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Identification of weed plants excluding the uptake of heavy metals

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Abstract

Using the field pot-culture and sample-analysis method, 54 weed species belonging to 20 families and 31 weed species belonging to 17 families were systematically examined as to whether they can exclude the uptake of heavy metals. After a systematic identification, it was determined that *Oenothera biennis* and *Commelina communis* were Cd-excluders and *Taraxacum mongolicum* was a Zn-excluder. *O. biennis* is a potential Cd-excluder, but also a potential Cu-excluder. The research raises the possibility of making a major breakthrough in the application of metal excluders for safe agro-production in the future.

Keywords: Weed species; Heavy metal; Contaminated soil; Excluder; Agricultural product; Ecological safety

1. Introduction

Increasing attention is being paid to environmental pollution and ecological unsafety caused by heavy metals and many corresponding measures have been applied to control the heavy metal contamination problem (Li et al., 2003; Zhou, 2003). However, a great deal of wastewater and drainage containing high levels of heavy metals is still being released into the soil environment as the economy develops (Zhou, 2000). In Western Europe countries, about 1 400 000 sites (large areas of land) are contaminated, many with heavy metals (ETCS, 1998). Tailings and waste residues of metal mines, usually hosting a large quantity of heavy metals, can easily contaminate local soils, groundwater and surface water by leaching, water erosion and wind erosion (Wong et al., 1999). Most plant species cannot survive on polluted sites due to toxic effects of heavy metals and natural landscapes with ecological value have also been destroyed in some areas (Wong, 2003). Thus, there is the urgent necessity to remediate contaminated agricultural soils, particularly in metalliferous mining areas.

Remediation technology such as vitrification, electrokinetics, vapour extraction, soil flushing and slurry-phase bioreactors has been applied to remediation of soils contaminated by heavy metals (Aar and Alshawabkeh, 1993; Chlopecha and Adriano, 1996). However, it is difficult to clean up thoroughly contaminated soils through permanent remediation owing to economic or other difficulties and in particular to deal with large-scale and heavy pollution of farmland (Pulford and Watson, 2003). Phytoremediation is a promising technology for remediating contaminated soils by metal hyperaccumulation in certain plants (Salt et al., 1995; Brooks et al., 1998). As an important mechanism, phytostabilization can reduce ecological risk of wind and water erosion of heavy metals, in particular through planting tolerant plants in contaminated sites (Chaney et al., 1997; Wong, 2003). However, many plants with the function of phytostabilization can induce ecological risk due to high levels of heavy metals in their aerial parts and metal re-deposition following leaf fall. Like hyperaccumulators for phytoremediation, the identification of plants which exclude heavy metals in soils is very important. Not only can metal excluders survive in highly polluted soils, but also their uptake of heavy metals is low even in the presence

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of high concentrations of heavy metals in soils (Baker, 1981; Wenzel et al., 2003).

Compared with crops, weed species often possess stress resistant properties, and can maintain growth under adverse water and fertilizer conditions. As a result of long-term natural selection, weed species have an extensive adaptive capacity, and play an important role in water and soil conservation and in improvement of soil fertility (Wei et al., 2003). If the functional genes with these excellent characteristics of weed species can be transplanted into tissues of a crop (Feng and Xie, 2000), it will be possible to harvest safe agricultural products from soils contaminated by heavy metals.

2. Materials and methods

2.1. Field pot-culture experiment

A pot culture experiment was carried out in an outdoor field of the Shenyang Ecological Experimental Station (41°31′N, 123°41′E), Chinese Academy of Sciences. The average annual temperature of the site is 5–9 °C and the annual precipitation is about 650–700 mm. The frostless season lasts 127–164 days/year. Weed species are widely distributed in fields of the station and can be easily gathered and used as test materials. Uncontaminated topsoil (0–20 cm) of meadow burozemeter used in the experiment was collected from the same field. The chemical analysis, which used common analysis methods (Lu, 2004), indicated that organic matter, pH value and cation exchange capacity (CEC) in the soil were 1.52%, 6.5 and 23.7 cmol/kg; and the baseline values for Cd, Pb, Cu and Zn were 0.15, 14.2, 12.4 and 39.9 mg/kg (dry weight), respectively.

