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RESEARCH ARTICLE

The evaluation of heavy metal accumulation and application of a comprehensive bio-concentration index for woody species on contaminated sites in Hunan, China

Xiulian Zhao · Jianfeng Liu · Xinli Xia · Jianmin Chu · Yuan Wei · Shengqing Shi · Ermei Chang · Weilun Yin · Zeping Jiang

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Abstract Fast-growing metal-accumulating woody plants are considered potential candidates for phytoextraction of metals. Shuikoushan mining, one of the biggest Pb and Zn production bases in China, presents an important source of the pollution of environment during the last 100 years. Over 150 km^2 of fertile soil have been contaminated by the dust, slag, and tailings from this mining. The goal of the present work has been to determine the content of Pb, Zn, Cd, and Cu in wild woody plants (18 species) naturally growing in this area. Two hundred five plant and soil samples from 11 contaminated sites were collected and analyzed. In addition, to assess the ability of multi-metal accumulation of these trees, we proposed a predictive comprehensive bio-concentration index (CBCI) based on fuzzy synthetic assessment. Our data suggest some adult trees could also accumulate a large amount of metals. Pb concentrations in leaves of *Paulownia fortunei* (Seem.) Hemsl. (1,179 mg/kg) exceeded the hyperaccumulation threshold (1,000 mg/kg). Elevated Pb concentrations (973.38 mg/kg) were also found in the leaves of *Broussonetia papyrifera* (L.) Vent., with a Pb bio-concentration factor of up to 0.701. Endemic species, *Zenia insignis* Chun exhibited huge potential for Zn and Cd phytoextraction, with the highest concentrations of Zn (1,968 mg/kg) and Cd (44.40 mg/kg), characteristic root nodules, and fast growth rates in poor soils. As for multi-metal accumulation ability, native species *B. papyrifera* was calculated to have the most exceptional ability to accumulate various metals simultaneously (CBCI 2.93), followed by *Amorpha fruticosa* L. (CBCI 2.72) and *Lagerstroemia indica* L. (CBCI 2.53). A trend of increasing metal from trunks to leaves (trunks<branches<leaves) and towards fine roots has been shown by metal partitioning between tissues. The proposed CBCI would allow for the selection of suitable trees for phytoremediation in the future.

Keywords Heavy metal pollution · Woody plants · Phytoextraction · Fuzzy synthetic evaluation · Comprehensive bio-concentration index

Introduction

Heavy metal contaminants are ubiquitous in industrialized societies. They are one of the most serious environmental problems currently facing mankind. Metal-polluted soils are receiving increasing attention because of their persistence and hazardous effects on the environment and human health (Bhargava et al. 2012; Khan et al. 2008; Peuke and Rennenberg 2005). Various physical and chemical remediation methods may be useful in the restoration of polluted soils of limited areas, but are usually very expensive (Sarma 2011; Douay et al. 2008; Glass 1999). Most of these methods have adverse effects on the properties of the soils, and render the soil useless for plant growth (Danh et al. 2009). Phytoextraction is an emerging technology that has received significant scientific and commercial

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X. Zhao · J. Liu · J. Chu · Y. Wei · S. Shi · E. Chang · Z. Jiang (✉)
Key Laboratory of Tree Breeding and Cultivation, State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, People's Republic of China
e-mail: Jiangzp@caf.ac.cn

X. Zhao · X. Xia · W. Yin
College of Biology, National Engineering Laboratory for Tree Breeding, Beijing Forestry University, Beijing 100083, People's Republic of China

attention because of its elegance and the vast extent of contaminated areas (McGrath and Zhao 2003; Pedron et al. 2009). However, continuous phytoextraction requires perennial, easily manageable plants that could accumulate large amounts of metal pollutants in their aerial tissues while producing high biomass (Maric et al. 2013). Very few available hyperaccumulators meet these criteria (Baker et al. 2000). As an alternative to herbaceous hyperaccumulators, fast-growing, metal-accumulating woody plants have been considered as potential candidates for the development of feasible phytoextraction (e.g., Pulford and Watson 2003; Unterbrunner et al. 2007; Puschenreiter et al. 2010).

