).

## Phytovolatilization

Phytovolatilization could be recognized as the absorption of contaminants from the soil, transforming them into volatile forms and their transpiration into the atmosphere (Jadia&Fulekar, 2009). Phytovolatilization has been applied with some contaminants as mercury; volatile inorganic chemicals as selenium and arsenate; along with volatile organic compounds e.g., trichloroethene. Phytovolatilization has a feature of releasing toxic pollutant into the atmosphere after transforming them into less toxic forms. However, precipitation may cause the redeposition of the polluting substances again in aquatic systems (Nikolic&Stevovic, 2015). Some organic volatile compounds like 1,4-dioxane have been found to effectively removed by phytovolatilization (Ferro et al., 2013). Genetically modified plants like Arabidopsis thaliana, Nicotianatabacum, and Liriodendron tulipifera have been accounted to be used in mercury phytovolatilization from polluted soils (Ali et al., 2013). These plants are cloned with a gene called mercuric reductase “merA”. Another bacterial gene called organomercuriallyase (merB) was reported to be used in methyl-mercury detoxification. Though both genes can be used for mercury detoxification, merB cloned plants are safer as this gene prevents the incorporation of methyl-mercury into the food chain. Phytovolatilization could be applied for selenium detoxification through the assimilation of metallic selenium into organic seleno-amino acids (seleno-cysteine and seleno-methionine). Seleno-methionine is further biomethylated to dimethylselenide which is lost in the atmosphere via volatilization (Terry et al., 2000). Effeciency of Selenium phytovolatilization is more efficient especially when Brassica species are used (Banuelos et al., 1997).

There are different mechanisms by which plant root systems participate successfully in the phytovolatilisation of toxic metals such as 1) a lower water table, 2) advection with gas fluxes because of alternations in diel water tables, 3) high soil permeability, 4) hydraulic chemical redistribution, 5) advection of VOCs with water toward the surface, and 6) interception of rainfall that would otherwise infiltrate the soil causing VOCs to be diluted and withdrawn away from surface (Limmer&Burken, 2016).

## Rhizofiltration

Rhizofiltration is the use of plants in absorption, concentration, and deposition of contaminants from aqueous sources in their roots (Schwitzguebel, 2002). Rhizofiltration could be used mainly in the purification of wastewater, extracted groundwater, and surface water containing lower concentrations of contaminants. The ideal plant species for rhizofiltration should have deep and highly branched roots possessing high ability to absorb toxic metals from solution throughout a long time. These plants should also be characterized by intensive biomass production (1.5 kg/ month. m2 of water surface) (Dushenkov et al., 2002). During rhizofiltration, the plant roots take up contaminant and relay it into other plant parts, according to the contaminant type, its concentration, and the plant species. Rhizofiltration is achieved by the help of specific compounds synthesized inside the roots, that drive heavy metals to be accumulated in the plant body. The adsorption of contaminants on the root surfaces is attributed to root secretions and soil pH (Krishna et al., 2012). The widely used plant species in rhizofiltration include the Indian mustard, sunflower, and maize (Brooks & Robinson, 1998). After metal rhizoextraction from the polluted sites, the used plants can be used for many purposes like energy production and metal chemical extraction of Ni, Cu, and Au (Verma et al., 2007; Kathi, 2015).

## Phytodegradation

Phytodegradation, also known as phytotransformation, is the involvement of plant metabolic processes in the internal or external breakdown of organic contaminants. In other words, the involvement of specific metabolic processes in the hydrolysis of organic compounds into simpler forms that can be easily absorbed by the plant (Suresh &Ravishankar, 2004). Some plant enzymes like peroxidase, nitroreductase, laccase, nitrilase, and dehalogenase are involved in the degradation of pollutants (Morikawa&Erkin, 2003).

Plants used in phytodegradation must be characterized by (a) highly branched roots for secreting a significant amount of enzymes, (b) tolerance to high pollutants levels, (c) fast growth rate, and (d) a relatively high biomass content (Wang & Chen, 2007). The microbes present in the root medium could enhance organic pollutants degradation in the soil. In the same time, secretion of exudates like carbohydrates, amino acids, and flavonoids by root surface could motivate microbial activity 10–100 folds, compared to the activity in bulk soils (Ali et al., 2013). Phytodegradation is more specific to organic pollutants because heavy metals have non-biodegradable nature. Recent studies are more concentrated on phytodegradation of many organic pollutants such as herbicides and insecticides.

## Role of transgenic plants in phytoremediation

As the phytoremediation of pollutants is a slow process and accumulation of toxic metabolites also leads to the cycling of these metabolites into the food chain. From the last few decades, experiments has been carried out to develop transgenic plants to overcome the inbuilt constraints of plant detoxification capabilities. Transgenic technology has been developed to enhance metal uptake, transport, and accumulation as well as plant tolerance capacity to abiotic stresses (Kärenlampi et al., 2000). Genetic engineering technology has successfully exploited in altering the biological functions of plants via modifications of primary and secondary metabolism and by developing new phenotypic and genotypic traits in order to comprehend and improve their phytoremediation properties (Fasani et al., 2018). Heavy metals were the first contaminants to be remediated by transgenic plants using tobacco plant which expressed a metallothionein gene to create higher tolerance for cadmium and Arabidopsis thaliana plant which overexpressed a reductase gene mercuric ion for better Hg tolerance ( Misra et al., 1989). Similarly, transgenic A. thaliana plants expressing SRSIp/ArsC and ACT 2p/γ-ECS together showed high tolerance to As. These plants accumulated 4- to 17-fold greater fresh shoot weight and 2- to 3-fold more arsenic per gram of tissue than wild plants or transgenic plants expressing γ-ECS or ArsC alone ( Dhankher et al., 2002). Example of other genes that can be used for developing transgenic plants for metal tolerance and phytoremediation are shown in Table 3.Though, the risks of escaping genes from transgenic plants have been found negligible (Zhu et al., 1999). One of the possible risks associated with transgenic application is the biological transformation of metals into chemical species that are easily bioavailable. This will enhance exposure of various wildlife and human beings to toxic heavy metals. Another aspect of concern could be the uncontrolled distribution of transgenic plants owing to higher fitness of such plants in the particular climatic conditions and/or interbreeding with populations of wild relatives (Dhankher et al., 2002). Also, transgenic approach enhance exposure of humans and wildlife to metals through increased metal concentration in plant edible parts, or volatilization. These risks have to be assessed and weighed not only against the benefits of the technique, but also against the non-targeted risks. Undoubtedly, the plants to be employed for

