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Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator *Solanum nigrum* L.

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Abstract

Remediation of heavy metal contaminated sites using hyperaccumulators presents a promising alternative to current environmental methodologies. In the pot-culture experiment, the effects of Cd, and Cd–As on the growth and its accumulation in the Cd-hyperaccumulator (*Solanum nigrum* L.) were determined. No reduction in plant height and shoot dry biomass was noted when the plants were grown at Cd concentration of $\leqslant$25 mg/kg. The contents of Cd in the stems increased from 122 to 387 mg/kg with increasing Cd, with the Cd transfer factor and bioaccumulation factor being >1.0. The plant can be classified as a Cd-hyperaccumulator. Growing in the presence of 10 mg/kg Cd and 50 mg/kg As, the plant height and shoot dry matter yields did not decrease significantly ($p > 0.05$) compared to that at 10 mg/kg Cd, however the stem Cd content increased by 28%. It was also observed that *S. nigrum* used exclusion strategy to reduce As uptake in the roots and restricted translocation into the shoots, resulting in As contents of the plant being root > leaf > stem > seed. The Cd accumulation capacity coupled with its relatively high As tolerance ability could make it useful for phytoremediation of sites co-contaminated by Cd and As.

Keywords: *Solanum nigrum* L.; Cd-hyperaccumulator; Phytoremediation; Combined pollution

1. Introduction

Arsenic (As) and cadmium (Cd) are of highly bioactive and toxic elements, their presence at elevated levels in soils and drinking water is threatening food safety and human health (Kang and Jin, 2004; Geng et al., 2005). They adversely affect biological activities as a teratogen, carcinogen or mutagen as well as having detrimental effects on the digestive system, respiratory system and immune system (Zhou and Huang, 2000; Zhou and Song, 2004; Liao et al., 2005). Large areas of cultivated land in many countries have been contaminated by As and Cd due to agricultural and industrial practices such as application of pesticides and chemical fertilizers, waste water irrigation, precipitation from heavy coal combustion, and smelter wastes and residues from metalliferous mining (Boisson et al., 1999; Zhou and Huang, 2000; McGrath et al., 2001; Xie et al., 2006; Verma et al., 2007). Recognition of the ecological and human health hazards of some toxic pollutants has led to development of reliable and cost-effective technologies such as bioremediation capable of reducing As and Cd in soils and wastes to environmentally acceptable levels (Zhou and Song, 2004; Kertulis-Tartar et al., 2006; Yoshida et al., 2006).

Phytoremediation can be defined as the use of plants including trees and grasses, to remove, destroy, or sequester hazardous contaminants from media, such as soil, water, and air (Chaney et al., 1997; Salt et al., 1998; Prasad,

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2003), is gaining a lot of importance in recent years since it is a cost effective, promising technology, as well as a desire 'green', sustainable process (Wei and Zhou, 2004a; Zhou and Song, 2004). Plants with metal resistance mechanisms based on exclusion can be efficient for phytostabilization technologies (Wei et al., 2005b). Hyperaccumulating plants, in contrast, may become useful for extracting toxic elements from the soil and thus decontaminate and restore fertility in polluted areas (Barceló and Poschenrieder, 2003). Hyperaccumulators are plants that have an innate capacity to absorb metal at levels 100 times greater than average plants (Baker and Brooks, 1989; Yang et al., 2004; Zhou and Song, 2004). They are often found in metal-rich regions where those traits probably give them a competitive advantage (Ma et al., 2001; Sun et al., 2005; Gonzaga et al., 2006).

Hyperaccumulators are defined based on the following characteristics: (1) shoots metal concentrations (threshold values) are >10,000 mg/kg dry weight of shoots for Zn and Mn, 1000 mg/kg for Co, Cu, Ni, As and Se, and 100 mg/kg for Cd (Baker and Brooks, 1989; Ma et al., 2001; Zhou and Song, 2004); (2) bioconcentration factor (ratio of metal concentration in plant to soil) is greater than 1.0, sometimes reaching 50–100 (Brooks, 1998; Cluis, 2004); (3) translocation factor (ratio of metal concentration in shoots to roots) is greater than 1.0 (Wei and Zhou, 2004a,b).

