Principles and Technologies of Phytoremediation for Metal-Contaminated Soils: A Review

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1 Introduction

Soil contamination with heavy metals has become a worldwide challenge, leading to losses in agriculture and hazardous health effects as they enter the food chain. This problem may be partially solved by the emerging phytoremediation technology. The cost-effective plant-based approach to remediation takes the advantage of the remarkable ability of plants to concentrate heavy metals in their tissues from the environment. However, the so-called phytoremediation has not achieved a lot partly due to the lack of basic information regarding the fundamental mechanisms employed by metal accumulators. The application of metal accumulators is also obstructed by metal accumulators. The application of metal accumulators is also obstructed by some of their inherent defects, such as small biomass, slow growth rate, and poor adaptability. Hence, before their practical application, the mechanisms responsible for the abnormal ability for metal tolerance and accumulation should be elucidated. Alternatively, efforts should be paid to explore new accumulators with ideal biological traits including high biomass and fast growth rate.

Phytoremediation is a plant-based approach to remediate contaminated soil, including phytoextraction, phytostabilization, phytovolatilization, phytodegradation, phytoavoidation, and phytoremediation coupled with agro-production. It is based on the ability of plants to concentrate specific contaminants (mainly heavy metal and organic pollutants) from the environment and volatilize, degrade, or metabolize various molecules in their tissues. Compared with physical and chemical remediation methods, phytoremediation is much more cost-effective

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and environmentally friendly and therefore has a promising application prospect (Long et al. 2001; Hu et al. 2014).

It is widely acknowledged that phytoextraction is an effective approach to remove heavy metals from soils (Hu et al. 2014); plants for phytoextraction are defined as hyperaccumulators. There are more than 700 hyperaccumulators found in the world (Li et al. 2011a, b, c), most of which still have some problems to be settled before applying into practice due to their low biomass and poor adaptability. The technologies of enhancing phytoremediation efficiency, phytoremediation for complex contaminated soils, and phytoremediation coupled with agro-production are required to be developed (Yang et al. 2003). Since last twenty years, we aimed at solving these problems, by screening and identifying metal hyperaccumulators and accumulators like *S. alfredii* Hance, *Elsholtzia splendens*, *E. argyi*, willows, *Ricinus communis*, *Brassica rapa*, etc. (He et al. 2003; Huang et al. 2011; Peng and Yang 2007; Xing et al. 2012; Yang et al. 2003). In addition, we also identified metal low accumulators of rice and vegetables as a supplement for safe crop production (Yang et al. 1998; Diao et al. 2005; Cao et al. 2010; Chen et al. 2010; Zhuang et al. 2009). Till now, serious experiments have been conducted to understand the mechanisms of metal hyperaccumulation by plants and form a systematic knowledge of phytoremediation in theory and practice.

In contrast to the extensive experimental studies, little attention has been paid to a systematic summary. Thus this paper summarizes the results of comprehensive experimental programs by us, as a guide to the further development of phytoremediation. Topics include screening and identification of metal hyperaccumulators and low accumulators, mechanisms of metal (Cd, Pb, Cu, Zn) hyperaccumulation and accumulation by plants, microbe- and chemical-assisted phytoremediation, phytoavoidation of heavy metal contamination in crops, and technologies of phytoremediation coupled with agro-production for complex contaminated soils.

2 Identification of Metal Hyperaccumulators/Accumulators

2.1 Zn/Cd Hyperaccumulator and Pb Accumulator: S. alfredii *Hance*

Through our survey and screening in different mined areas, we firstly found an extraordinary Zn/Cd-hyperaccumulating ecotype of *S. alfredii* Hance in an old Zn/Pb mining region in Quzhou, Zhejiang Province (Yang et al. 2002) (Fig. 1). This plant has been identified as a Zn/Cd co-hyperaccumulator. It can be healthily grown under Zn supply levels of 240 mg/L (Long et al. 2001) and Cd supply level of 400 μmol/L (Yang et al. 2004). Shoot Zn concentration can reach up to 2.9% DW under soil culture (Yang et al. 2005a, b). And shoot Cd concentration can reach to 9000 mg/kg under solution culture (Yang et al. 2004) while up to 989 mg/kg under

Fig. 1 Mined ecotype of *S. alfredii* Hance Zn/Cd hyperaccumulator and Pb accumulator

soil culture (Xiong et al. 2008). In addition, we found that *S. alfredii* Hance is also a lead accumulator (He and Yang 2007); it showed best growth at Pb supply level of 160 mg/L in solution culture and at Pb supply of 400 mg/kg under soil culture. Shoot Pb concentration in both solution and soil culture conditions could reach over 500 mg/kg (He and Yang 2003).

To date, more than 400 species of hyperaccumulators belonging to 45 families have been identified, of which about 75% are Ni hyperaccumulators (Reeves and Baker 2000; Chaney et al. 2005). So far, about 18 species of Zn hyperaccumulators and 5 species of Cd hyperaccumulators have been identified. They were found to mainly colonize area with high Zn, Cd, and Pb present in soils due to historical mining activity and the subsequent contamination of top soil with mine spoil rich in heavy metals and mainly distributed in Europe and Australia (Baker et al. 2000). *S. alfredii* Hance as a Zn/Cd hyperaccumulator is the first observation in China. It could tolerate higher Zn and Cd in growth media and be able to hyperaccumulate in its shoots as well as *Thlaspi caerulescens*. In comparison, *S. alfredii* Hance has its characteristics of faster growth rate, larger biomass, asexual reproduction, and being a perennial plant, and the plants can cover nearly 100% of the land surface. Besides, *S. alfredii* Hance could propagate three to four times a year when the environmental conditions are favorable. According to field survey, shoot dry matter of *S. alfredii* Hance was as high as 4.5 tons/ha. Therefore, there is a great potential in using *S. alfredii* Hance to remediate Cd-/Zn-/Pb-contaminated soils. The identification of *S. alfredii* Hance provides an excellent plant material to study the mechanism of plant hyperaccumulation for heavy metals and a potent new plant species to remediate heavy metal-polluted soils via hyperaccumulators.

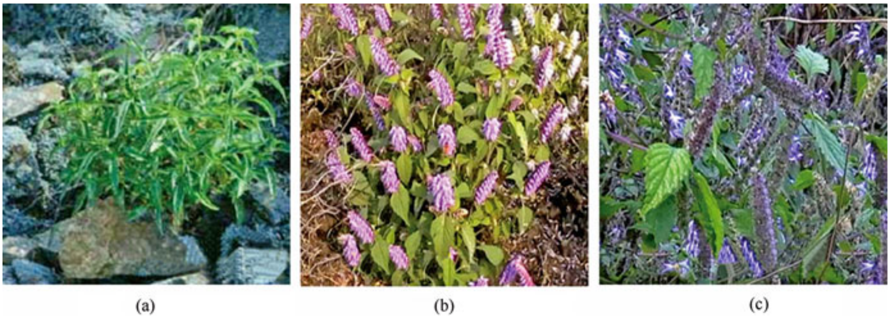


Fig. 2 Mined ecotypes of *E. splendens* and *E. argyi*, Cu and Pb accumulators. (**a**) *E. splendens*. (**b**) *E. splendens*. (**c**) *E. argyi*

2.2 *Copper and Lead High Accumulator Plants:* Elsholtzia

We firstly discovered Chinese native Cu- and Pb-tolerant and accumulating plants, *Elsholtzia splendens* (Haizhou Elsholtzia) and *E. argyi* (Purple Flower Elsholtzia) (Fig. 2), which are endemic to Cu and Pb/Zn mine waste deposits, respectively (Jiang et al. 2004a, b). *E. splendens* (Haizhou Elsholtzia) has a local nickname of "copper flower" because its growth is confined to highly Cu-contaminated soils. It has been identified as a Chinese native Cu-tolerant plant species and copper high accumulator (Yang et al. 1997). Both *Elsholtzia* plants belong to the family of Labiatae.

Previously, we conducted a wide investigations on phytoremediation of Cu soils using *E. splendens* and *E. argyi*. Under solution culture, both *Elsholtzia* plants can tolerate Cu levels of over 300 μmol/L and Pb level over 100 μmol/L. The Cu accumulation in stems can reach over 1000 mg/kg (Jiang et al. 2005, 2008; Islam et al. 2007b; Yang et al. 2000). Islam et al. (2008) showed that Pb concentration of *E. argyi* reached to over 300 mg/kg in leaves and 1800 mg/kg in stems grown at 200 μmol/L Pb. *E. splendens* displayed certain tolerance at 100 μmol/L Pb treatment. Lead concentration in stems and leaves of *E. splendens* reached 1600 and 381 mg/kg, respectively (Zhang et al. 2011a, b). Field experiments were conducted in a complex contaminated soil with metal total levels of 1500 mg/kg Cu, 2000 mg/kg Pb, 1500 mg/kg Zn, and 21 mg/kg Cd, respectively. The results showed that both *Elsholtzia* plants can highly tolerate complex metal toxicity and obtained dry matter yield of 9–12 tons/ha. And *E. argyi* had higher dry matter yields and greater ability of accumulating Pb than *E. splendens*. In contrast, *E. splendens* is superior in Cu accumulation (Peng and Yang 2007). Both *E. splendens* and *E. argyi* can effectively clean up lower Cu-contaminated water, with the former being better than the latter (Tian et al. 2008). These two accumulators provide good remediation plant materials for cleaning Cu and Pb from soils. Moreover, plants of *Elsholtzia* are Chinese traditional medicine in common use, and volatile oil which was distilled from their shoots plays important roles in antisepsis and antibiosis, such as *Bacillus* and *Coccus*. It has already been reported that *E. splendens* has remarkable resistance to epiphyte (Peng and Yang 2007; Xu et al. 2015). Hence, the medicinal value of the two species should reduce the cost of phytoremediation greatly. Therefore, E. splendens and *E. argyi* are potentially good materials for phytoremediation in practice. Considering its high biomass and fast growth, we proposed that *E. splendens* has a great potential for phytoremediation.

2.3 Cadmium High Accumulator Plants: Willow Clones (Salix)

Willow clones (39 clones) were obtained from the National Willow Germplasm Resources of the Jiangsu Academy of Forestry, Nanjing, China. Systematic studies were conducted to screen and identify Cd-tolerant and accumulating willow (*Salix* spp.) clones. The results showed a large difference in Cd tolerance and accumulation (Fig. 3). Shoot tolerance indexes (TIs) varied between 0.09 and 1.85, and root TIs varied between 0.27 and 1.99 among clones. The large differences in Cd concentration ranged from 64.7 to 663.7 (mg/kg, DW) in leaves, from 118.0 to 308.4 (mg/kg, DW) in stems, and even high in roots among clones (Yang et al. 2003). These results indicated that it is possible to identify Cd high accumulator willow clones for phytoremediation of soils and waters.

2.4 Cadmium Accumulator Plant: Ricinus communis

Castor (*R. communis*) species belongs to Euphorbiaceae family, a fast-growing C_3 plant, native to tropical Africa. It is an industrial crop because of its oil quality and quantity for plant-based industries for making eco-friendly paints and coatings used in chemical industry. Castor attracted attention because of its ability to grow in heavily polluted soil together with its capacity for metal ion accumulation and fast growth rate. In addition, castor is an industrial crop with multiple nonfood uses and an excellent rotation and companion crop. It has economic advantage as a cash crop in modern agriculture along with remediation of heavy metal-contaminated soils. At soil Cd level of 0.42 mg/kg, *R. communis* varied largely in the uptake and accumulation of Cd, with mean concentrations of 1.22, 2.27, and 37.63 mg/kg DW for Cd in leaf, stem, and root. The total uptake of Cd varied from 66.0 to 155.1 μg/pot. *R. communis* has great potential for removing Cd from contaminated soils attributed to its fast growth, high biomass, strong absorption, and accumulation for Cd (Huang et al. 2011) (Fig. 4).

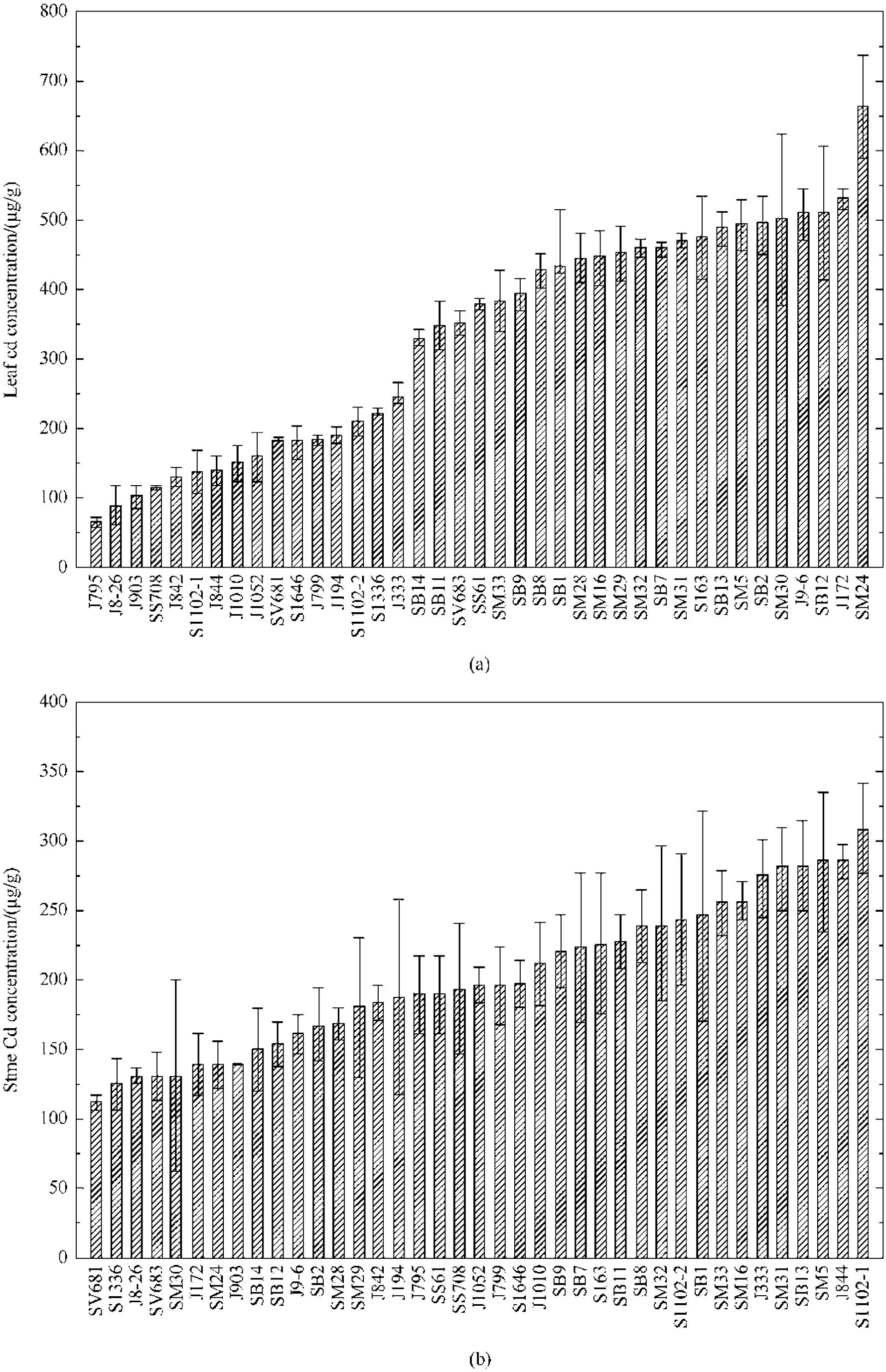


Fig. 3 Cd accumulation (μg/g DW) in (**a**) leaves and (**b**) stems of 39 willow clones exposed to 10 μmol/L Cd for 35 days

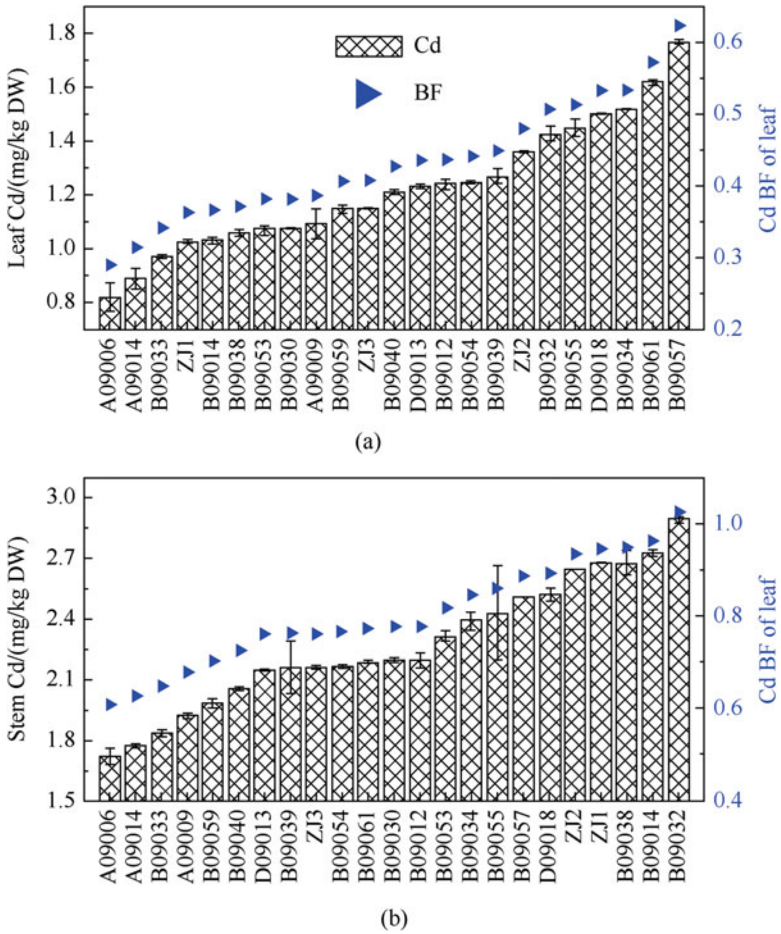


Fig. 4 (**a**) Cd accumulation in leaves of 23 genotypes of *R. communis*. (**b**) Cd accumulation in stems of 23 genotypes of *R. communis*