**Table 3.** Some transgenic plants used in phytoremediation of heavy metals

| Gene | Source | Target plant species | Heavy metals | Effects | References |
|---|---|---|---|---|---|
| *RhlA, RhlB* | *Pseudomonas aeruginosa* | *Nicotianatabacum* | Al | Increased Al tolerance. | (Brichkova et al., 2007) |
| *As(III) vacuolar antiporter ACR3* | *Pterisvittata* | *Arabidopsis thaliana* | As | Increase As tolerance and translocation to the shoot. | (Chen et al., 2013) |
| *PgIREG1* | *Psychotriagabriellae* | *Arabidopsis thaliana* | Ni | Ni tolerance and accumulation. | (Merlot et al., 2014) |
| *Selenocysteinelyase* | *Musmusculus* | *Arabidopsis thaliana* | Se | Enhanced Se tolerance. | (Pilon et al., 2003) |
| *MerA, MerB* | Gram-negative bacteria | *Arabidopsis thaliana* | Hg | Improved Hg tolerance | (Seth, 2012) |
| *IlMT2b* | *Iris lactea var. chinensis* | *Arabidopsis thaliana* | Cu | Increased Cu concentration and reduced H2O. | (Deng, 2015) |
| *OsMT2c* | *Oryza sativa* L. | | | Improved tolerance to Cu stress and increased ROS scavenging ability. | (Liu et al., 2015b) |
| *GshI* | *Escherichia coli* | *Populustremula P. alba* | Cd | Enhanced Cd uptake and accumulation in aerial parts. Reduced concentrations of O2, and H2O2. | (He et al., 2014) |
| *SpMTl* | *Sedum* | *Sedum* | | Elevated SpMTL | (Peng et al., |

| | | | | | |
|---|---|---|---|---|---|
| | *plumbizincicola* | *plumbizincicola* | | transcript level might contribute to the trait of Cd hyperaccumulation and hypertolerance. | 2017) |
| *γ-Glutamyl-cysteine synthetase glutathione synthetase GCS-GS operon* | *Streptococcus thermophilus* | *Beta vulgaris* | | *S.thermophilus* enhanced Cd tolerance compared with wild types. | (Liu et al., 2015a) |
| *Glutathione synthetase 1* | *Escherichia coli* | *Brassica juncea* | Zn | Enhanced Zn tolerance and accumulation. | (Bennett et al., 2000) |
| *AtZIP1, AtMTP1* | *Arabidopsis thaliana* | *Manihotesculenta* | | Higher Zn accumulation. | (Schachtman, 2015) |

phytoremediation, should not go through the food/feed chain. Meanwhile, sterile cultivars can be utilized for transformation to prevent inadvertent dispersion of the transgene. Modern technologies tend to focus on developing maker-genes free transgenic plants (Yau&Jr, 2013), thus leading to more applicable genetically modified crops, and more tremendous opportunities for their use.

## Role of microbes in phytoremediation

Phytoremediation can be potentially used in elimination of soil organic and inorganic pollutants and has been considered to be the most powerful technique as it inexpensive and more applicable compared with usual remediation methods. Nevertheless, expanding its application suffers from numerous problems, like lower biomass production, phytotoxicity, and volatilization of some contaminants during transpiration (Li et al., 2004). The solution to such problems lies in using microbe-assisted phytoremediation. Numerous earlier studies suggested that rhizosphere microorganisms could efficiently enhance phytoremediation efficacy (Gerhardt et al., 2009). For example, arbuscularmycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) were explored for their possible utilization in phytoremediation. These microorganisms improved plant growth rate, confined metals harmful effects, and increased trace elements uptake (Glick, 1995).

PGPR and endophytic microbes possess the ability to reduce heavy metals stress on host plants through: (a) chelating heavy metals via the produced organic matters (Dobbelaere et al., 2003), (b) adjusting soil heavy metals content (Lebeau et al., 2008), (c) secretion of 1-amino-cyclopropane-1-carboxylate (ACC) deaminase by PGPR, which catalyze ACC (ethylene prerequisite) to α-ketobutyrate and ammonium (Glick, 2003), and (d) production of auxins that improve heavy metals bioavailability and uptake by the plant (Zaidi et al., 2006). Metal-resistant endophytes found in many metal-hyperaccumulatorplants, play an essential role during their endurance and growth in heavy metal-contaminated soils. Their stimulatory action occurs through different mechanisms as nitrogen fixation, solubilization of minerals, phytohormone and siderophore synthesis, use of 1-aminocyclopropane-1-carboxylic acid as the only N source, and alteration of supplement components (Glick et al., 1999). Ma et al (2011) established that Alyssum serpyllifolium inoculated with Pseudomonas sp. A3R3 markedly enhanced Ni content in the plant tissues. Additionally, Beolchini et al (2009) found that that zinc, copper, mercury and cadmium mobility had increased by 90% after the inoculation of iron/sulphur oxidizing and iron-reducing bacteria. They accredited this to synergistic metabolism and coupled action of both bacterial species. Examples of some PGPR and AMF that can be used in phytoremediation are shown in Table 4.