So far, more than 400 species of natural metal hyperaccumulators belonging to 45 families have been documented in the world, but hyperaccumulation of Cd and As are a rare phenomenon in the plant kingdom (Zhou and Song, 2004). Compared with crops, weed plants display their characteristics of strong endurance to adverse environmental conditions, and high capacity to absorb water and fertilizers (Gardea-Torresdey et al., 2004; Wei et al., 2005a; Wei and Zhou, 2006). In this sense, weed is an important type of ideal natural resources for the remediation of contaminated soils. Black Nightshade herb is a very common species in a field in north China. It is tolerant to adverse environment, fast growing and with high biomass, under feasible environmental conditions, its biomass could increase rapidly (Wei et al., 2005a). So it could fill a gap of known hyperaccumulating plants and it has the potential for phytoremediation of metal contaminated soils (Zhou and Song, 2004). The objectives of this study was to examine the growth response and uptake, distribution, and accumulation of As and Cd by *Solanum nigrum* in response to As–Cd combined pollution.

2. Methods

2.1. Field site and soil characterization

The field pot-culture experiment was located at the Shenyang Ecological Experimental Station (41°31′N and 123°41′E). It belongs to the temperate zone with a semi-moist continental climate, 5–9 °C average annual temperature, 520–544 kJ/cm^2 total annual radiation, 650–700 mm average annual precipitation, and 127–164 days frostless duration per year. The coldest month (average −14 °C) is in January and the warmest month (average 24 °C) is in July. Burozem soil located in the station is a relatively clean soil based on the National Soil-Environmental Quality Standards of China (NSEQSC, GB 15618, 1995). Soil samples were collected from the surface (0–20 cm) from a field without pollution of heavy metals (Wei and Zhou, 2006). The main soil parameters were listed as follows: soil pH was 6.56; CEC was 12.26 g/kg; Total N, P, and K were 0.91, 0.40, and 183.00 g/kg, respectively; the concentrations of Cd, Cu, Zn, Pb and As were 0.17, 32.9, 28.1, 11.1 and 10.4 mg/kg, respectively.

2.2. Experimental procedure

Plastic pots of 15 cm in height and 20 cm in diameter were used. Air-dried soil of 2.5 kg was sieved through a 4 mm sieve, and then placed into each pot after mixed with Cd and As.

According to Liao et al. (2005), the concentration of As in the areas near the industrial districts of Chenzhou City, Southern China was 11.0–1217 mg/kg. According to Wei and Chen (2002), high arsenic levels in the Shimeng area was up to 129–3831 mg/kg. The concentration of soil Cd in the Zhangshi sewage irrigation area in the west Shenyang suburb was 7–25 mg/kg (Wen and Shi, 2005). In particular, the concentration of soil Cd in an old smeltery, northeast China was up to 11.19–197.28 mg/kg (Cui et al., 2007). Thus, three levels of Cd (10, 25, and 50 mg/kg) and As (0, 50, and 250 mg/kg) were used in this experiment, resulting in a total of nine treatments. The three Cd treatments will be referred to as 10, 25, and 50 mg/kg, and six Cd/As treatments will be referred to as Cd-10As-50, Cd-10As-250, Cd-25As-50, Cd-25As-250, Cd-50As-50, and Cd-50As-250. One control that had no Cd and As was included. Cadmium and arsenic were applied as $CdCl_2 \cdot 2.5H_2O$ and $Na_2HAsO_4 \cdot 7H_2O$, and then incubated for four weeks. A petri dish was placed under each pot to collect potential leachate during the experiment.

Three seedlings of *S. nigrum* of similar size, which were about three weeks old, 3–4 cm height with 2–3 leaves were transplanted into each pot. To simulate field conditions, the plants were grown under open field conditions and no fertilizer was added. Loss of water was made up using tap water (no Cd and As detected) to sustain 75% of the field water holding capacity. The plants were harvested after 70 days when they reached their physiological maturity. They were washed thoroughly first with running tap water followed by distilled water, and dried at 100 °C for 10 min, then at 70 °C in an oven until completely dry.

