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Potential of weed species applied to remediation of soils contaminated with heavy metals

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Abstract: To screen out a series of ideal plants that can effectively remedy contaminated soils by heavy metals is the main groundwork of phytoremediation engineering and the first step of its commercial application on a large scale. In this study, accumulation and endurance of 45 weed species in 16 families from an agricultural site were *in situ* examined by using the pot-culture field experiment, and the remediation potential of some weed species with high accumulation of heavy metals was assayed. The results showed that *Solanum nigrum* and *Conyza canadensis* can not only accumulate high concentration of Cd, but also strongly endure to single Cd and Cd-Pb-Cu-Zn combined pollution. Thus 2 weed species can be regarded as good hyperaccumulators for the remediation of Cd-contaminated soils. Although there were high Cd-accumulation in *Artemigia selengensis*, *Znula britannica* and *Cephalanoplos setosum*, their biomass was adversely affected due to action of heavy metals in the soils. If the problem of low endurance to heavy metals can be solved by a reinforcer, 3 weed species can be perhaps applied commercially.

Keywords: weed species; heavy metal; contaminated soil; phytoremediation; hyperaccumulation; endurance

Introduction

Phytoremediation of soils contaminated with heavy metals means the direct use of green plants, root excretion and rhizospheric microorganisms to *in situ* extract, volatilize and immobile or detoxify heavy metals, including phytoextraction, phytovolatilization, rhizofiltration and phytostabilization of heavy metals in soils(Salt, 1995; Licht, 1995; Chaney, 1997; Reeves, 2000). In particular, more and more attention has paid to phytoextraction and phytostabilization with the development of phytoremediation technology (Salt, 2000; Zhou, 2002a; 2002b; Wong, 2003). Phytoextraction is an important mechanism in which hyperaccumulators can strongly absorb heavy metals in soils and to promote upward translocation of heavy metals in plant tissues. After having harvested, mature hyperaccumulators containing high concentrations of heavy metals are incinerated or composted to be recycled. This procedure may be carried out so repetitious as to quietly decrease contents of heavy metals in soils to an unavailable level for living organisms. Phytostabilization is in principle the use of secretion from plant roots to chelate and precipitate heavy metals, thereby reducing the bioavailability of heavy metals and weakening adverse effects on ecosystems. In fact, it is important to reestablish the vegetation in soils where natural plants can not survival due to high concentrations of heavy metals through using hyper-tolerant plants, which can greatly inhibit the disperse of polluted soils resulted mainly from wind erosion, the transportation of exposed surface soils and leaching of heavy metals to groundwater.

Phytoremediation is at the groping stage(Sung, 2003). In particular, a unitive systematic method to screen out effective plants for the remediation of soils contaminated with heavy metals is still scarce till today, thus affecting the advance of riddling plants with remediation functions. In the world, the sum of known plants in the world is up to 500000 species containing 250000 spermatophyte species or so(Li, 1988). Ample plant resources can provide a sound basis for the selection of remediation plants. In this study, potential of some weed species applied to remediation of soils contaminated with heavy metals was evaluated by using the pot-culture simulative field experiment, and metal-endurant and accumulative characteristics of weed species were discussed so as to establish a warehouse of phytoremediation resources.

1 Materials and methods

1.1 Experimental site

The phytoremediation experiment was arranged at the Shenyang Network Station of Ecological Study(41°31′N, 123°41′E). This is a temperate zone with semi-moist continental climate. The average annual temperature is 5—9℃, the coldest and warmest months are January and July, with mean temperature of −14℃ and 24℃, respectively. The annual precipitation is about 650—700 mm, the annual accumulative temperature with higher than 10℃ is 3100—3400℃, and the total annual radiation is 520—544 kJ/cm^2. There is no frost in 127—164 d in each year.

Plenty of weed species are widely distributed in the site and can be used easily. The tested soil is meadow brown

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earth which naturally distributed in the site. The surface(0—20 cm) soil samples were taken. Its main properties are listed in Table 1.