3 Mechanisms of Metal Hyperaccumulation and/or Accumulation

3.1 Overview of Zn and Cd Hyperaccumulation Mechanisms

Pioneer researches on *S. alfredii* Hance as a potential zinc (Zn)/cadmium (Cd) hyperaccumulator were initiated in 2002 by Prof. Dr. Xiaoe Yang. In a field survey on an ancient Pb/Zn mine in Quzhou, Zhejiang Province, China, *S. alfredii* Hance was found prevailing and grew well in this area. The lab analysis showed that Zn concentration in the shoots ranged from 4134 to 5000 mg/kg DW (Yang et al. 2002) and Cd concentration in shoots could reach to 9000 mg/kg DW (Yang

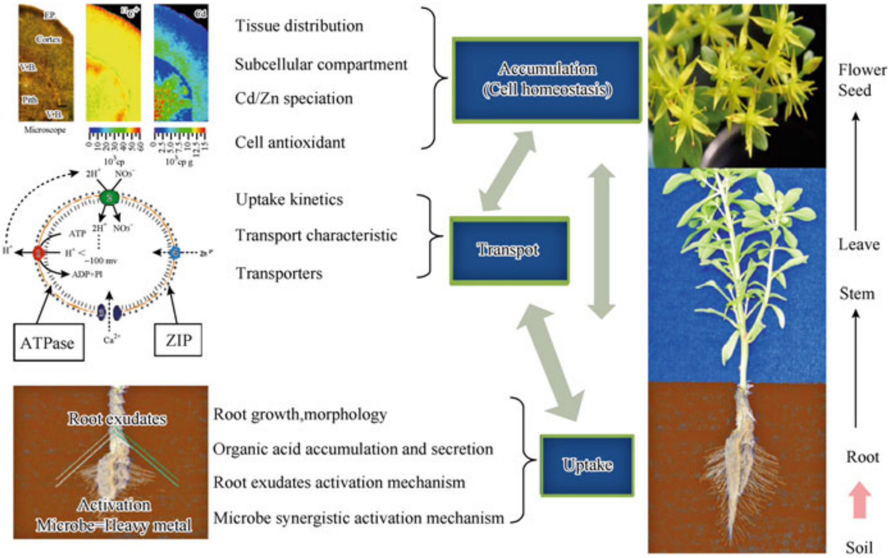


Fig. 5 Mechanisms of Cd/Zn hyperaccumulation in *S. alfredii* Hance

et al. 2004a), indicating that *S. alfredii* Hance was a promising Zn/Cd hyperaccumulator. Subsequently, a series of greenhouse experiments and field surveys consistently confirmed the remarkably high accumulation, translocation, and tolerance abilities of *S. alfredii* Hance under multiple heavy metal conditions (Ni et al. 2004a, b; Yang et al. 2004, 2006a, b; Long et al. 2006).

Researches in the past decades on hyperaccumulation process were mainly focused on three processes: ① rhizospheric activation and uptake potential, ② translocation and transport component, and ③ compartmentation and detoxification. Our past researches developed an explanation theory of "end-point control" for metal hyperaccumulation in plants (Fig. 5). It seems that metal hyperaccumulation is mainly driven by shoot accumulation capacity and root uptake potential and regulated by transporters. Shoot accumulation capacity is associated mainly with metal compartmentation, speciation, and cell antioxidation, while root uptake potential is mainly related to root tolerance, exudation, absorption, and endophytomicrobe synergistic. Metal transporters play an important role in regulating metal sequestration, translocation, and cell homeostasis. However, the whole network of metal hyperaccumulation in plant is not fully understood.

3.1.1 Uptake Potential and Rhizospheric Activation

The rhizosphere is the main interface of plants and the environment and the forefront for plants to take up heavy metals; thus it plays a fundamental role in the hyperaccumulation process. Soil pH is an important factor controlling metal availability in the rhizosphere. Rhizosphere soil pH was reduced by 0.5–0.6 units, as compared to bulk soil after HE *S. alfredii* Hance growth (Li et al. 2011a). The reduced soil pH could significantly increase soil Zn and Cd bioavailability in the rhizosphere and thus facilitate plant Zn and Cd uptake. In contrast, there was no obvious change in rhizosphere pH with NHE *S. alfredii* Hance (Li et al. 2011a, b). HE *S. alfredii* Hance root was reported to be able to exudate more dissolved organic matter (DOM) in the rhizosphere than that of NHE (Li et al. 2012a, b), and HE-DOM had greater ability to form complexes with Zn and Cd than NHE-DOM owing to the higher proportion of hydrophilic fraction (Li et al. 2011a, b). The greater DOM in the rhizosphere of HE *S. alfredii* Hance might be the main reason for the reduction of pH. By the addition of citric acid and tartaric acid, short-term (2 h) root uptake of ^{109}Cd increased significantly (Lu et al. 2013a). Besides, elevated CO_2 increased the uptake of Cd/Zn of HE *S. alfredii* Hance because of root growth promotion, lower pH, and higher photosynthetic carbon uptake rate (Li et al. 2013a, 2014, 2015a, b). Therefore, HE *S. alfredii* Hance is able to extract more Zn and Cd from soil pools that are unavailable to nonhyperaccumulator plants.

The hyperaccumulation of *S. alfredii* Hance was also related to its root morphology characteristics. It was shown that Zn concentration in shoot was positively correlated with root length, surface area, and volumes of *S. alfredii* Hance (Li et al. 2005a). With addition of Zn and Cd, the root volume and average diameter in NHE *S. alfredii* Hance were largely reduced, but they were significantly increased in HE *S. alfredii* Hance (Li et al. 2005a, b, 2009). The differential responses of *S. alfredii* Hance root to Zn and Cd between the two populations indicated a higher tolerance of HE *S. alfredii* Hance to heavy metal stress and accordingly favored its growth and Zn/Cd accumulation. *S. alfredii* Hance roots showed an initial rapid linear Zn absorption followed by a slower linear phase. Compared with NHE, Zn influx was threefold higher in HE, while Zn transporters were lower in NHE than in HE (Km value of 20.43 and 34.83 μmol/L) (Li et al. 2005a), which supports the enhanced root uptake in *S. alfredii*. Although the Km of ^{109}Cd influx into roots of both ecotypes were similar, the V_{max} was twofold higher in the HE compared to NHE *S. alfredii* Hance (Lu et al. 2008). The Cd uptake in HE was three to four times higher than the implied Cd uptake calculated from transpiration rate, and inhibition of transpiration rate in the HE had no essential effect on Cd accumulation in shoots of the plants (Lu et al. 2009), indicating that there were high-affinity transporters for HE to uptake a large amount of Zn and Cd. Several *ZIP* family genes have been cloned and found to respond Zn and Cd stress. But, the detailed identification and analysis are still under way.

3.1.2 Translocation and Transport Components

After the uptake of Cd and Zn, HE *S. alfredii* Hance can transport the heavy metal to xylem and shoots faster than NHE *S. alfredii* Hance. A ^{65}Zn2+ efflux experiment revealed 2.7-fold and 3.7-fold more Zn accumulation in root vacuole of NHE than that of HE *S. alfredii* Hance for short term and long term, respectively. The half time for Zn efflux from root vacuole was estimated to be 40% shorter in HE, indicating a 1.8-fold faster efflux from HE root vacuole than that of NHE (Li et al. 2005a). The rate of root-to-shoot translocation of ^{109}Cd in the HE was>10 times higher than NHE and HE shoots that accumulated dramatically higher ^{109}Cd content (Lu et al. 2008). Cell wall was an important site for Zn and Cd compartmentation in *S. alfredii* Hance root. The root cell walls showed similar Zn adsorption ability in both HE and NHE *S. alfredii* Hance, but they differed in desorption characteristics (Li et al. 2007a, b, c, d). Compared to NHE, Zn was bound more loosely to root cell walls in HE, whereas Cd was retained more tightly in the NHE due to more free pectic acid residues in the NHE root cell wall (Li et al. 2014). Two *MTP* genes were cloned from HE and NHE *S. alfredii* Hance and named as *SaMTP1* and *SnMTP1*, respectively. The two cloned *MTP* genes were mainly localized to the tonoplast and induced under Zn treatment but not influenced by Cd treatment. *SnMTP1* gene was found mainly expressed in the root and was induced under high Zn stress, while *SaMTP1* gene expression was induced in shoots but not roots by Zn level (Zhang et al. 2011a). The relative low expression of *SaMTP1* gene in HE root might be attributed to the reduced root sequestration into vacuoles, thus making Zn more mobile for xylem loading. Recently, we found that *SaHMA3* gene plays an important role in Cd translocation and localization into vacuoles (Zhang et al. 2015). However, the Cd sequestration transporters still need to be studied further.

3.1.3 Compartmentation and Detoxification

In HE *S. alfredii* Hance, Zn concentration increased in the order of root<leaf<stem (Li et al. 2006). HE *S. alfredii* Hance had the higher ability to remobilize Zn from mature leaves to mesophyll cells surrounding the phloem of new leaves (Lu et al. 2013b), but Zn saturation occurred in HE leaf mesophyll cells and stem vascular bundles relatively earlier, while epidermal layers served as a more important storage site for Zn accumulation in HE *S. alfredii* Hance (Tian et al. 2009). However, the difference was that mesophyll and vascular cells were more important storage sites for Cd storage of HE *S. alfredii* Hance (Tian et al. 2011a). Besides, Cd shared the similar storage sites with Ca (Tian et al. 2011a), and Cd uptake and translocation in HE *S. alfredii* Hance were positively associated with Ca pathway (Lu et al. 2010), which might be due to the increasing glutathione (GSH) content after Ca application (Tian et al. 2011b). At the subcellular level, it was observed that Zn was mostly distributed in cell walls and associated with malate in stems and complex with malate in *S. alfredii* Hance leaves (Lu et al. 2014), but aqueous Zn still occupied the highest proportion (>55.9%) of Zn speciation (Lu et al. 2013b). However, a larger proportion of Zn was found to be stored in organelles in NHE *S. alfredii* Hance than HE *S. alfredii* Hance (Li et al. 2006), which might partly explain its relative sensitivity to high Zn exposure. Extended X-ray absorption fine structure spectroscopy analysis showed that Cd in the stems and leaves of the

HE was mainly associated with oxygen ligands and malic acid (Tian et al. 2011a). Besides, a positive co-relationship of Zn distribution with P or S was revealed in HE *S. alfredii* Hance leaves and stems through SRXRF analysis (Tian et al. 2009). However, this relationship was not evident in NHE *S. alfredii* Hance. Chelation was suggested as a main strategy for heavy metal detoxification or the long-distance transportation in plants. Detailed analysis of Zn and Cd speciation might be useful to verify the detoxification mechanism of Zn and Cd.

Overaccumulation of hydrogen peroxide (H_2O_2) and superoxide radical (O^{2-}) was proposed as the main reason for phytotoxicity caused by excessive Zn and Cd, and much higher H_2O_2 was induced by Zn-treated leaf than Cd-treated one (Chao et al. 2008). Research showed that >500 μmol/L Zn in solution caused a marked increase in antioxidant enzyme activities, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX). However, in NHE, >10 μmol/L Zn was sufficient to activate the responses of antioxidant enzyme (Jin et al. 2008b). The different Zn concentration initiated antioxidant responses which reflected the higher tolerance ability of HE compared to NHE *S. alfredii* Hance. GSH and ascorbic acid (AsA) contents were found to be increased with Zn or Cd treatment in both HE and NHE *S. alfredii* Hance (Chao et al. 2008; Jin et al. 2008a, 2009), but the total S and GSH contents in shoots were higher under Cd treatment than under Zn treatment (Chao et al. 2008). Furthermore, a dose-dependent decrease in oxidized glutathione and marked increase in reduced glutathione and nonprotein thiols were observed in root tips of HE *S. alfredii* Hance but were not seen in the NHE *S. alfredii* Hance plants after Cd exposure (Tian et al. 2012). Besides, the expression level of *GSH1* in HE *S. alfredii* Hance shoots and roots also increased as Cd level increases but decreased in NHE *S. alfredii* Hance (Liang et al. 2014). It was presumed that GSH might directly bind with heavy metals or participate as a signal molecule in HE *S. alfredii* Hance (Jin et al. 2008a, c).

3.2 Mechanisms of Cu and Pb Accumulation by Plants

3.2.1 Uptake and Accumulation

E. splendens, a native Chinese Cu-tolerant and accumulating plant species, has been identified to be tolerant to high Cu concentration and has great potential in remediating contaminated soils (Jiang et al. 2004a, b). *E. splendens* can hyperaccumulate copper in its shoots with copper concentration up to 1133 and 3417 mg/kg wt when exposed to 500 and 1000 μmol/L Cu in nutrient solution, respectively. Copper uptake efficiency and translocation efficiency were dependent on Cu concentration in nutrient to maintain the concentrations of other essential nutrients, except potassium, within the range considered sufficient for normal growth of higher plants (Yang et al. 2002). *E. splendens* has the greater capacity to absorb Cu in roots from water and translocate Cu from roots to shoots (Tian et al. 2008).

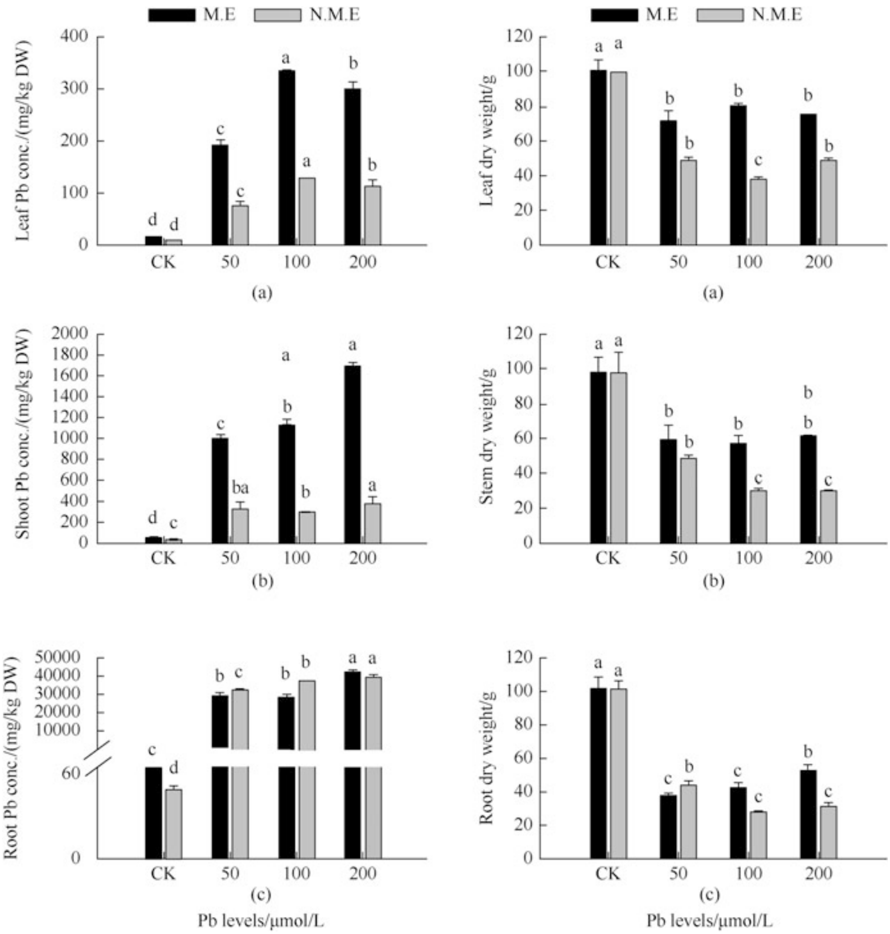


Fig. 6 Growth response and Pb uptake and accumulation in mined ecotype (ME) and non-mined ecotype (NME) of *E. argyi*

It was found that in the field experiment *E. splendens* can co-tolerate the concentration of 1500 mg/kg Cu in soil (Peng and Yang 2007). After exposure to 500 μmol/L Cu for 8 days, about 1000 mg/kg Cu was accumulated in the stem and 250 mg/kg Cu in the leaf of *E. splendens* (Peng et al. 2005a, b). And *E. splendens* can accumulate Cu up to 11.7 mg/plant if grown on the Cu smelter (Jiang et al. 2003).

