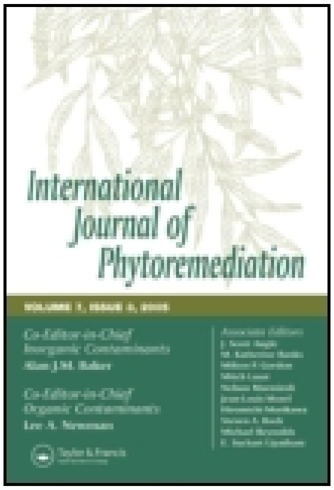
This article was downloaded by: [National Science Library]
On: 27 July 2015, At: 02:43
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: 5 Howick Place, London, SW1P 1WG



Click for updates

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

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

Kai Luo[a], Tingting Ma[a], Hongyan Liu[b], Longhua Wu[a], Jing Ren[b], Fengjiao Nai[b], Rui Li[b], Like Chen[ac], Yongming Luo[ad] & Peter Christie[a]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

[b] College of Resources and Environmental Engineering, Guizhou University, Guiyang, China

[c] University of Chinese Academy of Sciences, Beijing, China

[d] Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China

Published online: 06 Mar 2015.

To cite this article: Kai Luo, Tingting Ma, Hongyan Liu, Longhua Wu, Jing Ren, Fengjiao Nai, Rui Li, Like Chen, Yongming Luo & Peter Christie (2015) Efficiency of Repeated Phytoextraction of Cadmium and Zinc from an Agricultural Soil Contaminated with Sewage Sludge, International Journal of Phytoremediation, 17:6, 575-582, DOI: 10.1080/15226514.2014.935286

To link to this article: http://dx.doi.org/10.1080/15226514.2014.935286

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.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

International Journal of Phytoremediation, 17: 575–582, 2015
Copyright © Taylor & Francis Group, LLC
ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/15226514.2014.935286

Taylor & Francis
Taylor & Francis Group

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

KAI LUO[1,2], TINGTING MA[1], HONGYAN LIU[2], LONGHUA WU[1], JING REN[2], FENGJIAO NAI[2], RUI LI[2], LIKE CHEN[1,3], YONGMING LUO[1,4], and PETER CHRISTIE[1]

[1]*Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China*
[2]*College of Resources and Environmental Engineering, Guizhou University, Guiyang, China*
[3]*University of Chinese Academy of Sciences, Beijing, China*
[4]*Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, China*

Downloaded by [National Science Library] at 02:43 27 July 2015

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 graceolens* 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

Address correspondence to Longhua Wu, Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, P.O. Box 821, 71 East Beijing Road, Nanjing 210008, Jiangsu Province, China. E-mail: lhwu@issas.ac.cn

(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 (Phaenark *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 remediate 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 long-term 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 Huangmiaoshi 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. graceolens* monoculture (A) and *S. plumbizincicola* intercropped with *A. graceolens* (S+A). The pot experiment was carried out in the glasshouse at the Institute of Soil Science, Chinese Academy of Sciences (ISSCAS), 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. graceolens* 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 weighting 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,

Downloaded by [National Science Library] at 02:43 27 July 2015

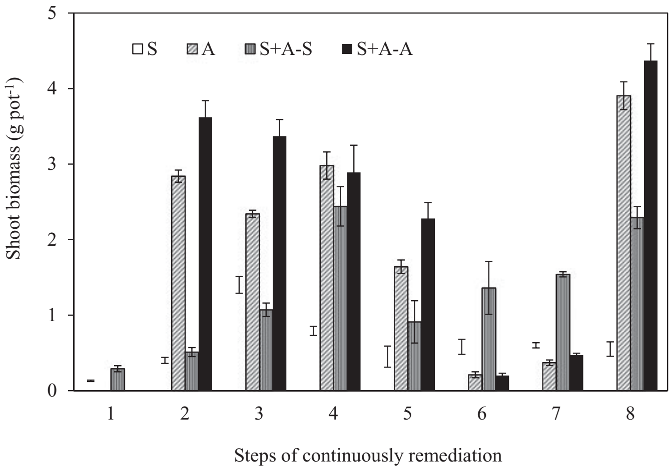


Fig. 1. Shoot biomass in the different treatments (g pot^{-1}), A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S+A, *S. plumbizincicola* intercropped with *A. graceolens*. Each value is the mean of four replicates ± standard derivation (SD).

