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Efficiency of Repeated Phytoextraction of Cadmium and Zinc from an Agricultural Soil Contaminated with Sewage Sludge

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Long-term application of sewage sludge resulted in soil cadmium (Cd) and zinc (Zn) contamination in a pot experiment conducted to phytoextract Cd/Zn repeatedly using Sedum plumbizincicola and Apium graveolens in monoculture or intercropping mode eight times. Shoot yields and soil physicochemical properties changed markedly with increasing number of remediation crops when the two plant species were intercropped compared with the unplanted control soil and the two monoculture treatments. Changes in soil microbial indices such as average well colour development, soil enzyme activity and soil microbial counts were also significantly affected by the growth of the remediation plants, especially intercropping with S. plumbizincicola and A. graveolens. The higher yields and amounts of Cd taken up indicated that intercropping of the hyperaccumulator and the vegetable species may be suitable for simultaneous agricultural production and soil remediation, with larger crop yields and higher phytoremediation efficiencies than under monoculture conditions.

Keywords: Cd and Zn contamination, Sedum plumbizincicola, intercropping, repeated phytoextraction, BIOLOG test

Introduction

Anthropogenic heavy metal pollution of soils has become a very important environmental problem worldwide and has led to considerable public concern and calls for urgent action in many regions including parts of China (Garbisu and Alkorta 2003). By the end of 2011 the area of agricultural soils in China contaminated with heavy metals reached one fifth of the total cultivated land area and resulted in a total reduction in grain output of over one billion tonnes (Xi et al. 2011).

Sewage sludge application to agricultural land is a worldwide practice combining sludge disposal and benefits to soils and plants including soil physical properties (Moodley and Hughes 2005), increasing soil organic matter content (Rengasamy et al. 1980; USEPA 1983), and supplying major and trace plant nutrients (Heil and Barbarick 1989; Basta et al. 2000; Tsakou et al. 2001, 2002). However, many sludges also contain inorganic and organic contaminants that may have detrimental effects on soils and plants when supplied in excessive amounts (Singh and Agrawal 2007; Achiba et al. 2009; Masto et al. 2012). The repeated application of sewage sludge may lead to the accumulation of heavy metals and organic pollutants such as antibiotics, antibiotic resistance genes and persistent organic pollutants in the soil, with negative effects on crop yields and potential contamination of the human food chain (Chaney 1990; Moreno et al. 1997; Qiao et al. 2003; Iwegbue et al. 2007; Kidd et al. 2007; Jamali et al. 2009; Hao et al. 2011; Nabulo et al. 2011; Zhu et al. 2013).

Heavy metals are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta 2001) so metal-accumulating plants offer numerous advantages for phytoextraction because they can actually extract metals from the polluted soils and thus theoretically render them clean (metal-free) (Garbisu and Alkorta 2001). Phytoremediation, especially the use of hyperaccumulators to extract, sequester, and/or detoxify pollutants, has been reported to be an environmental friendly, effective, non-intrusive, inexpensive, aesthetically pleasing, and socially acceptable technology to remediate polluted soils (Alkorta and Garbisu 2001). Zinc
(Zn) is generally considered to have relatively low toxicity but cadmium (Cd) is highly toxic to animals and humans and the two heavy metals are often present as combined contaminants in metal-polluted soils (Wu et al. 2012b). Hyperaccumulators of Cd and Zn have been known for some considerable time and have been discovered in many parts of the world (Phuena et al. 2009; Vasiliadou and Dordas 2009; Xu et al. 2009; Liu et al. 2011; Saraswat and Rai 2011; Li et al. 2012; Mok et al. 2013). However, their practical use in the field has been limited by factors such as limited growth seasons and slow growth rates as found for Athyrium yokoscense and Arabis gemmifera in Japan (Kubota and Takenaka 2003). In situ phytoremediation by intercropping alfalfa (Medicago sativa L) with tall fescue (Festuca arundinacea Schreb.), Salix caprea and Arabidopsis halleri indicates that intercropping is a promising bioremediation strategy (Wieshammer et al. 2007; An et al. 2011; Sun et al. 2011; Gomes et al. 2013).