E. argyi can tolerate external Pb levels up to 200 μmol/L in nutrient solution. The plants can healthily grow in metal complex contaminated soils with Pb over 3000 mg/kg. Lead concentration in leaf, stem, and root increased with lead supply levels and reached 350, 1600, 4000 mg/kg, respectively, when exposed to Pb level of 100 μmol/L (Fig. 6). ME exhibited higher tolerance to excessive levels of Pb in the growth medium, and Pb concentrations in the leaves and stem of ME were 2.6

and 4.5 times, respectively, higher than those of the NME (Ejaz et al. 2008). And in *E. splendens*, lead concentration in roots, stems, and leaves could reach 45, 183.6, 1657.6, and 380.9 mg/kg, respectively (Zhang et al. 2011b).

Lead concentrations in accumulator and non-accumulator ecotypes of *S. alfredii* Hance were positively correlated with root length, root surface area, and root volumes (Li et al. 2005a, b). The critical Pb concentration tolerance of *S. alfredii* Hance was 1000 μmol/L, and Pb concentration in different plant parts was in the order of root>stem>leaf (Xiong et al. 2004). Pb concentrations in the shoots of the accumulating ecotype of *S. alfredii* Hance were 1198.1 mg/kg at 0.2 mM treatment level of Pb (Liu et al. 2008a, b, c). The Pb concentration in roots and shoots of accumulating ecotype (AE) of *S. alfredii* Hance increased with the increase in Pb treatment, at 200 μmol/L Pb, and the maximum Pb concentration after 5 days was 53,775 mg/kg DW in roots and 2506 mg/kg DW in shoots (Gupta et al. 2010). The plantlets of *Pfaffia glomerata* also showed significant lead accumulation in roots (1532 μg/g DW) with a low root-to-shoot lead translocation (Gupta et al. 2011).

Lead uptake kinetics, xylem loading, and the contribution of low root zone temperatures and transpiration streams toward Pb uptake by the ME and NME of *E. argyi* were studied. For xylem loading of Pb, both AE and NAE showed a continuous increment with time and reached the maximum at 12 h; xylem loading of Pb by ME plants was higher than NME. Uptake of Pb was significantly and adversely affected by low transpiration streams (TS); however the overall reduction was much lower in AE than in NAE plants. Pb uptake was less reduced by low root zone temperatures (RZT) in AE than in NAE plants. It is suggested that apart from the passive uptake of Pb, the mined ecotype of *E. argyi* has an active component working simultaneously for the uptake of Pb (Islam et al. 2007a, b).

Lead uptake characteristics of *S. alfredii* Hance were studied in hydroponics (Liu and Yang 2008). The results indicated that under low Pb treatment level (10 μmol/L), the concentrations of measured Pb uptake by accumulating ecotype of *S. alfredii* Hance (AE) were higher than the non-accumulating ecotype (NAE) plants. It seems that active Pb uptake prevailed in AE plants at low external Pb treatment level; in contrast, passive uptake may be dominant in NAE at this treatment level. At Pb level of 50 μmol/L, active uptake may prevail in both ecotypes of *S. alfredii* Hance. After treating with metabolic inhibitor (CCCP), in AE plants, active uptake occupied 33.0%, 18.6%, and 20.5% of the total uptake amounts when exposed to Pb levels of 10, 25, and 50 μmol/L, respectively, while in NAE plants these were 18.3%, 14.2%, and 9.8%, respectively. In a low-temperature (4 °C) experiment, a similar trend as metabolic inhibitor was noted as active uptake percentages of AE plants were always higher than that of NAE at all treatment levels. It seemed that both active and passive Pb-uptake components coexist in *S. alfredii* Hance plants, with their relative contribution higher in AE than in NAE, especially at low Pb levels.

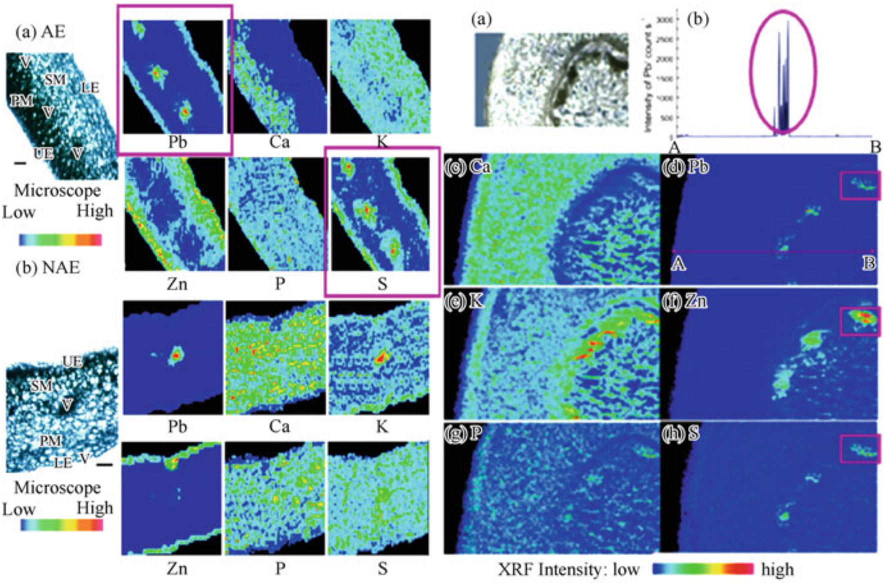


Fig. 7 Lead cellular distribution in leaf (left) and stem (right) sections of *S. alfredii* Hance

3.2.2 Distribution and Compartmentation

In *Elsholtzia* plants, Cu and Pb distribution in different plant organs is root>stem>leaf. The root accumulates the highest Cu and Pb, while the stem can hyperaccumulate Pb and Cu when exposed to high levels. Leaf contained the lowest concentration of Cu and Pb. For *S. alfredii* Hance, Pb distribution was also root>stem>leaf. The accumulating ecotype (AE) of *S. alfredii* Hance distributed more Pb in the stem and leaf than the NAE (Liu et al. 2008a, b, c). Pb was mostly accumulated in the roots; however only the accumulating ecotype *S. alfredii* Hance showed significant Pb accumulation in shoots (Gupta et al. 2010). And there were also high concentrations up to more than 1000 mg/kg of Pb which were transported to the shoots (Fig. 7).

With sequential chemical extraction and enzymatic methods, we found that Cu was mainly bound with cellulose and lignin on the cell wall both in roots and leaves. Water-soluble and acid-soluble fractions of Cu also accounted for a large proportion of total Cu present in cell, respectively. Pb accumulated mainly on the cell walls of the roots, stems, and leaves in *S. alfredii* Hance (He et al. 2003). Micro-XRF analysis of the stem and leaf cross section revealed that Pb was mostly restricted in the vascular bundles and epidermis tissues of both stem and leaf of *E. splendens* (Zhang et al. 2011b). We also found a very low mobility of Pb out of vascular bundles which was largely retained in the cell walls during transportation in plants of *S. alfredii* Hance (Tian et al. 2010), Pb distribution related closely to S distribution (Fig. 7). Further study revealed that the soluble Pb-EDTA complex could be transported and accumulated within the plants of *S. alfredii* Hance, but EDTA does not increase the internal mobility of Pb (Tian et al. 2011a, b, c).

3.2.3 Detoxification

Copper localization in cell walls and chloroplasts could mainly account for the high detoxification of Cu in *E. splendens* (Peng et al. 2005a, b). Also, some physiologically adaptive responses to Cu toxicity and the antioxidant enzyme system were involved in Cu detoxification in leaves of *E. splendens* observed by Peng et al. 2006. The activities of antioxidative enzymes SOD, XAT, GPX, and APX in leaves followed a similar pattern that all of them remained lower in treatments of 50 and 100 μmol/L Cu supply compared to those with Cu treatments lower than 50 μmol/L. The activities of these enzymes dramatically increased when plants grew in nutrient solution with Cu higher than 100 μmol/L. However, SOD and CAT seem to be more important in antioxidative stress initiated by Cu toxicity for their activities were always much higher than those of GPX and APX. The results implied that the oxidative stress was ROS dominantly in the given conditions. MDA content and electrolyte leakage in leaves were lower in treatments of 50 and 100 μmol/L Cu supply compared to those in other Cu treatments. The GSH content and Gr in 50 and 100 μmol/L Cu supply were higher than those of control or that in 500 μmol/L Cu supply. At 500 μmol/L Cu level, Cu detoxification could be attributed to the elevated activities of antioxidant enzymes such as GPX, APX, CAT, SOD, and GR in *E. splendens*; at 50 μmol/L Cu, the relationship between Cu detoxification and the antioxidant system in the plant leaf cell was maintained such that no marked Cu toxicity was exhibited (Peng et al. 2006). The results showed that Cu detoxification capacity of *E. splendens* depends on the balance between the oxidative-defense capacity and excessive Cu-induced oxidative stress.

The roles of free amino acids and organic acids played in Cu tolerance and accumulation were evaluated. Aspartate concentrations in leaves were negatively correlated to Cu concentrations both in leaves and in solution. This might be due to the metabolic enhancement and the accelerated turnover of Asp in cells under Cu stress. Concentrations of histidine and γ-aminobutyric correlated to Cu concentrations both in leaves and in solution. Glutamate concentrations in leaves were negatively correlated to GABA concentrations in leaves. Histidine might be involved in Cu uptake, and hyperaccumulation in *E. splendens* of γ-aminobutyric might enhance the ability of the plants to tolerate toxic Cu by signaling. The depletion of Glu in leaves was due to the enhanced synthesis of GABA under Cu stress. As far as we know, it was the first time that γ-aminobutyric was found to be involved in heavy metal tolerance and hyperaccumulation (Yang et al. 2005a, b). The role of His played in Cu hyperaccumulation has not yet been reported in any reliable document.

Citric acid concentrations in leaves were negatively correlated to Cu concentrations in leaves. Exterior supply with 250 μmol/L citric acid plus 250 μmol/L Cu significantly decreased Cu concentrations in roots, stems, and leaves compared to those supplied with only 250 μmol/L Cu. The plants exposed to Cu plus citric acid grew better than with addition of Cu only in terms of root elongation and shoot height as well as biomass yield. This result implied that citric acid enhanced Cu tolerance through declining Cu uptake by *E. splendens*. Citric acid may complex with Cu in nutrient solution and thus decrease the availability of Cu.

The presence of EDTA or Zn alleviated Pb phytotoxicity through changes in *S. alfredii* biomass, root morphology, and chlorophyll contents (Liu et al. 2008a, b, c). The alleviation of Pb toxicity by Zn was mainly due to improved plant defense against oxidative stress by competing with Pb for binding to critical cell constituents in *E. argyi* (Islam et al. 2011). In molecule research, other mechanisms of lead detoxification mentioned are sequestration of cells and the role of antioxidant enzymes and antioxidants. Large amounts of lead deposit in the cell walls of plants, preventing the damage of insoluble substance in the cell (Huang et al. 2008a, b; Zheng et al. 2007). Lead was predominantly restricted to the vascular bundles of both leaf and stem of the accumulator ecotype (Tian et al. 2010). These results indicated that the distribution of Pb and cell walls of the accumulating ecotype *S. alfredii* Hance could mainly account for the high tolerance to Pb (He et al. 2003) (Figs. 8 and 9). Enzymatic and nonenzymatic antioxidants play a key role in the detoxification of Pb-induced toxic effects in *S. alfredii* Hance (Gupta et al. 2010). In the absence of PCs, GSH may play an important role of detoxification mechanism in both ecotypes of *S. alfredii* Hance under Pb stress (Gupta et al. 2010). However, GSH played a role in lead detoxification of Pb in the shoots of *S. alfredii* Hance (Huang et al. 2008a, b; Zheng et al. 2007). Pb tolerance exhibited by accumulating ecotype plants could be mainly attributed to the maintenance of their growth, activity of antioxidant enzymes, as well as integrity of cell organelles, especially membranous organelles, in response to Pb toxicity (Liu et al. 2008a, b, c).

3.2.4 Root Exudation and Metal Mobility

Phytoextraction of Cu by *E. splendens* increased with increasing Cu levels in solution, EDTA addition, and application of organic manure at an appropriate rate (Jiang and Yang 2004; Peng et al. 2005a, b). Peng (2005) found that the increased extractability of Cu has mainly attributed to the rhizospheric acidification and chelation by dissolved organic matter (DOM), thus resulting in elevating Cu uptake and accumulation by *E. splendens* (Peng et al. 2005a, b).

We studied the effects of Pb on root exudation of organic acids (OAs) and the role of these OAs in soil metal mobility and phytoextraction by *E. argyi* (Ejaz and Yang 2008). The results showed that the application of different Pb treatments induced the exudation of lactic acid (LA) with comparatively more induction in the AE. The Pb and Cu extractability of root exudates collected from. *E. argyi* under different Pb toxicity levels was tested using two types of soils, i.e., Zhuji mined soil (ZMS) and Sanmen mined soil (SMS). The results showed that the overall metal extractability of the root exudates of AE was higher than NAE. The metal mobilization of various organic acids identified in the root exudates was evaluated to be

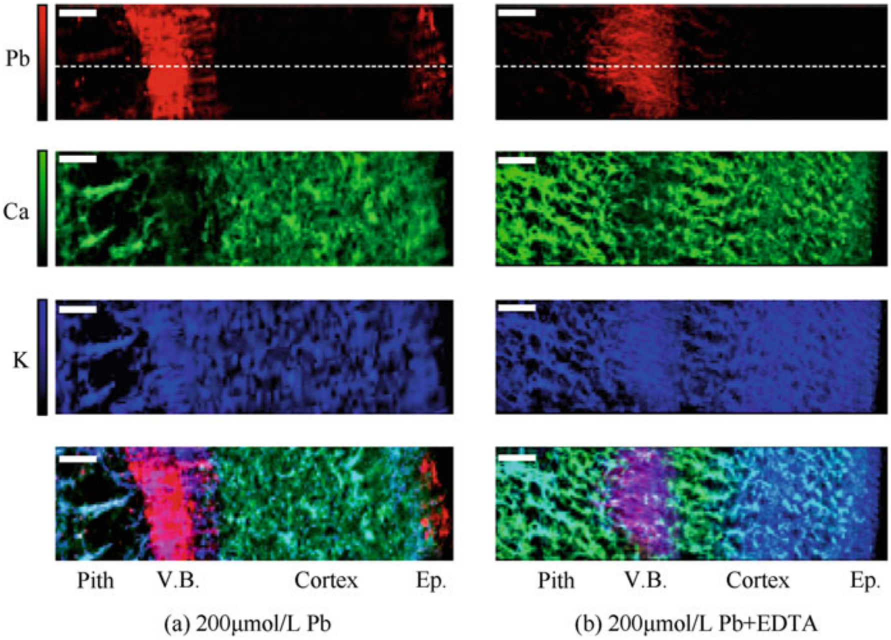


Fig. 8 Effects of **a** Pb and **b** Pb + EDTA application on the distribution of Pb across the stem of *S. alfredii* Hance (Tian et al. 2010)

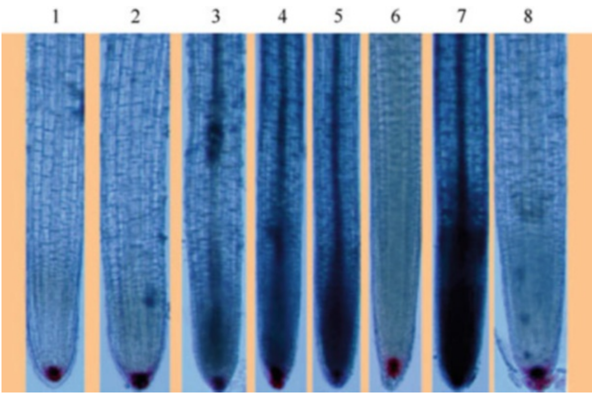


Fig. 9 Effect of lead and EDTA on cell death in accumulating ecotype of *S. alfredii* Hance root. Viability of cells was visualized by Evan's blue staining. The treatments are as follows: control (*1*), 25 μmol/L Pb (*2*), 50 μmol/L Pb (*3*), 100 μmol/L Pb (*4*), 200 μmol/L Pb (*5*), 200 μmol/L Pb + 200 μmol/L EDTA (*6*), 400 μmol/L Pb (*7*), +200 μmol/L EDTA (*8*)

decreased in the order OA>TA; LA>MA for Pb and OA>TA; MA>LA for Cu, respectively. In SMS, these decreased in the order OA>LA>MA>OA for Pb and MA>TA>LA>OA for Cu, respectively. Pot experiment showed that application of OAs brought significant increase in the shoot Pb and Cu concentrations of *Elsholtzia*, especially Pb accumulation in the shoots treated with LA. It is suggested that in *E. argyi*, MA may help the plants to take up exceptionally higher concentration of Pb and Cu from the growth medium but restricts them only up to roots, while LA might be involved in metal mobility in the rhizosphere and help root-to-shoot translocation of these metals. We found that there is a synergistic effect produced by IAA in combination with EDTA on the Pb uptake by *S. alfredii* Hance (Liu et al. 2007). In addition, increasing Zn/Pb levels or citric acid or acetic acid at higher concentrations ($>10^{-3}$ mol/L) could also enhance Pb uptake (Yang et al. 2006a, b, c, 2010).

