J. Mt. Sci. (2019) 16(9): 2079-2095 e-mail: jms@imde.ac.cn http://jms.imde.ac.cn
https://doi.org/10.1007/s11629-017-4752-x

Recent research progress in geochemical properties and restoration of heavy metals in contaminated soil by phytoremediation

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Citation: Fu, JT, Yu DM, Chen X, et al. (2019) Recent research progress on geo-chemical properties of heavy metals and their restoration in contaminated soil by phytoremediation. Journal of Mountain Science 16(9). https://doi.org/10.1007/s11629-017-4752-x

Abstract: Heavy metals are widely distributed contaminants in natural environments and their potential threats to human health have attracted worldwide concerns due to the food chain. Therefore, great efforts have been made to reduce them to a safe level in soil. Compared with the traditional methods, the method using plants to remove them has been accepted as a feasible and efficient way. Herein, the geochemical behavior of heavy metals and the restoration methods with phytoremediation were reviewed. In addition, issues on heavy metal speciation as well as its influencing factors, phytoremediation mechanism, phytoremediation effect and vegetation selection principle used for phytoremediation were discussed.

Received: 9 November 2017
1st Revision: 11 April 2018
2nd Revision: 27 May 2018
Accepted: 12 July 2018

Keywords: Heavy metals; Geochemical properties; Phytoremediation; Hyperaccumulator

Introduction

Heavy metals (Hg, Cd, Pb, As, Cu, Zn, Sn, Cr, etc) are defined as metals with density over 5 g/cm^3 (Hu et al. 2015; Jiang et al. 2015; Li and Zu 2016; Bhunia et al. 2018; Alvarez-Vázquez et al. 2019; Asharf et al. 2019). Amongst them, Hg, Cd, Pb, and As are non-essential heavy metals for animals and vegetation, while Cu and Zn are essential heavy metals. Heavy metals have adverse effects on animal health even at a lower level (Tepanosyan et al. 2017). For example, heavy metals make vegetation produce reactive oxygen species (ROS), such as superoxide radicals, peroxide and hydroxyl ion (Shahzad et al. 2018). ROS has destructive effects on membranes and other macromolecules through lipid peroxidation (Shahzad et al. 2018). Furthermore, heavy metals can replace essential metals in cells and enzymes, leading to severe physiological and metabolic dysfunction and even vegetation death (Nwaichi & Dhankher 2016).

Heavy metals have long-term toxicity to biosphere and can not be eliminated by biodegradation. Thus, governments are taking measures to remedy heavy metal-contaminated lands to reduce their toxic effects to an accepted level (Sarwar et al. 2017; Asharf et al. 2019). The measures used to remedy heavy metal-contaminated soils include soil replacement, chemical immobilization, soil leaching and other traditional ways. However, these traditional methods have many drawbacks, such as high cost, low efficiency, secondary contaminations, disturbance to soil texture, and damages to local environment (Salt et al. 1995; Vangronsveld et al. 1995; Wieshammer et al. 2007; Marques et al. 2009; Weber et al. 2011; Koptsik 2014; Huang et al. 2015). Recently, scholars tend to use vegetation to remedy contaminated soils, namely phytoremediation (Davidson et al. 2009; Vamerali et al. 2010; Laghlimi et al. 2015; Nwaichi et al. 2016). Phytoremediation refers to the removal of pollutants from contaminated soils or degradation of pollutants to non-hazardous matters by plants growing in contaminated lands (Teofilo et al. 2010), as illustrated in Figure 1. Phytoremediation includes phytoextraction, phytostabilisation, phytotransformation, and phytofiltration (Vamerali et al. 2010; Antonella et al. 2014; Laghlimi et al. 2015). Phytoextraction refers to that heavy metals are absorbed and concentrated by plant roots, thus reducing the levels of heavy metals in contaminated soils (Laghlimi et al. 2015). Phytostabilization or phytostabilisation refers to that the mobility or bioavailability of heavy metals is reduced by plants growing in heavy metal contaminated soils to avoid infiltrating into water to pollute groundwater and food chains, or heavy metals with higher toxicity are transformed into another speciation with lower toxicity by plants (Koptsik 2014; Laghlimi et al. 2015). Phytotransformation refers to that plants absorb contaminants from their growth environments and

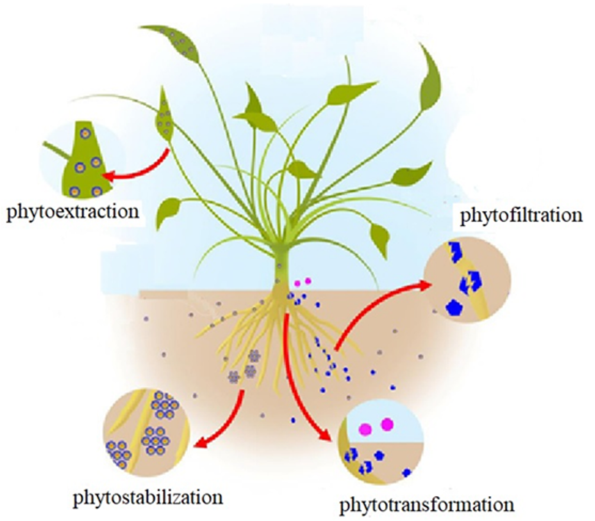

Figure 1 Schematic representation of phytoremediation approaches (modified from Parmar and Singh 2015) Phytostabilization: the immobilization or precipitation of contaminants from heavy metal contaminated soil, thus decreasing their availability; Phytofiltration: uses plant roots to absorb contaminants from their growing environment; Phytoextraction: the uptake and concentration of metals from contaminated soil into the plant tissue and their subsequent removal from the plants; Phytotransformation: the uptake of contaminants by plants from their growing environments and conversion into organic compounds with less toxic or nontoxic effects.

convert them into organic compounds with less toxicity or non-toxicity (Parmar and Singh 2015). Phytofiltration refers to the removal of environmental pollutants by plants or microorganisms related rhizosphere (Anamika et al. 2015; Laghlimi et al. 2015).

The objective of the paper is to summarize recent findings and progresses in phytoremediation. Based on these newly published studies, new research status and progress were discussed. Moreover, some views on the geochemical behavior of heavy metals including speciation, phytoremediation mechanism, and phytoremediation plant selection were proposed. This review can be used as a handbook for the researchers in this area.