Table 1 Physical and chemical properties of the tested soil

pH	Organic matter, %	CEC, cmol(+)/kg	Background concentration, mg/kg dry weight			
			Cd	Pb	Cu	Zn
6.5	1.52	23.7	0.15	14.2	12.4	38.9

1.2 Experimental method

The phytoremediation trial of single Cd pollution and Cd-Pb-Cu-Zn combined pollution were carried out using a pot-culture method by adding Cd, Pb, Cu and Zn to pots according to the contaminative status of heavy metals in soils and their polluting trends levels in the northeast of China (Zhou, 2002; Gu, 2002). There were 3 treatments: (1) CK(control), no external heavy metals were added; (2) level T_1, only Cd was filled and its added concentration was 10 mg/kg; (3) level T_2, Cd, Pb, Cu and Zn were added together, their added concentrations were 10, 1000, 400 and 1000 mg/kg, respectively. The added concentration was 10, 2, 1 and 2 times as many as the third grade criterion value of the National Soil-Environmental Quality Standard of China (GB15618, 1995). The forms of heavy metals which were respectively added to pot soils are $CdCl_2 \cdot 2.5H_2O$, $Pb(CH_3COO)_2 \cdot 3H_2O$, $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$ as solid GR reagents.

The tested soil samples were carefully sieved through a 4-mm mesh to remove stones and plant materials, fully mixed with the above-mentioned pollutants in plastic pots ($\Phi = 20$ cm, $H = 15$ cm), quietly equilibrated for 2 weeks. Total 45 weed species which belong to 16 families were taken from the station. The identical plant seedlings were transplanted to pots with the treatment of CK, T_1, and T_2 on April 10 to May 25, 2002. There were 2—6 seedlings in each pot according to the size of plants, but the number of seedlings is the same for the same plant in the 3 treatments. Plants in pots grew in field without a defilade. Losses of water by evaporation from pots were made up daily with tap water(no Cd, Pb, Cu and Zn was detected) to keep the soil moist. No fertilizer was added. There were triplicates for each treatment. Plants grew in the pots were harvested after their physiology maturity.

1.3 Determination of heavy metals and error analysis

After having harvested, intact plant roots were rinsed with tap water. Roots, separated stems, leaves and inflorescences were washed with deionized water. Plant samples were dried at 105℃ for 5 min and then at 70℃ in a oven till the weight of plant samples were unchangeable. Dried plant samples were digested with $HNO_3/HClO_4$ (v/v = 87%/13%) and concentrations of heavy metals were determined using the atomic absorption spectrophotometry (Ince, 1999).

The average of three replicates for each treatment and standard deviation(SD) were calculated using the Microsoft Excel 2000 on a computer. Significant differences among treatments, metal-accumulation and overground biomass were tested by using the least significant difference(LSD).

2 Results

2.1 Diagnostic standards of hyperaccumulators

Usually, the growth and development of plants surviving in soils containing high concentrations of heavy metals is adversely inhibited, and their botanic characteristics such as leaf color, plant height and fruiting could be changed to a great extent (Gao, 1986; Sun, 2001). In particular, the overground biomass of plants was severely decreased. Even so, there are still a lot of plants that can grow normally in some polluted soils and their biomass cannot be reduced either, which denoting these plants may have some tolerant mechanism to heavy metals. According to changes in overground biomass of plants, tested plants can be divided into two types of hyperaccumulators: (1) strongly tolerant plants, when a decrease in overground biomass of plants is not obvious compared with CK; (2) weak tolerant plants, where there is significantly reduction in overground biomass of plants.

The ratio of metal concentration in a plant(mg/kg, dry weight) to initial metal concentration in a soil(mg/kg, dry mass) is called bioaccumulation coefficient(*BC*), indicating metal-accumulative capability of a plant. A plant with high *BC* values of heavy metals especially in overground tissues would have strong accumulation capability of heavy metals, and could be applied to effective remediation of soils contaminated with heavy metals when phytoextraction mechanisms occur, besides the overground biomass of plants can easily be harvested. Only when concentration of a heavy metal in a plant is higher than that in a soil, particularly when concentration of a heavy metal in a soil is lower than the maximum concentration that a plant could accumulate from the soil, the efficiency of phytoremediation can be accepted(Salt, 1995; 2000). Thus, we can infer that the *BC* value in overground parts including stems, leaves and inflorescences of highly accumulative plants or hyperaccumulators should be higher than 1. Otherwise, they belong to lowly accumulative plants.