2.3. Plant and soil analysis

The plant and soil samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v). The concentrations of heavy metals were determined using the atomic absorption spectrophotometry (WFX-120) method (Sun et al., 2005). The arsenic concentration was determined using the hydrogen generation atomic fluorescence spectroscopy (Wei and Chen, 2002, 2006).

2.4. Statistical analysis

All treatments were replicated three times in the experiments. The means and standard deviations (SD) were calculated by the Microsoft Office Excel 2003 on a computer. Statistical analysis was carried out using one-way analysis of variance using SPSS10.0. When a significant difference was observed between treatments ($p < 0.05$ or $p < 0.01$), multiple comparisons were made using the LSD test.

3. Results and discussion

3.1. Effect of different Cd and As levels on plant growth

During the 70-day experiment, *S. nigrum* survived in the soil spiked up to 50 mg/kg Cd, showing no visual Cd toxicity symptoms such as necrosis and whitish-brown chlorosis. At 10 mg/kg Cd, no impact was observed on plant growth of all parameters measured (Fig. 1). At 25 mg/kg Cd, plant biomass was reduced in both shoots and roots. However, at 50 mg/kg Cd, both plant height and biomass were reduced significantly ($p < 0.05$). The results indicated that *S. nigrum* tolerated Cd at 10 mg/kg, however, levels at 25–50 mg/kg impacted plant growth as measured by plant height and biomass.

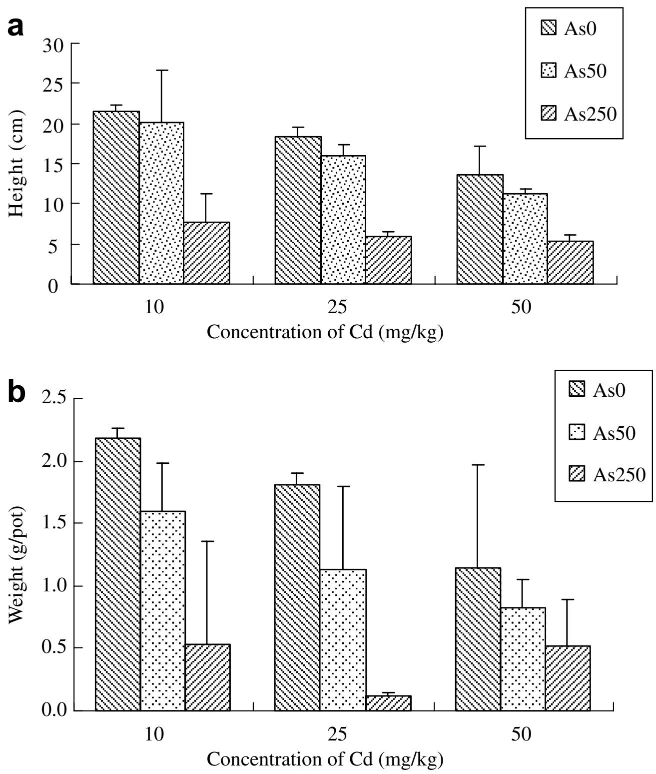

Fig. 1. Effects of Cd and As (mg/kg) on the growth of *S. nigrum*: (a) plant height and (b) shoot dry weight.

As showed in Fig. 1a, the height of the plants decreased significantly ($p < 0.01$) growing in As–Cd spiked soils compared to the control, except for the plants in treatment of Cd-10As-50 (10 mg/kg Cd and 50 mg/kg As) with the average height being 20.2 cm. For a given Cd level, the plant height reduced significantly ($p < 0.05$) with increasing As concentrations, especially at the concentration of 250 mg/kg As. The statistical analysis showed that the combined toxic effects on the plant height were not significantly ($p > 0.05$) different with increasing Cd concentration at 250 mg/kg As, and the height was much lower than the control and other treatments.

