International Journal of Phytoremediation

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bijp20

PHYTOREMEDIATION OF SOIL CONTAMINATED WITH CADMIUM, COPPER AND POLYCHLORINATED BIPHENYLS

Longhua Wu a, Zhu Li a,b, Cunliang Han a, Ling Liu a, Ying Teng a, Xianghui Sun a, Cheng Pan a, Yujuan Huang a, Yongming Luo a & Peter Christie c

a Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, PR China
b Graduate School of the Chinese Academy of Sciences, Beijing, PR China
c Agri-Environment Branch, Agri-Food and Biosciences Institute, Newforge Lane, Belfast, UK

Accepted author version posted online: 18 Oct 2011. Published online: 02 Feb 2012.

To cite this article: Longhua Wu, Zhu Li, Cunliang Han, Ling Liu, Ying Teng, Xianghui Sun, Cheng Pan, Yujuan Huang, Yongming Luo & Peter Christie (2012) PHYTOREMEDIATION OF SOIL CONTAMINATED WITH CADMIUM, COPPER AND POLYCHLORINATED BIPHENYLS, International Journal of Phytoremediation, 14:6, 570-584, DOI: 10.1080/15226514.2011.619227

To link to this article: http://dx.doi.org/10.1080/15226514.2011.619227

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.
PHYTOREMEDICATION OF SOIL CONTAMINATED WITH CADMIUM, COPPER AND POLYCHLORINATED BIPHENYLS

Longhua Wu,1 Zhu Li,1,2 Cunliang Han,1 Ling Liu,1 Ying Teng,1 Xianghui Sun,1 Cheng Pan,1 Yujuan Huang,1 Yongming Luo,1 and Peter Christie3

1Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, PR China
2Graduate School of the Chinese Academy of Sciences, Beijing, PR China
3Agri-Environment Branch, Agri-Food and Biosciences Institute, Newforge Lane, Belfast, UK

A pot experiment and a field trial were conducted to study the remediation of an aged field soil contaminated with cadmium, copper and polychlorinated biphenyls (PCBs) (7.67 ± 0.51 mg kg⁻¹ Cd, 369 ± 1 mg kg⁻¹ Cu in pot experiment; 8.46 ± 0.31 mg kg⁻¹ Cd, 468 ± 7 mg kg⁻¹ Cu, 323 ± 12 µg kg⁻¹ PCBs for field experiment) under different cropping patterns. In the pot experiment Sedum plumbizincicola showed pronounced Cd phytoextraction. After two periods (14 months) of cropping the Cd removal rates in these two treatments were 52.2 ± 12.0 and 56.1 ± 9.1%, respectively. Total soil PCBs in unplanted control pots decreased from 323 ± 11 to 49.3 ± 6.6 µg kg⁻¹, but with no significant difference between treatments. The field microcosm experiment intercropping of three plant species reduced the yield of S. plumbizincicola, with a consequent decrease in soil Cd removal. S. plumbizincicola intercropped with E. splendens had the highest shoot Cd uptake (18.5 ± 1.8 mg pot⁻¹) after 6 months planting followed by intercropping with M. sativa (15.9 ± 1.9 mg pot⁻¹). Liming with S. plumbizincicola intercropped with M. sativa significantly promoted soil PCB degradation by 25.2%. Thus, adjustment of soil pH to 5.56 combined with intercropping with S. plumbizincicola and M. sativa gave high removal rates of Cd, Cu, and PCBs.

KEY WORDS: soil, Cd, Cu, PCBs, mixed contamination, phytoremediation

INTRODUCTION

Soil pollution represents a risk to human health in various ways including contamination of food crops grown in polluted soils, contamination of groundwater or surface waters used as sources of drinking water or direct ingestion of contaminated soil (Muchuweti et al. 2006; Guney et al. 2010). Methods for remediating polluted soils are therefore of current interest. Classical remediation techniques such as soil washing, excavation, and chelate extraction are all labor-intensive and costly (Wu et al. 2010). Phytoremediation, the use
of plants to remove contaminants from the soil, is a relatively environmentally friendly technology which may be useful because it can be carried out in situ at relatively low cost with no secondary pollution and with the topsoil remaining intact (Jadia and Fulekar 2009).

However, a major drawback of phytoremediation is that a given plant species typically remediates a very limited number of pollutants. In practice, very few soils are contaminated with only one type of pollutant and multiple contamination of soils is common. For example, a soil may be contaminated with a number of potentially toxic elements together with persistent organic pollutants (Xu et al. 2009a, 2009b). It is therefore difficult to remove all pollutants from a soil using one plant species, and sometimes the pollutants may be toxic to the plants selected for remediating one target pollutant but the plants may be sensitive to other contaminants present in the soil.

There have been several studies on the effects of multiple pollutants on plant growth and absorption or degradation of contaminants by plants (Wang and Zhou 2005; Zhu et al. 2007). However, it is difficult to draw general conclusions from these studies because the combinations and concentrations of the pollutants and the plant species employed are different (Liu et al. 2008). Organic pollutants can change the speciation of metals in contaminated soil in which plants are grown (Chen et al. 2004). Several plant species which can accumulate or tolerate soil contaminants can be used to remediate polluted soils. For example, combined plant cultivation was found to enhance the dissipation of PAHs in contaminated soils (Maila et al. 2005; Cheema et al. 2010).