**Table 4.** Effects of some microbes against respective heavy metals in different plant species

| Microbe | Target Plant species | Heavy metal | Effects | References |
|---|---|---|---|---|
| | | PGPR | | |
| *Pseudomonas fluorescens G10 and Microbacterium sp. G16* | *Brassica napus* | Pb | Enhanced Pb uptake from Pb-amended soils. | (Sheng et al., 2008) |
| *Azotobactorchroococum and Rhizobium leguminosarum* | *Zea mays* L. | | Increased Pb accumulation. | (Hadi & Bano, 2010) |
| *Bacillus thuringiensis GDB-1* | *Alnus firma* | As | Enhanced As accumulation. | (Babu et al., 2013) |
| *Rhodococcuserythropolis NSX2* | *Sedum plumbizincicola* | Cd | Improved plant growth and Cd uptake. | (Liu et al., 2015c) |
| *Polygonumpubescens* | *Brassica napus* | Cd, Pb and Zn | Enhanced Cd, Pb, and Zn tolerance and accumulation. | (Jing et al., 2014) |
| *Pseudomonas veronii* | *S. alfredii* | Zn | Enhanced plant growth and Zn tolerance. | (Long et al., 2013) |
| *acillusthuringiensis* 002, *Bacillus fortis* 162, *Bacillus subtilis* 174, and *Bacillus farraginis* 354 | *Althea rosea* | Ni | Enhanced Ni accumulation. | (Khan et al., 2017) |
| | | AMF | | |
| *Gigaspora*, *Glomus*, *Scutellospora*and *Acaulospora* species | 22 different pioneering plant species from 11 families. The most common species were in the familiesFabaceae, Asteraceae and Poaceae | Cd | The majority of the pioneer species presented high mycorrhization levels and showed Cd stabilization. | (Carrillo-gonz & Guti, 2009) |
| *Glomusintraradices, Glomusetunicatum,* and *Glomusclaroideum* | *Agrostiscapillaris* | As, Cu, Pb, and Zn | *A. capillaris* inoculation helped the development of plants in the amended mine tailing substrate. Furthermore, it enhances P uptake, favoring the accumulation of proteins in the plant´s shoots and roots. | (Neagoe, et al. 2014) |

| | | | | |
|---|---|---|---|---|
| *Claroideoglomusclaroideum* | *Origanummajorana* L. | Cd and Pb | *C. claroideum* inoculation enhanced the removal of Cd and Pb from a contaminated area. | (Hristozkova et al., 2015) |
| *Funneliformismosseae*, *Glomusmosseae* | *Festucaarundinacea, Schedonorusarundinaceus* | Ni | Inoculation with AMF enhanced Ni tolerance. | (Shabani & Sabzalian, 2016) |
| *Rhizophagusclarus* | *Canavaliaensiformis* | Cu | *R. clarus* inoculation along with grape bagasse vermicompost treatment improved the efficiency of the phytoremediation of Cu-contaminated areas. | (Almeida et al., 2015) |

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## References

Ali, H., Khan, E., & Sajad, M.A. (2013). Phytoremediation of heavy metals-concepts and applications. *Chemosphere*, 91, 869–881.

Alkorta, I., Hern'andez-Allica, J., Becerril, J. M., Amezaga, I., Albizu, I., & Garbisu, C. (2004). Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. *Reviews in Environmental Science and Biotechnology*, 3, 71–90.

Atma, W., Larouci, M., Meddah, B., Benabdeli, K., & Sonnet, P. (2017). Evaluation of the phytoremediation potential of *Arundo donax* L. for nickel-contaminated soil. *International Journal of Phytoremediation*, 19, 377–386.

Babu, A. G., Kim, J. D., & Oh, B. T. (2013). Enhancement of heavy metal phytoremediation by *Alnus Firma* with endophytic *Bacillus thuringiensis gdb-1*. *Journal of Hazardous Materials*, 250, 477–483.

Banuelos, G. S., Ajwa, H. A., Mackey, B., Wu, L., Cook, C., Akohoue, S., & Zambruzuski, S. (1997). Evaluation of different plant species used for phytoremediation of high soil selenium. *Journal of Environmental Quality*, 26, 639–646.

Bennett, L. E., Burkhead, J. L., Hale, K. L., Terry, N., Pilon, M., & Pilon-Smits, E. A. (2000). Bioremediation and biodegradation analysis of transgenic indian mustard plants for phytoremediation of metal-contaminated mine tailings. *Journal of Environmental Quality*, 32, 432–440.

Beolchini, F., Dell'Anno, A., De Propris, L., Ubaldini, S., Cerrone, F., & Danovaro, R. (2009). Auto-and heterotrophic acidophilic bacteria enhance the bioremediation efficiency of sediments contaminated by heavy metals. *Chemosphere*, 74, 1321–1326.s

Bonanno, G., & Vymazal, J. (2017). Compartmentalization of potentially hazardous elements in macrophytes: insights into capacity and efficiency of accumulation. *Journal of Geochemical Exploration*, 181, 22-30.

Brama, M., Gnessi, L., Basciani, S., Cerulli, N., Politi, L., Spera, G., Mariani, S., Cherubini, S., d'Abusco, A. S., Scandurra, R., &Migliaccio, S. (2007). Cadmium induces mitogenic signaling in breast cancer cell by an ERα-dependent mechanism. *Molecular and cellular endocrinology*, 264, 102–108.

Brichkova, G. G., Shishlova, A. M., Maneshina, T. V, & Kartel, N. A. (2007). Tolerance to aluminum in genetically modified tobacco plants. *Cytology and Genetics*, 41, 151–155.