1 Heavy Metal Speciation and Influencing Factors

1.1 Heavy metal speciation

Total heavy metal concentration can be used as an index to partially reflect heavy metal contaminations in environment due to its poor reflection in mobility and implication in environmental quality assessment (Rao et al. 2008; Nemati et al. 2011). Generally, the properties of environmental heavy metals are mainly dependent on their speciation, and every species is accompanied with their reactions, rather than on the total heavy metal concentration (Kang et al. 2017). Thus, the properties of heavy metals are not largely determined by its total content in soil but by their speciation. Heavy metal speciation in soil is divided into exchangeable, carbonate, iron and manganese oxides, organic and residual speciation, respectively (Sundaray et al. 2011; Pizarro et al. 2016). Exchangeable speciation is easily transferred and absorbed by plants, and tends to be absorbed to clay, humus and other components. Carbonate speciation is sensitive to pH because heavy metals are precipitated on carbonate to form insoluble sediments, which are sensitive to acid. Heavy metals in iron and manganese oxides are stable and not easily absorbed by plants (Zhang et al. 2014), because their larger specific surface area had excellent capacity in absorbing heavy metals. Under oxidation conditions, some organic matters in heavy metal-organic speciation are degraded and some heavy metals combined with organic matters are dissolved. Residual speciation is relatively stable, because heavy metals exist in the lattices of silicate, original and secondary mineral in soil (Rao et al. 2008; Li and Zu 2016). Considering the abundance of every speciation, the mobility of heavy metals exhibit the following orders: exchangeable > carbonate > iron and Mn oxides > organic > residual (Ashraf et al. 2012).

1.2 The influencing factors of heavy metals speciation in soils

Due to the influence of various factors on heavy metal speciation, the existing forms of heavy metals in soil are much more complex (Zhang et al. 2014). These factors include total heavy metal content in soil, soil pH, soil organic matter content, soil texture, cation exchange capacity, redox potential, the interaction between elements and microorganism (Zhang et al. 2014; Qian et al. 1996). The correlation coefficient between heavy metal speciation and the total heavy metal content shows that the bioavailability of heavy metals to plants increase with the increase of total heavy metal content (Li and Zu 2016). For example, the residual speciation of Cr, Cu, Ni, Pb, Zn and Co, water soluble speciation, carbonate sediment speciation, iron and manganese oxides and residual speciation of Pb, Cd and Zn are positively correlated with their total content (Zhang et al. 2014; Li and Zu 2016). Soil pH plays an important role in the formation of heavy metal in carbonate speciation, because the increase of soil pH increases negative charge on clay surface, hydrous oxid, humic matters and other components, thus increases the absorption of heavy metals, and finally decreases the levels of heavy metals in soil (Bradl et al. 2004; Zhang et al. 2014). The increase of soil pH increases the stability of soil humus - metal complex in soil, and ultimately reduces soluble heavy metal. On the contrary, the decrease of pH leads to the dissolution of carbonate, releasing heavy metals precipitated on carbonate into soil, and H_2CO_3 produced form metabolism and other acid materials further reduce soil pH, therefore promoting the absorption of heavy metal by plants (Li and Zu 2016). For example, Park et al. (2013) found that humic acid increases the dissolubility of heavy metals in combined speciation and ultimately increases the mobility and solubility of heavy metals by adding humic acid to soil contaminated by Pb, Cu, Co and Ni. Zhong et al. (2009) observed that acid rain promotes transformation of Cd in soil from residual speciation to exchangeable speciation.

Soil organic matters refer to all types of organic matter, such as animal or plant residues, microorganisms as well as organic matter synthesized or decomposed by microorganisms. Soil organic matters interact with heavy metals in soil to form compounds, thus affect the mobility and bioavailability of heavy metals in soils (Różański et al. 2018; Shan et al. 2019). Humus is the main component of organic matters. The increase of humus leads to transformation of heavy metals from dissolved state to organic speciation, and heavy metals in organic speciation are released into soil because organic matters are degraded under strong oxidation conditions, increasing the mobility and bioavailability of heavy metals in soil (Rieuwerts et al. 1998). For example, Tong et al. (2014) analyzed the effect of organic fertilizers on

Pb speciation by applying organic fertilizer to Pb-contaminated soil, and found that proper dose of organic fertilizers reduce Pb concentration in exchangeable speciation and carbonate speciation while increase Pb concentration in iron and manganese oxides, organic sulfide complex and residual speciation. Moreover, Chao et al. (2012) demonstrated that biochar in soil can convert exchangeable heavy metals into reducible, oxidizable, and residual speciation. The reason is attributed to that some soil acidic materials are neutralized by biochar and alkaline groups (like OH^-, SiO_3^{2-}, and CO_3^{2-}) in soil solution, which promote the formation of hydroxide, silicate and carbonate of insoluble heavy metals and decline the concentration of exchangeable heavy metals. Moreover, similar conclusions have been obtained by Zhu et al. (2015) and Abdel-Fattah et al. (2015).

In terms of cation exchange capacity (CEC) and soil texture, soil CEC is mainly related to negative charge on soil colloid surface, which is correlated with clay (particle) content, humus content and soil pH (Rieuwerts et al. 1998; Ashraf et al. 2012; Kang et al. 2017; Shan et al. 2019). Pb concentration in water soluble, organic, residual speciation and total Pb concentration in soil are positively correlated with soil CEC (Zu and Li 2016) as well as Cd and Zn in carbonate speciation. Moreover, Cu concentration in residual speciation and total Zn concentration are significant positively correlated with clit content in soil, Cd concentration in iron and manganese oxide speciation, and clay content in soil (Fernandes 1997).

In terms of redox potential, heavy metal speciation in sediments changes from one speciation to another under different redox potential conditions. For example, Cd in organic speciation is relatively stable compared with that in other speciation under deoxidation condition, while it can be converted into water soluble state, exchangeable speciation, or dissolved combined state under oxidizing conditions, which can be easily absorbed by plants (Fernandes 1997; Li and Zu 2016).