In Zhejiang province, east China, the disassembly of electronic products over a long period of time has resulted in the pollution of cultivated land with potentially toxic elements and organic pollutants, especially copper, cadmium and PCBs (Xu et al. 2009a, b). Copper can have a positive effect on plant growth at low concentrations but is potentially harmful at higher concentrations (Gaetke and Chow 2003). Cadmium is a non-essential element and is toxic to plants and animals (Deckert 2005). PCBs are a class of persistent organic pollutants in soils and pose an environmental and human health risk (Gilbert 2004). These pollutants in combination in soil may exert toxic effects. There have been few phytoremediation studies on soils with combined contamination by copper, cadmium and polychlorinated biphenyls (PCBs).

*Sedum plumbizincicola* is a hyperaccumulator that has been shown to have a remarkable capacity to extract zinc and cadmium from contaminated soils (Wu et al. 2008). *Elsholtzia splendens* is regarded as a copper tolerant and accumulating plant species (Tang et al. 2001) and *Medicago sativa* has been found to degrade PCBs in contaminated soil (Xu et al. 2010). *Houttuynia cordata* is a popular vegetable in the coastal areas of Zhejiang province and it grows well with high PCBs contents in roots on PCB-contaminated soil (Teng et al. 2008). These four plant species were selected for a glasshouse pot experiment and a field microcosm experiment with different intercropping combinations. The aim was to study the combined effects of plant species on the removal (Cd and Cu) or degradation (PCBs) of soil contaminants and to determine a suitable intercropping regime for the phytoremediation of soil contaminated with these pollutants.

**MATERIALS AND METHODS**

**Soil Characterization and Design of the Pot Experiment**

Soil from the top 15 cm of the soil profile was collected from an agricultural field contaminated with heavy metals and organic contaminants from the disassembly of electronic components.
products in Zhejiang province, east China. A sub-sample of the fresh soil was freeze dried for the determination of PCBs. The remaining soil was air dried, passed through a 2-mm nylon sieve and stored at room temperature prior to the pot experiment and chemical analysis for heavy metals. The soil type is Hortic Anthrosols according to the World Reference Base for Soil Resources (WRB) international standard taxonomic soil classification system. The soil pH was 4.56 (soil: H2O 1:2.5) and the soil organic carbon, total N, total P, and total K were 36.5, 2.10, 0.60, and 19.7 g kg$^{-1}$, respectively. Soil aqua regia-extractable Zn, Cd, and Cu concentrations were 369 ± 1, 7.67 ± 0.51, and 179 ± 5 mg kg$^{-1}$, respectively, and the mean concentration of PCBs in the soil was 323 ± 12 µg kg$^{-1}$ and mainly consisted of three and four chloride congeners, 41.8 and 44.6% respectively.

The pot experiment was conducted in a glasshouse located in Nanjing with two growth periods from May to December 2008 and from April to December 2009. In 2008 six treatments were established: (1) unplanted control with no plants (NP); (2) S. plumbizincicola monoculture (Sed); (3) M. sativa monoculture (Med); (4) H. cordata monoculture (Hou); (5) M. sativa intercropped with H. cordata (Med+Hou); (6) S. plumbizincicola intercropped with H. cordata (Sed+Hou). Of the three plant species, only S. plumbizincicola produced enough biomass to be harvested. This may have been due to late transplanting of seedlings and possibly the inability of M. sativa to survive under the relatively low soil pH conditions. The treatments were therefore modified in 2009 as follows: (1) unplanted control (NP); (2) unplanted control with addition of lime [NP(Ca)]; (3) M. sativa monoculture with addition of lime [Med(Ca)]; (4) E. splendens monoculture (Els); (5) S. plumbizincicola monoculture (Sed); and (6) S. plumbizincicola intercropped with E. splendens (Sed+Els). The quantity of lime used was 5 g kg$^{-1}$ soil (oven dry basis) and the soil was equilibrated for one week after liming before transplanting and sowing of seeds and the pH was 5.86. Air-dried soil equivalent to 1.2 kg (oven dry basis) was placed in each 15-cm-diameter pot. Each pot had six drainage holes (1 cm diameter) in the bottom and a white tray was placed under the pot for watering and four replicates of each treatment were arranged in a fully randomised design. Considering the pot areas, six seedlings of S. plumbizincicola were transplanted in each pot and twelve seeds of the other species were sown in each pot and thinned to six seedlings two weeks after sowing. Three plants of each species were selected for the intercropping treatments. Deionised water was applied daily to each tray to maintain the soil water holding capacity at about 70% by weight. There were four replicate pots of each treatment.