Sedum plumbizincicola has been screened for the in situ phytoremediation of soils contaminated by both Cd and Zn and has shown great potential in repeated phytoextraction (Wu et al. 2006; Liu et al. 2011; Li et al. 2014). Use of phytoremediation alone can be time-consuming but it might be possible to enhance the remediation efficiency by intercropping with other remediation plant species (Llugany et al. 2012; Ma et al. 2012; Gupta et al. 2013). Celery has been found to be a highly tolerant vegetable to some heavy metals such as Hg, Cd, Zn and Pb and its ability to ameliorate soil contaminated with both Cd and Zn has been observed and employed for several years in our laboratory (Li and Zhang 2012; Liu et al. 2012; Liu et al. 2013; Nai et al. 2013).

The aim of the present study was to investigate intercropping phytoremediation effects in a soil contaminated with both Cd and Zn collected from a vegetable field to which sewage sludge applications had been made. The four treatments comprised control pots with no plant, S. plumbizincicola and celery intercropping, S. plumbizincicola in monoculture, and celery in monoculture. Phytoextraction was conducted eight successive times with eight consecutive cropping periods of the intercropping and monoculture treatments. The amounts of Cd and Zn taken up by the shoots of the two plant species from the soil in each pot, the shoot biomass of both plant species and soil microbial parameters in the different treatments were investigated to compare the remediation efficiency of the different treatments with the aim of devising an optimum planting strategy for long-term practical remediation of soil with Cd and Zn combined contamination.

Materials and Methods

Experimental Soil

The soil was collected from a vegetable field to which sewage sludge had been applied as an agricultural fertilizer for 13 years in Cixi city, Zhejiang province, east China. Soil samples were collected from the top 15 cm of the soil profile, air-dried at room temperature and screened through a 2 mm nylon sieve. The soil type is Hortic Anthrosols according to the World Reference Base for Soil Resources (WRB) international standard taxonomic soil classification system. The total Cd and Zn concentrations were 0.573 and 605 mg kg\(^{-1}\), respectively.

Experimental Plants

The remediation plant species were Sedum plumbizincicola, a Cd and Zn hyperaccumulator (Wu et al. 2006) which was selected because of its excellent performance in previous remediation studies (Zhao et al. 2011; Liu et al. 2012; Ma et al. 2012; Nai et al. 2013). Seeds of S. plumbizincicola were sown in trays with a matrix of vermiculite and a layer of perlite on the surface using Hoagland’s nutrient solution (Ma et al. 2013). Seedlings were ready for use when they had six true leaves. Seeds of Huangmiaoashi celery (Apium graveolens) were purchased from Jiangsu Academy of Agricultural Sciences in Nanjing, Jiangsu province. Celery seedlings were cultured using non-polluted soil in the glasshouse. After pre-incubation, seedlings of similar height with healthy well developed roots were selected for transplanting.

Phytoremediation Experiment

The four treatments comprised a control (CK), S. plumbizincicola monoculture (S), A. graveolens monoculture (A) and S. plumbizincicola intercropped with A. graveolens (S+A). The pot experiment was carried out in the glasshouse at the Institute of Soil Science, Chinese Academy of Sciences (ISS-CAS), Nanjing. Plastic pots (15 cm height × 10 cm diameter) contained 1 kg soil (oven dry basis) per pot and there were four replicates of each treatment. The pots were arranged in a fully randomized design and re-randomized every week. Six seedlings in total were transplanted in each pot for the monoculture treatments, three seedlings of A. graveolens and three seedlings of S. plumbizincicola in the intercropping treatment, and the control pots remained unplanted. During the experiment urea and potassium dihydrogen phosphate (analytical grade) were applied as fertilizers and deionized water was added daily to maintain the soil water content at about 70% of water holding capacity (WHC). From June 10th 2010 to January 10th 2013 seven remediation crops were grown for repeated phytoextraction for 84, 55, 131, 70, 86, 142, and 84 days, and the eighth crop grew for 70 days from March 12th 2013 to June 15th 2013.