Brooks, R. R., & Robinson, B. H. (1998). Aquatic phytoremediation by accumulator plants. *Plants that hyperaccumulate heavy metals: Their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining.*

Brouwer, M., Hoexum-brouwer, T., & Cashon, R. E. (1993). A putative glutathione-binding site in CdZn-metallothionein identified by equilibrium binding and molecular-modelling studies. *Biochemical Journal*, 294, 219–225.

Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety*, 45, 198–207.

Carrillo-gonz, R., & Guti, M. C. (2009). Natural attenuation in a slag heap contaminated with cadmium : the role of plants and arbuscular mycorrhizal fungi. *Journal of Hazardous Materials*, 161, 1288–1298.

Chen, Y., Xu, W., Shen, H., Yan, H., Xu, W., He, Z., & Ma, M. (2013). Engineering arsenic tolerance and hyperaccumulation in plants for phytoremediation by a pvacr3 transgenic approach. *Environmental Science & Technology*, 47, 9355-9362.

Cobbett, C. S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology*, 123, 825–832.

Cunningham, S. D., & David, W. O. (1994). Promises and Prospects of Phytoremediation. *Plant physiology*, 4, 4–8.

Das, S., & Goswami, S. (2014). A study on cadmium phytoremediation potential of water lettuce, *Pistia stratiotes* L. *Bulletin of Environmental Contamination and Toxicology*, 92, 169–174.

Gu, C. S., Liu, L. Q., Deng, Y. M., Zhu, X. D., Huang, S. Z., & Lu, X. Q. (2015). The heterologous expression of the iris lactea var. chinensis type 2 metallothionein ilmt2b gene enhances copper tolerance in *Arabidopsis thaliana*. *Bulletin of Environmental Contamination and Toxicology*, 94, 247–253.

Dhankher, O. P., Li, Y., Rosen, B. P., Shi, J., Salt, D., Senecoff, J. F., Sashti, J. F., & Meagher, R. B. (2002). Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthetase expression. *Nature Biotechnology,* 20, 1140–1145.

Dobbelaere, S., Vanderleyden, J., & Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews In Plant Sciences*, 22, 107–149.

Dushenkov, S., Kapulnik, Y., Blaylock, M., Sorochisky, B., Raskin, I., & Ensley, B. (1997). Phytoremediation : a novel approach to an old problem. In *Studies in Environmental Science* (Vol. 66, pp. 563-572). Elsevier 563–572.

Dzantor, E. K., & Beauchamp, R. G. (2002). Phytoremediation, Part I: Fundamental basis for the use of plants in remediation of organic and metal contamination. *Environmental Practice*, 4, 77–87.

Ensley, B. D. (2000). Phytoremediation of toxic metals: using plants to clean up the environment. Edited by I. Raskin and B. Ensley, John Wiley & Sons, Inc., New York.

Evans, K. M., Gatehouse, J. A., Lindsay, W. P., Shi, J., Tommey, A. M., & Robinson, N. J. (1992). Expression of the pea metallothionein-like gene PsMTA in *Escherichia coli* and *Arabidopsis thaliana* and analysis of trace metal ion accumulation : Implications for PsMT A function. *Plant Molecular Biology,* 20, 1019–1028.

Evans, L. J. (1989). Chemistry of metal retention by soils. *Environmental Science & Technology*, 23, 1046-1056.

Fasani, E., Manara, A., Martini, F., Furini, A., & DalCorso, G. (2018). The potential of genetic engineering of plants for the remediation of soils contaminated with heavy metals. *Plant, Cell & Environment*, 41, 1201–1232.

Ferro, A. M., Kennedy, J., & LaRue, J. C. (2013). Phytoremediation of 1, 4-dioxane-containing recovered groundwater. *International Journal of Phytoremediation*, 15, 911–923.

Fijakowski, K., Kacprzak, M., Grobelak, A., & Placek, A. (2012). The influence of selected soil parameters on the mobility of heavy metals in soils. *Inżynieria i Ochrona środowiska*, 15, 81–92.

Foyer, C. H., & Noctor, G. (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell*, 17, 1866–1875.

Fuentes, A., Lloréns, M., Sáez, J., Soler, A., Aguilar, M. I., Ortuño, J. F., & Meseguer, V. F.  (2004). Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere,* 54, 1039–1047.

Gaitán-Solís, E., Taylor, N. J., Siritunga, D., Stevens, W., & Schachtman, D. P. (2015). Overexpression of the transporters at zip1 and at mtp1 in *Cassava* changes zinc accumulation and partitioning. *Frontiers In Plant Science,* 6, 492.

Garcia-Hernandez, M., Murphy, A., & Taiz, L. (1998). Metallothioneins 1 and 2 have distinct but overlapping expression patterns in *Arabidopsis*. *Plant Physiology*, 118, 387–397.

Gaur, A., & Adholeya, A. (2004). Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Current Science*, 528–534.

Gerhardt, K. E., Huang, X.- .D, Glick, B. R., & Greenberg, B. M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Science*, 176, 20–30.

Glass, D. J. (1999). Current market trends in phytoremediation. *International Journal of Phytoremediation*, 1, 1–8.

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology*, 41, 109-117.

Glick, B. R. (2003). Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances*, 21, 383–393.

Glick, B. R., Patten, C. L., Holguin, G., & Penrose, D. M. (1999). Biochemical and genetic mechanisms used by plant growth promoting bacteria. World Scientific.

Grison, C. M., Mazel, M., Sellini, A., Escande, V., Biton, J., & Grison, C.  (2015). The leguminous species *Anthyllis vulneraria* as a zn-hyperaccumulator and eco-zn catalyst resources. *Environmental Science and Pollution Research,* 22, 5667–5676.

Guo, J., Xu, W., & Ma, M. (2012). The assembly of metals chelation by thiols and vacuolar compartmentalization conferred increased tolerance to and accumulation of cadmium and arsenic in transgenic *Arabidopsis thaliana*. *Journal of hazardous materials*, *199*, 309-313.