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/HNO_3 (by volume) according to McGrath and Cunliffe (1985) and in shoot subsamples after digestion with 3: 2 HCl/HNO_3 (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.* 2012a) 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 (Stpniewska *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). BIOLOG 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. graveolens* (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

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

Downloaded by [National Science Library] at 02:43 27 July 2015

Downloaded by [National Science Library] at 02:43 27 July 2015

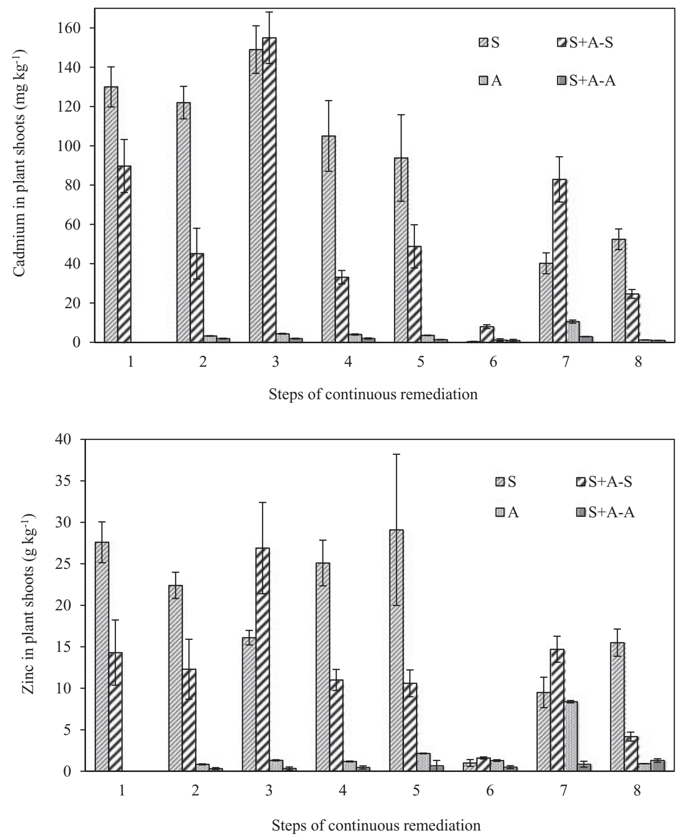


Fig. 2. Shoot concentrations of Cd and Zn in the three planted treatments, CK, the control; A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S + A, *S. plumbizincicola* intercropped with *A. graceolens*. Each value is the mean of four replicates ± standard derivation (SD).

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

Table 2. Soil physicochemical properties under the different treatments

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

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.

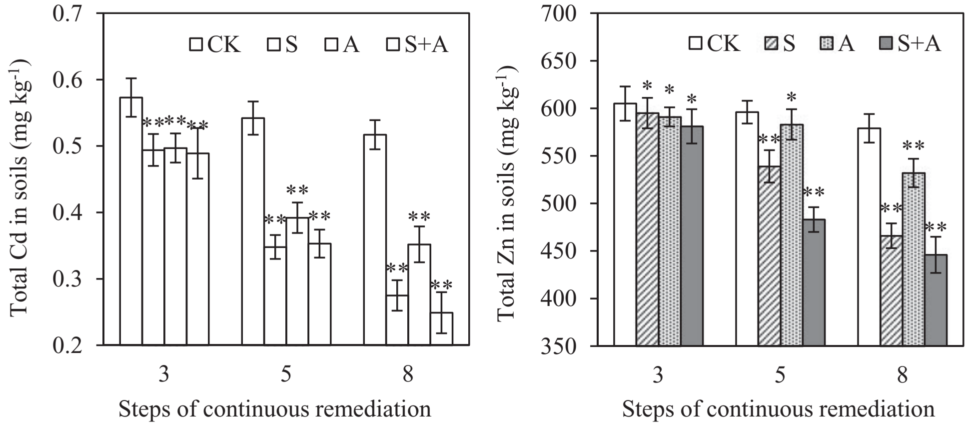


Fig. 3. Soil total Cd and Zn in different treatments, CK, the control; A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S + A, *S. plumbizincicola* intercropped with *A. graceolens*. Each value is the mean of four replicates ± SD. * in columns denote significant difference at $p 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

Downloaded by [National Science Library] at 02:43 27 July 2015

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.