Fig. 1b shows the dry weight of plant shoots under combined contamination of As and Cd. It is revealed that the dry weight of shoots were significantly ($p < 0.05$) decreased under the various treatments compared to the control. There were no significant differences in shoot biomass at 50 mg/kg As compared to the control, and it did not reduce significantly with increasing Cd supply at 50 mg/kg As. Compared to the dry shoot yields at 10 mg/kg and 25 mg/kg Cd, it did not increase significantly when plants were grown at 10 mg/kg Cd and 50 mg/kg As, the results above indicate that *S. nigrum* had the high capability to tolerate Cd and As in contaminated soils, in particular, there should be the similar plant growth responses reported by Fayiga and Ma (2005).

The growth and biomass of green plants including hyperaccumulators can be promoted by addition of chelate compounds, chemical fertilizers or nutrient elements such as nitrogen and phosphorus (Alkorta et al., 2004; Gardea-Torresdey et al., 2004; Singh and Ma, 2006). Our previous field study (Wei and Zhou, 2004b; Wei et al., 2005a) indicated that there was a big biomass of the Cd-hyperaccumulator *S. nigrum* growing under field conditions with high fertility, for example, total soil N was up to 0.127%. In other words, *S. nigrum* could display its characteristic of tolerating to adverse environment, fast growing and high biomass under field conditions. However, the dry aboveground biomass in this pot-culture experiment was certainly very low because of possible lack of nutrient materials and ill environmental conditions without exchange of water and nutrient materials like field conditions, in particular, localization of the pot-culture conditions. In order to avoid influences from chemical fertilizers and other factors (Wang et al., 2001), the plants in this study were grown under pot-culture conditions and no fertilizer was added. To a certain extent, the pot-culture experiment with low soil nitrogen may be responsible for the low biomass of the hyperaccumulator.

3.2. Hyperaccumulative characteristics of *S. nigrum*

The accumulation of Cd in the *S. nigrum* stems increased with increasing Cd concentrations in the soil

(Tables 1 and 2). They reached 122, 208 and 387 mg/kg (dry weight), respectively, at single Cd treatments of 10, 25, and 50 mg/kg, and were in the order of stem > leaf > root > seed, and shoot > root. They all exceeded the critical level for a Cd-hyperaccumulator (Baker, 1981; Brooks, 1998; Zhou and Song, 2004).

The tolerant mechanisms of Cd tolerant plants have been reported previously (Zhou, 1994; Küpper et al., 2000; Zhao et al., 2002). They included two strategies: exclusion and accumulation (Zhou and Song, 2004). With the accumulation strategy, plants accumulated high amounts of Cd in the tissue, with only a small amount of Cd being stored in the roots and the rest being all translocated to the shoots.

In addition to total metal content, both the bioaccumulation factor (BF) and the translocation factor (TF) need to be considered while evaluating hyperaccumulators. The bioaccumulation factor is defined as the ratio of metal concentration in the plant to metal concentration in the soil. Hyperaccumulating plants are those that have a BF > 1.0 (Cluis, 2004). In the present experiment, the concentrations of Cd in stems and leaves were as high as 208 and 192 mg/kg, respectively, at Cd concentration of 25 mg/kg. The corresponding BF was then 8.33 and 7.68, respectively (Table 2), showing high efficient accumulation of Cd from soil by this plant.

The transfer factor is defined as the ratio of the metal concentration in the stems to that in the roots, which is used to measure the effectiveness of a plant in translocating Cd from roots to shoots. The presence of Cd did not affect the capability of plants to transport Cd from roots to shoots significantly ($p > 0.05$). They were 1.92, 1.66, and 1.44, respectively (Table 2), all being >1.0. These ratios suggest that the mechanism of metal tolerance in these populations and the high metal translocation from the roots to the shoots are vital characteristics for plants to be used in phytoextraction techniques (Baker, 1981; Zhang et al., 2002; Fayiga and Ma, 2006).