In terms of the interaction between elements, the interaction between elements and ions leads to the change of bioavailability of heavy metals, thus affecting the absorptivity of heavy metals. For example, Wang et al. (2011) analyzed the behavior of Cu and Cr in Cu and Cr contaminated soil, and found that Cu at low level transforms Cr to exchangeable speciation, and Cr at low level transforms Cu to residual speciation, while high concentration of Cr or Cu leads to the opposite effect. Moreover, Cd inhibits N uptake, absorption, transport, translocation, and metabolization, resulting nutrient deficiency in plants, and Cr, Cd and Co inhibit the absorption and uptake of Cu to plants (Li and Zu 2016).

In terms of microorganism, Tabak et al. (2005) found that microorganisms can convert dissolved Cr into sedimentary $Cr(OH)_3$.

2 Mechanism of Phytoremediation in Heavy Metal-Contaminated Soil

2.1 The bioavailability of heavy metals

The bioavailability of heavy metals is one of the research hotspots of reclamation of heavy metal contaminated lands (Olaniran et al. 2013). The absorption of heavy metal is the first step of phytoremediation, and largely depends on the bioavailability of heavy metals in soil (Olaniran et al. 2013; Park et al. 2013). Bioavailability means that heavy metals have the property of speciation, which can be easily absorbed by plants or has toxic effect on plants (Park et al. 2013). Higher bioavailability of heavy metals not only means higher absorptivity and toxicity to living things, but also is essential for successful phytoremediation (Rajkumar et al. 2009). Actually not all the heavy metals can be absorbed by plants, but only a fraction of heavy metals with certain speciation can be absorbed by plants, especially heavy metals with elevated bioavailability (Olaniran et al. 2013). Related studies have shown that bioavailability depends on total heavy metal concentration, physical and chemical attributes, valence state, additive, and pH (Rieuwerts et al. 1998).

The increase of total heavy metal concentration leads to the increase of bioavailability (Zhong et al. 2008; Wang et al. 2011; Simposon 2012; Zhang et al. 2014). For example, Zhong et al. (2008) demonstrated that total concentration of Cd is linearly correlated with its bioavailability, and Wang et al. (2011) reported the highly significant correlation between bioavailablility of Cd, Pb and As and their total concentrations. However, some scholars have doubted this review. For example, Rieuwerts et al. (1998) believed that the increase of total heavy metals does not always mean the increase of bioavailability, which depends on the ratio of their solid phase to liquid phase, consistent with the results of Alexander (2006), Alvarenga et al. (2009) and Marcin et al. (2014). Thus, the contradictory view needs to be further investigated.

In terms of physical and chemical attributes, heavy metals in water soluble speciation with ionic state and soluble organic complex have the highest bioavailability, followed by those in iron and manganese oxide speciation, and those in residual speciation have the lowest bioavailability (Tong et al. 2014). Moreover, the bioavailability of heavy metals is related to their molecular form (Reeder et al. 2006). For example, the bioavailability of trivalent Cr that is largely precipitated in soil, is lower than that of hexavalentv Cr mainly in chromate (Hossner et al. 1998; Reeder et al. 2006). Hungerford and Linder (1983) discovered that ferrous ion has higher bioavailability compared with ferric ion.

In terms of additives such as chelating agents and exogenous fertilizers, Suthar et al. (2014) found that the bioavailability of Pb and Cd is significantly increased after the application of exogenous ethylenediaminetetraacetic acid (EDTA) to Pb and Cd contaminated soil contaminated. Huang et al. (2014) investigated the effect of exogenous Pb on its mobility and bioavailability, and confirmed that exogenous Pb is active and has potential bioavailablity for plants. Teofilo et al. (2010) reported that low molecular weight organic acids can improve the solubility and bioavailability of heavy metal due to its heavy metal chelating properties.

In terms of plant species, Pizarro et al. (2016) investigated the bioavailability of trivalent and tetravalent As in carrot (*Daucus carota*), quinoa (*Chenopodium*) and beet (*Beta vulgaris*), and found that the bioavailability of As in *D. carota* and *B. vulgaris* is 100%, and that of *Chenopodium* is 40%.

In terms of the influence of pH on the bioavailability of heavy metals, scholars believe that variation of pH affects the speciation of heavy metals and further affects the absorptivity of heavy metals by plants (Rieuwerts et al. 1998). For example, Lu and Yan (2007) investigated the effect of humic acid secreted by *Kandelia candel* L. roots on the bioavailability of Cu, Zn, Pb and Cd, and found that heavy metal concentration in rhizosphere sediment is larger than that in non-rhizosphere sediment, which is attributed to that low molecular weight of humic acid secreted by root of *K. candel*, especially citric acid can accelerate the dissolution of heavy metals.

2.2 Mechanism of the absorption of heavy metals by plants

2.2.1 The absorption of heavy metals by plants

Apoplast absorption and infiltration into cell membrane are the main pathways for heavy metals to migrate from root system surface to its inside (Li and Zu 2011). Apoplast absorption refers to the radial migration of heavy metals through the channel between cell wall and intercellular space penetrating cortex and cortical sediments. Seregin et al. (2004) observed that Cd and Pb radially transport via the appoplast in maize roots. Infiltration of heavy metal across plasma membrane into cell refers to that heavy metals infiltrate across cytoplasm aggregate and then sediment in cell. Wu et al. (2005) investigated the absorption characteristics of heavy metal sensitive barley and heavy metal tolerant barley to Cd, and found that Cd content in cytoplasm of sensitive barley is significantly higher than that of tolerant barley, and Cd content in cell wall of tolerant barley is relatively higher than that of sensitive barley. Based on the absorption characteristic of heavy metal by roots, plant absorption of heavy metals can be divided into three groups, including heavy metal excluder, heavy metal indicator and heavy metal accumulator (Memon and Schröder 2009). Heavy metal excluder can inhibit the transfer of heavy metals from the underground parts to the aboveground parts. For example, *Oxyira isnensis* Hemsl can survive normally in Cd, Pb and Zn contaminated soil, heavy metals content in the underground part is lower than that in the aboveground part, and heavy metals in the growing soil is higher than that in the underground parts (Li et al. 2013). Heavy metal indicator can accumulate heavy metals in the aboveground part with the concentration in the aboveground part approximately equal to that in the growth environment. Therefore, heavy metal concentration in the indicator planted environment can be reflected by determining heavy metals in the aboveground part of heavy metal indicator. Heavy metal accumulating plants, namely hyperaccumulators, can accumulate heavy metals in the aboveground part, and heavy metal level in the aboveground part is higher than that in the growth environment (Memon and Schröder 2009). Danh et al. (2014) found that *Pteris vittata* (Chinese brake fern) can accumulate and store arsenate and arsenite, most of which are translocated in leaves with up to 9677 mg/kg dry weight while only a small part remain in roots.