According to the National Soil-Environmental Quality Standard of China (NSEQSC), a single Cd treatment (T_1) and a joint Cd, Pb, Cu and Zn treatment (T_2) were designed, corresponding to the current levels in soils contaminated by heavy metals in northeast China (Zhou et al., 2002; Gu and Zhou, 2002). In the treatment T_1, a concentration of 10 mg/kg of Cd was spiked into the pot soil. In the treatment T_2, the concentrations of Cd (10 mg/kg), Pb (1000 mg/kg), Cu (400 mg/kg) and Zn (1000 mg/kg) were spiked. The spiked concentrations of Cd, Pb and Zn were 10 times, twice and twice the third grade criterion values (1 mg/kg for Cd, 500 mg/kg for Pb, and 500 mg/kg for Zn dry mass) of NSEQSC (CEPAS, 1995). The spiked Cu concentration was equal to the third grade criterion values for Cu (400 mg/kg dry mass). The chemical forms of the spiked heavy metals were $CdCl_2{\cdot}2.5H_2O$, $Pb(CH_3COO)_2{\cdot}3H_2O$, $CuSO_4{\cdot}5H_2O$ and $ZnSO_4{\cdot}7H_2O$, as solid reagents (analytical grade). Meanwhile, the treatment without external heavy metal addition was regarded as the control (CK).

In the spring season of 2002, 54 weed species from 20 families were selected from fields in the station. The tested topsoil sample was sieved through a 4 mm mesh to remove stones, roots and other plant materials, well mixed with the spiked chemical reagents into plastic pots (¢ = 20 cm, H = 15 cm), and equilibrated for 2 weeks. Weed seedlings at identical growth phases were transplanted into the pots with treatments CK, T_1 and T_2 from April 10 to May 25. There were 2–6 seedlings in each pot according to the size of the plants. The same numbers of transplanted seedlings were transplanted in the same treatment pot. The plants were grown in fields. Loss of water by evaporation from pots was replaced by tap water (no detection of Cd, Pb, Cu and Zn) in order to maintain the soils at 80% of field capacity. No fertilizer was added into the pot soils. All the treatments were replicated three times. Weed plants grown in these pots were harvested at maturity or when the frost occurred.

2.2. Sample-analysis experiment in a Pb–Zn mining area

The investigated Pb–Zn mining area is situated in Qingchengzi (40°41′N, 123°37′E), Fengcheng County, Liaoning Province, China. The site meteorology is similar to the pot site. In the mining area, secondary forest, sparse brushwood and some transplanted trees are the main vegetation and marble and micacite are the main host rocks. Brown soil (burozem) is widely distributed. The main host rocks are marble and micacite, and the grade of Pb–Zn minerals (galena and sphalante) is up to 70–80%. Cadmium is an associate metal, mainly compounded within the crystal lattice of sphalante and its average grade is about 0.034% (Zhou, 1989).

In view of the basic trait that accumulation of heavy metals in plants increases positively with increasing concentration of heavy metals in soils (Schwartz et al., 2003), it was necessary to collect soil and plant samples in contaminated soils rich in heavy metals in order to identify characteristics of a plant excluding heavy metals. Usually, concentrations of heavy metals in mining areas are often far above the values in natural soils (Long et al., 2002). In principle, we can get plants with the function of heavy metal exclusion in a mining area because some plants can still survive in polluted soils around mining areas.

The weed species distributing around the Qingchengzi Pb–Zn mining area were collected together with the soil adhering to roots from July to September 2002. Exclusion characteristics of 31 weed species belonging to 17 families were examined in this experiment.

2.3. Determination of heavy metals and data processing

Roots, stems, leaves and inflorescences of harvested plant samples were rinsed with tap water to remove dirt, and carefully washed with deionized water. The samples were then dried at 105 °C for 5 min, then at 70 °C in an oven until completely dry. The dried plant samples were ground to a powder. Collected soil samples were air-dried and ground using a mortar and pestle and passed through a 0.149 mm sieve. The plant and soil samples were digested with a solution containing 87% of HNO_3 and 13% of $HClO_4$ (Ince, 1999). The concentrations of heavy metals in digested solutions were determined using an atomic absorption spectrophotometer (AAS), model of AAS Hitachi 180-80, and a 1.3 nm spectral band width is used. The wavelengths for Cd, Pb, Cu and Zn are 228.8, 283.3, 324.8 and 213.8 nm, respectively.

The average of three replicates for each treatment and standard deviation (S.D.) were calculated using Microsoft Excel software and significant differences in dry weights between the roots and above-ground parts (the sum of stems, leaves, and inflorescences) of plants were analyzed statistically by the least significant difference (LSD) for multiple comparisons.