Guo, P., Wang, T., Liu, Y., & Xia, Y. (2014). Phytostabilization potential of evening primrose (*Oenothera glazioviana*) for copper-contaminated sites. *Environmental Science and Pollution Research*, 21, 631-640.

Gupta, A. K., & Sinha, S. (2007). Phytoextraction capacity of the plants growing on tannery sludge dumping sites. *Bioresoures Technology* 98, 1788–1794.

Guzzi, G., & La Porta, C. A. M. (2008). Molecular mechanisms triggered by mercury. *Toxicology* 244, 1–12.

Hadi, F., & Bano, A. (2010). Effect of diazotrophs (*Rhizobium* and *Azatebactor*) on growth of maize (*Zea mays* L.) and accumulation of lead (Pb) in different plant parts. *Pakistan Journal of Botany*, 42, 4363-4370.

Hamon, R., & McLaughlin, M. (2003). Food crop edibility on the OK Tedi/Fly river flood plain. Report for OK Tedi Mining Ltd.

Hassinen, V. H., Tervahauta, A. I., Schat, H., & Karenlampi, S. O. (2011). Plant metallothioneins-metal chelators with ROS scavenging activity?. *Plant Biology,* 13, 225–232.

He, J. L., Li, H., Ma, C. F., Zhang, Y. L., Polle, A., Rennenberg, H., et al. (2014). Overexpression of bacterial c-glutamylcysteine synthetase mediates changes in cadmium influx , allocation and detoxification in poplar. *New Phytologist*, 205, 240-254.

Hinchman, R. R., Negri, M. C., & Gatliff, E. G. (1996). Phytoremediation: using green plants to clean up contaminated soil, groundwater and wastewater. (pp. 1-13). In *Proc. International Topical Meeting on Nuclear and Hazardous Waste Management*, Spectrum.

Hinsinger, P., Gobran, G. R., Gregory, P. J., & Wenzel, W. W. (2005). Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phytologist*, 168, 293–303.

Hosh, M.G., & Singh, S. P. (2005). A Review on phytoremediation of heavy metals and utilization of its by products. *Asian Journal of Energy & Environment,* 6, 214–231.

Hristozkova, M., Geneva, M., Stancheva, I., Boychinova, M., & Djonova, E. (2015). Aspects of mycorrhizal colonization in adaptation of sweet marjoram (*Origanum majorana* L.) grown on industrially polluted soil. *Turkish Journal of Biology,* 39, 461–468.

Ignatius, A., Arunbabu, V., Neethu, J., & Ramasamy, E. V. (2014). Rhizofiltration of lead using an aromatic medicinal plant *Plectranthus amboinicus* cultured in a hydroponic nutrient film technique ( NFT ) system. *Environmental Science and Pollution Research*, 21, 13007-13016.

Jadia, C. D., & Fulekar, M. H. (2009). Phytoremediation of heavy metals: recent techniques. *African Journal of Biotechnology,* 8.

Jing, Y. X., Yan, J. L., He, H. D., Yang, D. J., Xiao, L., Zhong, T., Yuan, M., Cai, X. D., & Li, S. B. (2014). Characterization of bacteria in the rhizosphere soils of polygonum pubescens and their potential in promoting growth and cd, pb, zn uptake by *Brassica napus* and their potential in promoting growth and cd, pb, zn uptake by *Brassica napus*. *International journal of phytoremediation*, 16, 321-333.

Jones, D. L., Hodge, A., & Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist,* 163, 459–480.

Kärenlampi, S., Schat, H., Vangronsveld, J., Verkleij, J. A. C., van der Lelie, D., Mergeay, M., & Tervahauta, A. I. (2000). Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environmental Pollution*, 107, 225-231.

Kathi, S. (2016). Bioenergy from phytoremediated phytomass of aquatic plants via gasification. In *Bioremediation and Bioeconomy* (pp. 111-128). Elsevier.

Khalid, S., Shahid, M., Niazi, N. K., Murtaza, B., Bibi, I., & Dumat, C. (2017). A comparison of technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration,* 182, 247–268.

Khan, W. U., Yasin, N. A., Ahmad, S. R., Ali, A., Ahmed, S., & Ahmad, A. (2017). Role of ni-tolerant *Bacillus* Spp. and *Althea rosea* L. in the phytoremediation of ni-contaminated soils. *International Journal of Phytoremediation* ,19, 470–477.

Krishna, M. P., Varghese, R., & Mohamed, A. A. (2012). Depth wise variation of microbial load in the soils of midland region of Kerala: a function of important soil physicochemical characteristics and nutrients. *Indian Journal of Education and Information Management*, 1, 126–129.

Kumar, B., Smita, K., & Cumbal, L. (2013). Plant mediated detoxification of mercury and lead. *Arabian Journal of Chemistry*, 10, 2335-2342.

Kumar, B. P. A. N., Dusheknov, V., Motto, H., & Raskin, I. (1995). Phytoextraction:the use of plants to remove heavy metals from soils. *Environmental Science &Technology,* 29, 1232–1238.

Kumar, K., Gupta, N., Kumar, A., Reece, L. M., Singh, N., Rezania, S., & Khan, S. A. (2018). Mechanistic understanding and holistic approach of phytoremediation : A review on application and future prospects. *Ecological Engineering*, 120, 274-298.

Laghlimi, M., Baghdad, B., Hadi, H. El., & Bouabdli, A. (2015). Phytoremediation mechanisms of heavy metal contaminated soils : a review. *Open Journal of Ecology*, 5, 375.

Lebeau, T., Braud, A., & Jézéquel, K. (2008). Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. *Environmental Pollution*, 153, 497-522.