2.2.2 Mechanisms of plant tolerance to heavy metal stress

Exposed to heavy metal contaminated environment for a long time, plants have evolved a set of mechanism to alleviate and detoxify the adverse and toxic effects of heavy metals to continue their normal growth, physiological, metabolic behavior. The alleviation and detoxification mechanisms of plant response to heavy metals can be divided into the following aspects, including absorption rejection, combination-inactivation, compartmentalization, and metabolic detoxification.

Absorption rejection: Absorption rejection refers to that heavy metals in soil are immobilized in the environment by rhizosphere derived from metabolic process, thus reducing the absorption of heavy metal to plants (Hashimoto et al. 2011). Absorption rejection of plants is related to rhizospheric microorganisms, and root exudates that are responsible for decreasing the absorption of heavy metals. Absorption rejection is also related to plant species, heavy metal speciation and soil attributes. Vogel-Mikus et al. (2006) found that arbuscular mycorrhizae fungi (AMF) can alleviate the adverse effect of Cd on plant growth. Other studies have shown that mycorrhizal fungi can reduce metal penetration into plants cells by releasing metal chelating agents, such as citric acid and oxalic acid, siderophores, phenolic compounds and phosphate ions, absorbing heavy metals on surface of fungal cells through sedimentation of sulphides and hydrated iron oxides, immobilizing and accumulating inside fungal structures, arbuscules, vesicles and hyphae (Miransari 2011).

Combination-inactivation: Combination-inactivation refers to the inactivation of heavy metals absorbed by plants through binding to cell wall or cell membrane. Cell wall binding refers to the binding of heavy metals with main components of cell wall such as pectin, cellulose, semi-cellulose and lignin. Mehes-Smith et al. (2013) reported that a great number of heavy metals accumulate in cell wall, due to the binding of divalent metal cation and trivalent metal cation with functional groups, such as -COOH, -OH and -SH in plant cells. Jiang and Liu (2010b) confirmed that -OH in pectin can bind to Pb in cells to tolerate Pb toxicity. Similar results have been reported by Bringezu et al. (1999), Meyers et al. (2008), Solanki and Dhankhar (2011), Colzi et al. (2012) and De et al. (2012).

Cell membrane is selective in heavy absorption. The extent of heavy metals entering cell is decreased through the combination-inactivation effect of plants, and heavy metals are absorbed from plants to reduce the effect of heavy metals on metabolic behavior through transportation by cation-efflux transport proteins in plasmalemma (Huang and Xin 2013). For example, heavy metals infiltrate into cell wall to bind to pectin, amino acid, saccharides, and carboxyl, amidogen, hydrosulphonyl and phenolic groups in cell membranes, forming stable chelates (Yuan et al. 2007). Steffens (1990) found that polypeptide can bind heavy metals (Zn and Cu) to form metal-bind polypeptide to reduce the toxicity of heavy metals.

Compartmentalization/sequestration: Compartmentalization also named sequestration is another way for plants to avoid or tolerate heavy metal stress. Compartmentalization refers to that the absorbed heavy metals are transported to some certain positions in plants to be separated from active target molecules to avoid cell damage (Li and Zu 2016). Compartmentalization can be achieved in vacuole and epidermis cell, subepidermal cell layer, and epitrichoderm (Leitenmaier and Küpper 2013; Li and Zu 2016). For example, Davies et al. (1991) found the increase of Zn in vacuole in *Festuca rubra* roots. Krämer et al. (2000) observed that *Thlaspi goesingense* is a Ni hyperaccumulator and can accumulates nearly two times more Ni in its vacuole than the non-accumulator under Ni exposure conditions. Chen et al. (2006) reported that *Pteris vittata* is a As accumulator, and accumulates most of the absorbed As in vacuoles, with 78% of foliar As in vacuoles and 61% of plant As in vacuoles, indicating that vacuoles play an important role in As compartmentalization. Jiang and Wang (2008) reported the increase of Zn compartmentalization in the vacuole or apoplast. Guo et al. (2012) reported that transgenic *Arabidopsis* under Cd and As stresses alleviates the toxicity by thiol chelating toxic metals and vacuolar compartmentalization. Leitenmaier and Küpper (2013) discovered that hyperaccumulators avoid the adverse effect of heavy metal stress by sequestering them into vacuoles

Metabolic detoxification: As heavy metals enter plant cells, the metabolic mechanism has been mobilized to overcome the adverse effects (Nies and Silver 2007). Metabolic mechanism refers to that heavy metals infiltrate cell wall or cell membrane into inside, and react with protein, glutathione, oxalic acid, citric acid, and malic acid in protoplasm, forming complex chelates to alleviate the toxicity. Metabolic detoxification is achieved by detoxifying metabolic substance, including metallothionein (MT), phytochelatins (PC), polyamines (Pas), antioxidant enzyme system, abscisic acid (ABA), heat shock protein (HSP), and other metal chelating ligands (Li and Zu 2016). Metallothionein (MT), a cysteine-rich protein with low-molecular weight, is ubiquitous non-enzymatic protein and has an unusual amino acid composition. It does not contain aromatic amino acids, and one third of its residues are cysteines with high hydrosulphonyl content, so it is easily to form low toxic and even non-toxic compounds by binding to heavy metals (Klaassen et al. 1999).