Sampling and Analysis

All shoots were harvested and collected by cutting just above ground level. Fresh shoots were washed with tap water and deionized water and blotted with paper tissues before weighing the fresh biomass. Samples were then transferred into paper bags to destroy enzymes at 105 °C in the oven for 30 minutes and oven dried at 80 °C. About 200 g of soil were collected from each pot, mixed thoroughly, and divided into two portions, one of which was selected for microbial analysis and the other air dried at room temperature and sieved through 0.15- and 0.85-mm sieves. Soil physical and chemical properties comprising water content, pH value, organic matter (OM) content, electronic conductivity, available N,
available P, available K, total N, total P, and total K were determined.

Total Cd and Zn concentrations in soil subsamples were determined after digestion with 4:1 HCl/HNO₃ (by volume) according to McGrath and Cunliffe (1985) and in shoot subsamples after digestion with 3:2 HCl/HNO₃ (by volume) according to Li et al. (2013). Cd was determined with a Varian SpectrAA 220Z spectrophotometer equipped with a graphite furnace (Wu et al. 2012) and Zn by flame atomic absorption spectrophotometry (Varian SpectrAA 220FS). The detection limits of the two spectrophotometers were 0.03 mg L⁻¹ and 0.05 mg L⁻¹ for Cd and Zn, respectively. Blank controls and national standard reference material GSV-2 were included in each batch of samples analysed.

Catalase activity was measured by titration with potassium permanganate and urease activity was determined using the phenol/sodium hypochlorite colorimetric method (Stpniweska et al. 2009; Yan et al. 2013). Soil bacteria and fungi were enumerated using dilution plate counting on LB medium and Martin’s medium (Martin 1950; Rayner et al. 1990). BILOGO tests and calculations were conducted following Ma et al. (2012) and were performed according to the method of Atlas (1984) and Garland and Mills (1991) for the determination of average well colour development (AWCD) and other soil microbial indices.

Data Analysis

Data are presented as mean ± standard derivation and were analysed by one-way analysis of variance using SPSS version 16.0 for Windows. Differences in mean biomass, heavy metal concentrations or metal uptake between treatments were tested by Duncan’s multiple range test at the 5% level.

Results and Discussion

Shoot Biomass and Cd and Zn Uptake

Shoot Biomass

Figure 1 shows that the shoot biomass of both species were much higher after intercropping with eight successive crops compared with results of the monoculture treatments. This indicates that intercropping S. plumbizincicola (S) and A. graceolens (A) may increase the aboveground yields of both plant species (p < 0.05).

Plant biomass is particularly important in the field but it is not the only critical factor as shown in studies of Thlaspi caerulescens (Keller et al. 2003). It was found that to extract 50% of the total Cd and Zn present in the soil investigated within 10 years, shoot metal concentrations of 45 mg kg⁻¹ Cd or 10 mg kg⁻¹ Zn would be required at a biomass production of 10 t ha⁻¹, assuming a linear decrease in soil metals (Kayser et al. 2000). The success of phytoremediation hinges on the selection of plant species and soil amendments that maximize contaminant removal (Ebbs et al. 1998). In our study after eight repeated phytoextraction steps there was no discernible reduction in shoot biomass of either plant species in monoculture or intercropping and this indicates that there were no negative continuous cropping effects occurring over the long term with repeated cropping. During the growth of the fifth crop the shoot biomass of the monocultures showed a slight decline but this might be an expected result of continuous cropping with poor growth and low biomass production (Wu et al. 2000). However, it has been recorded that S. plumbizincicola can continuously extract Cd and Zn from contaminated