Lee, S., Ji, W., Lee, W. S., Koo, N., Koh, I. H., Kim, M. S., & Park, J. S. (2014). Influence of amendments and aided phytostabilization on metal availability and mobility in Pb/Zn mine tailings. *Journal of Environmental Management*, 139, 15-21.

Li, J. T., Liao, B., Dai, Z. Y., Zhu, R., & Shu, W. S. (2009). Phytoextraction of Cd-contaminated soil by carambola (*Averrhoa carambola*) in field trials. *Chemosphere,* 76, 1233–1239.

Li, T. Q., Yang, X. E., & Long, X. X. (2004). Potential of using *Sedum alfredii* Hance for phytoremediating multi-metal contaminated soils. *Journal of Soil and Water Conservation,* 18, 79–83.

Limmer, M., & Burken, J. (2016). Phytovolatilization of organic contaminants. *Environmental Science & Technology*, 50, 6632-6643.

Liu, D., An, Z., Mao, Z., Ma, L., & Lu, Z. (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.

Liu, J., Shi, X., Qian, M., Zheng, L., Lian, C., Xia, Y., & Shen, Z. (2015). Copper-induced hydrogen peroxide upregulation of a metallothionein gene, osmt2c, from *Oryza sativa* L. confers copper tolerance in *Arabidopsis thaliana*. *Journal of Hazardous Materials,* 294, 99–108.

Liu, W., Wang, Q., Wang, B., Hou, J., Luo, Y., Tang, C., & Franks, A. E. (2015). Plant growth-promoting rhizobacteria enhance the growth and cd uptake of Sedum plumbizincicola in a cd-contaminated soil. *Journal of Soils And Sediments*, 15, 1191–1199.

Long, X. X., Chen, X. M., Wong, J. W. C., Wei, Z. B., & Wu, Q. T. (2013). Feasibility of enhanced phytoextraction of zn contaminated soil with zn mobilizing and plant growth promoting endophytic bacteria.” *Transactions of Nonferrous Metals Society of China*, 23, 2389–2396.

Lorestani, B., Cheraghi, M., & Yousefi, N. (2011). International journal of phytoremediation the potential of phytoremediation using hyperaccumulator plants: a case study at a lead-zinc mine site. *International Journal of Phytoremediation*, 14, 786-795.

Lotfy, S. M., & Mostafa, A. Z. (2018). Phytoremediation of contaminated soil with cobalt and chromium Phytoremediation of contaminated soil with cobalt and chromium. *Journal of Geochemical Exploration*, 144, 367-373.

Ma, Y., Rajkumar, M., Luo, Y., & Freitas, H. (2011). Inoculation of endophytic bacteria on host and non-host plants-effects on plant growth and Ni uptake. *Journal of Hazardous Materials,* 195, 230–237.

Mahar, A., Wang, P., Ali, A., Awasthi, M. K., Lahori, A. H., Wang, Q., Li, R., & Zhang, Z. (2016). Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. *Ecotoxicology and Environmental Safety*, 126, 111-121.

Mallmann, F. J. K., dos Santos Rheinheimer, D., Ceretta, C. A., Cella, C., Minella, J. P. G., Guma, R. L., Filipović, V., Oort, F. V.,& Šimůnek, J. (2014). Soil tillage to reduce surface metal contamination–model development and simulations of zinc and copper concentration profiles in a pig slurry-amended soil. *Agriculture, Ecosystems & Environment*, 196, 59-68

Marschner, H. (2011). Marschner's mineral nutrition of higher plants. Academic press.

Martinez, V. D., Vucic, E. A., Becker-Santos, D. D., Gil, L., & Lam, W. L. (2011). Arsenic exposure and the induction of human cancers. *Journal of Toxicology,* 2011.

Maser, P., Thomine, S., Schroeder, J. I., Ward, J. M., Hirschi, K., et al. (2001). Phylogenetic relationships within cation transporter families of *Arabidopsis*. *Plant Physiology*, 126, 1646-1667.

Massanyi, P., Lukáč, N., Uhrin, V., Toman, R., Pivko, J., et al. (2007). Female reproductive toxicology of cadmium. *Acta Biologica Hungarica*, 58, 287-299.

Mccormack, M. L., & Guo, D. (2014). Impacts of environmental factors on fine root lifespan. *Frontiers In Plant Science*, 5, 205.

Melo, E. E. C., Costa, E. T. S., Guilherme, L. R. G., Faquin, V., & Nascimento, C. W. A. (2009). Accumulation of arsenic and nutrients by castor bean plants grown on an As-enriched nutrient solution. *Journal of Hazardous Materials*, 168, 479–483.

Merkl, N., Schultze-Kraft, R., & Infante, C. (2005). Phytoremediation in the tropics-influence of heavy crude oil on root morphological characteristics of graminoids. *Environmental Pollution*, 138, 86–91.

Merlot, S., Hannibal, L., Martins, S., Martinelli, L., Amir, H., Lebrun, M., & Thomine, S. (2014). The metal transporter pgireg1 from the hyperaccumulator *Psychotria gabriellae* is a candidate gene for nickel tolerance and accumulation. *Journal of Experimental Botany*, 65, 1551–1564.

Migeon A, Blaudez, D., Wilkins, O., Montanini, B., Campbell, M. M., et al. (2010). Genome-wide analysis of plant metal transporters, with an emphasis on poplar. *Cellular and Molecular Life Sciences*, 67, 3763–3784.

Misra, S., & Gedamu, L. (1989). Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants. *Theoretical and Applied Genetics,* 78, 161–168.

Mitch, M. L. (2002). Phytoextraction of toxic metals: a review of biological mechanism.*Journal of Environmental Quality*, 31, 109–120.

Morikawa, H., & Erkin, O. C. (2003). Basic processes in phytoremediation and some applications to air pollution control. *Chemosphere,* 52, 1553–1558.