Many plants cope with high level of heavy metals by binding to phytochelatins (PCs) and then forming complexes inside the cells (Yang et al. 2005a, b). PCs play an important role in eliminating toxic effects of heavy metals in cytosol (Zhang et al. 2018). PCs are ubiquitous in cytosol, but the content is low. PCs belong to the family of metal – complexing peptides (Anamika et al. 2015), and are transported into the vacuole as a complex once they are to heavy metals. PCs consist of three amino acids including glutamine (Glu), cystine (Cys), and glycine (Gly). They are structurally related to the tripeptide glutathione (GSH), and are synthesized by GSH enzymatic reaction (Yang et al. 2005 a, b). Relevant investigations have shown that PCs participate in the detoxification of heavy metal and improve plant tolerance to heavy metals (Chen and Goldsbrough 1994). Zhang et al. (2010) found that PCs serve as an intercellular Cd detoxification mechanism in shoots rather than roots in *Sedum alfredii*. Schat et al. (2002) investigated the effect of application of the g-glutamylcysteine synthetase inhibitor, L-buthionine-[S,R]-sulphoximine (BSO), and phytochelatins (PCs) on the tolerance of *Silene vulgaris* (Moench) Garcke, *Holcus lanatus* L. and *Agrostis castel-lana* Boiss. et Reuter (all of them hypertolerant species) stressed by Cu, Cd, Zn, As, Ni, and Co, and found that PC-based sequestration is not essential for the tolerance and hypertolerance to these heavy metals. Plant sensitivity to Cd and As is significantly increased by BSO, and PC-based sequestration is essential for these plants. Inouhe et al. (2000) investigated the cellular response of *Solanum tuberosum* L. and *Vigna angularis* to different concentrations of Cd (0, 50, 100 and 200 μM), and found that *S. tuberosum* cells produce PCs to improve its tolerance to different concentrations of Cd, however *V. angularis* cells can not produce PC in response to Cd, indicating that PCs in *V. angularis* cells are not involved in Cd-induced detoxification. Therefore, whether PCs in all plants play a role in heavy metals induced detoxification or not needs to be further investigated (Inouhe et al. 2000).

Cellular free radical produced in the process of plants coping with heavy metals is harmful to cell macromolecules and leads to redoxidation of plasma membrane, meanwhile antioxidant systems including include antioxygens and antioxidant enzymes are activated to scavenge free radical (Li and Zu, 2016). Antioxygens include glutathione, r-glutamyl-cysteingl-glycine (GSH), ascorbic acid (AsA), and vitamin E. GSH is a general defense substance and semi-chemical substance in plants, and is responsible for protecting plants from injury of membrane lipid and chelating with heavy metals to detoxify the adverse effects (Sun et al. 2013). Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), superoxide dismutase (POD), ascorbate peroxidase (ASA-POD), and glutathion peroxidase (GR) (Ling et al. 2011). SOD converts free radical into H_2O_2 and O_2, CAT and POD further convert H_2O_2 into H_2O, thereby reducing the toxic effects. ASA-POD and GR are key enzymes in removing H_2O_2. Under heavy metal stresses, free radicals in plants induce denaturation of macromolecules such as protein and nucleic acid as well as peroxidation of membrane lipid. Subsequently, plant antioxidant enzymes are activated to protect plants from further damage. Thus, the activities of SOD, POD, GSH are activated to scavenge free radicals triggered by heavy metals. SOD, CAT and POD in heavy metal tolerant plants maintained at the general level (Sun et al. 2013; Li and Zu 2016). Sun et al. (2013) investigated the effects of of Cr and S contents on AsA-GSH recycle in *Brassica chinensis*, and observed that Cr-S in moderation levels can promote the activation of APX, DHAR, GR and MDHAR, and maintain a higher reduction capacity such as AsA/DHA ratio and GSH/GSSH ratio) as well as higher levels of AsA and GSH to ensure effective recycling of AsA-GSH to scavenge the excessive H_2O_2 in cells, thereby reducing the injury to membrane lipid.

2.3 The effects of heavy metals on plants

In the process of restoration of heavy metal contaminated soil, plants are generally affected by heavy metals and then respond to the stresses, including plant growth, physiological and metabolic responses. Plant responses are different due to their different capacity in heavy metal tolerance. Therefore, plant tolerance to heavy metals is assessed by growth, physiological and metabolic responses (Rajkumar et al. 2009). Chami et al. (2014, 2015) selected root dry weight, shoot dry weight, root length and shoot length as indicators to analyze the effects of Ni, Pb and Zn on growth of *Sorghum bicolor* and *Carthamus tinctorius* by pot experiments, and the results showed that the growth of two species is influenced by these heavy metals. In *S. bicolor*, when Ni concentration is 5 mg/L, the shoot growth is promoted to a certain extent, and shoot dry weight and shoot length increases by 11% and 20% compared with control group, respectively. When the concentrations of Ni and Pb are over 5 mg/L and 100 mg/L, respectively, the parameters of *S. bicolor* decrease. When Zn concentration is over 10 mg/L, growth parameters including shoot dry weight, root dry weight, shoot length and root length decrease by 43%, 66%, 33% and 34%, respectively. In *C. tinctorius*, when Ni concentration is 5 mg/L, shoot dry weight and shoot length decrease by 67% and 64%, respectively. When Ni concentration is over 10 mg/L, *C. tinctorius* blights. When the concentrations of Pb and Zn are over 25 mg/L, *C. tinctorius* can not survive. To reveal the effects of Cu, Ni, Zn, Hg, Cr, Pb and Cd on germination of *Crambe abyssinica* on sandy cultivation medium, Hu et al. (2015) chose germination rate, relative root length, relative shoot length and relative seeding weight as test parameters to assess the effects of heavy metal on plant germination. The results showed that when the concentrations of Cu and Hg are above 0.7 mmol/L and 0.3 mmol/L, respectively, germination of *C. abyssinica* is significantly suppressed, however, a proper concentration of Cr can promote the germination of *C. abyssinica*. Moreover, these seven heavy metals decrease the relative root length, relative shoot length and relative seeding weight to different extents, and *C. abyssinica* shows the strongest tolerance to Cu, Zn, Hg, Cr, Pb and Cd, and weaker tolerance to Ni. Marchiol et al. (2004) assessed the tolerance of *B. napus* and *R. sativus* to heavy metals by pot experiments and found that *R. sativus* exhibits better tolerance than *B. napus*.