The total amount and distribution of Cd accumulated in *S. nigrum* under joint stress of Cd and As are shown in Tables 2 and 3. When As level was at 250 mg/kg, Cd concentration in the stems decreased by 16.5%, 49.4% and 73.6%, respectively, compared to no As. Compared to the low concentration of As (50 mg/kg), the content of Cd in the stems reduced by 32.6–73.5% at the high level treatment of As (250 mg/kg). The result showed that the high concentration of As had toxic effects on plant Cd uptake. However, As could facilitate plant uptake Cd from soils in the low concentration of Cd for the treatment at 10 mg/kg Cd and 50 mg/kg As, about 38.9% higher than that at 10 mg/kg Cd. The results above suggested that *S. nigrum* could rapidly and efficiently accumulate large amounts of Cd from the moderately contaminated soil.

Table 2 shows the BF and TF values of the plants under combined pollution of As and Cd. Both As and Cd did not affect the plant capability to uptake and transport. Especially, when *S. nigrum* growing at 10 mg/kg Cd and 50 mg/kg As, the hyperaccumulator had the highest TF

Table 1
Accumulation of Cd and As in the stems of *S. nigrum* growing in a soil spiked with Cd and As (mean ± SD)

Treatment (mg/kg)	Accumulation of Cd (mg/kg)			Accumulation of As (mg/kg)		
	Cd-10	Cd-25	Cd-50	Cd-10	Cd-25	Cd-50
As-0	122 ± 38.2b a	208 ± 78.1b a	387 ± 84.9a a			
As-50	170 ± 86.6b a	184 ± 78.4b a	385 ± 57.1a a	6.43 ± 1.69a a	8.27 ± 2.89a a	15.85 ± 3.74a a
As-250	106 ± 26.8a a	124 ± 16.5a a	102 ± 20.0a b	58.99 ± 2.1 b	121.08 ± 9.45a b	21.60 ± 7.89c a

Means followed by different letters differ at $p < 0.05$ (LSD test).

Table 2
Single Cd treatments and Cd–As joint stress on Cd accumulation in *S. nigrum*

Treatment (mg/kg)	Cd accumulation (mg/kg)			
	Root	Stem	Leaf	Seed
Cd-10	80 ± 15.5e	122 ± 38.2b	83.2 ± 8.4cd	14 ± 3.1b
Cd-10 + As-50	42 ± 7.0f	170 ± 86.6b	110 ± 23.2cd	11 ± 3.5b
Cd-10 + As-250	55 ± 18.2ef	106 ± 26.8b	57 ± 5.9d	15 ± 13.7b
Cd-25	122 ± 15.2cd	208 ± 78.1b	192.1 ± 47.9bc	20.1 ± 3.2b
Cd-25 + As-50	164 ± 10.5b	184 ± 78.4b	124 ± 13.7bcd	12 ± 4.5b
Cd-25 + As-250	130 ± 1.8bc	124 ± 16.5b	100 ± 2.9cd	11 ± 1.8b
Cd-50	337 ± 29.2a	387 ± 85.0a	356 ± 101.5a	52 ± 23.7a
Cd-50 + As-50	303 ± 38.3a	385 ± 57.1a	223 ± 28.5b	20 ± 3.0b
Cd-50 + As-250	88 ± 14.7de	102 ± 20.0b	90 ± 14.0cd	8.4 ± 1.0b
Sig. (*p*)	<0.001	<0.001	0.001	0.05
F	69.8	7.4	9.2	5.8

Table 3
Bioaccumulation and transfer factors of Cd and As in *S. nigrum* growing in a soil spiked with Cd, or Cd and As

Concentration (mg/kg)	Transfer factor		Bioaccumulation factor	
	Cd	As	Cd	As
Cd-10	1.92 ± 0.30ab		12.2 ± 3.82bcd	0.13 ± 0.02cd
Cd-10 As-50	2.07 ± 0.83a	0.17 ± 0.07ab	17.0 ± 11.56bc	0.18 ± 0.18bc
Cd-10 As-250	0.95 ± 0.07c		5.23 ± 1.60d	
Cd-25	1.66 ± 0.98abc	0.14 ± 0.09ab	8.33 ± 3.12cd	0.17 ± 0.06bcd
Cd-25 As-50	1.11 ± 0.42bc		18.5 ± 7.84b	
Cd-25 As-250	1.04 ± 0.18bc	0.19 ± 0.09ab	11.1 ± 0.22bcd	0.48 ± 0.09a
Cd-50	1.44 ± 0.09abc	0.25 ± 0.03a	7.74 ± 1.70cd	
Cd-50 As-50	1.30 ± 0.34abc	0.25 ± 0.1a	38.5 ± 5.71a	0.32 ± 0.07b
Cd-50 As-250	1.21 ± 0.43abc	0.09 ± 0.02b	10.3 ± 1.99bcd	0.09 ± 0.03d