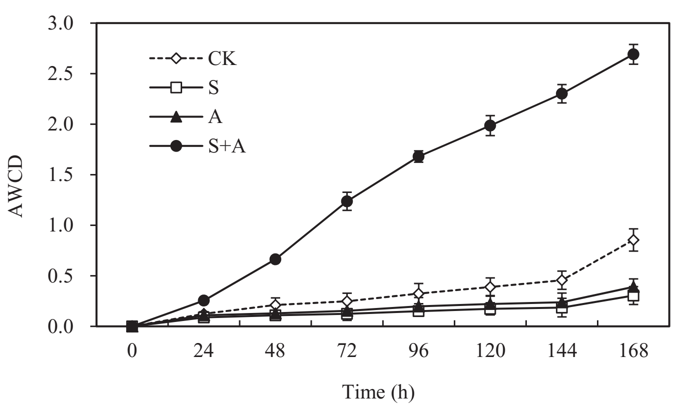


Fig. 4. Soil AWCD values in different treatments, CK, the control; A, *A. graceolens* monoculture; S, *S. plumbizincicola* monoculture; S + A, *S. plumbizincicola* intercropped with *A. graceolens*. Each value is the mean of four replicates ± SD.

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.

Table 3. Soil microbial counts and enzyme activities

Treatment	Bacterial count ($\times 10^6$)	Fungal count ($\times 10^5$)	Urease activity (mg NH_4^+-N per 100 g soil per 24 h)	Catalase activity (ml $KMnO_4$ per g soil)
CK	3.33 ± 1.30d	1.58 ± 0.40b	388 ± 92b	1.19 ± 0.33b
A	4.98 ± 0.31b	1.75 ± 0.39b	423 ± 12b	1.32 ± 0.28b
S	3.26 ± 0.94c	1.26 ± 0.15b	394 ± 7b	1.21 ± 0.19b
S + A	287 ± 32a	12.3 ± 5.4a	981 ± 46a	2.38 ± 0.45a

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.

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

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

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 denote significant difference at $p< 0.05$ level between treatments.

Downloaded by [National Science Library] at 02:43 27 July 2015

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 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; Stępniewska *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 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.

Funding

This research was supported by the Chinese National Natural Science Foundation (41271326 and 41461097) and the Chinese National High Technology Research and Development Program (2012AA06A204).

References

Achiba WB, Gabteni N, Lakhdar A, Laing GD, Verloo M, Jedidi N, Gallali T. 2009. Effects of 5-year application of municipal solid waste compost on the distribution and mobility of heavy metals in a *Tunisian calcareous* soil. Agr Ecosyst Environ 130:156–163.

Aciego Pietri JC, Brookes PC. 2008. Relationships between soil pH and microbial properties in a UK arable soil. Soil Biol Biochem 40:1856–1862.

Alkorta I, Garbisu C. 2001. Phytoremediation of organic contaminants in soils. Bioresource Technol 79(3):273–276.

An LY, Pan YH, Wang ZB, Zhu C. 2011. Heavy metal absorption status of five plant species in monoculture and intercropping. Plant Soil 345(1–2):237–245.

Atlas RM. 1984. Diversity of microbial communities. Adv Microbiol Ecol 7:1–47.

Basta NT, Zupancic RJ, Dayton EA. 2000. Evaluating soil tests to predict bermudagrass growth in drinking water treatment residuals with phosphorus fertilizer. J Environ Qual 29:2007–2012.

Chaney RL. 1990. Twenty years of land application research. Biocycle 31:54–59.

Classen AT, Boyl SI, Haskins KE, Overby ST, Hart SC. 2003. Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. FEMS Microbiol Ecol 44(3):319–328.

de Mora AP, Ortega-Calvo JJ, Cabrera F, Madejón E. 2005. Changes in enzyme activities and microbial biomass after "*in situ*" remediation of a heavy metal-contaminated soil. Appl Soil Ecol 28:125–137.

Ebbs SD, Kochian LV. 1998. Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian Mustard (*Brassica juncea*). Environ Sci Technol 32(6):802–806.

Gao Y, Zhou P, Mao L, Zhi YE, Zhang CH, Shi WJ. 2010. Effects of plant species coexistence on soil enzyme activities and soil microbial community structure under Cd and Pb combined pollution. J Environ Sci 22:1040–1048.

Garau G, Castaldi P, Santona L, Deiana P, Melis P. 2007. Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil. Geoderma 14:47–57.