2.4 Heavy metal hyperaccumulator

Compared with the above plant species, there are some special species growing in heavy metal enriched areas with significant potential in heavy metal tolerance (Brooks et al.1977, 1981). With the transplantation of this species in non-heavy metal areas, they show some inadaptable symptoms to some extents. Brooks et al. (1977) analyzed the capacity of shrubs *Homalium* and *Hybanthus* in Ni uptake, and found that two shrubs show great tolerance to Ni stress, absorb and accumulate large amounts of Ni from soil, with Ni concentration in their body exceeding 1000 μg/(g dry weight). Then, Brooks et al. (1977) proposed the definition of these special species and named them as hyperaccumulator. Baker and Brooks (1989), Li and Zu (2016) obtained the critical content of heavy metals in hyperaccumulators, as shown in Table 1. Moreover, the basic attributes of the hyperaccumulator have been presented as following: (1) Strong tolerance to heavy metals

Table 1 Critical content for heavy metals in hyperaccumulators

Heavy metal	Critical concentration (mg/kg)
Cd	100
Pb	1000
Cu	1000
Co	1000
Ni	1000
Zn	10000
Mn	10000
As	1000
Cr	1000
Hg	10

Note: Adapted from Li and Zu (2016).

Table 2 Maximum absorption of heavy metals by hyperaccumulators

Species	Heavy metal	MADHM (mg/kg)
Psycotria vanbermanni	Ni	37520
Psycotria glomerata	Ni	20250
Psycotriaosseana	Ni	12780
Garcinia bakeriana	Ni	7440
Streptantbus polygaloydes	Ni	14800
Tblaspi tatrense	Zn	20100
Cardaminopsis balleri	Zn	13620
Dicbapetalum gelonioides	Zn	30000
Viola calaminaria	Zn	10000
T. caerulescens	Zn	26000
Pteris vittata	As	22000
Vetiveria zizanioides	Pb	4069
Bidens maximovicziana)	Pb	2164
Pogonatherum crinitum	Pb	4639.4
Isachne globosa	Pb	6848
Arabis Paniculata	Zn	14769
Minuaritia verna	Zn	11400
Artemisia sacrorumvar	Zn	2858
Carex gentiles	Zn	1834
Typha orientalis	Zn	7819
Minuarti vernia	Pb	20000
Armeria maritime	Pb	1600
Agrostis tenuis	Pb	13490
Alyxia rubricalis	Mn	14000
Maytenus bureaviana	Mn	33750
Lecytbis allaria	Se	18200
Astragalus racemosus	Se	14920
Aeollantbus subacaulis	Cu	13700
Haumaniastrum robertii	Co	10232

Note: (1) Adapted from Marques et al. (2009), Reeves and Baker (2000), and Nie (2016); (2) MADHM refers to maximum absorption dosage to heavy metals.

refers to that plants have an exceptional attribute in heavy metal stress tolerance; (2) Biological accumulating coefficient (BAC) of hyperaccumulator to heavy metals is larger than 1, indicating that heavy metal concentration in plants is larger than that in the growth environment; (3) The growth of hyperaccumulator is not affected under the conditions, when heavy metal content in the medium is 10 -500 times of that under the general conditions, Moreover, the maximum absorption of heavy metals by hyperaccumulators is shown in Table 2; (4) Biological transfer coefficient (BTC) of hyperaccumulator to heavy metals is larger than 1, indicating that heavy metal content in the aboveground part is larger than that in the underground part. The explanation of BAC and BTC has been shown as following:

Biological accumulating coefficient (BAC) and Biological transfer coefficient (BTC) of phyeraccumulator are illustrated in the following:

$$BAC = \frac{M_{plant}}{M_{soil}} \quad (1)$$

$$BTC = \frac{M_{Aboveground}}{M_{underground}} \quad (2)$$

where, M_{plant} refers to concentration of a certain heavy metal in a plant, M_{soil} refers to concentration of the heavy metal in growing environment where the plant planted; $M_{\text{underground}}$ refers to concentration of the heavy metal in underground part of the plant. And larger BAC means stronger capacity of a plant in accumulating one or some certain heavy metals, greater BTC means stronger capacity of a plant in transferring the heavy metal absorbed.

At present, more than 700 hyperaccumulators have been identified, and most of them belong to plant families, as shown in Table 3. Hyperaccumulator have a selective attribute in heavy metal absorption, uptake, and transportation, indicating that different hyperaccumulators have different potential in the absorption, uptake, and transportation of heavy metal. For example, Brown et al. (1994) found that Cd concentration in foliage of *Thlaspi caerulescens* is 10800 mg/kg after grown in the field with 1020 mg/kg Cd for five weeks. Komar et al. (2001) observed that *Pteris vittata* not only has high efficiency in extracting arsenic from soils and transporting it to the above-ground part, but also converts As from As (V) to As (III) in the process of transporting arsenic from roots to leaves. Vázquez et al. (2015) found that *T. caerulescens* has significant potential in absorbing Zn. Lin et al. (2014) found that *Noccaea caerulescens* can accumulate Ni, Zn and Cd in soil, and considered *N. caerulescens* as Ni, Zn and Cd hyperaccumulator. Li et al. (2016) classified

Table 3 Main hyperaccumulators for different heavy metals

Heavy metals	Species and Families
Cd	*Thlaspi caerulescens; Noccaeacaerulescens; Pistia stratiotes; Beta vulgaris* L. var. cicla
Pb	*T. totundifolium; Helianthus annuus; Brassica nigra; Brassica juncea; Medicago sativa; Betula occidentalis; Noccaeacaerulescens; Pistia stratiotes;*
Cu	*Ipomoea alpine; Hanmaniastrum katangense; Pistia stratiotes;*
Co	*Hanmaniastrum robertii*
Ni	*Berkheya coddii; Berkheya zeyheri; Pentacalia (10 species); Helianthus annuus; Senecio coronatus; Alyssum (48 taxa, all in section Odontarrhena) ; Bornmuellera (6 taxa); Thlaspi (23 taxa); Buxus (17 taxa); Leucocroton (27 species); Phyllanthus (16 taxa); Phyllanthus chamaecristoides; Cleidion viellardii; Baloghia sp.; Homalium (7 species;); Xylosma (11 species) Psychotria douarrei; Noccaeacaerulescens; Pistia stratiotes;*
Ti	*Iberis intermedia*
Se	*Astragalus racemosa*
Zn	*T. caerulescens; T. calaminare; Noccaeacaerulescens; Pistia stratiotes;*
Mn	*Alyxia rubricaulis; Macadamia neurophylla;*
As	*Agrostis canina L.; Agrostis stolonifera L.; Agrostis tenuis Sibth.; Calluna vulgaris; Helianthus annuus; Holcus lanatus; Jasione montana L.; Pityrogramma calomelanos; Pteris biaurita L.; Pteris cretica; Pteris longifolia; Pteris quadriaurita; Pteris ryukyuensis; Pteris umbrosa; Pteris vittata; Reynoutria sachalinensis;*
Cr	*Pistia stratiotes;*
Hg	*Spatina plants; Pistia stratiotes;*

Note: Adapted from Brooks et al. (1977); Alkorta et al. (2004); Padmavathiamma and Li (2007); Rathinasabapathi (2010); Koptsik (2014); Jiang et al. (2015).