4 Microbe-Assisted Phytoremediation

4.1 Exploitation of Plant Growth-Promoting Endophytic Microbes

4.1.1 Screening and Identification of Endophytic Microbes from Hyperaccumulator

Eighty-five metal-resistant bacterial strains were isolated from rhizosphere soils and *S. alfredii* Hance plants grown in the old Pb/Zn mining area using mineral-minimal media containing 1-aminocyclopropane-1-carboxylic acid (ACC) as the sole nitrogen source and high levels of Zn, Cd, and/or Pb. Analyses of bacterial 16S ribosomal RNA genes (16S rDNA) revealed that the 85 strains were affiliated to 20 genera of *Proteobacteria* (55%), *Actinobacteria* (27%), *Firmicutes* (11%), and *Bacteroidetes* (7%) and that 45 strains (53%) might represent 15–17 novel species of eight genera. ACC deaminase structure genes (*acdS*) were amplified from 28 strains; ACC deaminase activities were detected from 21 of them in free-living states. The phylogenies of the *acdS* sequences from the other seven strains that did not show ACC deaminase activities were incongruent with those of their 16S rDNA; these *acdS* genes might be evolved through horizontal transfer. All the 85 strains showed differential resistance to high levels of Zn, Cd, and/or Pb. The percentages of the obtained bacterial strains with relatively higher metal resistance were positively correlated to the metal concentrations of the rhizosphere soil, root, and shoot tissues. This indicated the nature of selection of the high level of metals in soils and plants on bacterial metal resistance and adaptation. Most of the bacterial strains could produce indole acetic acids and siderophores, or solubilize mineral phosphate, and thus had potentials to promote plant growth and increase metal solubility from soils.

4.1.2 Colonization of Microbes in the Root of Plant

Inoculating endophytic bacteria was proven as a promising way to enhance phytoremediation.

S. alfredii Hance is a Zn and Cd co-hyperaccumulating plant species found in an old mining area in China. Four bacterial strains, *Burkholderia* sp. *SaZR4*,

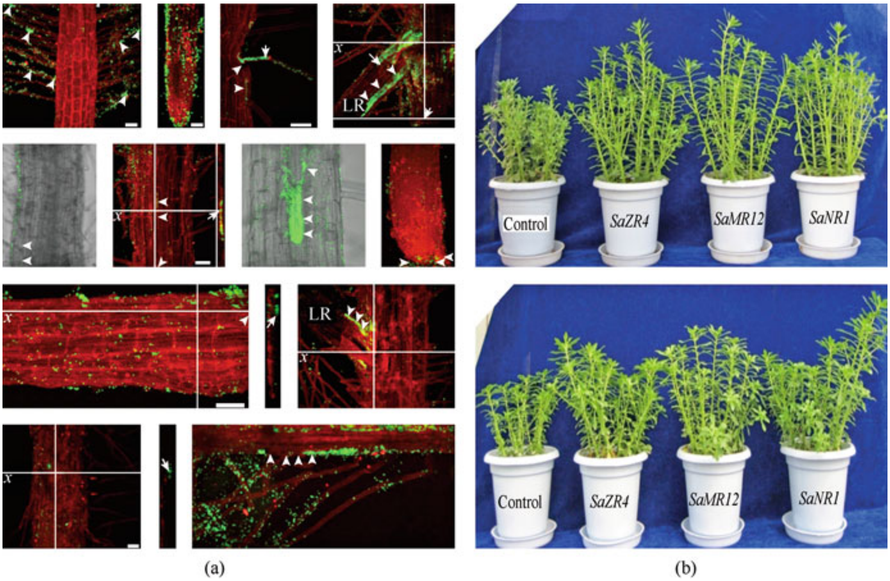


Fig. 10 **a** Localization of endophytic bacteria in roots and **b** effects of endophytic bacteria inoculation on growth of *S. alfredii* Hance

Burkholderia sp. *SaMR10*, *Sphingomonas* sp. *SaMR12*, and *Variovorax* sp. *SaNR1*, isolated from surface-sterilized *S. alfredii* Hance plants were used to investigate their endophytic nature and root colonization patterns and effects on phytoextraction of Zn and Cd. Laser scanning confocal microscopy revealed that *gfp*-tagged *SaZR4*, *SaMR12*, and *SaNR1* cells formed biofilms on roots and that *SaZR4* and *SaMR12* cells could invade root tissues (Zhang et al. 2013) (Fig. 10).

Fluorescence in situ hybridization (FISH) was also used to investigate *SaMR12* colonization in the root of oilseed and *S. alfredii* Hance. The results showed that *SaMR12* could successfully colonize in the inner tissues of root of oilseed and *S. alfredii* Hance. In the space of root cell, the bacteria cell could gather into a block and multiply abundantly (unpublished).

4.2 Effects of Endophytic Microbe on Metal Uptake and Phytoextraction

4.2.1 Effects of Endophytes on Growth and Metal Uptake of *S. alfredii* Hance

Soil experiments showed that endophytic bacterial strains *SaZR4*, *SaMR12*, and *SaNR1* increased the shoot biomass of *S. alfredii* Hance in two harvests and root biomass in the second harvest in the original Pb/Zn mined soil and a multi-metal-

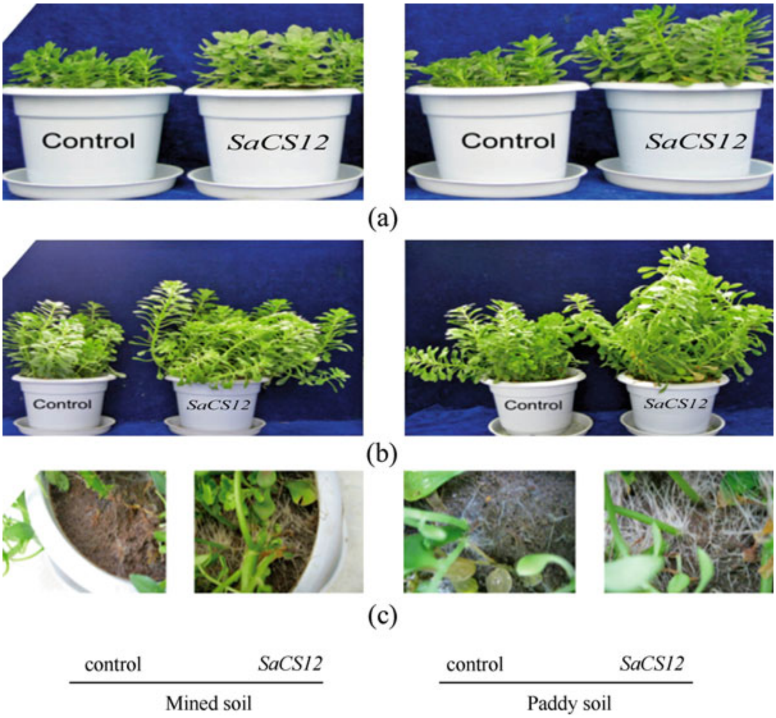


Fig. 11 Effects of *Fusarium oxysporum* on the *S. alfredii* Hance grown **a** after 1 month, **b** after 5 months, **c** in mined soil and paddy soil

contaminated paddy soil. The phytoextraction of Zn, Cd, Pb, and Cu was significantly enhanced in the two soils. Phytoextraction of Zn was approximately twofold after inoculation with *SaZR4* (Zhang et al. 2011a). Therefore, continual inoculation of endophytic bacteria to enhance phytoremediation by the perennial *S. alfredii* Hance is applicable.

The inoculation effects of the broad-spectrum endophytic fungus *Piriformospora indica* on hyperaccumulating and non-hyperaccumulating ecotype *S. alfredii* Hance (HE and NHE) plants were studied in a hydroponic system. NHE plants were stressed under treatment of 200 μmol/L Zn or 10 μmol/L Cd and injured under 400 μmol/L Zn or 20 μmol/L Cd, whereas HE plants were able to tolerate higher levels of Zn and Cd. *P. indica* colonized in the roots of the two ecotypes and promoted their root development, nitrogen and phosphorus uptake, and metal tolerance (Fig. 11). HE shoots accumulated 1.17–1.75-fold Zn or 1.83–2.21-fold Cd compared with the uninoculated controls. *P. indica* reduced Zn or Cd uptake and increased metal tolerance by NHE plants under 200 μmol/L Zn or 5–10 μmol/L Cd but was not able to reduce the toxic effects under 400 μmol/L Zn or 20 μmol/L Cd. *P. indica* thus has an application potential to enhance phytoextraction by HE *S. alfredii* Hance plants and metal tolerance by non-accumulating plants.

A heavy metal-resistant fungus that belonged to the *Fusarium oxysporum* complex was isolated from *S. alfredii* Hance. This *Fusarium* fungus was not pathogenic to plants but promoted host growth. Hydroponic experiments showed that 500 μmol/L Zn or 50 μmol/L Cd combined with the fungus increased root length, branches, and surface areas and enhanced nutrient uptake and chlorophyll synthesis, leading to more vigorous hyperaccumulators with greater root systems. Soil experiments showed that the fungus increased root and shoot biomass and *S. alfredii*-mediated heavy metal availabilities, uptake, translocation, or concentrations and thus increased phytoextraction of Zn (144% and 44%), Cd (139% and 55%), Pb (84% and 85%), and Cu (63% and 77%) from the original Pb/Zn mined soil and a multi-metal-contaminated paddy soil. Together, the nonpathogenic *Fusarium* fungus was able to increase *S. alfredii* Hance root systems and function, metal availability and accumulation, plant biomass, and thus phytoextraction efficiency. This study showed an indigenous culturable *Fusarium* fungus other than mycorrhizal fungi to enhance phytoextraction by hyperaccumulators and a new function and application potential for the worldwide distributed soil and plant-associated nonpathogenic *F. oxysporum* fungi. This study also showed a new avenue of microorganism-assisted phytoextraction for hyperaccumulators that are mainly non-mycorrhized Brassicaceae plants (Zhang et al. 2012).

4.2.2 Effects on Plant Growth and Metal Uptake of Rapeseed

Four plant growth-promoting bacteria (PGPB) were used as materials; among them two heavy metal-tolerant rhizosphere strains SrN1 (*Arthrobacter* sp.) and SrN9 (*Bacillus altitudinis*) were isolated from rhizosphere soil, while two endophytic strains SaN1 (*Bacillus megaterium*) and SaMR12 (*Sphingomonas*) were identified from roots of the Cd/Zn hyperaccumulator *S. alfredii* Hance. A pot experiment was carried out to investigate the effects of these PGPB on plant growth and Cd accumulation of *Brassica napus* (*B. napus*) plants grown on aged Cd-spiked soil. The results showed that the four PGPB significantly boosted oilseed rape shoot biomass production, improved soil and plant analyzer development (SPAD) value, and enhanced Cd uptake of plant and Cd translocation to the leaves (Fig. 12). The endophytic bacteria performed better in this respect than the rhizosphere bacteria. However, all four PGPB could increase seed Cd accumulation. Due to its potential to enhance Cd uptake by the plant and to restrict Cd accumulation in the seeds, SaMR12 was selected as the most promising microbial partner of *B. napus* when setting up a plant-microbe fortified remediation system (Pan et al. 2016).

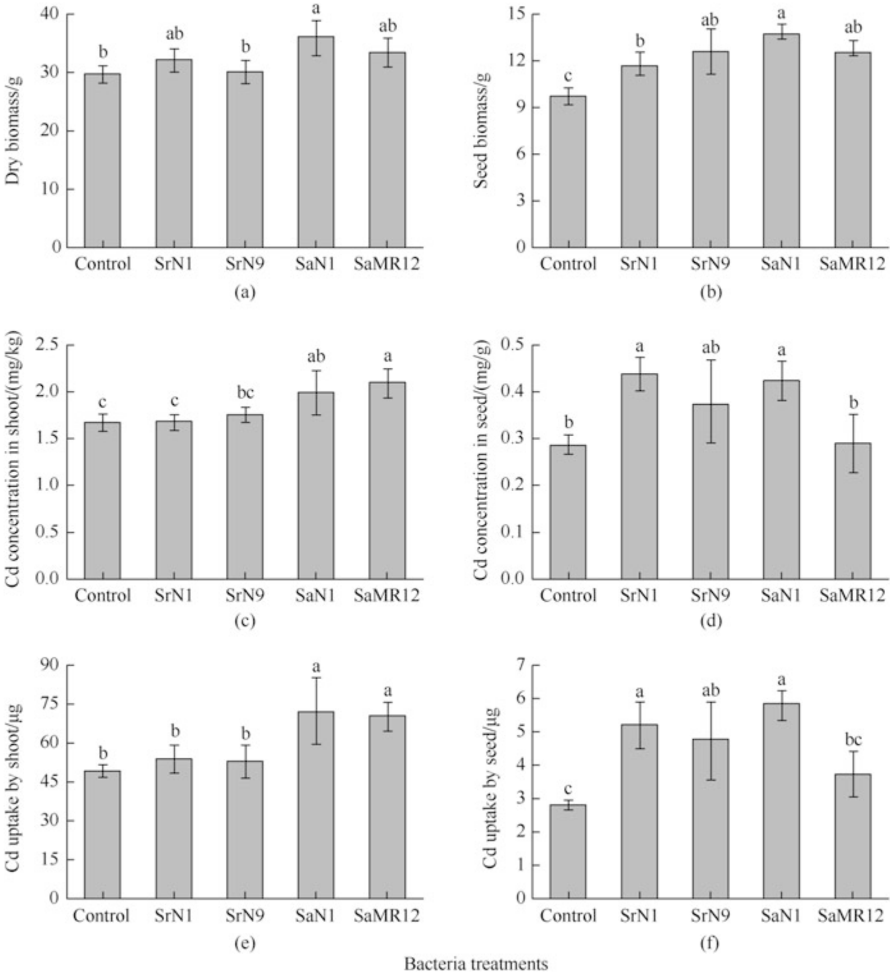


Fig. 12 The influences of PGPB as harvested at mature stage. (**a**) Biomass, (**b**) seed yield, (**c**) Cd concentration of shoot, (**d**) Cd concentration of seed, (**e**) Cd uptake of shoot, (**f**) Cd uptake of seed. Error bars represent SD from three individual replicates. The different letters on the error bars indicate significant difference among treatments at $p < 0.05$

4.3 Mechanisms of Endophytes Enhancing Metal Phytoextraction

4.3.1 Effects on Root Growth and Root Hair Development

Our serious studies showed that endophytic microbes can considerably enhance root growth and root hair development of the hyperaccumulator. It showed that by inoculating endophytic bacterium *Sphingomonas* SaMR12 to *S. alfredii* Hance,

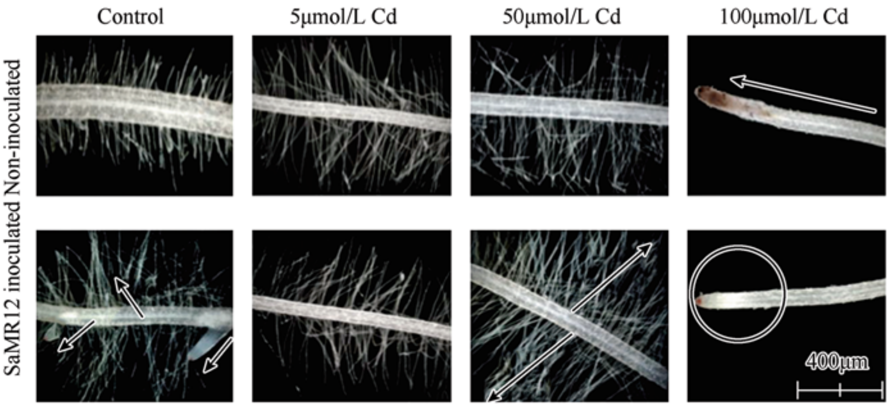


Fig. 13 Effects of endophytic bacteria SaMR12 on root hair development of *S. alfredii* Hance under different Cd levels

SaMR12 inoculation improved plant biomass, length of roots, number of root tips, and root surface area (Fig. 13). The study also showed that inoculation with SaMR12 could enhance Zn and Cd accumulation in *S. alfredii* Hance (Chen et al. 2014a, b).

4.3.2 Effects of Root Exudation of Organic Acids

Plant growth-promoting bacteria have many mechanisms to improve phytoremediation efficiency. Root secretion of oxalic, citric, and succinic acids was also increased after inoculation, which might alleviate the cadmium toxicity to plant or inhibit the rising trend of oxidative stress of plant. Our study indicated that SaMR12 improved cadmium bioavailability and absorption facility by increasing root-soil contact area and root organic acid secretion, and in the shoot, SaMR12 increased cadmium tolerance by alleviating the oxidative stress of plant, so as to enhance the capability of cadmium extraction by the plant (Chen et al. 2014b) (Fig. 14).

4.3.3 Effects on Expression of Metal Transporters

Compared with control, inoculation of SaMR12 significantly enhanced Cd accumulation of *S. alfredii* Hance under Cd stress. As a number of antioxidant enzymes (SOD, CAT, and POD) in *S. alfredii* Hance were not sensitive to the changes in Cd treatment levels, even SaMR12 had no significant effects on the antioxidant enzymes activities (Fig. 15). However, Cd exposure levels and SaMR12 influenced root concentration of H_2O_2 and GSH as well as the expression of related genes, such as PER1, ATPS, GS, and GSH1. Hence these results indicate that SaMR12 could

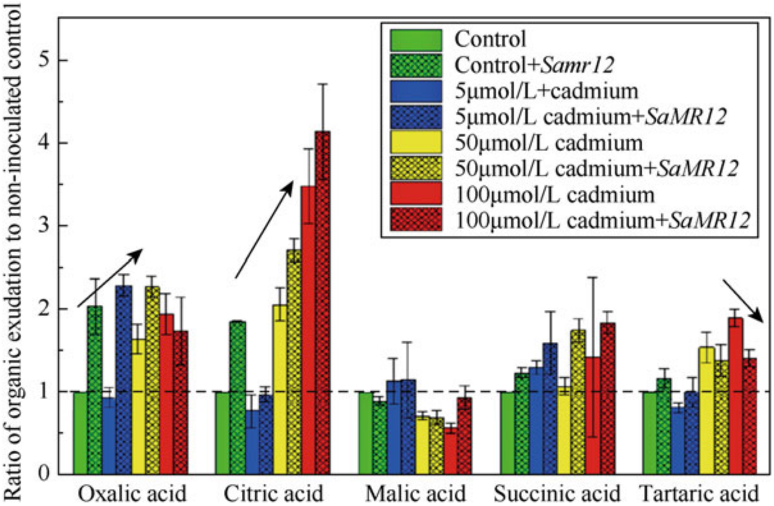


Fig. 14 Organic acid exudation as affected by SaMR12 and Cd treatment. Bars plot mean six SD of three replicate experiments. The level of each acid is normalized to the value obtained for the non-inoculated and unsupplemented treatment

enhance plant Cd tolerance and accumulation by increasing GSH concentration and related gene expression and reducing H_2O_2 concentration and root damage (Pan et al. 2016).