Based on the metal-endurance of weed plants and the *BC* values of heavy metals (Cd, Pb, Cu and Zn) in overground parts of weed plants, the tested weed species can be divided into four types: (1) strong endurance to heavy metals (overground biomass of a plant is not decreased significantly) and high accumulation of heavy metals(the *BC* value in overground parts of a plant is higher than 1); (2) inhibition of plant growth by heavy metals (overground biomass is significantly reduced) but high accumulation of heavy metals in plant tissues (the *BC* value of overground parts is higher than 1); (3) strong endurance to heavy metals (overground biomass of a plant is not decreased significantly) but low accumulation of heavy metals in a plant (the *BC* value of overground parts in a plant is lower than 1); (4) inhibition of plant growth by heavy metals (overground biomass of a plant is reduced significantly) and normal accumulation of heavy metals in a plant (the *BC* value in overground parts of a plant is lower than 1). Types 1 and 2 should belong to hyperaccumulators to an extent.

2.2 Weed plants with Cd hyperaccumulative characteristics

In the single Cd pollution treatment(T_1), there were 8 weed species whose overground biomass (including stem, leaf and florescence biomass) was not decrease significantly (Fig. 1). Compared with CK, the overground biomass of *Portulaca oleracea*, *Tephroseris subdentata*, *Peucedamum terebinthaceum*, *Conyza canadensis* and *Kalimeris integrifolia* was slightly increased ($p < 0.05$), about 0.27, 1.34, 1.59, 2.35 and 3.43 g/pot respectively. Correspondingly, root biomass of these weed plants was also increased. It seems that the five weed species had strong endurance or detoxification for Cd, and might play an important role in stimulating the growth of the weed plants even in this high level of Cd in the pot soil. The phenomenon was similar to the response of some hyperaccumulators to heavy metal contamination (Knight, 1997; Liu, 2002; Wei, 2003).

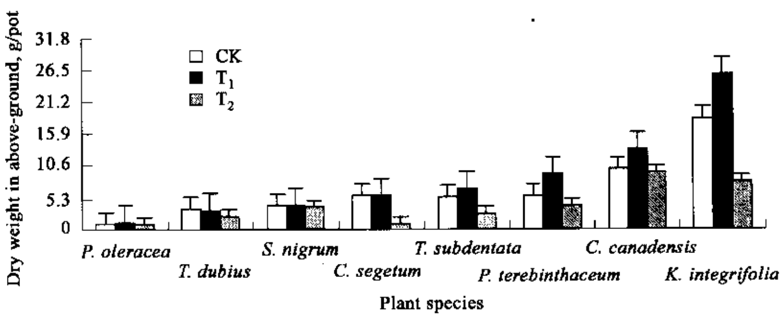

Fig.1 Dry overground biomass of weed plants with characteristics of strong endurance and high accumulation of Cd

As for the treatment of Cd-Pb-Cu-Zn combined pollution (T_2), the growth of *Tragopogon dubius*, *Cephalanoplos segetum*, *Tephroseris subdentata*, *Peucedamum terebinthaceum* and *Kalimeris integrifolia* was adversely inhibited. The decreased rate of overground biomass was up to 74.3%, 48.9%, 81.2%, 28.7%, 57.0% and 34.2%, respectively. Correspondingly, their root biomass was also decreased, though these species displayed strong endurance to Cd in treatment T_1. It can be seen that these species have weak endurance to heavy metals and the mechanism may be additive or synergetic effects of heavy metals on soil-plant systems (Zhou, 1995). Overground biomass of *Solanum nigrum* and *Conyza canadensis* was slightly decreased. It is showed by analysis of variance (LSD) that the difference of the decreased biomass was not significant. According to Salt *et al*. (Salt, 1995), Chaney *et al*. (Chaney, 1997) and Salt (Salt, 2000), it could be inferred from the experiment that *Portulaca oleracea*, *Solanum nigrum* and *Conyza canadensis* would be hyperaccumulators with basic characteristics of strong metal-endurance.