Garbisu C, Alkorta I. 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technol 77:229–236.

Garbisu C, Alkorta I. 2003. Basic concepts on heavy metal soil bioremediation. Eur J Min Proc Environ Prot 3:58–66.

Garland JL, Mills AL. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57(8):2351–2359.

Gomes HI, Dias-Ferreira C, Ribeiro AB. 2013. Overview of *in situ* and *ex situ* remediation technologies for PCB-contaminated soils and sediments and obstacles for full-scale application. Sci Total Environ 445–446(15):237–260.

Gupta AK, Verma SK, Khan K, Verma RK. 2013. Phytoremediation using aromatic plants: a sustainable approach for remediation of heavy metals polluted sites. Environ Sci Technol 47(18):10115–10116.

Hao XZ, Zhou DM, Wang YK, Shi FG, Jiang P. 2011. Accumulation of Cu, Zn, Pb, and Cd in edible parts of four commonly grown crops in two contaminated soils. Int J Phytorem 13(3):289–301.

Heil DM, Barbarick KA. 1989. Water treatment sludge influence on the growth of *sorghum–sudangrass*. J Environ Qual 18(3):292–298.

Hinojosa MB, Carreira JA, Rodríquez-Maroto JM, García-Ruíz R. 2008. Effects of pyrite sludge pollution on soil enzyme activities: ecological dose–response model. Sci Total Environ 369:89–99.

Iwegbue CMA, Emuh FN, Isirimah NO, Egun AC. 2007. Fractionation, characterization and speciation of heavy metals in composts and compost-amended soils. Afr J Biotechnol 6(2):67–78.

Jamali MK, Kazi TG, Arain MB, Afridi HI, Jalbani N, Kandhro GA, Shah AQ, Baig JA. 2009. Heavy metal accumulation in different varieties of wheat (*Triticum aestivum* L.) grown in soil amended with domestic sewage sludge. J Hazard Mater 164:1386–1391.

Jiang JP, Wu LH, Li N, Luo YM, Liu L, Zhao QG, Zhang L, Christie P. 2010. Effects of multiple heavy metal contamination and repeated phytoextraction by *Sedum plumbizincicola* on soil microbial properties. Europ J Soil Biol 46(1):18–26.

Kayser A, Wenger K, Keller A, Attinger W, Felix HR, Gupta SK, Schulin R. 2000. Enhancement of phytoextraction of Zn, Cd, and Cu from Calcareous soil: the use of NTA and sulfur amendments. Environ Sci Technol 34(9):1778–1783.

Keller C, Hammer D, Kayser A, Richner W, Brodbeck M, Sennhauser M. 2003. Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. Plant Soil 249(1):67–81.

Kidd PS, Dominguez-Rodriguez MJ, Diez J, Monterroso C. 2007. Bioavailability and plant accumulation of heavymetals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere 66:1458–1467.

Kubota H, Takenaka C. 2003. Field Note: *Arabis gemmifera* is a hyperaccumulator of Cd and Zn. Int J Phytorem 5(3):197–201.

Li J, Zhang Y. 2012. Remediation technology for the uranium contaminated environment: a review. Procedia Environ Sci 13:1609–1615.

Li NY, Fu QL, Zhuang P, Guo B, Zou B, Li ZA. 2012. Effect of fertilizers on Cd uptake of *Amaranthus hypochondriacus*, a high biomass, fast growing and easily cultivated potential Cd hyperaccumulator. Int J Phytorem 14(2):162–173.

Li Z, Wu LH, Hu PJ, Luo YM, Christie P. 2013. Copper changes the yield and cadmium/zinc accumulation and cellular distribution in the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. J Hazard Mater 261(15):332–341.

Li Z, Wu LH, Luo YM, Christie P. 2014. Dynamics of plant metal uptake and metal changes in whole soil and soil particle fractions during repeated phytoextraction. Plant and Soil 374: 857–869.

Liu CP, Luo CL, Xu XH, Wu CA, Li FB, Zhang G. 2012. Effects of calcium peroxide on arsenic uptake by celery (*Apium graveolens* L.) grown in arsenic contaminated soil. Chemosphere 86(11):1106–1111.

Liu L, Wu LH, Li N, Luo YM, Li SL, Li Z, Han CL, Jiang TG, Christie P. 2011. Rhizosphere concentrations of zinc and cadmium in a metal contaminated soil after repeated phytoextraction by *Sedum Plumbizincicola*. Int J Phytorem 13(8):750–764.