3. Results

3.1. Identification of weed species excluding heavy metals in the pot-culture experiment

The overground biomass of *Taraxacum mongolicum* (Compositae), *Plantago asiatica* (Plantaginaceae), *Commelina communis*

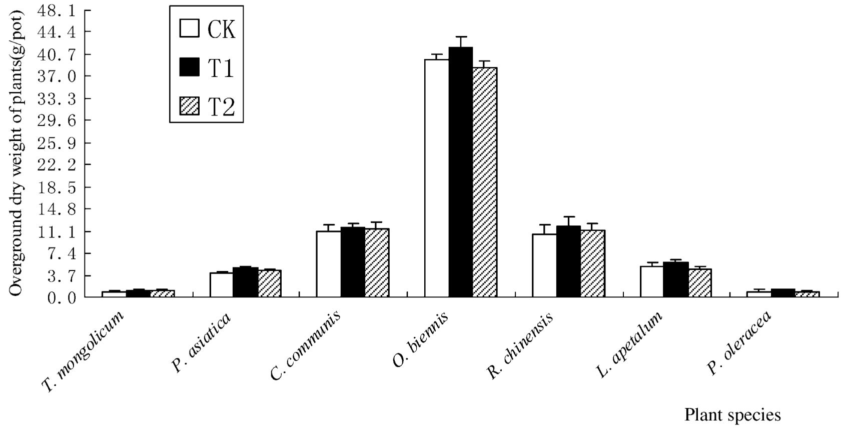

Fig. 1. Dry aboveground biomass of weed plants grown in a contaminated soil.

(Commelinaceae), *Oenothera biennis* (Onagraceae), *Ranunculus chinensis* (Ranunculaceae), *Lepidium apetalum* (Cruciferae) and *Portulaca oleracea* (Portulacaceae) in the treatments T_1 and T_2 were not decreased obviously ($p<0.05$) compared with CK, as shown in Fig. 1. Moreover, the overground biomass increment of *T. mongolicum*, *P. asiatica*, *O. biennis* and *P. oleracea* in the treatment T_1 was up to 0.16, 1.03, 2.92 and 0.23 g per pot ($p<0.05$), respectively. It seems that the 4 species had high Cd-tolerance and strong Cd-exclusion, maintaining the growth even under the high concentration of Cd in the tested soil. This phenomenon was similar to previous research on some excluders of heavy metal pollution (Wenzel et al., 2003). According to the lack of response to the single Cd or combined Cd–Pb–Cu–Zn pollution, the 7 weed plants can be considered as potential excluders.

The distribution of heavy metals in aboveground parts and roots of the potential excluders is listed in Table 1. The results indicate that *P. asiatica*, *C. communis* and *O. biennis* may qualify as Cd-excluders because the concentrations of Cd in aboveground parts of them were 0.29–0.78 mg/kg regardless of the high Cd concentration in the pot soil. Only *P. oleracea* can be regarded as a Pb-excluder because the concentration of Pb in aboveground parts of it was not detected. The concentrations of Cu in aboveground parts of *P. asiatica*, *O. biennis* and *R. chinensis* were not higher than 10 mg/kg; they can thus be classified as Cu-exluders. With the exception of *C. communis* and *L. apetalum*, the concentration of Zn in aboveground parts of the other plants was not higher than 100 mg/kg. We thus suggest that *C. communis* and *L. apetalum* should be considered as potential Zn-excluders.

3.2. Identification of weed species excluding heavy metals in the mining area

According to Table 2, the range of Cd, Pb, Cu and Zn concentrations in the soil samples from the Qingchengzi mining area were 1.01–32.1, 249.2–7793.8, 25.2–119.4 and 146.9–

Table 1
Distribution of heavy metals in tissues of some weed species with metal-excluding function