**Field Microcosm Experiment**

The field experiment was conducted in the same agricultural field from which the soil for the pot experiment was collected in Zhejiang province, east China, where the climate is subtropical monsoon with an annual precipitation of approximately 1480–1530 mm and an annual temperature of about 17°C. The soil physico-chemical properties were the same as in the pot experiment except for the mean concentrations of Cu, Cd, and Zn which were 468 ± 7, 8.46 ± 0.31, and 159 ± 7 mg kg$^{-1}$, respectively. The soil (top 15 cm) was removed from the field and roots and stones were removed before the soil was mixed thoroughly before use. In the treatments that included lime application the lime was added to the soil two days before transplanting and the soil pH after liming averaged 5.66. Open-ended PVC cylinders (height 30 cm, diameter 30 cm and protruding 10 cm above the soil surface to prevent surface cross-contamination) were arranged in a randomised block with three replicates except for the control and limed treatments which were set up in duplicate. S. plumbizincicola was
introduced as transplanted cuttings and *M. sativa* and *E. splendens* were sown as seed in the PVC cylinders. Seven treatments were set up: (1) unplanted control with lime (F-Ca); (2) unplanted control (F-NP); (3) *M. sativa* monoculture (12 plants per microcosm) with lime [F-Med(Ca)]; (4) *M. sativa* intercropped with *E. splendens* (6 plants of each species) and with lime [F-Med+Els(Ca)]; (5) *S. plumbizincicola* intercropped with *E. splendens* (4 plants of each species) [F-Sed+Els]; (6) *S. plumbizincicola* (4 plants) intercropped with *M. sativa* (6 plants) and with lime [F-Sed+Med(Ca)]; (7) *S. plumbizincicola* (3 plants), *M. sativa* (6 plants), and *E. splendens* (3 plants) intercropped and with lime applied [F-Sed+Med+Els(Ca)]. The experiment was conducted from April to October 2009.

**Soil and Plant Sampling**

Only the shoots were harvested in the pot experiment but both shoots and roots were harvested in the field experiment except for *S. plumbizincicola* whose roots were not harvested. All plant samples were washed with tap water and then rinsed three times with deionised water, oven dried at 50°C, weighed and finely ground with a stainless steel mortar.

Composite soil samples were prepared by mixing five random subsamples, air dried, and passed through a 100-mesh nylon sieve for heavy metal determination or through a 60-mesh stainless steel sieve for PCB analysis.

**Chemical Analysis**

**Determination of Heavy Metals in Soils and Plants.** Soil total heavy metal concentrations were determined by atomic absorption spectrophotometry (Varian SpectrAA 220FS, 220Z; Varian, Palo Alto, CA) after digestion of 0.25 g samples with 12 ml of HCl-HNO₃ (4: 1, v/v). Replicate samples, blanks, and a certified reference material (GBW07401, provided by the Institute of Geophysical and Geochemical Exploration, Langfang, Hebei province, China) were included in all analyses for quality control. Plant samples (0.5 g) were digested using a mixture of 6 ml HNO₃ and 4 ml HClO₄. A certified reference material (GBW07603, Institute of Geophysical and Geochemical Exploration, Langfang, Hebei province, China) was used for quality control. All chemicals used were of analytical reagent grade and the reference material results obtained by the methods described above were within the certified ranges of Cu, Zn, and Cd.

**Extraction and analysis of PCBs.** Samples (10 g soil, 2 g plant) were thoroughly mixed, soaked in 30 ml hexane/acetone (1:1 v/v) overnight, sonicated for 15 min (600 W power output) with a ultrasonicator (KQ-600DB, Ningbo Scientz Biotechnology Co., Ltd., China), centrifuged for 5 min at 1500 rpm and the supernatants were retained. This procedure was repeated twice using 20 ml of the same solvent and the supernatants were mixed together in a 120 ml glass vial and reduced to approximately dry with a rotary evaporator and then 5 ml hexane was added and reduced again to 2 ml. A 25 × 1 cm silica gel column filled with silica gel, alumina, acid silica gel and anhydrous Na₂SO₄ (2:2:1: 1 w/w) was used for purification. Before purification the column was washed with 10 ml hexane and the extract was transferred and eluted with 25 ml of hexane. The eluate was collected and concentrated to 5 ml for gas chromatographic (GC) analysis.

A Varian 3800 gas chromatograph (Varian, Santa Clara, CA) equipped with an electron capture detector (ECD) and auto injector was used for gas chromatographic analysis. The solution was separated on a 30 m × 0.25 mm i.d. CP-Sil 24CB capillary column with a film thickness of 0.5 µm. The injector and detector chamber temperatures were set at 260°C.
and 300°C respectively. The oven initial temperature was set at 180°C, held for 0.5 min, ramped to 260 at 30°C/min held for 18 min, and then 15°C min⁻¹ from 260°C to 270°C, kept 2 min. 1 µl sample was used for analysis, and high purity N₂ was used as the carrier gas. PCB congeners were identified by retention time matching to the standard mixture and quantified by using peak area integration. All these process were described in detail by Xu et al. (2010). The standard PCB mixture used (J & K Scientific Ltd., Beijing, China) ranged from lower chlorinated PCBs to higher chlorinated PCBs and included PCB8, PCB18, PCB28, PCB44, PCB52, PCB66, PCB77, PCB101, PCB105, PCB118, PCB126, PCB128, PCB138, PCB153, PCB170, PCB180, PCB195, PCB200, PCB206, and PCB209. Seven-point calibration was adopted and the R² values ranged from 0.989 to 0.997. The apparatus and method detection limit were 1.43–5.10 µg kg⁻¹ and 1.33–3.45 µg kg⁻¹, respectively. The spike recoveries were 72.0–109.8%.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Duncan’s multiple range test at the 5% level used to compare mean values with the SPSS software package version 16.0 for Windows. Data are presented as mean ± standard error of the mean (SEM).