Dysphania Ambrosioides as Pb and Zn accumulator. Although hyperaccumulators have excellent capacity in heavy metal tolerance and accumulation, their application are limited by the disadvantages of slow growth, dwarfish plant height, and undeveloped root system. However, it should be pointed out that a newly identified hyperaccumulator, *Antidesma montis-silam*, has very high Ni concentration in leaves, reaching 32700 μg/g (Nkrumah et al. 2017). Moreover, some Mn hyperaccumualtors belonging to genus *Antidesma* have been identified, which exhibit co-accumulation of Mn and Ni, and may work together to remedy Mn-Ni contaminated lands (Nkrumah et al. 2017).

3 Phytoremediation Assessment

Because there are many kinds of plants to control heavy metal contaminated land, the absorption of heavy metals by plants is quite different (Marques et al. 2009). Therefore, the phytoremediation ability of heavy metal contaminated soil can be evaluated by measuring heavy metal in soil (Zhuang et al. 2009). Murakami and Ae (2009) compared the ability of *Oryza sativa, Glycine max* (Linn.) Merr. and *Zea mays* L. in remedying soil slightly and moderately contaminated by Cu, Pb and Zn. The results showed *O. sativa* and *Z. mays* have a prefer selection in Cu absorption, while *G. max* has a prefer selection in Zn absorption. Tzvetkova et al. (2015) selected *Paulownia tomentosa* × fortunei-TF 01 and *Paulownia elongata* × fortunei- TF 01 as tested species under Pb, Cu, Zn and Cd stress conditions and quantified their extraction effects. The results showed that the aboveground part of *P. tomentosa* has a prefer selection in Pb and Zn than the underground part, while *P. elongata* has a prefer selection in Zn. Further study showed that the mobility and transportation of heavy metals are quite different, for example, the mobility of Zn and Cd is stronger than that of Cu and Pb. The stronger mobility and transportability of Zn and weaker mobility and transportability are attributed to the fact that Zn is essential for plant metalloenzymes and photosynthesis while Pb and Cd are toxic to plants. Through the application of EDTA, NH_4NO_3 and $(NH_4)_2SO_4$ to the filed, Zhuang et al. (2009) compared the absorption capacity of *S. bicolor* to Cu, Cd, Zu and Cu, and found that Pb concentration in *S. bicolor* leaves is higher, and the concentrations of Cu, Cd and Zn in stem are relatively higher. Moreover, EDTA is more capable of promoting the absorption of heavy metals than NH_4NO_3 and $(NH_4)_2SO_4$. Ishikawa et al. (2014) investigated the absorption effect of *Salix pseudolinearis* (FXM), *Salix pet-susu* (HB471, KKD) and *Salix sachalinensis* on Cd and Zn, and found that *S. pseudolineаris* have better absorption of Cd and Zn than other plants.

4 Selection Principles of Phytoremediation

Extreme high concentrations of heavy metals in heavy metal contaminated regions have stress effect on plants, leading to slow growth, blighting and even death of plants. It is a reasonable choice to choose suitable plants to remedy the contaminated lands. Based on relevant references, the following principles are obtained: (1) Candidate plants should survive in heavy metal contaminated environment and have excellent adaptability to heavy metal stresses; (2) Heavy metal concentration in plants is relatively higher than that in the growth environment; (3) Plants have excellent adaptability to local climate, strong resistance to adverse conditions and developed root system; (4) Plants should have a fast growth rate and large biomass (Garbisu et al. 2002; Koptsik 2014).

Following the principles, scholars have conducted in-situ or ex-situ phytoremediation experiments. Wang et al. (2016) assessed the effects of plant species on the removal and absorption of heavy metals from soil, and proposed BAC and BTC to plants. Tzvetkova et al. (2015) compared phytoremediation effects of *Paulownia tomentosa* × fortune and *Paulownia elongate* × fortune mono-planted on Cd, Cu, Pb and Zn contaminated soil under greenhouse conditions, and the results showed that BAC for *P. tomentosa to* Pb, Cu, Zn and Cd was 0.96, 2.52, 3.84 and 1.57, and the corresponding BTC was 1.82, 0.93, 1.1 and 2.68, and BAC for *P. elongate to* Pb, Cu, Zn and Cd was 0.93, 2.09, 3.37 and 2.68, and the corresponding BTC was 0.95, 0.96, 1.05 and 0.59, respectively. These results indicated that the two species can remedy heavy metal contaminated soil. In addition, Zhang et al. (2009), Linger et al. (2002), Chami et al. (2015) performed similar experiments and found that plants mono-planted plants absorbed one or some certain heavy metals, so the absorption efficiency is relatively low. Jiang et al. (2010) carried out intercropping method to remedy the soil contaminated with multi heavy metals and found that interplanted or intercropping may be a promising method to remedy heavy metal contaminated lands compared with the method of mono-planted plants (An et al. 2011).

5 Phytoremediation Practice

Woch et al. (2016, 2017) investigated the effect of old heaps waste contaminated with high concentrations of Cd, Pb and Zn on spontaneous plants as well as the community composition, and discovered that there is distinct difference between plant community composition and areas with different contamination extent. Similarly, Dietterich and Casper (2017) found that preliminary soil improvement has an important effect on plant community composition in heavy metal contaminated mountain area for nine consecutive years. To further investigate heavy metal concentration in plant organs, Stefanowicz et al. (2016ab) found that all the elements in herbs and tissues show significant differences, plant organs affect the concentrations of all elements except As and Fe, and species × organ interaction has an impact on the distribution of As, Ca, Cd, Mn and Zn in plants. This result can be used to improve the efficiency of phytoremediation. The above findings indicate that plant community may be a high efficient way for phytoremediation in terms of removing heavy metals by different plants.