Table 1. Amounts of Cd and Zn taken up by plants after 8 consecutive crops of repeated phytoextraction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st crop</th>
<th>2nd crop</th>
<th>3rd crop</th>
<th>4th crop</th>
<th>5th crop</th>
<th>6th crop</th>
<th>7th crop</th>
<th>8th crop</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd (μg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>16.9 ± 1.3b</td>
<td>48.6 ± 3.3a</td>
<td>209 ± 17a</td>
<td>83.1 ± 14.2a</td>
<td>42.2 ± 9.9a</td>
<td>0.20 ± 0.08b</td>
<td>24.1 ± 3.2b</td>
<td>28.8 ± 2.9b</td>
<td>453 ± 35b</td>
</tr>
<tr>
<td>A</td>
<td>ND</td>
<td>9.06 ± 0.41c</td>
<td>10.1 ± 0.5c</td>
<td>11.7 ± 0.9b</td>
<td>5.73 ± 0.26b</td>
<td>0.24 ± 0.13b</td>
<td>3.90 ± 0.29c</td>
<td>2.60 ± 0.13c</td>
<td>43.3 ± 1.8c</td>
</tr>
<tr>
<td>S + A</td>
<td>26.0 ± 3.9a</td>
<td>29.8 ± 4.9b</td>
<td>172 ± 10b</td>
<td>86.3 ± 6.1a</td>
<td>47.4 ± 6.9a</td>
<td>108 ± 9a</td>
<td>74.5 ± 6.5a</td>
<td>100 ± 6a</td>
<td>645 ± 35a</td>
</tr>
</tbody>
</table>

| Zn (mg kg⁻¹) |         |         |         |         |         |         |         |         |         |
| S         | 3.59 ± 0.32a | 8.94 ± 0.63a | 22.5 ± 1.2b | 19.8 ± 2.2b | 13.1 ± 4.1a | 0.58 ± 0.24b | 5.70 ± 1.10b | 8.50 ± 0.90b | 82.7 ± 7.1b |
| A         | ND      | 2.36 ± 0.19b | 3.10 ± 0.16c | 3.53 ± 0.17c | 3.51 ± 0.08b | 0.27 ± 0.02b | 3.10 ± 0.05c | 2.10 ± 0.03c | 18.0 ± 0.5c |
| S + A     | 4.15 ± 1.14a | 7.43 ± 1.28a | 29.9 ± 4.0a | 28.1 ± 2.2a | 11.1 ± 1.1a | 21.8 ± 1.2a | 13.9 ± 0.9a | 22.0 ± 1.5a | 138 ± 9a |

S. S. plumbizincicola monoculture; A. A. graceolens monoculture; S + A, S. plumbizincicola intercropped with A. graceolens. Each value is the mean of four replicates ± standard deviation (SD). ND, not detected. Different letters in columns denote significant difference at p < 0.05 level between treatments.
higher remediation efficiency. Negative effects of continuous cropping and thus to achieve species in the remediation process might help to alleviate theous harvests did not substantially influence the soil organic high densities of roots remaining in the pots from the previ-

soil and this may also benefit the soil microbial biomass and enzyme activities (Jiang et al. 2010). The root distribution was severely restricted, especially for long interval remediation. But thanks to the screening of soil after each remediation step, high densities of roots remaining in the pots from the previous harvests did not substantially influence the soil organic matter content or microbial activity, and this was intended to reflect field conditions. The introduction of two or more plant species in the remediation process might help to alleviate the negative effects of continuous cropping and thus to achieve higher remediation efficiency.

**Shoot Cd and Zn Concentrations**

The uptake of Cd and Zn by *S. plumbizincicola* intercropped with celery was significantly higher than in monoculture (*p* < 0.05) but celery showed the opposite effect except at the eighth crop (Table 1 and Figure 2). Generally the total amounts of the two target heavy metals in the two remediation plant species increased with increasing remediation time, for example during the 3rd crop which grew for 131 days and the maximum taken up by the hyperaccumulator was 209 ± 17 µg pot−1 in the intercropping treatment. One exception was the sixth crop when in a remediation period of 142 days less Cd and Zn were taken up except in the intercropping treatment. This might be due to the high nutrient application rates to the pots as a high salinity effect and another problem was that the temperature was too low in the winter season after transplanting, resulting in low soil microbial activities. The combination of these adverse effects resulted in a slow growth rate. After the harvest of this crop we set up all the pots as fallow for about one month. Seedlings were transplanted when the temperature increased to a suitable value (about 25 °C) and all the plants grew well.