RESULTS

Plant Biomass and Concentrations of Heavy Metals and PCBs

Pot experiment. No visible symptoms of toxicity were observed in S. plumbizincicola at either harvest and there was no biomass available of the other plant species in 2008 (Table 1). The yield of S. plumbizincicola was smaller at the first harvest than the second, but the plant metal concentrations were significantly higher at the first harvest. The metal concentrations in S. plumbizincicola seemed to decrease, especially Cd and Zn which decreased from 661, 709 mg kg⁻¹ for Cd, and 4766, 4888 mg kg⁻¹ for Zn at the first harvest, to 180, 164 mg kg⁻¹ for Cd, and 1275, 1163 mg kg⁻¹ for Zn at the second harvest (Table 1). This may have been due to a decline in the bioavailability of the heavy metals in contaminated soil after remediation (Jiang et al. 2010).

In the second growing period (year 2009) S. plumbizincicola grew poorly in monoculture with a low yield of 1.01 g pot⁻¹ (Table 1). The shoot concentrations of heavy metals and PCBs varied among the plant species used in the experiment (Table 1). Concentrations of Zn, Cd, Cu, and PCBs in S. plumbizincicola were notably higher than in the other plant species, with intermediate concentrations in M. sativa and lower values in E. splendens. E. splendens is a Cu tolerant species but in the present study E. splendens had lower Cu concentrations than did M. sativa, perhaps because of leaf fall before harvest. Cu concentrations in E. splendens were significantly higher in the leaves than the stems. One unexpected result was the very high concentration of Cu observed in S. plumbizincicola in monoculture and this may explain its low yield in monoculture. The effects of Cu on this plant species, its extraction efficiency and the associated plant physiological mechanisms merit future study.

Field microcosm experiment. All the plant species grew well under field conditions and the yields are listed in Table 2. Shoot and root Cu, Cd, Zn, and PCB concentrations are also shown in Table 2. Intercropping pattern had no effect on the uptake of heavy metals.
or PCBs under these experimental conditions. A similar result was obtained in the pot experiment and considerable differences in concentrations of heavy metals and PCBs among plant species also were found in the field experiment, with higher concentrations of Cu, Zn, and Cd in *S. plumbizincicola* than in the other plant species.

*M. sativa* had higher Cd than *E. splendens* and both *M. sativa* and *E. splendens* showed similar Cu accumulation. Both *M. sativa* and *E. splendens* had large yields and higher Cd and Cu concentrations in the roots than the shoots (Table 2), indicating that harvesting of roots needs to be considered when *M. sativa* and *E. splendens* are used for remediating soil contaminated with Cd and Cu.

### Heavy Metal and PCB Concentrations in Soil after Remediation

**Pot experiment.** After two periods of phytoextraction the metal concentrations in the soil showed varying decreasing trends (Table 3). *S. plumbizincicola* in monoculture and intercropped with *E. splendens* had significantly lower soil Zn and Cd concentrations compared to the unplanted control with and without liming. Compared to the unplanted control the decreases in Cd were 56.1 and 52.2% and in Zn were 17.3 and 14.8%, respectively. This may have been due to metal uptake by *S. plumbizincicola*, a known hyperaccumulator of Zn and Cd. *E. splendens* and *M. sativa* (with liming) both contributed to the removal of Cu from the contaminated soil.

Soil PCB concentrations after remediation in the treatments of NP, NP(Ca), Med(Ca), Els, Sed, and Sed+Els (Table 3) showed declines of 84.8, 89.6, 89.2, 84.6, 82.2, and 85.7% and with no significant difference among these treatments compared to the original study soil (323 µg kg⁻¹). However, there were no significant differences in the concentrations