We also explored the effects of endophytic bacterial SaMR12 on the expression of three metal transporter families at different Cd-treated levels in *S. alfredii* Hance. Our findings showed that elevated Cd treated level, within the threshold of toxicity, activated SaMR12 that resulted increased Fe, Zn, and Cu contents in shoots and elevated the expression of *SaIRT1*, *SaHMA2*, and *SaNRAMP3* in shoot and *SaHMA2* and *SaNARMP1* in root, suggesting that SaMR12 mainly improved the expression of some transporter genes to increase plant acquisition of essential elements and uptake of Cd, while at Cd stress circumstance, SaMR12 increased leaf chlorophyll concentration and Fe/Mg content in shoots and elevated the expression of *SaIRT1*, *SaZIP3*, *SaHMA2*, *SaHMA3*, and *SaNRAMP6* in shoot and *SaIRT1*, *SaZIP3*, *SaHMA3*, and *SaNARMP6* in roots, inferring that SaMR12 mainly increased plant Fe and Mg uptake and regulated the expression of metal transporter genes, so as to improve plant growth and Cd tolerance to enhanced Cd accumulation (Pan et al. 2016).

5 Chemical-Assisted Phytoremediation

We have studied some chemical-assisted phytoremediation technologies since 2002. The basic mechanisms are shown in Fig. 16.

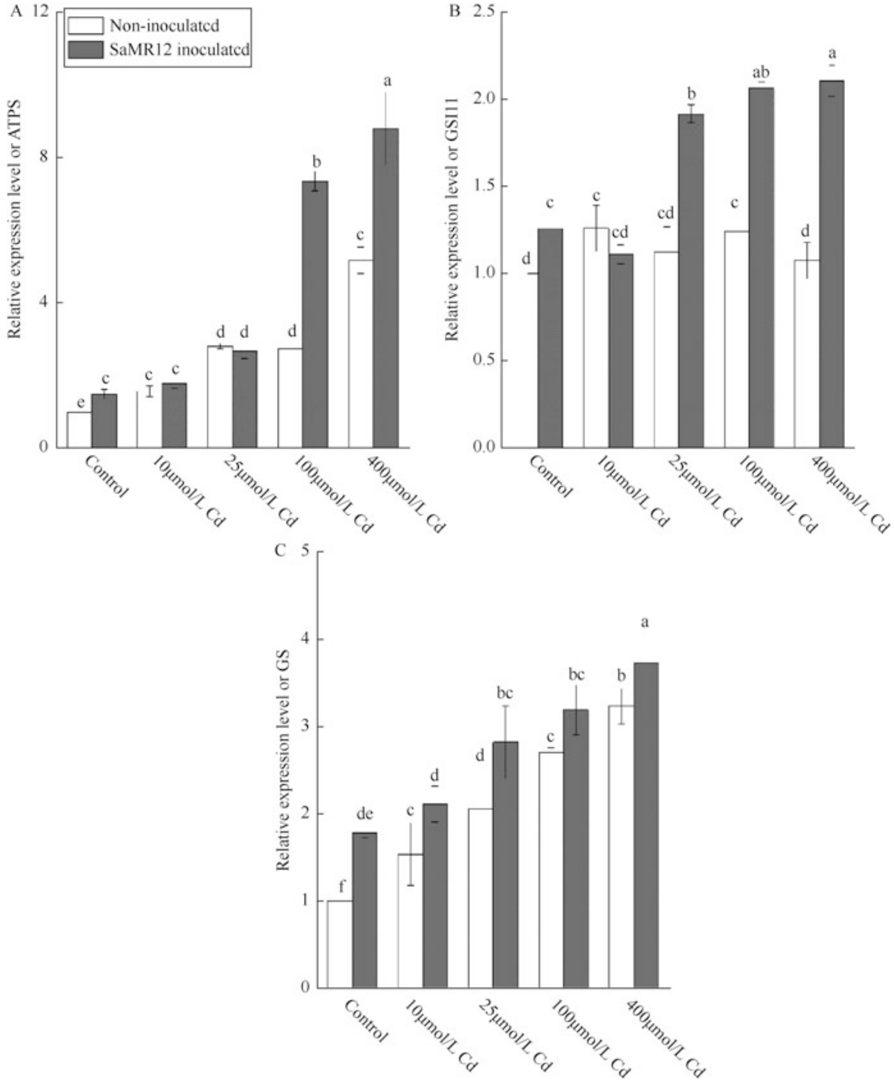


Fig. 15 Effects of Cd and SaMR12 on expression level of ATPS, GSH1 and GS genes. *Error bars* represent SD from three individual replicates. Different *letters* indicate significant difference at $p < 0.05$

5.1 Effects of Synthetic Chelators and Low Molecular Organic Compounds on Enhancing Phytoextraction

Some synthetic chelators and organic acids have positive effects on improving the phytoremediation efficiency of hyperaccumulators. Table 1 summarized some research results by us. We reported (Jiang et al. 2004a, b; Yang et al. 2005a) that

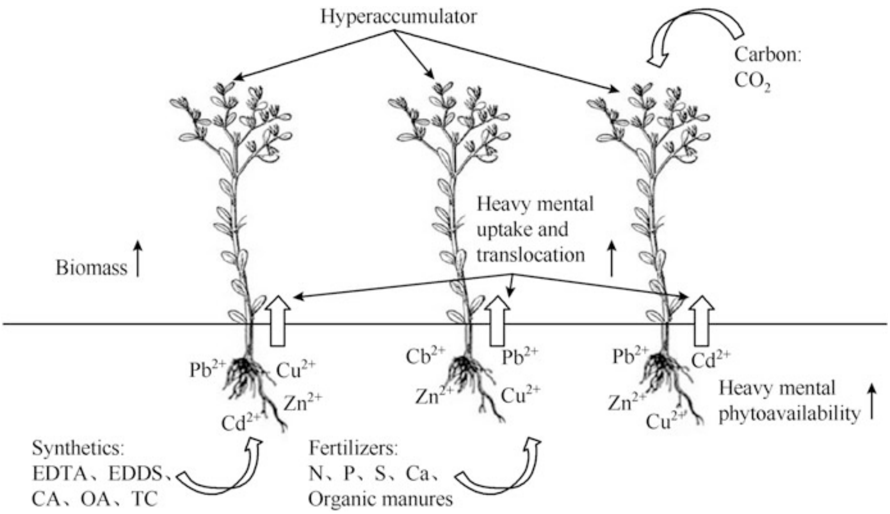


Fig. 16 Basic mechanisms for chemical-assisted phytoremediation of metal-contaminated soils

Table 1 Effects of synthetic chelators and organic acids on phytoremediation efficiency

Hyperaccumulator	Material	Findings	Reference
E. splendens	EDTA	The shoot Cu concentration of *E. splendens* increased fourfold in mined soil and eightfold in paddy soil	Jiang et al. (2004a, b)
	Citric acid	No remarkable effects when citric acid concentration<5.0 mmol/kg	Yang et al. (2005a, b)
S. alfredii Hance	EDTA	Synergistic effect of IAA/EDTA combination was found on the Pb uptake by *S. alfredii* Hance	Liu et al. (2007)
	EDDS	Better Pb accumulation efficiency in low-Pb soil	Wang et al. (2009)
	Oxalic acid	Root Zn accumulation efficiency was prominently increased	Fang et al. (2012)
	Citric acid	Cd accumulation was elevated after long-term application; root Zn accumulation efficiency was prominently increased	Fang et al. (2012) and Lu et al. (2013a, b)
	Tartaric acid	Cd accumulation was elevated after long-term application	Lu et al. (2013a, b)

addition of 2.5 mmol/kg EDTA increased the H_2O extractable Cu concentration in soil significantly (from 1.20 to 15.78 mg/kg in mined soil and from 0.26 to 15.72 mg/kg in paddy soil, in contrast with the control), leading to the shoot Cu concentration of *E. splendens* increasing fourfold in mined soil (MS) and eightfold in paddy soil (PS) compared with the control (Jiang et al. 2004a, b). The efficiency of Cu phytoextraction by *E. splendens* in polluted soils was increased mostly through adding 2.5–5.0 mmol/kg EDTA, while $<$5.0 mmol/kg citric acid (CA) had no remarkable effects (Yang et al. 2005a).

The phytoremediation efficiency of *S. alfredii* Hance is also influenced by synthetic chelators and exogenous low-molecular-weight organic acids. The application of CA or oxalic acid (OA) increased shoot Zn accumulation in non-hyperaccumulator ecotype (NHE) *S. alfredii* Hance significantly. But there is no significant effect in shoot Zn accumulation of hyperaccumulating ecotype (HE) *S. alfredii* Hance. However, root Zn accumulation efficiency was prominently increased in both ecotypes of *S. alfredii* Hance (Long et al. 2001). CA might be also involved in the processes of Cd uptake, translocation, and tolerance in *S. alfredii* Hance. Therefore, after long-term application of CA or tartaric acid (TA), Cd accumulation was elevated in *S. alfredii* Hance (Lu et al. 2013a, b).

EDTA was found effective for enhancing Pb accumulation in the shoots of *S. alfredii* Hance. And it acted as a chelating agent that assisted Pb to be transported from roots to shoots (Liu et al. 2008a, b, c). Further study by Liu et al. (2007) showed that there was a synergistic effect of IAA/EDTA combination on the Pb uptake by *S. alfredii* Hance. The treatment of 200 μmol/kg EDTA combined with 10 or 100 μmol/kg IAA increased the Pb accumulation in shoots of *S. alfredii* Hance significantly ($p < 0.05$) by 149.2% and 243.7% compared with 200 μmol/kg Pb treatment. Wang et al. (2009) reported that ethylenediaminedisuccinic acid (EDDS) could enhance Pb accumulation in *S. alfredii* Hance as well. And it had a better effect in the low-Pb soil (400 mg/kg) than in high-Pb soil (1200 mg/kg).

Besides these effects, similar effects are also found in EDDS for Cu and DTPA for Cu/Cd in the shoots of *S. alfredii* Hance (Liu et al. 2008a, b, c). Though some synthetic chelators could enhance the phytoextraction of heavy metals, the way of reducing potential environmental risks of synthetic chelators still need to be studied.

5.2 Effects of Mineral Fertilizers on Enhancing Phytoremediation

We have conducted a series of hydroponic experiments (Li et al. 2007a, b, c, d, 2008a, b; Lin et al. 2007; Zhu et al. 2010), so as to assess the effects of different nitrogen forms and concentration on *S. alfredii* Hance. The results showed that the application of inorganic nitrogen (urea) significantly ($p < 0.05$) promoted the phytoremediation efficiency of Cd compared with organic nitrogen (arginine) in hyperaccumulator *S. alfredii* Hance. Exogenous applications of inorganic nitrogen also markedly, increased shoot and root biomass as well as chlorophyll a and b content in leaves ($p < 0.05$) (Li et al. 2008). Furthermore, NH_4^+-N has greater ability to promote Cd accumulation in *S. alfredii* Hance than NO_3^--N (Li et al. 2008). The studies of nitrogen (urea) concentration effects showed that root and shoot growth of *S. alfredii* Hance was promoted consistently when nitrogen level is less than 16.0 mmol/kg and reached the peak at 16 mmol/kg, but it was restrained when nitrogen level was more than 32.0 mmol/kg (Li et al. 2007).

Except for N application, applying P appropriately enhanced Zn mobility, absorption, translocation from root to shoot, and accumulation in shoots of *S. alfredii* Hance (Sun et al. 2003; Huang et al. 2013). Hydroponic culture experiment showed that Zn contents and accumulations in leaves, stems, and roots of *S. alfredii* Hance were significantly increased by increasing P concentrations from 0.5 to 1.0 mmol/kg in nutrient solution (Sun et al. 2003). The pot experiment, conducted by Ni et al. (2004a, b), showed that Zn concentration and accumulation were peaked at 31 mg/kg dose; meanwhile the P concentration in shoots reached 2.09 mg/g DW, where they regarded as a potential index for P application in practice. Recent studies (Huang et al. 2012a, b) indicated that coupled with multiple cuttings, the application of P fertilizers could shorten the time needed for remediation of Zn-/Cd-contaminated soils. In Table 2, we could conclude that the highest phytoextraction efficiency of Zn and Cd was in the treatments of KH_2PO_4 and $NH_4H_2PO_4$ when P_2O_5 concentration was 352 mg/kg (Huang et al. 2012a, b) indicating that coupled with multiple cuttings, the application of P fertilizers could shorten the time needed for remediation of Zn-/Cd-contaminated soils (Fig. 17).

Different letters indicate significant difference among various treatments ($p < 0.05$); small letters are for different phosphorus application rates in the same phosphorus fertilizer treatments, and capital letters are for different phosphorus fertilizer treatments at the same application rate of P_2O_5.

Table 2 Zn and Cd extracted amount from *S. alfredii* shoots under various types of phosphate fertilizers (Huang et al. 2012a, b)

P rate (P_2O_5/(mg/kg))	P fertilizer type	Zn extraction amount/(mg/pot)	Cd extraction amount/(μg/pot)
0	KH_2PO_4	5.0 ± 0.64c	119.0 ± 14.43ab
	$Ca(H_2PO_4)_2$	5.0 ± 0.64c	119.0 ± 14.43ab
	NaH_2PO_4	5.0 ± 0.64c	119.0 ± 14.43ab
	$NH_4H_2PO_4$	5.0 ± 0.64c	119.0 ± 14.43ab
22	KH_2PO_4	4.5 ± 0.73cB	114.3 ± 2.85bB
	$Ca(H_2PO_4)_2$	26.4 ± 0.32bA	131.4 ± 9.66aA
	NaH_2PO_4	5.8 ± 0.67bcAB	99.9 ± 5.19bC
	$NH_4H_2PO_4$	6.6 ± 1.26cA	126.9 ± 4.90bcA
88	KH_2PO_4	7.6 ± 0.42bB	128.8 ± 4.03abB
	$Ca(H_2PO_4)_2$	27.1 ± 0.38bB	114.5 ± 9.05aBC
	NaH_2PO_4	7.6 ± 0.61bB	128.9 ± 2.13aB
	$NH_4H_2PO_4$	10.2 ± 1.46bA	147.8 ± 6.71abA
352	KH_2PO_4	12.8 ± 1.12aAB	143.3 ± 6.73aB
	$Ca(H_2PO_4)_2$	212.1 ± 0.85aB	123.4 ± 5.10aC
	NaH_2PO_4	11.1 ± 0.94aB	132.4 ± 4.74aC
	$NH_4H_2PO_4$	13.8 ± 0.69aA	162.6 ± 15.50aA

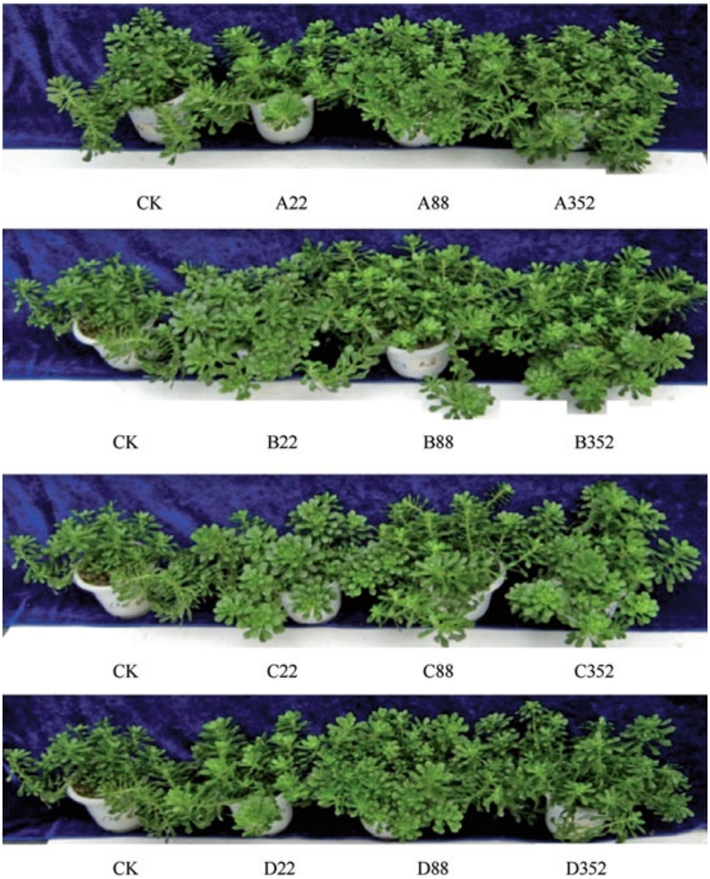


Fig. 17 Photographs of *S*. *alfredii* Hance at the third clipping after 120 days of growth (Huang et al. 2012a, b)

Sulfur application is another potential method to improve the phytoextraction efficiency of Cd in *S*. *alfredii* Hance. Cd uptake and translocation efficiency of *S*. *alfredii* Hance were increased with increasing S (K_2SO_4) supplies in hydroponic culture. And the relationship could be respectively described by logarithmic equation and linear equation. A significant increase was observed in biomass; Cd contents; amounts of Cd accumulated in leaves, stems, and roots; and total amounts of Cd accumulated in *S*. *alfredii* Hance when S supplies increased from 1.5 to 2.25 mmol/kg (Li et al. 2008).