BC values of Cd in overground parts of the 8 weed species were higher than 1 respectively (Table 2), which can regard them as high Cd-accumulation plants. However, the *BC* values of Pb, Cu and Zn in overground parts of the species were lower than 1, and their accumulative abilities of Pb, Cu and Zn were also weak. In treatment T_1, concentration of Cd in overground parts of *Solanum nigrum*, *Cephalanoplos segetum*, *Conyza Canadensis* and *Kalimeris integrifolia* was 31.8, 16.3, 18.82, and 25.04 mg/kg, respectively, which is significantly higher than that in the pot soil. Moreover, the concentration of Cd in overground parts was higher than that in roots, which denoting that the weed species had a strong potential of Cd-translocation from roots to overground parts (Knight, 1997; Lasat, 2001). Thus these weed species can belong to hyperaccumulator with basic characteristics of high Cd in overground parts. In particular, the *BC* value in overground parts of Solanum nigrum was up to 3.13, which showed strong extractability of Cd by the plant. Though the Cd-accumulative capability of *Portulaca oleracea*, *Tragopogon dubius*, *Tephroseris subdentata* and *Peucedamum terebinthaceum* was strong (all the overground *BC* values were higher than 1), the concentration of Cd in overground parts was lower than that in roots. In other words, the weed species is weak in Cd translocation potency from roots to overground parts, and their potential of phytoextraction would be limited, so that they do not hold basic characteristics of hyperaccumulators. In treatment T_2, concentration of Cd in *Kalimeris integrifolia* was lower than that in T_1 significantly ($p < 0.05$), for example, concentration of Cd in roots and overground parts was 17.71 and 25.04 mg/kg in treatment T_1, but 12.78 and 20.19 mg/kg in treatment T_2, which may be attributed to antagonistic effects of Pb, Cu and Zn in soil-plant systems (Zhou, 1995). On the contrary, concentration of Cd in *Cephalanoplos segetum*, *Tephroseris subdentata* and *Conyza canadensis* for treatment T_2 was higher than that for treatment T_1 significantly ($p < 0.05$), which may be due to additive or synergistic effects of Cd, Pb, Cu and Zn (Zhou, 1995). This should also be the result of active absorption of heavy metals by plants on the basis of their endurance and accumulation (Zhou, 1995; Zhang, 1996).

Table 2 Weed species with strong endurance and high accumulation of Cd

Plant species/ families	Treatment	Total Cd, mg/kg			Total Pb, mg/kg		Total Cu, mg/kg		Total Zn, mg/kg		Time, d
		OP(1)	*BC*(2,3)	Root	OP	Root	OP	Root	OP	Root	
Tragopogon	CK	0.11		0.21	2.5	3.0	10.6	14.9	25.9	19.3	92
dubius	T_1	11.73	1.15	11.94							
Compositae	T_2	10.98	1.08	11.44	17.9	189.6	56.8	81.4	188.5	389.5	
Cephalanoplos	CK	0.19		0.07	1.5	0.1	5.5	5.3	28.8	17.0	128
segetum	T_1	16.30	1.61	2.37							
Compositae	T_2	32.53	3.20	4.85	32.3	76.5	15.0	38.5	49.3	107.8	
Tephroseris	CK	0.73		0.21	3.1	1.6	2.6	6.8	13.9	15.1	60
subdentata	T_1	12.45	1.22	17.75							
Compositae	T_2	19.06	1.87	23.58	15.1	206.3	12.7	92.3	275.3	240.3	
Conyza	CK	0.23		0.01	1.6	0.3	3.3	13.1	22.2	19.4	138
canadensis	T_1	18.82	1.85	9.04							
Compositae	T_2	22.68	2.23	18.00	24.9	84.3	15.1	54.5	179.8	96.4	
Kalimeris	CK	0.26		0.26	nd	0.5	3.7	9.8	29.2	14.1	158
integrifolia	T_1	25.04	2.46	17.71							
Compositae	T_1	20.19	1.98	12.78	1.7	65.3	4.2	12.6	30.5	15.7	
Portulaca	CK	nd(4)		0.23	nd	nd	6.1	9.9	24.2	54.5	118
oleracea	T_1	13.99	1.37	92.82							
Portulaceae	T_2	13.28	1.31	91.89	nd	3.2	12.9	17.4	75.0	107.4	
Solanum	CK	1.29		0.13	1.2	0.8	6.8	31.1	47.2	103.2	116
nigrum	T_1	31.80	3.13	27.76							
Solanaceae	T_2	31.61	3.11	27.49	51.7	213.7	11.4	79.8	73.8	380.4	
Peucedamum	CK	0.15		0.26	4.1	4.9	4.4	7.3	26.1	33.4	70
terebinthaceum	T_1	10.85	1.06	14.75							
Umbelliferae	T_2	10.99	1.08	14.33	16.8	135.5	6.4	63.1	178.9	209.2	