In order to improve the efficiency of intercropping of plants in removing heavy metals from soil, scholars carried out experiments to investigate the practical effects of intercropping or interplanting models in removing heavy metals, and further analyzed the corresponding absorption efficiency of heavy metals.

By planting *Digitaria sanguinalis*, *Vetiveria zizanioides* and *Cassia occidentalis* in deserted mining area with Mn, Pb and Zn contaminated soil, Liu et al. (2014) investigated the practical effect of three species on mining area restoration and obtained their BAC and BTC. The results showed that BAC and BTC of three species to Pb are less than 1, BAC of *V. zizanioides* to Mn and Zn is 0.9 and 0.4, less than 1, and the corresponding BTC is 3.7 and 1.1, both BAC and BTC of *D. sanguinalis* and *C. occidentalis* to Mn and Zn are larger than 1, so the two species are hyperaccumulators to Mn and Zn. Marchiol et al. (2004) compared the remedy effects of *Brassica napus* (cv. Kabel), *Brassica juncea* (cv. Vitasso), *Raphanus sativus* (cv. Rimbo) and *Brassica carinata* (cv. BRK13) on Cd, Cr, Cu, Ni, Pb and Zn contaminated land in Italy by pot experiments, and discovered that BAC of these species are larger than 1, but BTC of them are less than 0.5. These results indicated that these species can store the absorbed heavy metals in the underground part and can not transfer them in the aboveground part, so it is unavailable to remove these heavy metals by harvesting the aboveground biomass. Hou et al. (2012) investigated cropping patterns (*Pinus massoniana* + *Lespedeza bicolor*, *P. massoniana* + *L. bicolor* + *Vetiveria zizanioides*, *P. massoniana* + *local flood turf*, *Liquidambar formosana* + *local flood turf*, *Eucalyptus robusta* + *local flood turf*, and *P. massoniana* + *E. robusta* + *local flood turf*) on phytoremediation using field experiments in Fujian, China, and cluster analysis was used to compare the remedy effects of the selected cropping patterns in stabilization on soil agglomerations, soil nutrient, and heavy metal reduction. The result showed that *P. massoniana*+ *L. bicolor*+*V. zizanioides*, *L. formosana* + *local flood turf* and *P. massoniana* + *E. robusta* + *local flood turf* are easy to restore soil.

6 Conclusion and Recommendation

6.1 Conclusion

(1) Heavy metal speciation and the influencing factors

The mobility, bioavailability and toxicity of heavy metals are determined by their own speciation. For example, the toxicity of Zn in organic state is more serious than that in other states, and the toxicity of As in tri-valence is more serious than that in tetravalent. The toxicity and bioavailability of heavy metals in exchangeable state, in organic state and in bound-state sulfide are more serious than those in other speciation. The reasons are attributed to the following aspects. Heavy metals in these speciation have stronger mobility, and heavy metal speciation is influenced by soil pH, organic content in soil, soil texture, additive, and microorganism. Lower pH converts heavy metals in residual speciation, in carbonate speciation, in bound-state sulfide speciation into exchangeable speciation with more mobility. In the process of heavy metal transformation from one speciation into other speciation, the bioavailability, absorption and uptake of heavy metals are alerted.

(2) Mechanism of phytoremediation of heavy metal contaminated soil

Mechanism of phytoremediation of heavy metal contaminated soil involves heavy metal absorption mechanism and heavy metal tolerance mechanism. Heavy metal absorption mechanism involves apoplast absorption and infiltration into cell membrane. Heavy metal tolerance mechanism involves absorption rejection, combination-inactivation, compartmentalization and metabolic detoxification.

(3) Plants selection and planting pattern

Among the identified species in terms of absorption efficiency of heavy metal absorption, hyperaccumulators seem to be preferred candidates with strong tolerance to heavy metal stress and great potential in absorbing heavy metals from soils. However, most of hyperaccumulators have the shortcomings of slow growth, smaller biomass, undeveloped root systems. Moreover, most of hyperaccumulators have selective absorption for one or more heavy metals.

6.2 Recommendation

(1) Heavy metal speciation and the influencing factors

So far, many measures have been taken to immobilize or fix heavy metals by transforming their speciation from high mobility to lower mobility to reduce the bioavailability and toxicity. However, the mobility, bioavailability and toxicity of heavy metals depend on the speciation and on the external environment. Once the external environments alert, the speciation may be re-transformed and the immobilized or fixed heavy metals may be released into the environment. Therefore, the removal of heavy metals from the environment may be a priority concept rather than immobilizing or fixing.

(2) Mechanism of phytoremediation of heavy metal contaminated soil

Mechanism of phytoremediation of heavy metal contaminated soil is the core of phytoremediation, but phytoremediation is a result of many factors, especially for the species planted in multiple heavy metals contaminated lands. Therefore, further investigation should be focused on molecular biology. Such as, the response of membrane transporting protein to heavy metals in the process of alleviates the toxicity of heavy metals.

(3) Plants selection and planting pattern

Plants with fast growth, big biomass and excellent characteristic in heavy metal tolerance and absorption are the ideal species. However, many plants with fast growth, big biomass and excellent characteristic in heavy metal tolerance exhibit disappointing performance in heavy metal absorption. Thus, it may be a good idea to use transgenic technology to remedy these species, but gene safety in terms of gene flow should be considered. Interplanted species may be a high efficient way to remove heavy metals, but the interaction between species should be clearly investigated.

(4) Root-system interaction between plants and heavy metal contaminated soil

Phytoremediation is a promising way to remove heavy metals from the environment. Therefore, the investigation of root-heavy metals system should also be prioritized, because the absorption of heavy metals occurs in root – heavy metals system, and root is the first organ affected by heavy metals.

Acknowledgements

The project has been financially supported by the Special Fund of Shaanxi Education Department (18JK0172), and the Initial Funding of Talent in Shaanxi University of Technology (SLGQD2017-02). And authors are grateful for Hu Xia-song, Han Wen-xia and Zeng Fang-ming from Qinghai Institute of Salt Lakes, Chinese Academy of Sciences for giving essential help of the manuscript.

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