---

**Table 1** Shoot yield of *S. plumbizincicola* and shoot concentrations of heavy metals and PCBs in the pot experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (g pot⁻¹)</th>
<th>Cu (mg kg⁻¹)</th>
<th>Cd (mg kg⁻¹)</th>
<th>Zn (mg kg⁻¹)</th>
<th>PCBs (µg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year 2008</strong>&lt;br&gt;1st crop&lt;br&gt;Sed</td>
<td>4.97 ± 1.72b</td>
<td>45.7 ± 3.0a</td>
<td>661 ± 22a</td>
<td>4766 ± 583a</td>
<td>—</td>
</tr>
<tr>
<td>Sed+Hou</td>
<td>3.47 ± 0.89b</td>
<td>36.7 ± 2.1ab</td>
<td>709 ± 76a</td>
<td>4888 ± 751a</td>
<td>—</td>
</tr>
<tr>
<td>2nd crop&lt;br&gt;Sed</td>
<td>12.5 ± 1.5a</td>
<td>32.2 ± 2.5b</td>
<td>180 ± 36b</td>
<td>1275 ± 206b</td>
<td>128 ± 24</td>
</tr>
<tr>
<td>Sed+Hou</td>
<td>14.9 ± 1.0a</td>
<td>30.1 ± 2.0b</td>
<td>164 ± 4b</td>
<td>1163 ± 36b</td>
<td>183 ± 30</td>
</tr>
<tr>
<td><strong>Year 2009</strong>&lt;br&gt;Med</td>
<td>14.0 ± 1.3</td>
<td>14.7 ± 3.0c</td>
<td>8.37 ± 2.03b</td>
<td>111 ± 15b</td>
<td>88.9 ± 29.3b</td>
</tr>
<tr>
<td>Els</td>
<td>32.6 ± 3.3</td>
<td>7.30 ± 4.21d</td>
<td>0.84 ± 0.01c</td>
<td>69.1 ± 23.4b</td>
<td>45.9 ± 14.9b</td>
</tr>
<tr>
<td>Sed</td>
<td>1.01 ± 0.60</td>
<td>254 ± 72a</td>
<td>132 ± 40a</td>
<td>1908 ± 545a</td>
<td>411 ± 90a</td>
</tr>
<tr>
<td>Sed+Els (Sed)</td>
<td>3.77 ± 0.57</td>
<td>18.9 ± 0.4b</td>
<td>161 ± 57a</td>
<td>1555 ± 469a</td>
<td>236 ± 76a</td>
</tr>
<tr>
<td>Sed+Els (Els)</td>
<td>15.8 ± 2.2</td>
<td>9.40 ± 1.68d</td>
<td>4.52 ± 1.63b</td>
<td>84.8 ± 9.2b</td>
<td>77.8 ± 38.0b</td>
</tr>
</tbody>
</table>

“Sed+Els (Sed)” denotes *S. plumbizincicola* in the treatment of *S. plumbizincicola* intercropped with *E. splendens*, and “Sed+Els (Els)” denotes *E. splendens* in the treatment of *S. plumbizincicola* intercropped with *E. splendens*.

Values are means ± SEM.

The difference in concentrations in PCBs in shoots for different plant species in different treatments within each year were determined by Duncan’s multiple range test, and significant differences (p < 0.05) are indicated by different letters in each column.
Table 2: Plant biomass (g DM pot\(^{-1}\)), heavy metals (mg kg\(^{-1}\)) and PCBs (µg k g\(^{-1}\)) concentrations in the microcosm field experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass</td>
<td>PCBs</td>
<td>Cu</td>
</tr>
<tr>
<td>F-Med(Ca)</td>
<td></td>
<td></td>
<td>35.2±0.7</td>
</tr>
<tr>
<td>F-Med+Els(Ca)</td>
<td>M. sativa</td>
<td>18.0±0.1</td>
<td>139±5a</td>
</tr>
<tr>
<td></td>
<td>E. splendens</td>
<td>52.4±1.5</td>
<td>140±4a</td>
</tr>
<tr>
<td>F-Sed+Els</td>
<td>S. plumbizincicola</td>
<td>16.2±3.6</td>
<td>122±1b</td>
</tr>
<tr>
<td></td>
<td>E. splendens</td>
<td>31.8±5.6</td>
<td>161±7a</td>
</tr>
<tr>
<td>F-Sed+Med(Ca)</td>
<td>S. plumbizincicola</td>
<td>12.7±2.4</td>
<td>136±6ab</td>
</tr>
<tr>
<td></td>
<td>M. sativa</td>
<td>21.0±2.0</td>
<td>122±5b</td>
</tr>
<tr>
<td>F-Sed+Med+Els</td>
<td>S. plumbizincicola</td>
<td>10.5±1.5</td>
<td>137±9ab</td>
</tr>
<tr>
<td></td>
<td>M. sativa</td>
<td>21.3±1.3</td>
<td>123±8ab</td>
</tr>
<tr>
<td>Els(Ca)</td>
<td>E. splendens</td>
<td>40.7±10.5</td>
<td>150±2a</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
“(Ca)” denotes treatments in which soil pH was adjusted with lime.