Liu XM, Song QJ, Tang Y, Li WL, Xu JM, Wu JJ, Wang F, Brookes PC. 2013. Human health risk assessment of heavy metals in soil–vegetable system: a multi-medium analysis. Sci Total Environ 463–464(1):530–540.

Llugany M, Miralles R, Corrales I, Barceló J, Poschenrieder C. 2012. *Cynara cardunculus* a potentially useful plant for remediation of

Downloaded by [National Science Library] at 02:43 27 July 2015

Downloaded by [National Science Library] at 02:43 27 July 2015

soils polluted with cadmium or arsenic. J Geochem Explor 123:122–127.

Ma TT, Luo YM, Christie P, Teng Y, Liu WX. 2012. Removal of phthalic esters from contaminated soil using different cropping systems: A field study. Europ J Soil Biol 50(5–6):76–82.

Ma Y, Rajkumar M, Luo YM, Freitas H. 2013. Phytoextraction of heavy metal polluted soils using *Sedum plumbizincicola* inoculated with metal mobilizing *Phyllobacterium myrsinacearum* RC6b. Chemosphere 93(7):1386–1392.

Martin JP. 1950. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci 69:215–232.

Masto RE, Sunar KK, Sengupta T, Ram LC, Rout TK, Selvi VA, George J, Sinha AK. 2012. Evaluation of the co-application of fly ash and sewage sludge on soil biological and biochemical quality. Environ Technol 33(8):897–905.

McGrath SP, Cunliffe CH. 1985. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. J Sci Food Agric 36:794–798.

McGrath SP, Lombi E, Gray CW, Caille N, Dunham SJ, Zhao FJ. 2006. Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. Environ Pollut 141(1):115–125.

Mok HF, Majumder R, Laidlaw WS, Gregory D, Baker AJM, Arndt SK. 2013. Native Australian species are effective in extracting multiple heavy metals from biosolids. Int J Phytorem 15(7):615–632.

Moodley M, Hughes JC. 2005. The effects of a polyacrylamide–derived water treatment residue on the hydraulic conductivity, water retention and evaporation of four contrasting South African soils and implications for land disposal. Water Sci Technol 54(5): 227–234.

Moreno JL, Garcia C, Hernandez T, Ayuso M. 1997. Application of composted sewage sludges contaminated with heavy metals to an agricultural soil: effect on lettuce growth. Soil Sci Plant Nutr 4:565–573.

Nabulo G, Black CR, Young SD. 2011. Trace metal uptake by tropical vegetables grown on soil amended with urban sewage sludge. Environ Pollut 159(2):368–376.

Nai FJ, Wu LH, Liu HY, Ren J, Liu WX, Luo YM. 2013. Effects of intercropping *Sedum plumbizincicola* and *Apium graceolens* on the soil chemical and microbiological Properties under the contamination of zinc and cadmium from sewage sludge application. Chin J Appl Ecol 24(5):1428–1434.

Phaenark C, Pokethitiyook P, Kruatrachue M, Ngernsansaruay C. 2009. Cd and Zn accumulation in plants from the padaeng zinc mine area. Int J Phytorem 11(5):479–495.

Qiao XL, Luo YM, Christie P, Wong MH. 2003. Chemical speciation and extractability of Zn, Cu and Cd in two contrasting biosolids-amended clay soils. Chemosphere 50:823–829.

Rayner MH, Sadler PJ, Scawen MD. 1990. NMR studies of a bacterial cell culture medium (LB Broth): cyclic nucleosides in yeast extracts. FEMS Microbiol Lett 68(1–2):217–221.

Rengasamy P, Oades JM, Hancock TW. 1980. Improvement of soil structure and plant growth by addition of alum sludge. Commun Soil Sci Plant Anal 11(6):535–545.

Saraswat S, Rai JPN. 2011. Prospective application of *Leucaena Leucocephala* for phytoextraction of Cd and Zn and nitrogen fixation in metal polluted soils. Int J Phytorem 13(3):271–288.

Singh RP, Agrawal M. 2007. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants. Chemosphere 67:2229–2240.

Stpniewska Z, Wolińska A, Ziomek J. 2009. Response of soil catalase activity to chromium contamination. J Environ Sci 21(8):1142–1147.