Species	Treatment	Total Cd (mg/kg)		Total Pb (mg/kg)		Total Cu (mg/kg)		Total Zn (mg/kg)		Culture time (days)	Mature
		AG	Root	AG	Root	AG	Root	AG	Root		
T. mongolicum	CK	0.22	0.03	1.8	2.1	5.5	10	11.1	38.6	70	No
	T1	32.64	8.04								
	T2	31.79	7.35	24.2	49.2	22	35.3	34.4	62.4		
P. asiatica	CK	0.1	0.42	1.6	3.5	3	20.5	8.5	34.5	60	Yes
	T1	0.7	14.08								
	T2	0.78	14.38	13	55.6	8.4	25.4	92.9	98.2		
C. communis	CK	0.16	0.17	2.7	0.7	4.9	4.6	47.8	41.4	150	Yes
	T1	0.64	5.41								
	T2	0.55	6.74	20.8	82	12.1	39.5	214.5	216.6		
O. biennis	CK	nd	nd	2.4	0.1	3.9	2.6	16.3	15.8	112	Yes
	T1	0.3	1.43								
	T2	0.29	1.7	6.1	38.7	5.4	15.4	22	27.4		
R. chinensis	CK	0.01	0.22	2.2	3.2	5.3	18	20.9	27.9	93	Yes
	T1	2.58	23.08								
	T2	2.35	24.17	6.5	87.1	6.1	101.7	86.3	255.2		
L. apetalum	CK	0.15	0.2	1.4	2.2	1.1	2	12	26.6	61	Yes
	T1	3.36	9.7								
	T2	3.91	8.21	18.7	72.2	10.6	31.5	217.8	296.4		
P. oleracea	CK	nd	0.23	nd	nd	6.1	9.9	24.2	54.5	118	Yes
	T1	13.99	92.82								
	T2	13.28	91.89	nd	3.2	12.9	17.4	75	107.4		

AG, aboveground parts of a plant; nd, not detected.

Table 2
Concentrations of heavy metals in the soil of the mining area and the values of NSEQSC (mg/kg)

Sampling no.	Total Cd	Total Pb	Total Cu	Total Zn
1	1.22	697.3	61.4	146.6
2	4.23	1469.3	67.1	899.3
3	11.6	1481.5	98.9	1142.9
4	4.29	1555.3	66.7	952.7
5	3.59	698.5	25.2	1322.5
6	29.03	7793.8	108.1	188.6
7	3.59	698.5	25.2	1322.5
8	2.63	618	65.6	619.2
9	18.38	2120.4	84.7	2740.7
10	4.12	657	26.6	1004.8
11	1.01	249.2	32.3	361.4
12	3.72	1387.9	58.5	643.2
13	32.1	8958.9	119.4	79.2
14	11.6	1481.5	98.9	1142.9
15	1.31	720.9	61.4	493.6
16	1.11	393.1	31.5	300.8
17	2.47	599.1	63.5	518.1
The first grade criterion values	0.2	35	35	100
The second grade criterion values	0.3	250	50	200
The third grade criterion values	1	500	400	500

2740.7 mg/kg, respectively. The mining area contaminated soil can be classified into 3 types: (1) single Cd pollution, for example, soil samples 11 and 16; (2) combined Cd–Pb pollution, including soil samples 1, 6, 13 and 15; (3) combined Cd–Pb–Zn pollution, all the other soil samples. However, no soil could be classed as Cu-polluted because the concentration of Cu in the soil did not exceed the value of NSEQSC (CEPAS, 1995).

The uptake of heavy metals by plants corresponding to the above-mentioned soil samples collected from the mining area was analyzed and examined. The analytical results are listed in Table 3. These indicate that the concentration of Cd in aboveground parts of *C. communis*, which was regarded as Cd-excluder on the basis of the pot-culture experiment, was still low, only 0.310.37 mg/kg. Similar, the concentration of Cd in aboveground parts of *O. biennis*, which was regarded as Cd-excluder in the pot-culture experiment, was also low, down to 0.12–0.79 mg/kg. However, the Cd-excluding characteristic of *P. asiatica*, which was regarded as Cd-excluder by the pot-culture experiment, is doubtful because the concentration of Cd in its aboveground parts was 0.3–14.54 mg/kg. Undoubtedly, *O. biennis* and *R. chinensis* from the mining area can be still regarded as potential Cu-excluders because the concentration of Cu in their aboveground parts was low, only 1.6–2.9 and 7.9–8.3 mg/kg, respectively. Moreover, the Zn-excluding characteristic of *T. mongolicum* from the mining area could be further validated because the concentration of Zn in its aboveground parts was only 23.1–58.2 mg/kg. Unfortunately, the concentration of Pb in all the collected plant samples was high. Thus the identification of a Pb-excluder was not possible in the mining area.