The difference in the concentrations of Cu, Zn, Cd, and PCBs, respectively, in roots and roots were determined by Duncan’s multiple range test, and significant differences (p < 0.05) are indicated by different letters in each column.
Table 3 Concentrations of heavy metals and PCBs in soil after remediation in the pot experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heavy metals (mg kg(^{-1}))</th>
<th>PCBs (µg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu</td>
<td>Cd</td>
</tr>
<tr>
<td>NP</td>
<td>369 ± 1a</td>
<td>7.79 ± 0.53a</td>
</tr>
<tr>
<td>NP(Ca)</td>
<td>369 ± 3a</td>
<td>6.71 ± 0.80a</td>
</tr>
<tr>
<td>Medi(Ca)</td>
<td>350 ± 6c</td>
<td>7.60 ± 0.20a</td>
</tr>
<tr>
<td>Els</td>
<td>352 ± 2bc</td>
<td>6.58 ± 0.18a</td>
</tr>
<tr>
<td>Sed</td>
<td>361 ± 3ab</td>
<td>3.42 ± 0.50b</td>
</tr>
<tr>
<td>Sed+Els</td>
<td>345 ± 1c</td>
<td>3.72 ± 0.27b</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

The differences in the concentrations of Cu, Zn, Cd, PCBs and congeners, respectively, in soil after remediation in the pot experiment were determined by Duncan's multiple range test, and significant differences (p < 0.05) are indicated by different letters in each column.

“NP” denotes the treatment of unplanted control; “NP(Ca)” denotes the treatment of unplanted control with addition of lime; (3) “Medi(Ca)” denotes the treatment of *M. sativa* monoculture with addition of lime; “Els” denotes the treatment of *E. splendens* monoculture; “Sed” denotes the treatment of *S. plumbizincicola* monoculture; “Sed+Els” denotes the treatment of *S. plumbizincicola* intercropped with *E. splendens*. 
of PCBs among the six treatments. Disturbance such as soil mixing during the preparation of the pot experiment might have had a large effect on degradation of PCBs by changing plant growth conditions, soil structure and the micro-environment of soil micro-organisms and further research is required on the mechanisms of high PCB degradation rates under pot experiment conditions.

**Field microcosm experiment.** Under field conditions the soil Cu concentrations in the intercrop between *E. splendens* and *S. plumbizincicola* were significantly lower than in the unplanted controls, irrespective of liming of the control, but the other treatments showed no significant decrease in soil Cu (Table 4). Three treatments including *S. plumbizincicola* (intercropped with *M. sativa* and *E. splendens* with lime, intercropped with *M. sativa* and lime, and intercropped with *E. spendens* without lime) showed significant decreases in soil Cd. However, *S. plumbizincicola* showed less uptake of Cd in the former treatment because its yield was lower than in the latter two treatments, with decreases in soil Cd in these three treatments of 25.5, 43.9, and 46.2%, respectively. Only *M. sativa* intercropped with *S. plumbizincicola* with lime and *E. splendens* intercropped with *S. plumbizincicola* without lime showed significant decreases in soil Zn compared to the unplanted control without lime, and the decreases were 10.0 and 12.5%, respectively (Table 4).

Concentrations of soil PCBs in the field experiment after remediation are listed in Table 4. Adding lime to unplanted soil promoted a decrease in PCBs of 25.2%. *M. sativa* monoculture, *M. sativa* intercropped with *E. splendens*, *M. sativa* intercropped with *E. splendens* and *S. plumbizincicola*, and *M. sativa* intercropped with *S. plumbizincicola* (all with lime) showed declines in PCBs of 7.6, 20.1, 47.7, and 42.8%, respectively, compared to the unplanted control soil with lime and the latter two were significant (p < 0.05). The unplanted controls (with and without lime) and the *M. sativa* monoculture with lime showed similar concentrations of soil PCB congeners but they had significantly higher concentrations of Tetra-Cl, Penta-Cl, and ≥Hexa-Cl than did the other treatments.

**Plant Heavy Metal Uptake**

The total uptakes of Cu, Cd, and Zn by plant shoots in the pot and by plants in the microcosm field experiments are shown in Fig. 1. In the pot experiment, shoot uptake of Cu, Cd, and Zn from the soil of *S. plumbizincicola* and *E. splendens* intercropped with *S. plumbizincicola* was significantly higher than that of *E. splendens* monoculture and *M. sativa* with lime. Shoot Cu uptake (Fig. 1a) was lower than that the decrease in soil Cu (Table 3). Leaf fall and the unaccounted-for Cu in the roots may help explain this discrepancy. In the field experiment there were additional uncontrolled factors and no statistically significant differences were found in plant Cu uptake over all treatments. The largest Cd and Zn uptakes were 18.5 ± 1.8 and 131 ± 27 mg kg⁻¹ in the intercrop between *S. plumbizincicola* and *E. splendens* (Fig. 1b).