Means followed by the same letter are not significantly different at $p = 0.05$ ($n = 3$) according to LSD test. The statistical analysis was a one-way ANOVA.

value and its BF value was higher than that under single treatment of Cd, up to 2.07 and 17.0, respectively. The fact that high metal concentrations were present in the shoots but not in the roots may indicate that efficient root-to-shoot transport system is important for metal tolerance and account for hyperaccumulation (Zhang et al., 2002; Singh and Ma, 2006). The results showed that the enhancement effect of As on Cd uptake occurred only when plants were grown at suboptimal Cd supply levels, just as the report of Zhou (1994). Similarly, higher accumulation of As took place in combined treatment of As–Pb, As–Zn, and As–Ni (Fayiga and Ma, in press). In another study, Fayiga and Ma (2006) also found that As uptake in *Pteris vittata* was increased using phosphate rock to immobilize metals in soils. This knowledge helps to rehabilitate the co-contaminated sites by Cd and As using some strengthening measures.

3.3. As uptake and partitioning in the plant under Cd and As

The total amount of As and its distribution in *S. nigrum* are shown in Fig. 2, the As content was in sequence root > leaf > seed, and >75% of the As uptake by the plant was concentrated in the roots. Furthermore, the As BF and TF values were very low (Table 2), showing *S. nigrum* inefficiently uptake of As from contaminated soil and had tolerance mechanism towards As, by restricting As entry into the roots (Gonzaga et al., 2006; Kertulis-Tartar et al., 2006).

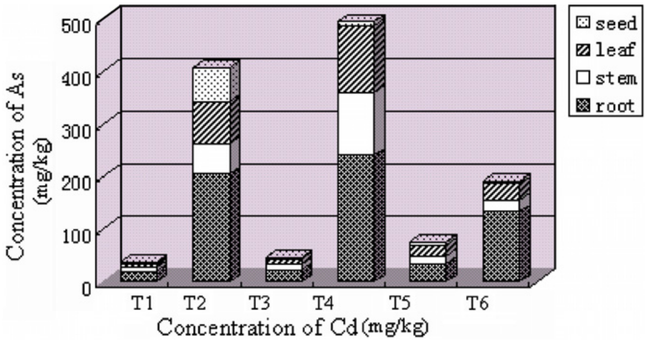

Fig. 2. Accumulation of As in *S. nigrum* growing in a soil spiked with Cd and As. *Note*: Key to metal applications (mg/kg): T1 = Cd-10/As-50; T2 = Cd-10/As-250; T3 = Cd-25/As-50; T4 = Cd-25/As-250; T5 = Cd-50/As-50; T6 = Cd-50/As-250.

Uptake of As by the hyperaccumulator increased with increasing soil As. In other words, there was a significant ($p < 0.01$) correlation between As uptake by the plant in various treatments and total soil As. The result was consistent with the report by Fayiga and Ma (2005). In another study, Gonzaga et al. (2006) even found that there was a good correlation between As uptake in various treatments and various soil arsenic fractions including total As, As fractionation and water-soluble As both in the rhizosphere and bulk soil.

It is revealed from Table 1 that a certain level of Cd can facilitate As uptake by *S. nigrum*. When the concentration of As was 50 mg/kg, the content of As in the stems increased with Cd. It reached the maximum at the treatment of 25 mg/kg Cd and 250 mg/kg As. However, there was significantly ($p < 0.01$) decreased As under the combined effect of 50 mg/kg Cd and 250 mg/kg As. This means that the ability of metal uptake by the plants not only rests with the concentrations of heavy metals but also relates to their combinations (Zhou, 1995).