There are other potential mineral fertilizer-assisted phytoremediation methods, such as applying appropriate concentration of exogenous Ca^{2+} to *S*. *alfredii* Hance to improve its ability of accumulating more Zn (Huang et al. 2008a, b) and so on. All of these findings revealed that using mineral fertilizers to assist phytoremediation has a promising future.

Table 3 Effects of elevated CO_2 treatment on total Cd uptake, bioconcentration factor, phytoextraction efficiency, and postharvest available Cd in the soil (Li et al. 2012a)

Soil	CO_2 level	Total Cd uptake/ (mg/pot)	Bioconcentration factor	Phytoextraction efficiency/%	Postharvest available Cd/(mg/kg)
CK	Ambient	0.24 ± 0.01	76.6 ± 2.9	15.25 ± 1.07	0.08 ± 0.01
	Elevated CO_2	0.33 ± 0.02*	78.6 ± 1.8	20.47 ± 1.80*	0.06 ± 0.02
Cd5	Ambient	1.92 ± 0.09	61.4 ± 2.1	11.58 ± 1.29	1.43 ± 0.04
	Elevated CO_2	2.86 ± 0.19*	71.9 ± 6.6*	17.18 ± 1.88*	0.99 ± 0.15*
Cd50	Ambient	11.88 ± 1.05	48.0 ± 3.1	9.50 ± 0.89	17.61 ± 1.43
	Elevated CO_2	16.78 ± 1.39*	55.7 ± 3.9*	13.43 ± 1.06*	13.83 ± 2.04*

*Significant differences ($p < 0.05$) between ambient and elevated CO_2 treatments

5.3 *Effects of Carbon and Organic Manures on Enhancing Phytoremediation*

In recent years, we have examined the effects of elevated CO_2 on improving the phytoremediation efficiency of Cd/Zn hyperaccumulator *S. alfredii* Hance. As Li et al. (2012) reported, after 60 days of growth, the total Cd uptake per pot and Cd phytoextraction efficiency of *S. alfredii* Hance were significantly ($p < 0.05$) increased in elevated (800 μL/L) CO_2 treatment compared with ambient (350 μL/L) CO_2 treatment, which could be seen from Table 3. Meanwhile, the shoot and root biomass of elevated CO_2-treated *S. alfredii* Hance was increased by 24.6–36.7% and 35.0–52.1%, respectively.

Various studies (Li et al. 2013a, b, 2015a, b) showed that elevated CO_2 could change soil microenvironment and increase bioavailability of Cd and Zn through generating more efficient dissolved organic matter (DOM) into the rhizosphere (Li et al. 2013a, b) and forming more (8.01% for Cd and 8.47% for Zn, respectively) Cd/Zn-DOM complexes (Li et al. 2014). These changes facilitated metal uptake by the hyperaccumulator *S. alfredii* Hance. What is more, elevated CO_2 increased Pn (105–149%), Pn_{max} (38.8–63.0%), and AQY (20.0–34.8%) of *S. alfredii* Hance significantly ($p < 0.05$) and, on the contrary, reduced dark respiration and photorespiration, indicating that elevated CO_2 promoted the growth of *S. alfredii* Hance *by* increasing photosynthetic carbon uptake rate and photosynthetic light-use efficiency (Li et al. 2015a, b). These studies indicated that using elevated CO_2 may be a potential way to improve phytoremediation efficiency of Cd-/Zn-contaminated soil by *S. alfredii* Hance.

We also studied the effects of organic manures and found that the application of pig manure vermicompost (PMVC) enhanced the remediation of Cd and PAH co-contaminated soil by *S. alfredii* Hance, which inferred to be resulted from increased root exudates by PMVC (Wang et al. 2012a, b). Another pot experiment conducted by Peng et al. (2005a, b) showed that application of organic manure at the rate of 5.0% increased shoot Cu concentration by four times in the *E. splendens* grown on the PS and consequently elevated shoot Cu accumulation by three times compared with the control.

6 Phytoavoidation of Heavy Metal Contamination in Crops

6.1 Metal Lower Accumulation by Crops

Phytoavoidation is the reduction of organic and inorganic pollutant uptake into edible parts of crops by using immobilization measures together with low-accumulator cultivars. It is very important to select and cultivate pollution-safe cultivars (PSC) because most of the heavy metal-polluted lands are being used for agricultural purposes. The feasible move to reduce the heavy metal health risk in human through food is the assortment of pollution-safe cultivars. The concept of pollution-safe cultivars (PSC) came from Yu et al. (2006) and Liu et al. (2010a, b) who proposed that crop species and their cultivars differ genetically in accumulation of particular heavy metal, the cultivar which accumulates low concentration in their edible parts which is safe for consumption even when grown in contaminated soil. Many pot and field screening experiments have been done on various crops to investigate low and high accumulators of different heavy metals. Therefore, low heavy metal-accumulating cultivars in several crop species have been well documented, including rice (Arao and Ae 2003; Arao et al. 2003; Cao et al. 2010), wheat (Zhang et al. 2000, 2002), barley (Chen et al. 2007), maize (Kurz et al. 1999), peanut (McLaughlin et al. 2000), and Chinese cabbage (Liu et al. 2010a, b; Wang et al. 2014). We also reported that the Hangzhouyoudonger, Aijiaoheiye 333, and Zaoshenghuajing cultivars of Pak choi (*Brassica chinensis* L.). Chen et al. (2012) found Cd-PSCs safe for consumption when grown in low Cd-contaminated soils ($\leq$1.2 mg/kg). Similarly, We (2016) identified low co-accumulator genotypes of Chinese cabbage of Cd and nitrate grown in moderately combined Cd and nitrate-contaminated field soil, without posing risk to food safety. In the field culture experiment, uptake of Pb was investigated among spinach cultivars to screen out low accumulators of Pb or Pb-safe cultivars for food safety (Unpublished) (Fig. 18).

The data on the mechanism of low accumulation of heavy metal in different crops is relatively scarce. However, among pak choi cultivars in low Cd-accumulating cultivars, the variations in the shoot Cd concentration were attributed to the differential distribution of Cd within the plant which could be the speculated elementary mechanism of low Cd accumulation of pak choi (Wang et al. 2014). The diverse tolerance of Cd by *B. chinensis* and *B. pekinensis* might be explained partially by the accumulation of nonprotein thiols (NPT) in the shoots of

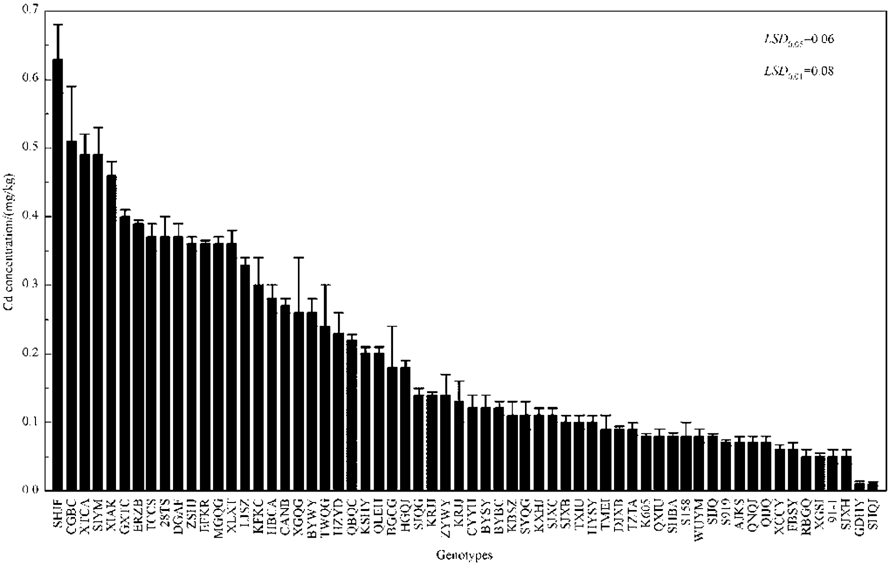


Fig. 18 Cadmium concentrations in 62 Chinese cabbage genotypes grown on the Cd-NO_3^- combined contaminated soil. Plants were harvested after 8 weeks of growth. All data were means of four replications; *bars* indicate standard errors of the mean value. The differences among the cultivars are analyzed by the *LSD* method (Tang et al. 2016)

these two plants (Liu et al. 2007). Sun et al. (2015) also found novel transporter genes HvZIP3 and HvZIP8 in low Cd-accumulating cultivar of barley which were identified as being related with low-grain Cd accumulation. Ueno et al. (2010) reported a gene OsHMA3 from the low Cd-accumulating cultivar of rice which was responsible for the restricted translocation of Cd but not other micronutrients from the roots to the aboveground tissues, and their overexpression caused the low accumulation of Cd by selective sequestration of Cd into the root vacuoles.

6.2 Amendments for Enhancing Phytoavoidation

There are different remediation techniques; chemical immobilization is one of them that decrease the concentration of dissolved pollutants by precipitation or sorption (Dayton and Basta 2001) which supports the phytoavoidation of heavy metals in contaminated soil. For the immobilization of heavy metals in contaminated soils, various inorganic (Yang et al. 2003; Park et al. 2011a; He et al. 2013; Tang et al. 2015) and organic amendments (Madejón et al. 2006; Park et al. 2011b) have been applied. Inorganic amendments that have successfully immobilized the heavy metals include rock phosphate, apatite, hydroxyapatite, liming agents, iron and manganese oxides, and oxyhydroxides (Keller et al. 2005). In this review we focus on organic amendments as they are cost-effective adsorbents and besides immobilization of pollutants also supply plant nutrients and enhance the water retention capacity of soil (Tang et al. 2016). Various studies have assessed the impact of soil organic amendments on immobilization of heavy metals in contaminated soils, but here we summarize the biochar amendments of soils for reducing availability and uptake of heavy metals in the soil-plant system. Biochar has multiple applications and benefits when used as soil amendment such as C sequestration (Kuzyakov et al. 2009; Woolf et al. 2010), reduction in greenhouse gas emission (Joseph et al. 2010; Singh et al. 2010a, b), soil as well as water remediation, immobilization of pollutants (Ahmad et al. 2014; Mohan et al. 2014), and soil fertilization (Chan et al. 2008a, b; Van Zwieten et al. 2010).

Numerous studies have been done on land remediation through biochar, but recently researcher's emphasis is on immobilization of pollutants especially heavy metals in crops grown in different agricultural soils. In soil-plant system Cd immobilization was carried out through biochar which was derived from fecal matter, cow manure, *Prosopis juliflora*, and coffee husk (Woldetsadik et al. 2016), biochar from water hyacinth and rice straw (Yin et al. 2014; Lou et al. 2011), biochar from compost (Yousaf et al. 2016, 2017), and nut shield and plum stone magnetic biochar (Trakal 2016; Trakal et al. 2014, 2016) were used. Biochar composed from chicken manure, mushroom soil, corn cobs, corn stover, mixed grass, hop grass, switch grass, pine mulch, pine bark, and cow manure was used for immobilizing mercury (Hg) in soil (Liu 2016; Gong et al. 2012), whereas for co-contaminated soil with Cd, Pb, Cr, Zn, and Cu, pepper stem-derived biochar which effectively reduced the concentration of all metals in soil was used (Park et al. 2016). But there is no published work or data on phytoavoidation of heavy metals in agricultural soils through organic amendments.

Recently, we conducted a pot experiment to evaluate the impact of maize stalk-, bamboo-, and cow manure-derived biochar as a soil amendment on cadmium immobilization and uptake by two contrasting Cd-accumulating genotypes of *Brassica chinensis* L. (pak choi). The maximum reduction of Cd availability in soil was recorded at 54%, and accumulation in shoots of low accumulator was 41% with maize stalk biochar (Fig. 19), compared to control which did not receive amendment. With bamboo biochar and cow manure-derived biochar, the availability of soil Cd was reduced by 45% and 39%, while Cd concentration in shoots of low-accumulator genotype was reduced by 35% and 27%, respectively (Khan et al., unpublished).

Similarly, we conducted a greenhouse experiment in which the cow manure-derived biochar was applied as soil amendment at different application rates in Cd-contaminated red acidic soil on which contrasting low- and high-accumulating genotypes of pak choi were grown. The maximum immobilization of Cd in soil was observed at 70% and its accumulation in shoots at 68% with 6% biochar application rate, compared to the control which did not receive amendment. In addition to immobilization of Cd contents, cow manure biochar also enhanced shoot dry biomass and availability of essential trace metals (Khan et al. Unpublished), whereas the application of humic acid as soil amendment in different Cd-contaminated soils also has the potential to reduce the availability of Cd in Haplic Udic Isohumosol and Udic Ferrisol soil by 15–19% and accumulation in

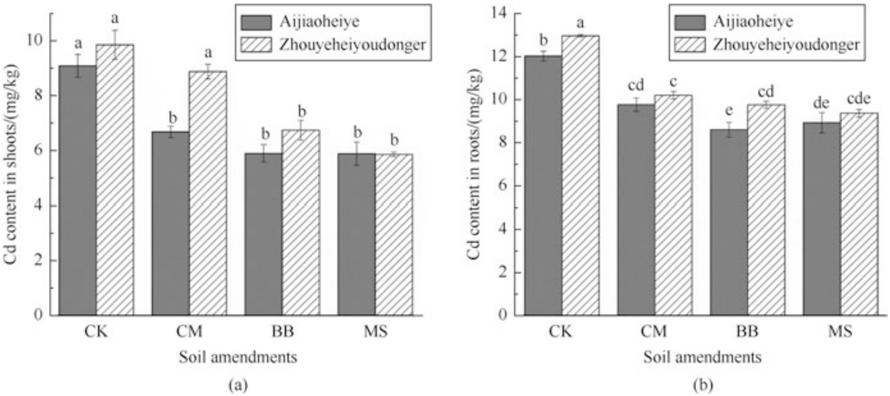


Fig. 19 Effects of different biochar on **a** shoots and **b** roots of *Brassica chinensis* L.

shoots by 25–34% in both high- and low-accumulating genotypes of pak choi compared to the control which did not receive amendment. Experimental findings revealed that application of humic acid increased the bioavailability of Mn and Zn in the soil-plant system (Khan et al., Unpublished). It has been concluded that organic amendments (biochar and humic acid) positively reduced Cd availability in soil and both low and high Cd accumulator genotypes of *B. chinensis* L. grown in different agricultural soils of China. These amendments also keep crops safe from health risks by enhancing biomass and essential trace metals in soils and plants.

7 Phytoremediation Coupled with Agro-production (PCA)

Soil contamination in China tends to be severe and complicated. Soil contamination by heavy metals and persistent organic pollutants or secondary salinization has been accelerated in China during the past decade because of rapid urbanization and industrialization. The output of heavy metals and organic pollutants into the environment will stay in a comparatively high level for a long period.

7.1 Phytoremediation of Cd-DDT Co-contaminated Soils Coupled with Crop Production

Environmental pollution of the soil-crop system is very complex due to the coexistence of old and new pollutants, as well as inorganic and organic compounds. Especially soil pollution caused by heavy metals and persistent organic pollutants (POPs) is a widespread concern. In recent years, the frequent outbreak of environmental pollution accidents due to soil-water pollution puts forward urgent demand for remediation and restoration of contaminated soil.

In this research, the plant-microbe remediation of Cd and DDT was carried out under pot and field experiments to identify bioremediation strategy of Cd-DDs co-contaminated soil and to investigate the mechanism for enhancing bioremediation efficiency. Cadmium (Cd) and dichlorodiphenyltrichloroethane (DDT) or its metabolite residues DDD/DDE (DDT, DDE, and DDD are collectively called DDs) are frequently detected in agricultural soils and agricultural products, posing a threat to human health. The plant-microbe remediation of Cd and DDT was carried out under pot and field experiments to identify bioremediation strategy of Cd-DDs co-contaminated soil and to investigate the mechanism for enhancing bioremediation efficiency.

7.1.1 Plant Genotypic Differences in Co-accumulation of Cd and DDTs

Zhu et al. (2012) compared the ability of 21 genotypes of *Cucurbita pepo* ssp. in mobilizing and uptake of Cd and DDs (p, p′-DDT, o, p′-DDT, p, p′-DDD, and p, p′-DDE) in the co-contaminated soil. The plant genotypes varied greatly in the uptake and accumulation of Cd and DDs, with mean concentrations of 0.26–1.12, 0.49–2.25, 1.04–4.84, and 1.61–7.72 mg/kg DW for Cd and 338.4–793.2, 1619–1812, 1273–3548, and 3396–12,811 ng/g DW for DDs in leaf, stem, and root, respectively (Fig. 20). The TF and BAF values were 0.79 and 1.68 for Cd and 0.60 and 1.54 for DDs, respectively. These results indicate that *Cucurbita pepo* cv. "Tebiexuan mibenwang" has a low ability to absorb and accumulate Cd and DDs from the contaminated soils, but *Cucurbita pepo* cv. "Riben Hongtianmi" has great potential for accumulating Cd and DDs from moderately co-contaminated soil (Cd $\leq$1.50 mg/kg, DDs $\leq$1.00 mg/kg).