Sun MM, Fu DQ, Teng Y, Shen YY, Luo YM, Li ZG, Christie P. 2011. *In situ* phytoremediation of PAH-contaminated soil by intercropping alfalfa (*Medicago sativa* L.) with tall fescue (*Festuca arundinacea* Schreb.) and associated soil microbial activity. J Soils Sediments 11(60):980–989.

Tripathi S, Chakraborty A, Chakrabarti K, Bandyopadhyay BK. 2007. Enzyme activities and microbial biomass in coastal soils of India. Soil Biol Biochem 39:2840–2848.

Tsakou A, Roulia M, Christodoulakis NS. 2001. Growth of cotton plants (*Gossypium hirsutum*) as affected by water and sludge from a sewage treatment plant: I. Plant phenology and development. Bull Environ Contam Toxicol 66:735–742.

Tsakou A, Roulia M, Christodoulakis NS. 2002. Growth of flax plants (*Linum usitatissimum*) as affected by water and sludge from a sewage treatment plant. Bull Environ Contam Toxicol 68:56–63.

U.S. Environmental Protection Agency (USEPA). 1983. *Process design manual. Land application of municipal sludge*. EPA–625/1–83–016, Cincinnati, Ohio, USA.

Vasiliadou S, Dordas C. 2009. Increased concentration of soil cadmium effects on plant growth, dry matter accumulation, Cd, and Zn uptake of different tobacco cultivars (*nicotiana tabacum* L.) Int J Phytorem 11(2):115–130.

Wang Y, Shen QR, Shi RH. 1996. Soil microbial biomass and its ecological effects. J Nanjing Agr Univ 19(4):45–51.

Wieshammer G, Unterbrunner R, García TB, Zivkovic MF, Puschenreiter M, Wenzel WW. 2007. Phytoextraction of Cd and Zn from agricultural soils by *Salix* ssp. and intercropping of *Salix caprea* and*Arabidopsis halleri*. Plant Soil 298(1–2):255–264.

Wu FZ, Zhao FY, Liu YY. 2000. On the reasons of continuous cropping obstacles in vegetable facility gardening. J Northeast Univ 21(3):241–247.

Wu LH, Cheng MM, Li Z, Ren J, Shen LB, Wang SF, Luo YM, Christie P. 2012a. Major nutrients, heavy metals and PBDEs in soils after long-term sewage sludge application. J Soils Sediments 12:531–541.

Wu LH, Zhou SB, Bi D, Guo XH, Qin WH, Wang H, Wang CJ, Luo YM. 2006. *Sedum plumbizincicola*, a new species of the *Crassulaceae* from Zhejiang, China. Soils 38(5):632–633.

Wu QT, Hei L, Wong JWC, Schwartzc C, Morelc JL. 2007. Co-cropping for phyto-separation of zinc and potassium from sewage sludge. Chemosphere 68:1954–1960.

Wu WF, Nan ZR, Wang SL, Zhou T, Liao Q, Li CC. 2012b. Effects of Cd-Zn combined pollution on the growth of carrot in oasis soils of arid areas and the migration and enrichment of Cd and Zn. J Lanzhou Univ (Nat Sci) 48(2):1–8.

Xi JF, Yu XZ, Zhou LX, Li DC, Zhang GL. 2011. Comparison of soil heavy metal pollution in suburb fields of different regions. Soils 43(5):769–775.

Xu LS, Zhou SB, Wu LH, Li N, Cui LQ, Luo YM, Christie P. 2009. Cd and Zn tolerance and accumulation by *Sedum Jinianum* in East China. Int J Phytorem 11(3):283–295.

Yan JL, Quan GX, Ding C. 2013. Effects of the combined pollution of lead and cadmium on soil urease activity and nitrification. Procedia Environ Sci 18:78–83.

Zak JC, Willig MR, Moorhead DL. 1994. Functional diversity of microbial communities: A quantitative approach. Soil Biol Biochem 26(9):1101–1108.

Zhao B, Shen LB, Cheng MM, Wang SF, Wu LH, Zhou SB, Luo YM. 2011. Effects of intercropping *Sedum plumbizincicola* in wheat growth season under wheat-rice rotation on the crops growth and their heavy metals uptake from different soil types. Chin J Appl Ecol 22(10):2725–2731.

Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, Hashsham SA, Tiedje JM. 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. PNAS 110(9):3435–3440.