3.4. Joint effects of Cd and As absorption in roots and shoots

The large amounts of heavy metals, accumulated in the aboveground parts of a hyperacccumulator, were favorable to shift out metals from soil by harvesting aboveground parts and appropriate treatment and to reach the aim of ecological remediation of contaminated soils by heavy metals (Zhou and Song, 2004; Fayiga and Ma, 2005). In the meantime, a phytomining operation would entail planting a hyperaccumulator over a low-grade ore body or mineralized soil, followed by harvesting and incineration of the biomass to a commercial bio-ore (Li et al., 2003). The dominating Cd uptake by *S. nigrum* was in the shoots, up to 72.7–95.6% in the whole plant (Fig. 3). The accumulation of Cd in the shoots markedly decreased ($p < 0.05$) with the concentration of As in soil when Cd in soil was at the same level except for that under the combined pollution of 10 mg/kg Cd and 50 mg/kg, the accumulation of Cd in

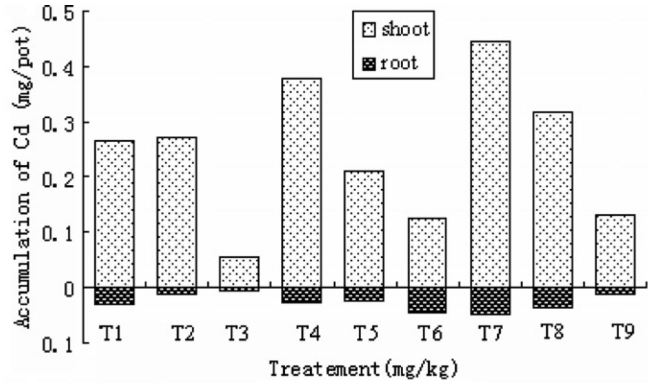

Fig. 3. Cd–As joint stress on Cd accumulation in *S. nigrum*. *Note*: Key to metal applications (mg/kg): T1 = Cd-10; T2 = Cd-10/As-50; T3 = Cd-10/As-250; T4 = Cd-25; T5 = Cd-25/As-50; T6 = Cd-25/As-250; T7 = Cd-50; T8 = Cd-50/As-50; T9 = Cd-50/As-250.

shoots were higher than that under the concentration of 10 mg/kg Cd, showing that As can affiliate Cd uptake and accumulation under a certain level of Cd and As.

The plant effective number (PEN) and the metal extraction ratio (MER) have been applied to evaluate the ability of remedying contaminated soil by a hyperaccumulator. PEN is defined that the number of plants needed to extract 1.0 g metal when the biomass of shoots and of total plants is considered (García et al., 2004). To remove 1.0 g of Cd from soil, more than 3691 shoots or more than 3530 entire plants would be needed when the concentration of Cd in soil was 10 mg/kg and the concentration of As in soil was 50 mg/kg.

MER is defined as the ratio of metal accumulation in the shoots to that in soil (Mertens et al., 2005), which is calculated as follows:

$$\mathrm{MER} = (C_{\mathrm{plant}} \times M_{\mathrm{plant}}/C_{\mathrm{soil}} \times M_{\mathrm{rooted\ zone}}) \times 100 \qquad (1)$$

where C_{plant} is the metal concentration in the harvested component of the plant biomass, M_{plant} is the mass of the harvestable aboveground biomass produced in one harvest, C_{soil} is the metal concentration in the soil volume, and $M_{\mathrm{rooted\ zone}}$ is the mass of the soil volume rooted by the species under study.

The extraction ratio of Cd at the different treatments of Cd and As was 1.07%, 1.08%, 0.22%, 0.60%, 0.33%, 0.20%, 0.35%, 0.25% and 0.11%, respectively. However, at the joint stress of Cd and As the extraction ratio of As was 0.007%, 0.006%, 0.006%, 0.001%, 0.007% and 0.003%, respectively. The results above indicated that *S. nigrum* had strong capability to uptake and accumulate Cd under single treatments of Cd and combined pollution of Cd and As, but it was the reverse to As absorption and accumulation.