The same research was done by Huang et al. (2011) on 23 genotypes of *R. communis* wherein *R. communis* genotypes varied largely in the uptake and accumulation of DDTs and Cd. These results indicate that *R. communis* has great potential for removing DDTs and Cd from contaminated soils attributed to its fast growth, high biomass, strong absorption, and accumulation for both DDTs and Cd.

7.1.2 Phytoremediation of Cd-DDT Co-contaminated Soils

Phytoremediation of Cd-DDT co-contaminated soils was studied using Cd hyperaccumulator plant *S. alfredii* Hance (SA) and DDT-degrading microbes (DDT-1) (Fang et al. 2012; Zhu et al. 2012). Initially, inoculation with DDT-1 was shown to increase *S. alfredii* Hance root biomass in a pot experiment. It was found that when *S. alfredii* Hance was applied together with DDT-1, the levels of Cd and DDs in the co-contaminated soil decreased by 32.1–40.3% and 33.9–37.6%, respectively. In the 18-month pot experiment, the levels of Cd and DDs in the co-contaminated soil decreased by 31.1% and 53.6%, respectively (Fig. 21). This result demonstrates that the integrated bioremediation strategy is effective for the remediation of Cd-DDs co-contaminated soils (Fig. 22).

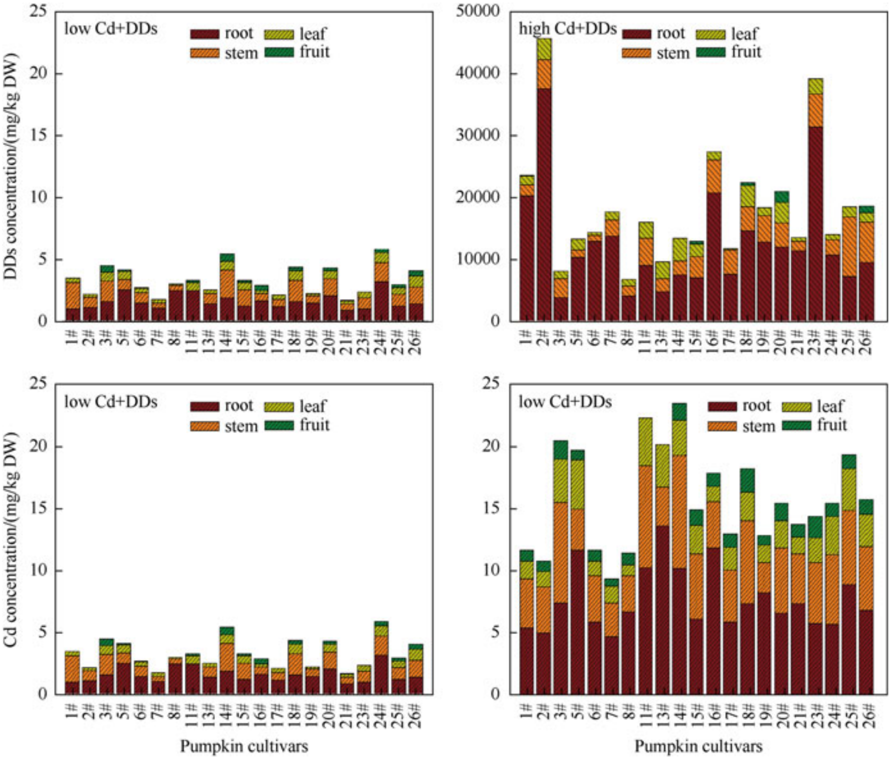


Fig. 20 Distribution of DDs and Cd in different parts of various pumpkin cultivars growing at low (1.35 Cd + 0.80 DDs mg/kg) and high (3.48 Cd + 3.30 DDs mg/kg) levels

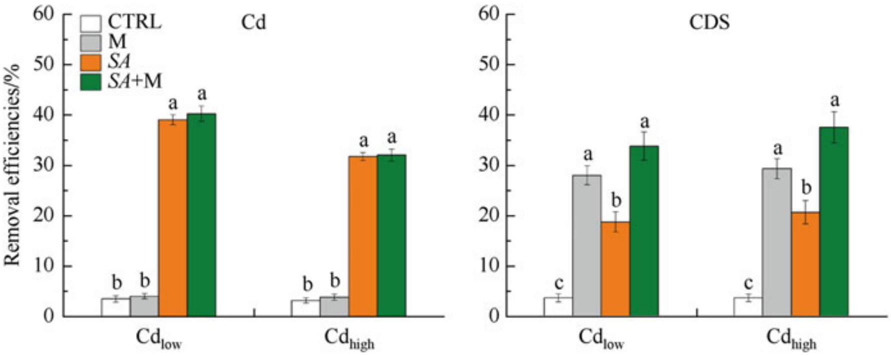


Fig. 21 Effects of *S. alfredii* Hance and DDT-1 strains on removal of Cd and DDs in low and high co-contaminated soils in pot experiment (low = 1.35 Cd + 0.80 DDs mg/kg; high = 3.48 Cd + 3.30 DDs mg/kg)

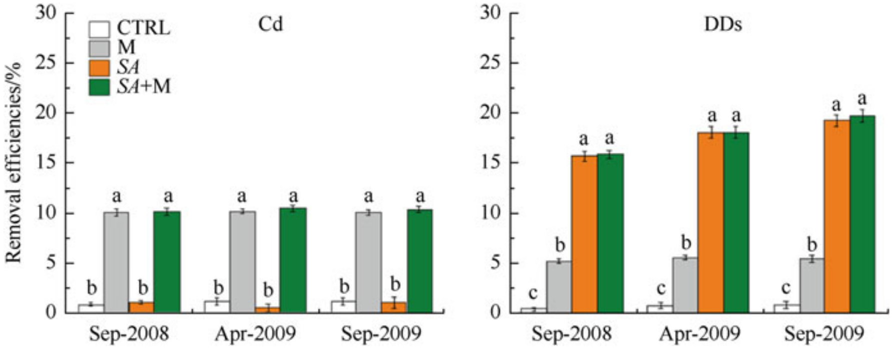


Fig. 22 Removal efficiency of Cd and DDs by *Sedum* and DDT-degrading microbes in field (Cd 0.53 + DDTs 0.72 mg/kg)

Rotation or intercropping technology, a widely accepted agronomical practice in China for 2000 years, can increase total crop yields and remediation efficiency through increased resource use efficiency. We found that co-cropping of *S. alfredii* Hance and *Brassica campestris* ssp. *chinensis* associated with DDT-1 increased the biomass and metal phytoextraction of *S. alfredii* Hance ($p < 0.05$), and also enhanced the root growth ($p < 0.05$), but had no significant effects on shoot biomass of *B. campestris* ssp. *chinensis*. The combined remediation strategy decreased the Cd concentration by 48.0–51.5% and 54.7–71.4% in the roots and shoots of *B. campestris* ssp. *chinensis*, respectively, and also corresponded to a decrease in root DDs concentration by 37%. The removal efficiencies were 30–46% for Cd and 36.8–42.7% for DDs. Laser scanning confocal microscopy revealed that gfp-tagged DDT-1 heavily colonized in rhizosphere soil and on rhizoplane of *S. alfredii* Hance. The soil diversity indices were higher in all of the planted treatments than in the unplanted control. Principal component analysis of bacterial T-RFLP data also revealed strong shifts in bacterial community composition with the planted treatments. The results of this study indicated that the co-cropping of *S. alfredii* Hance and *B. campestris* ssp. *chinensis* associated with DDT-1 appears to be a promising approach for the bioremediation of soils co-contaminated by Cd and DDs while simultaneously decreasing the pollutant concentration in edible part of *B. campestris* ssp. *chinensis* and hence maintaining product safety and reliability of this vegetable (Zhu 2012).

7.1.3 DDT-Degrading Microbes Under Successive Three Crops in Field

Co-cropping *S. alfredii* Hance and *Cucurbita pepo* cv. "Tebiexuan *mibenwang*" associated with DDT-1 increased the root (26.8%) and shoot (21.7%) biomass of cv. T. *mibenwang* ($P<0.05$) but had no significant effect on the root and shoot biomass of *S. alfredii* Hance. The combined remediation strategy did not significantly decrease the Cd concentrations in roots and shoots of both plants. However,

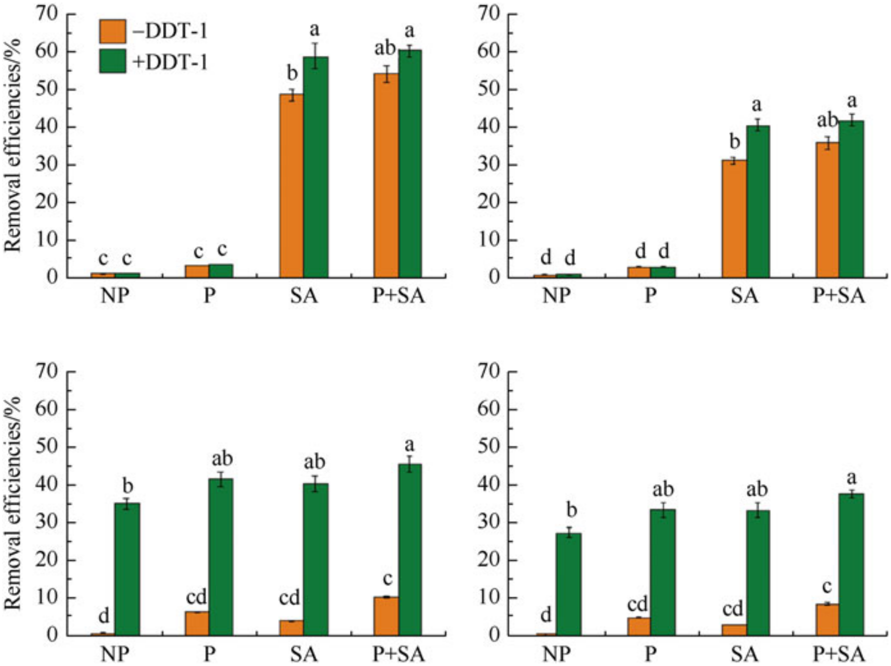


Fig. 23 Removal efficiency of Cd and DDTs by hyperaccumulator and low-accumulator co-planting with or without DDT-degrading microbes under successive three crops in field

for cv. *T. mibenwang*, the bioremediation strategy decreased the shoot DD concentration by 38.2–44.5% ($p < 0.05$) but had no significant effects on root DD concentration; for *S. alfredii* Hance, DDs concentration increased and decreased by 34.7–67.7% and 38.2–44.5% in roots and shoots, respectively (Fig. 23). The removal efficiencies were 41.9–60.7% for Cd and 37.5–45.2% for DDs. For roots of cv. *T. mibenwang*, Cd was largely retained in the cell walls and vessel tissue, and DDs were largely retained in the central cylinder and were adsorbed on root hairs, but for shoots, Cd was largely retained in the collenchyma tissue and vacuoles, and DDs were largely retained in vessel tissue, vacuoles, and glandular trichomes. Laser scanning confocal microscopy revealed that gfp-tagged DDT-1 heavily colonized on the rhizoplane of *S. alfredii* Hance and "Tebiexuan *mibenwang*." The soil diversity indices were higher in the treatment than in the unplanted control. The results of this study indicated that the co-cropping of *S. alfredii* Hance and cv. T. *mibenwang* "associated with DDT-1 appeared to be a promising approach for the bioremediation of soils co-contaminated by Cd and DDs, and decreasing the DDs concentration in shoot of cv. *T. mibenwang*" and ensuring product safety of the pumpkin (Zhu 2012).

7.2 Phytoremediation of Cd-PAHS Co-contaminated Soils Coupled with Crop Production

Cadmium (Cd) and polycyclic aromatic hydrocarbons (PAHs) are of particular concern due to their persistence; potentially carcinogenic, mutagenic, and teratogenic properties; and ubiquitous occurrence in the environment. So many regions were co-contaminated by heavy metals and PAHs, and environmentally friendly, low-cost, and in situ strategies are required.

Li et al. investigated the interaction between Cd and PAHs during the process of soil co-contamination and their effects on phytoremediation of Cd-PAHs co-contaminated soil by a Cd hyperaccumulator *S. alfredii* Hance (Li et al. 2011). Agronomic, chemical, and microbial strategies were also investigated to improve phytoremediation of Cd-PAHs co-contaminated soils (Wang et al. 2012a); the same research was also done on other organic pollutants (Fang et al. 2009; Huang et al. 2012a, b; Xiao et al. 2013).

7.2.1 The Dissipation of Benzo[a]pyrene (B[a]P) in Soil

The dissipation of benzo[a]pyrene (B[a]P) in soil was mainly due to microbial degradation (Gu et al. 2010). High Cd concentration (25 mg/kg) significantly inhibited the degradation of B[a]P in the soil, and the addition of pyrene (PYR) with an initial concentration of 250 mg/kg significantly promoted B[a]P degradation. Both desorbing and non-desorbing fractions of B[a]P contributed to B[a]P degradation in soil; however, desorbing fraction contributed more as compared to non-desorbing fraction (Fig. 24) (Wang et al. 2014).

7.2.2 Phytoremediation of Cd-PAHs Co-contaminated Soil

Wang investigated the growth of *S. alfredii* Hance and the removal effect of contaminants from Cd-PAHs co-contaminated soil via a pot experiment (Wang et al. 2012b). Elevated Cd level (6.38 mg/kg) increased the growth of *S. alfredii* Hance. The presence of PAHs decreased the stimulatory effects of Cd on plant biomass and Cd concentrations in shoots in Cd-spiked soil, thus decreasing Cd phytoextraction efficiency. Cadmium removal effect by *S. alfredii* Hance was from 5.8% to 6.7% in the low Cd concentration soil and from 5.7% to 9.6% of the high Cd soil after 60 days, respectively. The elevated Cd inhibited the removal rate of pyrene and the activity of dehydrogenase in soil. It may attribute to the decrease of the microbial activity in soil. The results demonstrate that the *S. alfredii* Hance could effectively extract Cd with PHE or PYR from Cd-contaminated soils, but PAHs had negatively affected the phytoextraction of Cd from Cd-contaminated soil (Fig. 25).

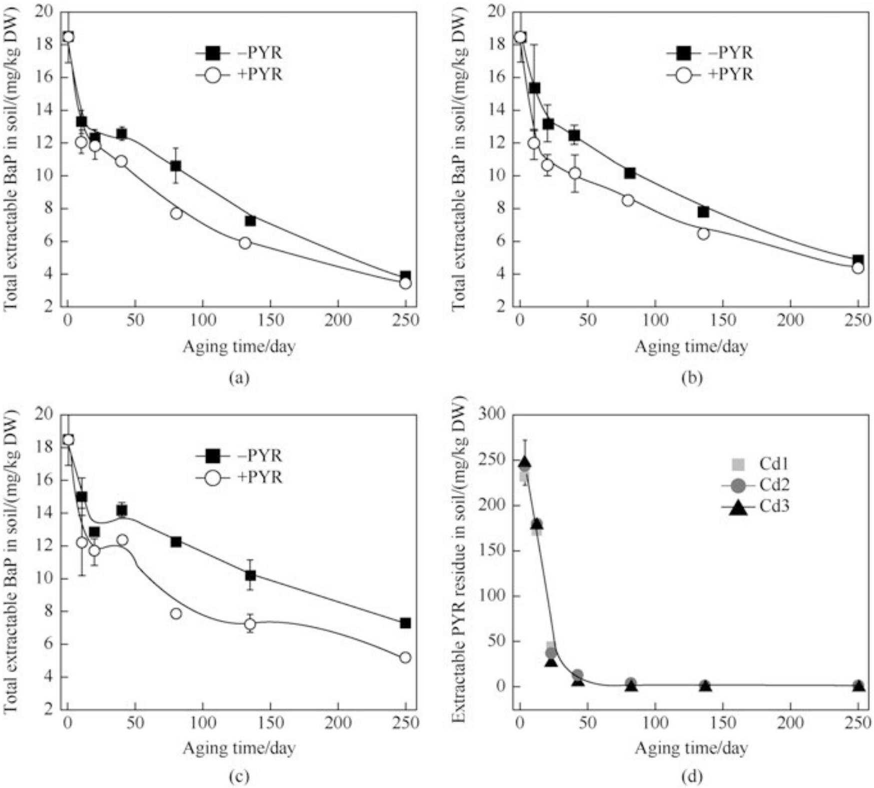


Fig. 24 Effects of pyrene on dynamic changes in total extractable B[a]P concentration in soils at different Cd concentrations; dynamic changes in extractable pyrene concentrations in soils at different Cd concentrations