Neagoe, A., Stancu, P., Nicoară, A., Onete, M., Bodescu, F., Gheorghe, R., & Iordache, V. (2014). Effects ofArbuscular mycorrhizal fungi on *Agrostis capillaris* grown on amended mine tailing substrate at pot, lysimeter, and field plot scales. *Environmental Science and Pollution Research*, 21, 6859-6876.

Neuberg, M. (2012). Removal of Al, Fe and Mn by *Pistia stratiotes* L. and its stress response. *Open Life Sciences*, 7, 1037-1045.

Nikolic, M., & Stevovic, S. (2015). Family *Asteraceae* as a sustainable planning tool in phytoremediation and its relevance in urban areas. *Urban Forestry & Urban Greening*,14, 782–789.

Otten, A. M., Alphenaar, A., Pijls, C., Spuij, F., & De Wit, H (1997). How to Apply in Situ Soil Remediation. In: In *situ soil remediation* (pp. 26-58). Springer Science & Business Media.

Ouyang, Y. (2002). Phytoremediation: modeling plant uptake and contaminant transport in the soil-plant-atmosphere continuum. *Journal of Hydrology*, 266, 66–82.

Padmavathiamma, P. K., & Li, L.Y. (2007). Phytoremediation technology: hyper-accumulation metals in plants. *Water, Air, and Soil Pollution*, 184, 105–126.

Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L., Lasat, M. M., et al. (2000). The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences*, 97, 4956–4960.

Peng, J. S., Ding, G., Meng, S., Yi, H. Y., & Gong, J. M. (2017). Enhanced metal tolerance correlates with heterotypic variation in spmtl, a metallothionein-like protein from the hyperaccumulator *Sedum* *plumbizincicola.Plant, Cell & Environment*, 40, 1368–1378.

Pichtel, J., & Anderson, M. (1997). Trace metal bioavailability in municipal solid waste and sewage sludge composts. *BioresourceTechnology*, 60, 223–229.

Pilon, M., Owen, J. D., Garifullina, G. F., Kurihara, T., Mihara, H., Esaki, N., & Pilon-Smits, E. A. (2003). Enhanced selenium tolerance and accumulation in transgenic *Arabidopsis* expressing a mouse selenocysteine lyase 1. *Plant Physiology*, 131, 1250–1257.

Qian, J. I. N., Shan, X., Wang, Z., & Tu, Q. (1996). Distribution and plant availability of heavy metals in different particle-size fractions of soil. *Science of the Total Environment*, 187, 131–141.

Rafati, M., Khorasani, N., Moattar, F., Shirvany, A., Moraghebi, F., & Hosseinzadeh, S. (2011). Phytoremediation potential of *Populus alba* and *Morus alba* for cadmium, chromuim and nickel absorption from polluted soil. *International Journal of Environmental Research*, 5, 961–970.

Rakhshaee, R., Giahi, M., & Pourahmad, A. (2009). Studying effect of cell wall's carboxyl-carboxylate ratio change of Lemna minor to remove heavy metals from aqueous solution. *Journal of Hazardous Materials*, 163, 165–173.

Rodriguez, L., Lopez-Bellido, F. J., Carnicer, A., Recreo, F., Tallos, A., & Monteagudo, J. M. (2005). Mercury recovery from soils by phytoremediation. In *Environmental Chemistry* (pp. 197-204). Springer, Berlin, Heidelberg.

Romeh, A. A., & Khamis, M. A. (2016). Potential of *Plantago major* L. for phytoremediation of lead-contaminated soil and water. doi: 10.1007/s11270-015-2687-9.

Romeiro, S., Lagoa, A. M. M. A., Furlani, P. R., de Abreu, C. A., de Abreu, M. F., Erismann, N. M. (2006). Lead uptake and tolerance of *Ricinus communis* L. *Brazilian Journal of Plant Physiology*, 18, 483–489.

Ross, S. M. (1994). Toxic metals in soil-plant systems. (p. 469). Chichester: Wiley.

Saad-Allah, K. M., & Elhaak, M. A. (2017). Hyperaccumulation activity and metabolic responses of *Solanum nigrum* in two differentially polluted growth habitats. *Journal of the Saudi Society of Agricultural Sciences*, 16, 227-235.

Salt, D. E., Blaylock, M., Kumar, N. P. B. A., Dushenkov, V., Ensley, B. D., Chet, I., & Raskin, I. (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Bio/technology*, 13, 468–474.

Sanders, T., Liu, Y., Buchner, V., & Tchounwou, P. B. (2009). Neurotoxic effects and biomarkers of lead exposure: a review. *Reviews on Environmental Health*, 24, 15-46.

Santana, N. A., Ferreira, P. A. A., Soriani, H. H., Brunetto, G., Nicoloso, F. T., Antoniolli, Z. I., & Jacques, R. J. S. (2015). Interaction between Arbuscular Mycorrhizal fungi and vermicompost on copper phytoremediation in a sandy soil. *Applied Soil Ecology*, 96, 172–182.

Sarma, H. (2011). Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *Journal of Environmental Science and Technology*, 4, 118–138.

Schnoor, J. L. (1997). Phytoremediation. Technology evaluation report. *Ground-Water Remediation Technologies Analysis Center. E Series TE-98-101.*

Schwitzguebel, J.- P. (2002). Hype or hope: the potential of phytoremediation as an emerging green technology. *Federal Facilities Environmental Journal*, 13, 109–125.

Shabani, L., & Sabzalian, M. R. (2016). Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in *Festuca arundinacea*. *Mycorrhiza*, 26, 67-76.

Sheng, X. F., Xia, J. J., Jiang, C. Y., He, L. Y., & Qian, M. (2008). Characterization of heavy metal-resistant endophytic bacteria from rape ( *Brassica napus* ) roots and their potential in promoting the growth and lead accumulation of rape. *Environmental Pollution*, 156,1164–1170.