4. Discussion

Excluder plants can normally survive in contaminated soil containing high levels of heavy metals and the contents of heavy metals accumulated in aboveground parts and roots of such plants are all very low or else only low heavy metal concentrations occur in above-ground parts even though the concentrations in roots may be very high. Some other excluder plants have been discovered. For example, the Ni-excluder plant—*Silene vulgaris* (Wenzel et al., 2003), the Ni-excluder crop—maize (*Zea mays* L.) (Seregin et al., 2003), the Cu-excluder—*Hyparrhenia hirta* (Poschenrieder et al., 2001) and the Co-excluder—*Armeria maritima*

Table 3
Distribution of heavy metals in weed species from the Pb–Zn mining area (mg/kg)

Plant species	Cd		Pb		Cu		Zn		Corresponding soil sample
	AG	Root	AG	Root	AG	Root	AG	Root	
T. mongolicum	2.11	0.93	71.7	212.2	11.5	8.9	23.1	127.2	1
T. mongolicum	5.18	2.20	51.7	128.4	5.8	7.3	58.2	35.9	2
P. asiatica	2.79	5.25	181.8	274.5	10.8	14	334.9	651	3
P. asiatica	0.30	0.31	81.3	269.4	13.9	16	70	90.1	4
P. asiatica	0.38	0.42	20.9	65.9	7.6	17.4	198	276.3	5
P. asiatica	14.5	15.0	72.9	344.7	17	18.4	140.1	179.4	6
C. communis	0.31	0.34	17.4	19.6	5.4	7.3	74.7	487.3	7
C. communis	0.37	1.62	73.6	199.8	5.7	7.1	152.2	177.2	8
O. biennis	0.79	1.22	16.7	50.5	2.7	3.8	151.8	212.7	9
O. biennis	0.15	0.21	42.4	43	2.9	3.5	36.6	43.9	4
O. biennis	0.17	0.27	5.5	78.4	1.6	2.4	41.4	64.3	10
O. biennis	0.12	0.15	8.2	83.2	2	1.7	45.5	62	11
R. chinensis	0.46	4.92	42.1	170.5	7.9	25.2	81.9	121.3	12
R. chinensis	5.66	44.5	542.2	1511	8.3	22.1	183	618.4	13
L. apetalum	2.90	3.17	67.6	90.2	3.3	4.1	288.5	334.1	14
L. apetalum	0.32	0.35	8	11.9	1.4	1.5	25	25.6	15
L. apetalum	0.68	1.06	19	35.1	2.6	1.6	124.4	190.1	10
L. apetalum	0.34	0.61	9.5	21.3	1.6	2.5	26.2	48.2	16
P. leracea	0.37	0.83	21.8	25.8	9.9	11.7	73.1	129.8	17

AG means aboveground parts of a plant.

(Brewin et al., 2003). If excluder plants were used for phytostabilization of heavy metals contaminated soils, the ecological risk caused by the re-deposition of dead foliage would be low, because of the low content in aboveground parts of excluder plants.

Crops have been bred for particular characteristics, aiming at high yield and their economic products including roots, stems, leaves, fruits and seed, etc. Thus a crop is a special type of plant that has different characteristics from plants in general. The effect of polluted soil on a crop is significantly different from other species. Even though the growth of a crop may not be inhibited, agricultural products harvested from polluted soil have a potential risk to humans due to the concentration of contaminants exceeding the food sanitation standard or relative environmental quality standard. Thus the adverse effects of polluted soil on crops are growth inhibition and contamination of their edible portions.

Excluder crops can normally grow on severely heavy metals contaminated soils and the content of heavy metals in agricultural products must not exceed the standards. If the exclusion mechanisms of excluder plants could be discovered and the exclusion genes in them located and transplanted to crops, many crops would have strong resistance and exclusion, and the products of agriculture would be safe when grown on land contaminated by heavy metals. Thus, the identification of exclusion resources is very important.

A breakthrough in excluder crop research in transgene breeding has been achieved. For example, Feng and Xie (2000) have transferred the shield gene, i.e. heavy metal exclusion gene in Chinese storehouse rat into the Cruciferae crop—*Brassica rapa* L. The transgene crop can accumulate Cd mostly in the root, so that it is not translocated aboveground parts such as stem, leaf and fruit. The results showed that the application of excluder plants has potential for economic use in contaminated soils and an excellent prospect for safe agro-production.

5. Conclusions

The term excluder plant was first documented in 1981 by Baker, but studies on this field are still limited (Wenzel et al., 2003). The results of this experiment showed that *T. mongolicum* is a potential Zn-excluder and *C. communis* is a potential Cd-excluder. Under certain conditions, *O. biennis* can simultaneously exclude the uptake of Cd and Cu, and *R. chinensis* can be regarded as a Cu-excluder. To screen excluder plants based on weed species could make a great breakthrough in phytostabilization and safe agro-production for soils contaminated by heavy metals.

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