A pot experiment was carried out to investigate the potential of enhanced phytoextraction of Cd by *S. alfredii* Hance and dissipation of PAHs in co-contaminated soil by the application of pig manure vermicompost (PMVC). Application of PMVC to co-contaminated soil increased the shoot and root dry biomass of *S. alfredii* Hance by 2.27-fold and 3.93-fold, respectively, and simultaneously increased Cd phytoextraction efficiency by 1.97-fold without inhibiting soil microbial quantity and enzyme activities. The highest dissipation rate of PAHs was observed in plant+PMVC treatment. The dissipation rates of PHE, PYR, and ANT were significantly increased by 0.26%, 3.21%, and 4.00%, respectively, as compared to the control. However, neither *S. alfredii* Hance nor PMVC enhanced PAHs dissipation when applied separately (Fig. 26). Abundant PAH degraders in soil were not significantly related to PAH dissipation rate. Plant+PMVC treatment significantly influenced the bacterial community structure. Enhanced PAHs dissipation in the plant+PMVC treatment could be due to the improvement of plant root growth, which may result in increased root exudates and subsequently change

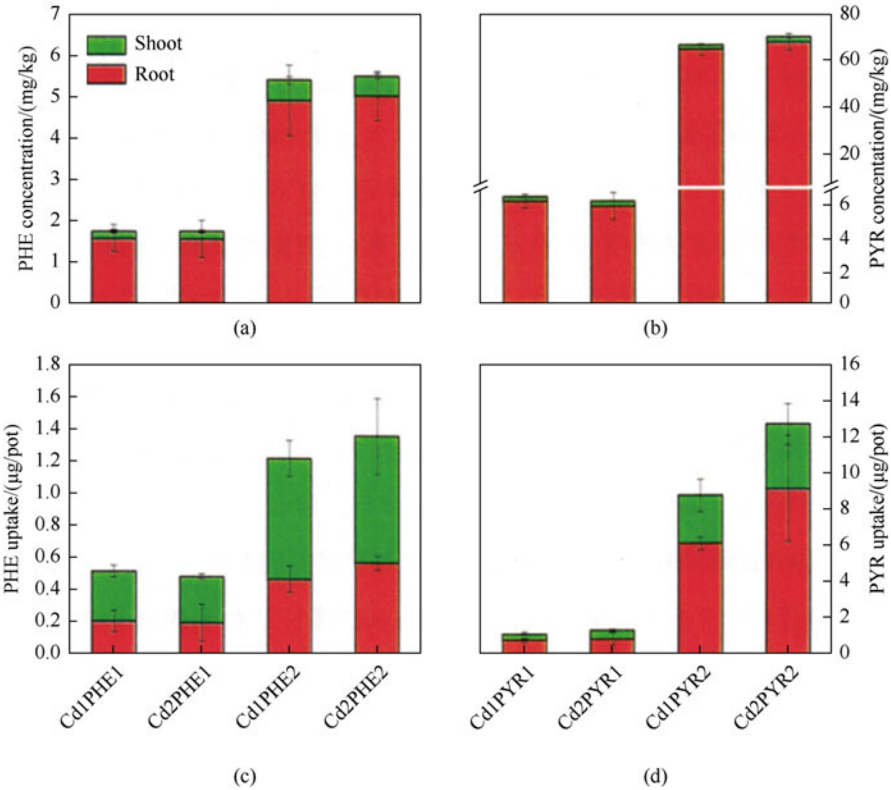
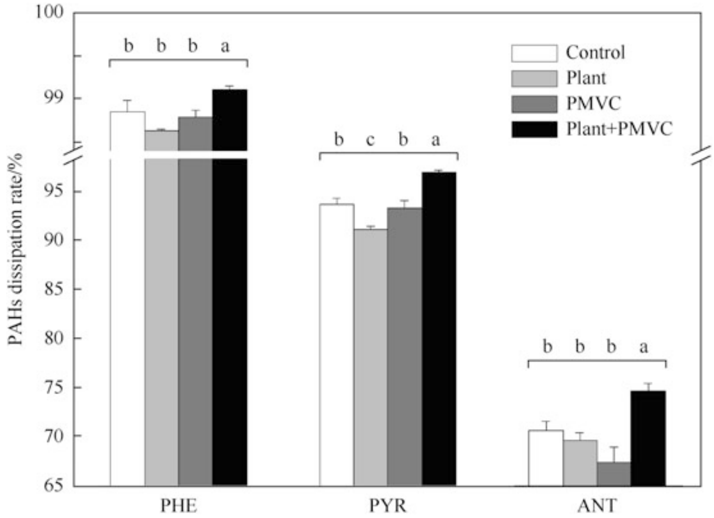


Fig. 25 Concentrations and total uptake of PHE and PYR in shoot and root of plants influenced by Cd and PAH treatments after 60 days of growth. Error bars show standard deviation ($n = 3$)

bacterial community structure to be favorable for PAHs dissipation; remediation of Cd-PAHs co-contaminated soil by *S. alfredii* Hance can be enhanced by simultaneous application of PMVC (Wang et al. 2012a).

Wang (2012) and Wang et al. (2013) investigated the potential for phytoextraction of heavy metals and rhizoremediation of PAHs in co-contaminated soil via a pot experiment by co-planting *S. alfredii* Hance with ryegrass (*Lolium perenne*) or castor (*Ricinus communis*). Co-planting with castor decreased the shoot biomass of S. alfredii Hance as compared to that in monoculture. Cadmium concentration in the shoot of *S. alfredii* Hance decreased by 64.3% and 47.4% in S-R (*S. alfredii* Hance and ryegrass) and S-C (*S. alfredii* Hance and castor) treatments, respectively, as compared to that in *S. alfredii* Hance monoculture. In contrast, Zn concentration in the *S. alfredii* Hance shoot increased by 19.8% and 25.6% in S-R and S-C treatments, respectively, as compared to that in *S. alfredii* Hance monoculture. Lead concentration in *S. alfredii* Hance shoot in S-R treatment was significantly greater as compared to that in *S. alfredii* Hance monoculture. Total removal of either Cd, Zn, or Pb by plants was similar across *S. alfredii*



	Plant	*PMVC*	*Plant×PMVC*
PHE%	n.s.	*	**
PYR%	n.s.	*	**
ANT%	*	n.s.	**

Fig. 26 PAHs dissipation rates in different treatments after 90 days. Means followed by different *letters*, with each PAH, are significantly different at $p < 0.05$ (based on LSD). *Error bars* show standard error ($n = 3$). The results of two-way analysis of variance (ANOVA) are shown below the graph, *$p < 0.05$, **$p < 0.01$, and *NS* not significant

Hance monoculture or co-planting with ryegrass or castor, except for the enhanced Pb removal in *S. alfredii* Hance and ryegrass co-planting treatment. Co-planting of *S. alfredii* Hance with ryegrass or castor significantly enhanced the PYR and ANT dissipation as compared to that in the control soil or *S. alfredii* Hance monoculture (Fig. 27).

A pot experiment was conducted to investigate the separate and combined effects of nonionic surfactant (Tween 80) and B[a]P-degrading bacterium on phytoremediation of soils co-contaminated with Cd and high-molecular-weight PAH (B[a]P) by *S. alfredii* Hance. Neither separate nor combined application of Tween 80 and B[a]P-degrading bacterium significantly affected plant growth and Cd uptake and accumulation by plant. Cadmium phytoextraction rate after 120 days of growth varied from 16.8% to 20.8% and from 6.4% to 7.6% in Cd-free and Cd-added soils, respectively. In general, the application of B[a]P-degrading bacterium can enhance B[a]P dissipation in soil. However, the effects of Tween 80 on B [a]P dissipation were influenced by the interaction between soil Cd concentration and plant. In the soil with high Cd concentration (4.71 mg/kg), the combined application of Tween 80 and B[a]P-degrading bacterium could remove B[a]P most effectively in both planted and unplanted treatments. These findings suggest that decontamination of Cd and B[a]P can be uptaken by *S. alfredii* Hance

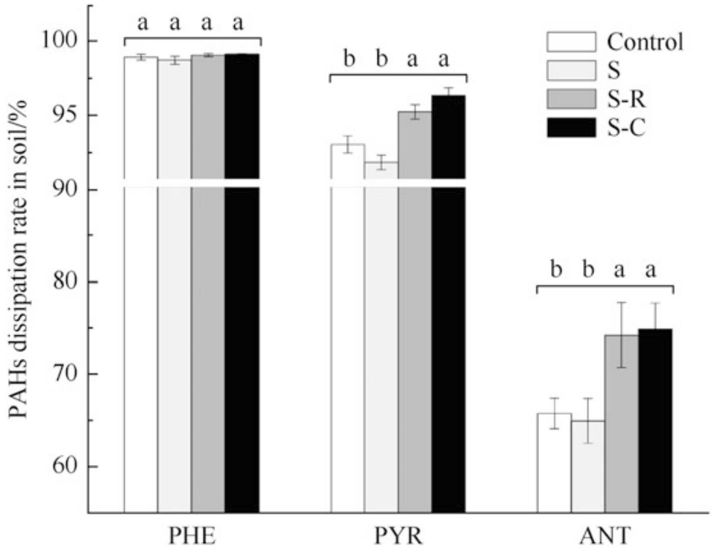


Fig. 27 PAHs dissipation rates in different treatments after 90 days. Means followed by different *letters* within each PAH are significantly different at $p < 0.05$ (based on LSD). *Error bars* show standard deviation ($n = 3$)

associated with B[a]P-degrading bacterium. However, the combined application of Tween 80 and B[a]P-degrading bacterium can remove B[a]P more efficiently from soil with relatively higher Cd concentration (4.71 mg/kg). The complicated interactions among Tween 80, B[a]P-degrading bacterium, and soil Cd concentration on the competition between B[a]P-degrading bacterium and indigenous microbial community need future investigations (Wang 2012).

7.3 Phytoremediation of Cd-Nitrate Co-contaminated Soil Coupled with Crop Production

In modern agriculture, repeated application of chemical fertilizers, especially nitrogen fertilizers, has resulted in excess nitrate in soils and subsequent accumulation of nitrate in crop plants. Nitrate could be stored in the vacuole of plants and enter the human body through food chain. Excess nitrate in food represents a threat to human health, as it results in human diseases such as methemoglobinemia and cancer. Moreover, excessive fertilizer application under protected cultivation of vegetables caused high accumulation of nitrate and cadmium in soil. Phytoremediation for Cd-nitrate co-contaminated soil has been much needed for both vegetable production and safety.

Table 4 Cadmium and nitrate concentrations in the shoots of seven safe Chinese cabbage genotypes

Genotypes	Cadmium/(mg/kg DW)	Nitrate/(mg/kg FW)
SIYM	0.49 ± 0.04	1071 ± 259.9
TCCS	0.37 ± 0.02	2165 ± 318.7
28TS	0.37 ± 0.03	2143 ± 112.2
DGAF	0.37 ± 0.02	1353 ± 215.3
CANB	0.27 ± 0.01	2179 ± 185.7
FBSY	0.06 ± 0.01	2037 ± 28.7
SJXH	0.05 ± 0.01	2915 ± 228.7

7.3.1 Genotypic Difference in Cd-Nitrate Co-accumulation

A field experiment was conducted to screen low co-accumulator genotypes of both Cd and nitrate from 62 Chinese cabbage genotypes grown in a moderately Cd (1.10 mg/kg) and nitrate (235.2 mg/kg) combined contaminated soil. Seven genotypes, i.e., *SIYM*, *TCCS*, *28TS*, *DGAF*, *CANB*, *FBSY*, and *SJXH*, were identified as low co-accumulators of Cd and nitrate based on their containing low Cd (<0.5 mg/kg DW) and nitrate (<3100 mg/kg FW) concentration in the edible parts even when grown in contaminated soils (Table 4). These genotypes were suitable for growing in slightly or moderately contaminated soils without risk to food safety (Tang et al. 2016).

7.3.2 Phytoremediation of Cd-Nitrate Co-contaminated Soils Coupled with Vegetable Safe Production

We used a novel phytoremediation system for decreasing Cd and nitrate in soil and vegetable shoots using endophytic microbe (M)-colonized Cd hyperaccumulator plant *S. alfredii* Hance (SA) in rotation with low-accumulator water spinach (*Ipomoea aquatica* Forsk.) and low-accumulator Chinese cabbage (*B. chinensis* L.) and through enhancement of CO_2 under protected cultivation. The treatments in first rotation were ① bok choy (CK), ② CK+CO_2, ③ *S. alfredii* Hance, ④ *Sedum* +CO_2, ⑤ *Sedum*+microbe (M), and ⑥ SA+CO_2+M. In second and third rotation, treatments ① and ② are planted with conventional water spinach and Chinese cabbage, while treatments ③–⑥ are planted with low-accumulator water spinach and Chinese cabbage, respectively. Two years of successive crop rotation in Cd, DTPA, Cd NO_3^- co-contaminated soil resulted in reduction of HMs by 56.5%, 62.7%, and 65.4%, respectively. Cd concentration in water spinach and Chinese cabbage shoots was 0.08 mg/kg and 0.01 mg/kg, respectively, and NO_3^- concentration in water spinach and Chinese cabbage shoots was 609.24 mg/kg and 419.62 mg/kg, respectively. All the concentration of contaminants in both vegetables were lower than the National Food Safety Standard of China (GB 2762—2012) and safe for consumption (Fig. 28). These findings suggest that *S. alfredii* Hance when applied together with CO_2 and M was the most effective for the remediation of Cd-NO_3 co-contaminated soils.

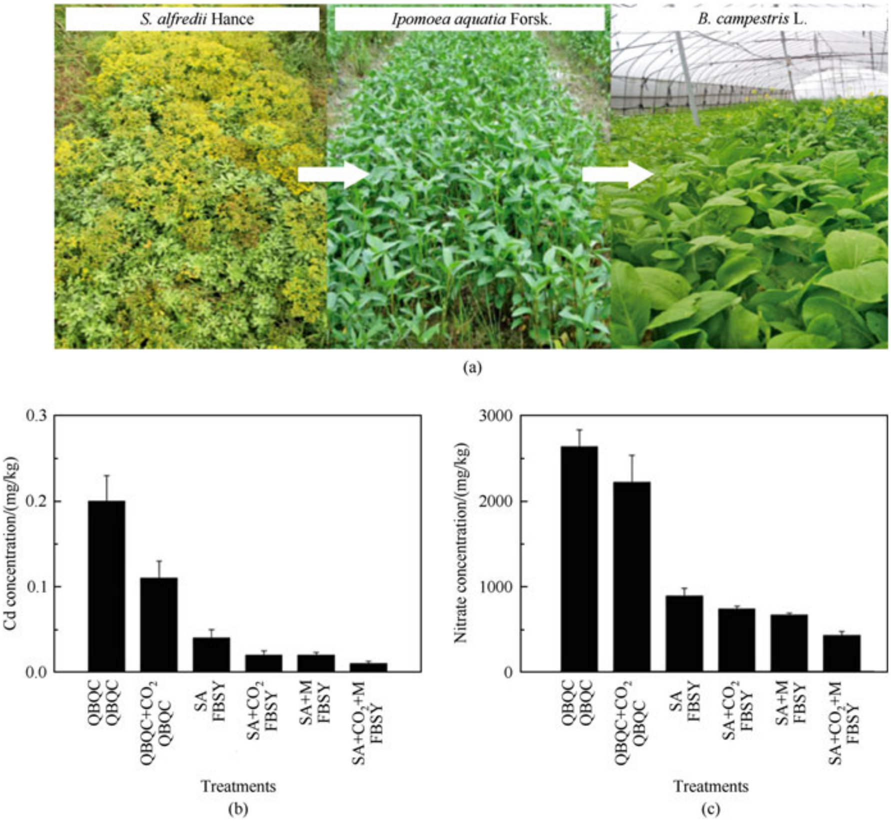


Fig. 28 Effects of phytoremediation of Cd-nitrate co-contaminated soil on Cd and nitrate accumulation in vegetable shoots after the first rotation of phytoremediation. QBQC-conventional cultivar, FBFY-lower accumulator cultivar of Chinese bok choy. The treatments in the first rotation were bok choy (CK), CK+CO_2, *S. alfredii* Hance (SA), *Sedum*+CO_2, *Sedum*+microbe (M), and SA+CO_2+M, respectively

7.3.3 Phytoremediation of Cd-Cu Co-contaminated Soil Coupled with Crop Production

Field experiments were conducted to study the effectiveness of phytoremediation of Cd-Cu co-contaminated soil coupled with vegetable production. We used several rotation and intercropping systems, like hyperaccumulator *S. alfredii* Hance intercropping with vegetable crop, accumulator rapeseed rotation with vegetable crops, accumulator *Elsholtzia splendens* co-cropping with vegetables, etc. After 1-year rotation or intercropping, the removal of soil Cd and Cu was at 16% and 12%, respectively (Fig. 29). One year after phytoremediation, the metal concentration in edible parts of vegetables was below the national food safety standard of

(a) (b)

(c)

Heavy metal in soil /(mg/kg)	Removal of heavy metals/%	
	Elsholtia	*Sedum*
0.63 (Cd)	0.87	16.40
46.6 (Cd)	12.08	0.05
178 (Zn)	1.29	4.38

(d)

Fig. 29 Phytoremediation of Cd-Cu co-contaminated soil. **a** Rotation of vegetable crop with Cd-accumulating rapeseed, **b** co-cropping of vegetable crops with Cu-accumulating *Elsholtzia*, **c** intercropping of *S. alfredii* Hance with crops, and **d** concentrations of heavy metal and their removal (%) by *Sedum* and *Elsholtzia*

China (GB 2762-2012). These results showed that it is possible and effective for phytoremediation of multi-metal-contaminated soil coupled with crop production.

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