Cadmium and Zn concentrations in fresh celery were 0.015 and 49.8 mg kg−1, below the national vegetable limit standards (0.2 mg kg−1 for Cd, 50 mg kg−1 for Zn). Thus intercropping of celery and *S. plumbizincicola* promoted the uptake of Cd and Zn by *S. plumbizincicola* and reduced their uptake by the celery shoots. In previous studies the metal hyperaccumulator intercropped with maize significantly increased the efficiency of Cd and Zn hyperaccumulation (Wu et al. 2007) and also produced low metal concentrations in the maize grains so as to achieve simultaneous crop production and soil remediation.

**Heavy Metal Concentrations in Soils**

**Changes in Soil Physical and Chemical Properties**

Repeated phytoextraction under the same planting pattern produced detectable changes in soil physical and chemical properties among the different treatments (Table 2). Soil pH did not differ between the control and intercropping pots. Soil electrical conductivity (EC) in planted treatments showed no significant difference among the sole and mixed cropping systems and there was no evidence of salinization. It was noted that soil organic matter (OM) content was higher in the intercropping treatment and this may have been due to higher microbial activity in this treatment. Planted treatments had significantly lower (*p* < 0.05) soil available nitrogen (N) than

![Fig. 2. Shoot concentrations of Cd and Zn in the three planted treatments, CK, the control; A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S + A, *S. plumbizincicola* intercropped with *A. graceolens*. Each value is the mean of four replicates ± standard derivation (SD).](image)

![Steps of continuous remediation](image)

![Steps of continuous remediation](image)

**Table 2.** Soil physicochemical properties under the different treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>EC (ms cm−1)</th>
<th>OM (g kg−1)</th>
<th>Available N (mg kg−1)</th>
<th>Available P (mg kg−1)</th>
<th>Available K (mg kg−1)</th>
<th>Total N (g kg−1)</th>
<th>Total P (g kg−1)</th>
<th>Total K (g kg−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>5.04 ± 0.34b</td>
<td>1.71 ± 0.14a</td>
<td>49.1 ± 2.0b</td>
<td>1993 ± 46a</td>
<td>196 ± 15b</td>
<td>823 ± 21b</td>
<td>4.02 ± 0.22a</td>
<td>6.18 ± 0.38a</td>
<td>20.3 ± 0.1a</td>
</tr>
<tr>
<td>A</td>
<td>5.67 ± 0.08a</td>
<td>0.50 ± 0.17bc</td>
<td>49.9 ± 3.0b</td>
<td>1320 ± 27c</td>
<td>157 ± 13c</td>
<td>398 ± 18d</td>
<td>2.96 ± 0.17b</td>
<td>5.14 ± 0.33b</td>
<td>17.3 ± 0.2b</td>
</tr>
<tr>
<td>S</td>
<td>4.47 ± 0.25c</td>
<td>0.84 ± 0.06b</td>
<td>50.2 ± 1.2b</td>
<td>1580 ± 33b</td>
<td>148 ± 18c</td>
<td>651 ± 16c</td>
<td>3.08 ± 0.19b</td>
<td>5.33 ± 0.26b</td>
<td>18.6 ± 0.2b</td>
</tr>
<tr>
<td>S + A</td>
<td>5.11 ± 0.13b</td>
<td>0.18 ± 0.12c</td>
<td>53.7 ± 0.9a</td>
<td>762 ± 10d</td>
<td>255 ± 9a</td>
<td>1547 ± 37a</td>
<td>2.60 ± 0.26b</td>
<td>3.15 ± 0.29c</td>
<td>17.1 ± 0.2b</td>
</tr>
</tbody>
</table>

CK, control; A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S + A, *S. plumbizincicola* intercropped with *A. graceolens*; EC, electrical conductivity; OM, organic matter. Each value is the mean of four replicates ± SD. Different letters in columns denote significant difference at *p* < 0.05 level between treatments.
Repeated Planting of *S. plumbizincicola* and/or Celery Extract Cd/Zn from Contaminated Soil

**Fig. 3.** Soil total Cd and Zn in different treatments, CK, the control; A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S + A, *S. plumbizincicola* intercropped with *A. graceolens*. Each value is the mean of four replicates ± SD. * in columns denote significant difference at *p* < 0.05 level compared with CK; ** in columns denote significant difference at *p* < 0.01 level compared with CK.

the control but the changes in soil available N varied greatly. Available phosphorus (P) and potassium (K) in the intercropping treatment were significantly higher than in other treatments (*p* < 0.05), possibly due to stimulation of P and K release by the roots of the two plant species when intercropped.