Note: (1) OP: overground parts; (2) *BC*: bioaccumulation coefficient in overground parts of a plant; (3) *BC* values of Pb, Cu and Zn in overground parts of a plant were omitted in treatment T_2 because they were lower than 1, respectively; (4) nd: not detected

To sum up, *Solanum nigrumand* and *Conyza canadensis* showed strong endurance to single Cd pollution and the Cd-Pb-Cu-Zn combined pollution and high accumulation of Cd. The phenomenon may be important and useful for the remediation of soils contaminated with Cd. However, it is pity that there is no basic characteristic of high Pb, Cu and Zn simultaneous accumulation, thus resulting in low remediation efficiency and limitation of cleanup processes when Cd-Pb-Cu-Zn combined pollution can occur.

2.3 Low-endurance weed species with high accumulation of Cd

BC values of Cd in overground parts of *Artemigia selengensis*, *Znula britannica* and *Cephalanoplos setosum* were higher than 1 respectively. In other words, these weed species can belong to Cd hyperaccumulators when accumulation of Cd in plants is considered. However, it is unfortunate that there is weak endurance to the harm of heavy metal treatments (T_1, T_2) on 3 weed species. Compared with CK, dry overground biomass was decreased at the rate of 19.1%—63.0%. In other words, their endurance to single Cd pollution and Cd-Pb-Cu-Zn combined pollution may be weak.

As for the accumulation of Pb, Cu and Zn, *BC* values in overground parts of the weed species were lower than 1, respectively. Thus, the ability of the plants accumulating Pb, Cu and Zn simultaneously was weak. We can infer that the remediation function of 3 species in removing Pb, Cu and Zn in contaminated soils may be confined, though they were Cd accumulators.

3 Discussion

Brooks *et al*. (Brooks, 1977) first used hyperaccumulators to name the plant that can accumulate Ni in stem more than 1000 mg/kg dry weight in 1977. After that, the concept was used to name a plant that can hyperaccumulate metals and its critical concentration standards of hyperaccumulators were revised many times. Now, main characteristics of hyperaccumulators are known as (Salt, 1995; 2000; Chaney, 1997): (1) the overground (stem or leave) concentration of heavy metals are 100 times those that occur in non-hyperaccumulative plants living in the same substrates. Critical contents of Mn, Ni, Co, Cu and Pb are 1000 mg/kg dry weight respectively, Cd 100 mg/kg, Au 1 mg/kg and Zn 10000 mg/kg; (2) concentrations of heavy metals in overground parts of plants are higher than those in roots; (3) plants lived in a soil containing high concentration of heavy metals cannot show a significant toxic symptom. Of course, ideal hyperaccumulators should be rapidly grown up in a short growth time, strong anti-disease, huge available biomass, hyperaccumulation of two or more than two types of heavy metals simultaneously, and so on. In fact, necessary characteristics of hyperaccumulators should include 2 aspects: (1) the overground biomass of a plant cannot reduce obviously, and (2) *BC* values of heavy metals in overground parts of a plant are higher than 1.