**DISCUSSION**

Efficiency of phytoextraction can be affected by numerous factors, such as soil properties, bioavailability, and amount of pollutants in soil, the toxicity of the contaminants to plants, the plant species selected for remediation, the environmental conditions of plant growth and agronomic practices. All these factors influence plant yield and/or the concentrations of pollutants in plants, and therefore influence the efficiency of phytoextraction (Hong et al. 2007; Li et al. 2009; Karami et al. 2010). In the present study the level of
Table 4  Concentrations of heavy metals and PCBs in soil after remediation in the microcosm field experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heavy metal (mg kg(^{-1}))</th>
<th>PCBs (µg kg(^{-1}))</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu</td>
<td>Cd</td>
<td>Zn</td>
<td>Di-Cl</td>
<td>Tri-Cl</td>
</tr>
<tr>
<td>F-NP</td>
<td>467 ± 9a</td>
<td>8.39 ± 0.29a</td>
<td>160 ± 7a</td>
<td>28.5 ± 3.9a</td>
<td>41.3 ± 2.1b</td>
</tr>
<tr>
<td>F-Ca</td>
<td>469 ± 8a</td>
<td>8.54 ± 0.26a</td>
<td>158 ± 8ab</td>
<td>13.9 ± 4.3c</td>
<td>31.6 ± 4.7b</td>
</tr>
<tr>
<td>F-Med(Ca)</td>
<td>452 ± 23ab</td>
<td>8.35 ± 0.67a</td>
<td>158 ± 6ab</td>
<td>14.0 ± 1.1c</td>
<td>23.7 ± 1.9b</td>
</tr>
<tr>
<td>F-Med+Els(Ca)</td>
<td>450 ± 11ab</td>
<td>8.16 ± 0.16a</td>
<td>153 ± 3ab</td>
<td>17.7 ± 0.4c</td>
<td>50.7 ± 3.6a</td>
</tr>
<tr>
<td>F-Sed+Els</td>
<td>425 ± 12b</td>
<td>4.51 ± 0.42c</td>
<td>140 ± 1c</td>
<td>21.6 ± 0.9ab</td>
<td>28.1 ± 2.5b</td>
</tr>
<tr>
<td>F-Sed+Med(Ca)</td>
<td>446 ± 18ab</td>
<td>4.71 ± 1.44c</td>
<td>144 ± 15bc</td>
<td>6.68 ± 0.26d</td>
<td>30.6 ± 4.3b</td>
</tr>
<tr>
<td>F-Sed+Med+Els(Ca)</td>
<td>454 ± 11ab</td>
<td>6.25 ± 0.28b</td>
<td>147 ± 7abc</td>
<td>7.62 ± 1.74d</td>
<td>23.0 ± 2.1b</td>
</tr>
<tr>
<td></td>
<td>Tetra-Cl</td>
<td>Penta-Cl</td>
<td>≥Hexa-Cl</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34.9 ± 2.8a</td>
<td>29.4 ± 1.8a</td>
<td>24.5 ± 2.0a</td>
<td>159 ± 9a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.4 ± 3.3a</td>
<td>22.0 ± 1.3a</td>
<td>19.3 ± 0.8a</td>
<td>119 ± 8b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.1 ± 4.2a</td>
<td>24.2 ± 1.7a</td>
<td>21.5 ± 0.2a</td>
<td>110 ± 9b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.3 ± 2.5b</td>
<td>11.5 ± 1.9b</td>
<td>7.20 ± 2.10bc</td>
<td>95.4 ± 9.6b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.3 ± 0.3b</td>
<td>13.6 ± 0.1b</td>
<td>8.77 ± 0.37c</td>
<td>82.3 ± 3.5bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.90 ± 0.70b</td>
<td>11.0 ± 1.0b</td>
<td>11.7 ± 1.3b</td>
<td>68.0 ± 6.6c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.21 ± 0.38b</td>
<td>11.1 ± 1.0b</td>
<td>13.2 ± 1.9b</td>
<td>62.2 ± 4.4c</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM.

The difference in the concentrations of Cu, Zn, Cd respectively, in soil of different treatments after remediation in the microcosm field experiment were determined by Duncan’s multiple range test, and significant differences (p < 0.05) are indicated by different letters in each column.
Cd in soil planted with *S. plumbizincicola* decreased significantly after remediation in both pot and field experiments (Tables 3 and 4). The Cd concentration data in the shoots of *S. plumbizincicola* also indicate the potential of this plant to phytoremediate Cd polluted soil as reported previously (Wu et al. 2008). Interestingly, the concentration of Cd in *S. plumbizincicola* was much higher than in previous studies (Li et al. 2009; Li et al. 2010) in which the average Cd concentrations in *S. plumbizincicola* were almost 30 and 400 mg kg$^{-1}$ when soil total Cd concentrations were 3.55 and 15.3 mg kg$^{-1}$, respectively,
and soil pH was above 7. In this study soil Cd concentrations were 7.67 mg kg\(^{-1}\) (pot experiment) and 8.46 mg kg\(^{-1}\) (field experiment) both of which were below 15.3 mg kg\(^{-1}\) at a soil pH of 4.56 (or 5.56 after liming). However, very high Cd concentrations in \(S.\) \textit{plumbizincicola} were detected at 709 mg kg\(^{-1}\) in the pot experiment and 1241 mg kg\(^{-1}\) in the field experiment (Tables 1 and 2) on average. This may be explained by the soil Cd level and the low soil pH. Some studies have examined the relationships between soil total Cd or pH and Cd bioavailability or plant Cd uptake. Sauve et al. (2000) concluded that total soil Cd and soil pH showed a relatively strong relationship with the free Cd\(^{2+}\) or dissolved Cd in the soil solution, which had a direct effect on Cd bioavailability. It was also reported that the soil metal concentrations have positive relationships with plant metal uptake, and decreasing pH can enhance phytoextraction (Wang et al. 2006). Kim et al. (2009) also found that low soil pH was strongly related to high phytoavailability of Cd, Cu, and Pb. Wang et al. (2006) found that soluble Cd and Zn increased greatly with decreasing soil pH, and lowering pH promoted growth and Cd and Zn uptake by \(T.\) \textit{caerulescens}. Thus, in the present study high Cd levels and low soil pH resulting from high bioavailability of Cd to plants may help explain the high Cd concentrations in \(S.\) \textit{plumbizincicola}. Significantly higher Cd concentrations in \(S.\) \textit{plumbizincicola} shoots were found in the field microcosm experiment compared to the pot experiment. The soil volume is limited and discontinuous in pots, leading to a substantial difference between pot and field experimental conditions for plant growth, with resulting differences in the distribution of roots, soil temperature where the roots do occur, and diurnal changes in water content. In the plastic pots the soil temperature under the surface 1 cm can be at 7.5 °C above ambient temperature (Watts 1975) and Gao et al. (1992) also found that the soil temperature in pots was more than 6.7°C higher than in the field (35.3°C) under natural conditions at 1:30 p.m. Soil water content decreased from 80 to 40% during the day. The high soil temperatures and decline in water content can have negative effects on plant physiology. Barber et al. (1988) reported that soil temperature and water content had significant effects on root growth. Moreover, under field conditions the soil is a continuum that can supply soluble metals soon after absorption by plants in the soil solution.