3.5. The potential of S. nigrum as Cd-hyperaccumulator and As-excluder

For a hyperaccumulator, usually plant biomass at time of seed maturity may be an end-point of a bioassay (Ernst and Nelissen, 2000; Yang et al., 2004). Plant aboveground biomass will not decrease significantly at the concentration of the critical value. Only when it exceeds the critical value, would plant growth be adversely inhibited. This will be followed by occurrence of chlorosis, reduction in plant height and decrease in aboveground biomass (Wei and Zhou, 2004b; Wei et al., 2005a). Compared to non-hyperaccumulators, the aboveground biomass of a hyperaccumulator does not decrease significantly growing in a soil contaminated with heavy metals, which is one of most important characteristics of a hyperaccumulator (Wei and Zhou, 2004b; Zhou and Song, 2004). This indicates that *S. nigrum* can be considered to be a species with potential for phytoextraction purposes in contaminated soils (García et al., 2004; Yoshida et al., 2006). This is because the high tolerance to metal and metalloid toxicity is an important characteristic required for phytoremediation of soils contaminated multiple-metals (Zhou, 1995; Yang et al., 2004).

Biomass production and metal accumulation of a plant is also related to its ecotypes or genotypes (Wang et al., 2000; Wierzbicka and Pielichowska, 2004; Geng et al., 2005; Yoshida et al., 2006). In this pot-culture experiment, the *S. nigrum* seedlings were digged from a field in the Shenyang Ecological Experimental Station. Their seeds collected from different plants had carried their different ecotypes or genotypes. To a certain extent, the big variations in biomass production and metal accumulation of the hyperaccumulator should be responsible for the issue of differences in individual plants, namely different ecotypes or genotypes. In view of the hyperaccumulator screened from ‘clean’ sites in the same area, their different seeds would show a few individual differences under similar treatment conditions because their tolerance and accumulative characters are hereditary.

A prerequisite for hyperaccumulators is the ability to efficiently tolerate high concentration of metals within the plant tissues and cells. Metal hyperaccumulating plants have the ability to solubilize metals from the soil matrix, efficiently absorb them into the roots and translocate them to the shoots (Pollard et al., 2002; Eapen and D’Souza, 2005). The toxicity of heavy metals decreased due to the function of vacuolar compartmentalization and complexation with organic acids such as metallothionein and phytochelation (Barceló and Poschenrieder, 2003; Alkorta et al., 2004), thus promoting absorption and accumulation of heavy metals in the plant. Basically, phytoremediation depends on high concentrations of the metal in plant biomass and production of a relatively large biomass. *S. nigrum* has high ability to uptake and accumulate Cd, the concentration of Cd in stems exceeded the Cd-hyperaccumulator threshold value (Table 1 and Fig. 3) at the different treatments, and the BF and TF values were greater than 1.0 that showed the plant had stronger endurance to Cd and could accumulate Cd (Table 2). However, the low concentration of As, BF and TF in *S. nigrum* expounded that it had the mechanism of tolerance and exclusion to As. Those results indicate that *S. nigrum* has a potential commercial and large scale application to remediation and treatment of contaminated sites by Cd and As.

4. Conclusions

As indicated in the results, the pot-culture experiment conducted with contaminated soils indicated that *S. nigrum* had the capability to tolerate high levels of Cd and As. Under the single Cd treatments the height and underground biomass of the plants did not reduce significantly at <25 mg/kg Cd, and meet the basic characteristics of a hyperaccumulator. Even at the combined contamination of 10 mg/kg Cd and 50 mg/kg As the height and the shoots of the plants showed no differences compared to single Cd concentration of 10 mg/kg, but the content of Cd in the shoots showed little increase. The content of As in *S. nigrum* showed that the plant could tolerate high concentration of As through exclusion mechanisms. Therefore, *S. nigrum* might be useful for the remediation of soil co-contaminated with Cd and As.

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