When plants live in a soil containing high concentrations of heavy metals, their overground biomass cannot obviously reduce. This is an important tolerant characteristic of hyperaccumulator distinguishing from a common plant. The prerequisite of phytoremediation should lie in the normal growth of remediation plants in a soil contaminated with heavy metals. The mechanism that overground biomass of a hyperaccumulator is not decreased and at the meantime hyperaccumulation of heavy metals may be due to the vacuolar compartmentalization and the chelation by some organic acids, which can reduce the inhibition of a plant by heavy metals(Ortiz, 1995; Huang, 1996; Kramer, 1996). Though some plants which can survive in a soil contaminated with heavy metals may complete their life cycle, their overground biomass would decrease significantly, usually showed as short plant height. Sometimes, biological characteristics such as color of leaves or flowers are changed(Kong, 1982). Though contents of heavy metals in a plant exceed known critical concentration standards of hyperaccumulator when a plant lives in a soil with higher concentration of a heavy metal than the criterion of hyperaccumulator even higher than several times, the content of a heavy metal in plants would be lower than the criterion if it lives in a soil with slightly lower heavy metal concentration than the criterion and show the same properties as a common plant, for the content of a heavy metal in plants is increased with the concentration in a soil (Zhou, 2001; Gu, 2002). Therefore, it is one of indispensable characteristics that overground *BC* value of plant is higher than 1 for hyperaccumulator distinguishing from a common plant, at least the *BC* value should be higher than 1 when the concentration of heavy metals in a soil can correspond to the criterion content of hyperaccumulator.

So far, it has reported that there were approximately 400 hyperaccumulators to accumulate unusually large amounts of metals. About 300 species hyperaccumulate Ni, 26 Co, 24 Cu, 19 Se, 16 Zn, 11 Mn, one Tl and one Cd(Brooks, 1998; Reeves, 2000). The remediation potential of the selected plants have been explored, such as *Thlaspi caerulescens*(Knight, 1997; Lasat, 2001; Lombi, 2002) and *Brassica juncea* (Salt, 1995; Su, 2002). Several hyperaccumulators have been discovered in China too, such as Cu hyperaccumulator *Elsholtzia splendens* (Jiang, 2002), and Zn hyperaccumulator *Sedum alfredii* (Yang, 2002). However, full-scale commercial application of phytoextraction is currently limited. The main shortcoming lies in the characteristics of known hyperaccumulators: lower biomass, longer growth time, inapplicability of mechanical culture on a large scale, and the limitation of advance on bioengineer hyperaccumulators. It is necessary to seek more ideal and more effective hyperaccumulators, so as to provide technological support for phytoremediation used to commercial application widely(Zhou, 2002a; Wei, 2003).

Mining activities result in a large amount of waste rocks and tailings which contain high concentration of heavy metals (Wong, 2003). For example, it is estimated that 3 million tons of tailings left from the mining activities contained about 470 kg of Cd, 37300 kg of Pb, 6800 kg of Hg, 20700 kg of As and 2600 kg of Tl in a famous abandoned gold mine tailings at Goldenville, Nova Scotia, Canada(Wong, 1999). In China, at Guangdong Fankou the No. 1 mine tailings, total Pb concentration is up to 34300 mg/kg, total Zn is 36500 mg/kg, and active Zn concentration is 1963 mg/kg (Lan, 1996). These mine drainage contains high heavy metals which can deposit on surface soils and seriously polluted soils and water through leaching, wind or water erosion, so the whole ecosystem is damaged. Therefore, establishing vegetation is essential to stabilize bare soils and minimize environmental pollution. The species with strong endurance to heavy metals are always the first choice(Wong, 2003; Wei, 2003).

Weed species, especially farmland grasses, are a type of exceedingly advanced flora under the stress of man-made or natural selection, and belongs to wild plant. Compared with crops, weed species possesses such properties as strong anti-stress and light-contest, and less soil water and low fertilizer can maintain their growth and development. They grow rapidly and have often high biomass. Through a long-time natural selection, they bear extensively adaptive capacity and vital force, and play an important role in water and soil conservation and soil improvement. Based on these characteristics, it is possible that weed species may have strong endurance and high accumulation of heavy metals, and can be used as pioneer plants of re-vegetation engineering for an abandon mine.

In this experiment, it was showed that *Solanum nigrum* and *Conyza canadensis* possessed basic characteristics of a Cd-hyperaccumulator. *Artemigia selengensis*, *Znula britannica* and *Cephalanoplos setosum* should be also hyperaccumulator if their biomass can be increased. When some strengthening measures such as added EDTA or fertilizer were taken, their remediation potential may be greatly improved. Of course, it is important to improve the selected hyperaccumulators by using biotechnological methods in the future.

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