In the present study the total PCB concentration in the unplanted control (F-NP) was 159 ± 9 mg kg\(^{-1}\) (Table 4), compared to the total PCBs at the beginning of the field microcosm experiment (323 ± 12 µg kg\(^{-1}\)), and the PCB degradation rate of 48.8% was higher than our former results of 5.4% for unplanted control (Xu et al. 2010). This may be because of the difference in PCB composition of the soils tested. In the present experiment the percentage of three chloride congeners in soil was 41.8% and that of four chloride congeners was 44.6%, but in the work of Xu et al. (2010) the soil PCBs were mainly five chloride congeners (36.3%) and four chloride congeners (41.0%), and the percentage of three chloride was only 10.0%. The degradation rate of PCBs decreases as chlorine substitution increases and high chloride congeners (≥5) can exhibit lower degradation rates under anaerobic than under aerobic conditions (Borja et al. 2005). Lower percentages of low chloride congeners might be a main explanation for the high soil PCB degradation rate. An additional factor was the long duration of the experiments. In the work of Xu et al. (2010) the experiment was conducted for only 90 days but the present field microcosm experiment lasted 180 days.

A decrease in soil PCBs during the phytoremediation process can result from plant uptake and metabolism from combined plant-microorganism effects in the root zone (Mackova et al. 2009). Xu et al. (2010) found an average decrease in PCBs of 36.0% when alfalfa
was grown compared to a decrease of only 5.4% in unplanted soil with lime. However, in the present study alfalfa (*Medicago sativa* L.) monoculture had no significant effect on the degradation of PCBs in the field (Table 4). This may be due to very high PCB degradation by soil microbes and addition of lime, and may have obscured the relatively low soil PCB removal effect of alfalfa plants. In our field microcosm experiment liming significantly decreased soil PCBs after plant growth for six months and in the unplanted controls lime addition gave a 25.2% decline in PCBs. Fuentes et al. (2006) found that application of lime increased soil pH, soil nitrate increased over time and microbial activity was enhanced. Addition of lime to soil can also increase soil microbial counts (Smolander et al. 1994) and this may enhance degradation of PCBs by soil microorganisms.

It has been reported that aboveground parts of plants can change belowground soil microbial communities by promoting rhizobacteria through the production of plant root exudates (Kowalchuk et al. 2002). Previous studies have also shown that mixtures of plant species growing together had much stronger effects on the decrease in PAHs than one plant species alone (Maila et al. 2005). In the present study there were combined effects on degradation of PCBs when different plant species were grown together. *S. plumbizincicola*, *M. sativa*, and *E. splendens* all together or in two-species intercropping combinations promoted the degradation or volatilization of high-chloride congeners compared to *M. sativa* in monoculture, unplanted soil without liming, or unplanted control soil with liming.

**CONCLUSIONS**

*S. plumbizincicola* showed substantial accumulation of Cd from a soil contaminated with multiple pollutants and strategies that included planting with *S. plumbizincicola* gave significant decreases in soil Cd. Both *M. sativa* and *E. splendens* showed similar uptake and accumulation of Cu, and liming and intercropping with *M. sativa* promoted the decrease in PCBs. *S. plumbizincicola* intercropped with *M. sativa* together with liming was a suitable plant growth model for remediating this multiple contaminated soil. The bioavailability of heavy metals after liming must be kept in mind because this will influence the efficiency of remediation when soil heavy metals are greatly depleted after repeated phytoremediation.

**ACKNOWLEDGMENTS**

This research was jointly supported by the Program of Innovative Engineering of the Chinese Academy of Sciences (KSCX2-YW-G-053), and the National Natural Science Foundation of China (40930739, 40871155, 40821140539).

**REFERENCES**