**Total Cd and Zn Concentrations in Soils of Different Treatments**

Figure 3 shows the removal of Cd and Zn from soils of different treatments and, particularly in the case of the intercropping treatment, significant decreases in both Cd and Zn in the soil occurred, indicating that intercropping promoted metal remediation efficiency. Comparing Figures 1 and 2, the total amount of Cd and Zn taken up by the plants was very similar to that removed from the soil, suggesting the establishment of dynamic equilibria of heavy metals between the amounts taken up by the plants and the amounts by which the soil metals decreased.

Shoot concentrations of Cd and Zn tended to decrease in the planted treatments compared to the control (*p* < 0.05), with a pronounced decrease (*p* < 0.01) in the intercropping treatment. After eight repeated remediation steps soil total Cd was 56.1, 41.6 and 55.9% lower in the celery monocrop, *Sedum* monocrop and intercropping treatment, respectively, than in the control, and corresponding values for total Zn were 19.5, 8.1 and 23.0%. In another study *T. caerulescens* was found to be useful in cleaning up soils moderately contaminated with Cd, with about 21.7 and 4.4% of the total soil Cd and Zn removed in one season when *T. caerulescens* was grown for 14 months in field trials (McGrath *et al.* 2006). More than twice as much Cd and Zn was removed in two crops in our study (especially in the intercropping treatment) compared with the remediation efficiency of *T. caerulescens* and this indicates some advantage of intercropping over sole cropping together with the prospect of achieving both high remediation efficiency and large crop yields on metal contaminated agricultural land.

**Changes in Soil Microbial Parameters**

**AWCD**

It is generally accepted that test samples with larger changes in AWCD may have a higher microbial carbon source utilization ability which may correspond with higher microbial abundance (Classen *et al.* 2003). The calculated AWCD values of the microbial population and community diversity are shown in Figure 4 which shows that the AWCD of the intercropped soil increased rapidly, indicating high microbial metabolic activity and abundance. In contrast, the lowest soil microbial metabolic activity was in the control. The highest numbers of bacteria and fungi in the intercropping treatment were associated with the highest AWCD. Although there was no dramatic increase in the two monoculture soils there was a slight increase in AWCD which also indicates some change in the soil environment which might favour the growth of the remediation plants.

**Fig. 4.** Soil AWCD values in different treatments, CK, the control; A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S + A, *S. plumbizincicola* intercropped with *A. graceolens*. Each value is the mean of four replicates ± SD.
Soil microbial activity is not only affected by the external environment and types of fertilizer but also by plant rhizosphere exudation which may also directly affect the quantity and types of soil microorganisms present (Wang et al. 1996). In the absence of plants the control had clearly lower microbial community diversity than the other treatments and this is consistent with the conclusion that the consecutive monoculture and intercropping had largely changed the soil microflora (Zak et al. 1994).

Table 3 shows that the number of bacteria in the intercropping soil was significantly higher than in the control, sole celery and sole Sedum by 86.2, 57.6 and 88.0 times respectively ($p < 0.01$). The soil bacterial numbers in sole celery were also significantly higher than in the control and sole Sedum ($p < 0.05$). Fungal abundance in the intercropping soil was significantly higher than in the control, sole celery and sole Sedum by 7.8, 7.0 and 9.8 times, respectively ($p < 0.01$). In the absence of plants over the long term, fungal numbers in the control soil were not significantly different from the two sole crops, which in turn did not differ from each other (all $p > 0.05$). The trends in the data reveal that intercropping had significantly promoted the soil microorganisms together with plant growth. After consecutive planting the microbial biomass of monoculture soil was significantly lower than of intercropping soil, reflecting a decline in soil quality with possible consequences for plant growth.