Sheoran, V., Sheoran, A. S., & Poonia, P. (2016). Factors affecting phytoextraction : a review. *Pedosphere*, 26, 148–166.

Sherene, T. (2010). Mobility and transport of heavy metals in polluted soil environment. In *Biological forum—an international journal* (Vol. 2, No. 2, pp. 112-121).

Shtangeeva, I., Laiho, J. V. P., Kahelin, H., & Gobran, G. R. (2004). Phytoremediation of metal-contaminated soils. In: *Symposia Papers Presented Before the Division of Environmental Chemistry, American Society Anaheim*, Calif, USA.

Singh, S. (2012). Phytoremediation: a sustainable alternative for environmental challenges.*InternationalJournal ofGreenand HerbalChemistry,* 1, 133–139.

Sinha, V., Manikandan, N. A., Pakshirajan, K., & Chaturvedi, R. (2016). International Biodeterioration & Biodegradation Continuous removal of Cr (VI) from wastewater by phytoextraction using *Tradescantia pallida* plant based vertical subsurface flow constructed wetland system. *International Biodeterioration & Biodegradation*, 119, 96-103.

Smolinska, B., & Rowe, S. (2015). The potential of *Lepidium sativum* L. for phytoextraction of Hg-contaminated soil assisted by thiosulphate. *Journal of Soils and Sediments,* 15, 393–400.

Soudek, P., Petrová, Š., & Vaněk, T. (2012). Phytostabilization or accumulation of heavy metals by using of energy crop *Sorghum* sp. In *3rd international conference on biology, environment and chemistry IPCBEE. IACSIT Press, Singapore*.

Sposito, G. (1983). The chemical forms of trace metals in soils. *Applied Environmental Geochemistry,* 123–170.

Suresh, B., & Ravishankar, G. A. (2004). Phytoremediation-a novel and promising approach for environmental clean-up. *Critical Reviews In Biotechnology*, 24, 97–124.

Tack, F. M. G., Vervaeke, P., Meers, E., & Vandecasteele,B.(2005). Phytoremediation/stabilisation of dredged sediment derived soils with willow. In *Geophysical Research Abstracts* (Vol. 7, p. 08101).

Tangahu, B. V., Abdullah, S., Rozaimah, S., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, 2011.

Tennstedt, P., Peisker, D., Böttcher, C., Trampczynska, A., & Clemens, S. (2009). Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. *Plant Physiology,* 149, 938-948.

Terry, N., Zayed, A. M., De Souza, M. P., & Tarun, A. S. (2000). Selenium in higher plants. *Annual Review of Plant Biology,* 51, 401–432.

Vamerali,T., Bandiera, M., & Mosca, G. (2010). Field crops for phytoremediation of metal-contaminated land. a review. *Environmental Chemistry Letters*, 8, 1–17.

Van Ginneken, L., Meers, E., Guisson, R., Ruttens, A., Elst, K., et al. (2007). Phytoremediation for heavy metal-contaminated soils combined with bioenergy production. *Journal of Environmental Engineering and Landscape Management*, 15, 227-236.

Vatamaniuk, O. K., Mari, S., Lu, Y. P., & Rea, P. A. (1999). AtPCS1, a phytochelatin synthase from *Arabidopsis*: isolation and in vitro reconstitution. *Proceedings of the National Academy of Sciences*, 96, 7110–7115.

Verma, V. K., Singh, Y. P., & Rai, J. P. N. (2007). Biogas production from plant biomass used for phytoremediation of industrial wastes. *BioresourceTechnology*, 98, 1664–1669.

Wang, G.- D., & Chen, X.- Y. (2007). Detoxification of soil phenolic pollutants by plant secretory enzyme. In *Phytoremediation* (pp. 49-57). Humana Press.

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *Isrn Ecology*, 2011.

Yang, J., Yang, J., & Huang, J. (2017a). Role of co-planting and chitosan in phytoextraction of As and heavy metals by *Pteris vittata* and *castor bean*–A field case. *Ecological engineering*, 109, 35–40..

Yang, J., Zheng, G., Yang, J., Wan, X., Song, B., Cai, W., & Guo, J. (2017b). Phytoaccumulation of heavy metals (Pb , Zn , and Cd) by 10 wetland plant species under different hydrological regimes.*Ecological engineering*, 107, 56–64.

Yang, L. (2008). Phytoremediation: an ecotechnology for treating contaminated sites. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, 12, 290-298.

Yang, Z., & Chu, C. (2011). Towards understanding plant response to heavy metal stress. In: *Abiotic stress in plants–Mechanisms and adaptations*, 10, 24204.

Yau, Y. Y., & Stewart, C. N. (2013). Less is more: strategies to remove marker genes from transgenic plants. *BMC biotechnology*, 13, 36.

Yoon, R. K. J., Jae, T. K., Gary, E. Y., & Kim, K. (2015). Bioavailability of heavy metals in soils: definitions and practical implementation — a critical review. *Environmental Geochemistry and Health*, 37, 1041–1061.

Zaidi, S., Usmani, S., Singh, B. R., & Musarrat, J. (2006). Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. *Chemosphere*, 64, 991–997.

Zhang S, Li, T., Huang, H., Zou, T. & Zhang, X.l. (2012). Cd accumulation and phytostabilization potential of dominant plants surrounding mining tailings. *Environmental Science and Pollution Research*, 19, 3879-3888.

Zhang, W., Pang, F., Huang, Y., Yan, P., & Lin, W. (2008). Cadmium exerts toxic effects on ovarian steroid hormone release in rats.*Toxicology Letters*, 182, 18-23.

Zhu, Y. L. (1999). Overexpression of glutathione synthetase in *Brassica juncea* enhances cadmium tolerance and accumulation. *Plant Physiology,* 119, 73–79.