**Soil Enzyme Activities**

Soil enzyme activities are often recommended as standard biological indicators for soil health and play important roles in plant growth (Hinojosa et al. 2008; Gao et al. 2010). Urease is important in the transformation of plant nutrients and catalase activity may be related to the metabolic activity of aerobic organisms as an indicator of soil fertility (Tripathi et al. 2007; Stepniewska et al. 2009), so that they are helpful in evaluating the efficiency of remediation treatment (Aciego Pietri and Brookes 2008). Urease and catalase activities showed no significant difference among the treatments, indicating that the activities of these enzymes were not directly responsible for the effects on plant growth or on the metal uptake efficiency of the remediation plants (Table 3). The control soil had the lowest urease and catalase activities and there were no significant differences in urease activity among the planted treatments ($p > 0.05$) (Table 3). Soil urease activity is not the main explanation for poor plant growth in the monoculture treatments but changes in catalase activity may have had some influence on plant growth in the intercropping system.

**Soil Microbial Indices**

Soil pollutants can affect key microbial processes and can decrease the numbers and activities of soil microorganisms so that changes microbial populations or activity can precede detectable changes in the physical and chemical properties of the soil (de Mora et al. 2005; Garau et al. 2007). From Table 4 the trends in Shannon H', Richness D and McIntosh U were similar to that of AWCD and the numbers of bacteria and fungi which were significantly higher in the intercropped pots. The removal of heavy metals from the soil of the intercropping treatment may have enhanced the living conditions of soil microorganisms so that the numbers, activities and diversity of soil microorganisms in this treatment increased substantially.

**Conclusions**

Phytoextraction of metal-contaminated soils is a long-term process. Under the restriction of pot experiment conditions, excessive fertilizer inputs, low temperatures, or other factors may have affected plant growth and phytoextraction efficiency. The results of this glasshouse study indicate that intercropping

### Table 3. Soil microbial counts and enzyme activities

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bacterial count ($\times 10^6$)</th>
<th>Fungal count ($\times 10^6$)</th>
<th>Urease activity (mg NH$_4^+$-N per 100 g soil per 24 h)</th>
<th>Catalase activity (ml KMnO$_4$ per g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>3.33 ± 1.30d</td>
<td>1.58 ± 0.40b</td>
<td>388 ± 92b</td>
<td>1.19 ± 0.33b</td>
</tr>
<tr>
<td>A</td>
<td>4.98 ± 0.31b</td>
<td>1.75 ± 0.39b</td>
<td>423 ± 12b</td>
<td>1.32 ± 0.28b</td>
</tr>
<tr>
<td>S</td>
<td>3.26 ± 0.94c</td>
<td>1.26 ± 0.15b</td>
<td>394 ± 7b</td>
<td>1.21 ± 0.19b</td>
</tr>
<tr>
<td>S + A</td>
<td>287 ± 32a</td>
<td>12.3 ± 5.4a</td>
<td>981 ± 46a</td>
<td>2.38 ± 0.45a</td>
</tr>
</tbody>
</table>

**Table 4. Diversity indices of microbial community in soils of different treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shannon H'</th>
<th>Richness D</th>
<th>McIntosh U</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>1.30 ± 0.11c</td>
<td>1.59 ± 0.51c</td>
<td>0.55 ± 0.23c</td>
</tr>
<tr>
<td>A</td>
<td>2.84 ± 0.17b</td>
<td>4.33 ± 0.68b</td>
<td>1.38 ± 0.47b</td>
</tr>
<tr>
<td>S</td>
<td>2.23 ± 0.64b</td>
<td>3.08 ± 0.87b</td>
<td>1.12 ± 0.43b</td>
</tr>
<tr>
<td>S + A</td>
<td>3.49 ± 0.23a</td>
<td>21.8 ± 1.3a</td>
<td>8.98 ± 0.52a</td>
</tr>
</tbody>
</table>

CK, control; A, A. graceolens monoculture; S, S. plumbizincicola monoculture; S + A, S. plumbizincicola intercropped with A. graceolens. Each value is the mean of four replicates ± SD. Different letters in columns denote significant difference at $p < 0.05$ level between treatments.
of the metal hyperaccumulator with a crop species such as celery can lead to both high remediation efficiency and large crop yields simultaneously in a heavy metal contaminated soil. However, field experiments need to be conducted to confirm the feasibility of this approach and to optimize the intercropping techniques.

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**References**


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