Numerous studies on this topic are reported in the literature; however, most of them report the use of poplar and willow for the remediation of Cd and Zn polluted soils, and compare either a limited number (<5) of species (Shi et al. 2011; Unterbrunner et al. 2007; Wang and Jia 2010) or a high number of clones from a single species (Di Lonardo et al. 2011; He et al. 2013). These studies were usually conducted in controlled hydroponic, pot or field conditions, using young cuttings of different species with short growth periods. Direct comparisons of metal concentrations between adult woody species are scarce. To better explore the metal accumulation properties of woody species, further field screenings are required.

As trees have varied ability to accumulate different metal pollutants, and most contaminated areas have combined pollution, it is necessary to assess the overall performance of trees in terms of metal accumulation for phytoremediation practice. Fuzzy synthetic assessment is an effective method for overall evaluation when multiple factors need to be considered (Chen et al. 2012). It is used broadly, for example to evaluate urban air quality (Onkal-Engin et al. 2004), for the estimation of methane generation rate constants for landfills (Garg et al. 2006), and for the assessment of the environmental lodging stress for maize planting (Mi et al. 2011). The continuum of values is between 0 (full non-membership) and 1 (full membership) in a fuzzy membership function. The fuzzy evaluation method has the advantage in combining results of several influencing indicators into one relative measurement index. No attempts to date have been made to evaluate the metal accumulation capabilities of plants by this method.

In this study, a field screening program was conducted in the Shuikoushan Pb/Zn mining and smelting area and surrounding areas in Hunan, China. Eleven sites were chosen based on the pollution situation of this area (Sun et al. 2011) and the distribution of the existing adult woody plants. Tissues of these woody species along with soils from their root zone were collected. Eighteen species were investigated, including 12 tree and 6 bush species. The concentrations of Pb, Zn, Cd, and Cu were analyzed in plants and soils. The aims of this screening program were as follows:

- Identify the accumulation status of the adult trees of the 18 species to determine the metal concentrations in their leaves and their bio-concentration factor.
- Propose a comprehensive bio-concentration index (CBCI) to assess the overall performance of the woody species in terms of multi-metal accumulation.
- Compare the comprehensive accumulation ability of these woody species, and assess their feasibility for phytoremediation.
- Explore the distribution regularities of metals in the different tissues of selected metal accumulator species for optimization of biomass production and phytoextraction.

Materials and methods

Description of sites and sampling procedure

The survey was conducted in the Shuikoushan Pb/Zn mining and smelting area and its surrounding region in Hengyang, Hunan, located between latitudes 26°25′58″–26°36′11″ and longitudes 112°22′34″–112°42′58″ (Fig. 1). The mine was created in 1896 and is still one of the biggest Pb and Zn production bases in China. It generates a significant quantity of dust, slag, and tailings every year, and contaminates the surrounding areas over several kilometers (Sun et al. 2011; Wei et al. 2009). This area falls in a subtropical zone with a warm, wet climate characterized by an annual average temperature of 17.8 °C and an average precipitation of 1,457.00 mm (Table 1).

Based on the pollution situation (Sun et al. 2011) and the distribution of the adult trees available in this area, 11 sites were chosen for investigation (Fig. 1). Each site had at least one woody species and each species had more than five adult individuals. The species investigated at each site are listed in Table 2. Leaves were collected randomly from the whole tree canopy (about 80 g DW) and mixed to a homogeneous sample in September 2012. Three to five replicates were used for each species at each site. We collected 102 plant samples from 18 woody species in total. The number of plant specimens at each site were 5 (site 1), 13 (site 2), 9 (site 3), 5 (site 4), 8 (site 5), 13 (site 6), 14 (site 7), 10 (site 8), 5 (site 9), 8 (site 10), and 12 (site 11). Soil sampling was conducted according to the S-shaped sampling method (He and Ma 2000). At each site, the sampled trees were circled and the circled area was regarded as sample area. The S-shaped belt transect was arranged according to the distribution of the sampled trees and occupy trees' localities as many as possible. Five points were equally distributed in the belt transect. Three soil samples were taken

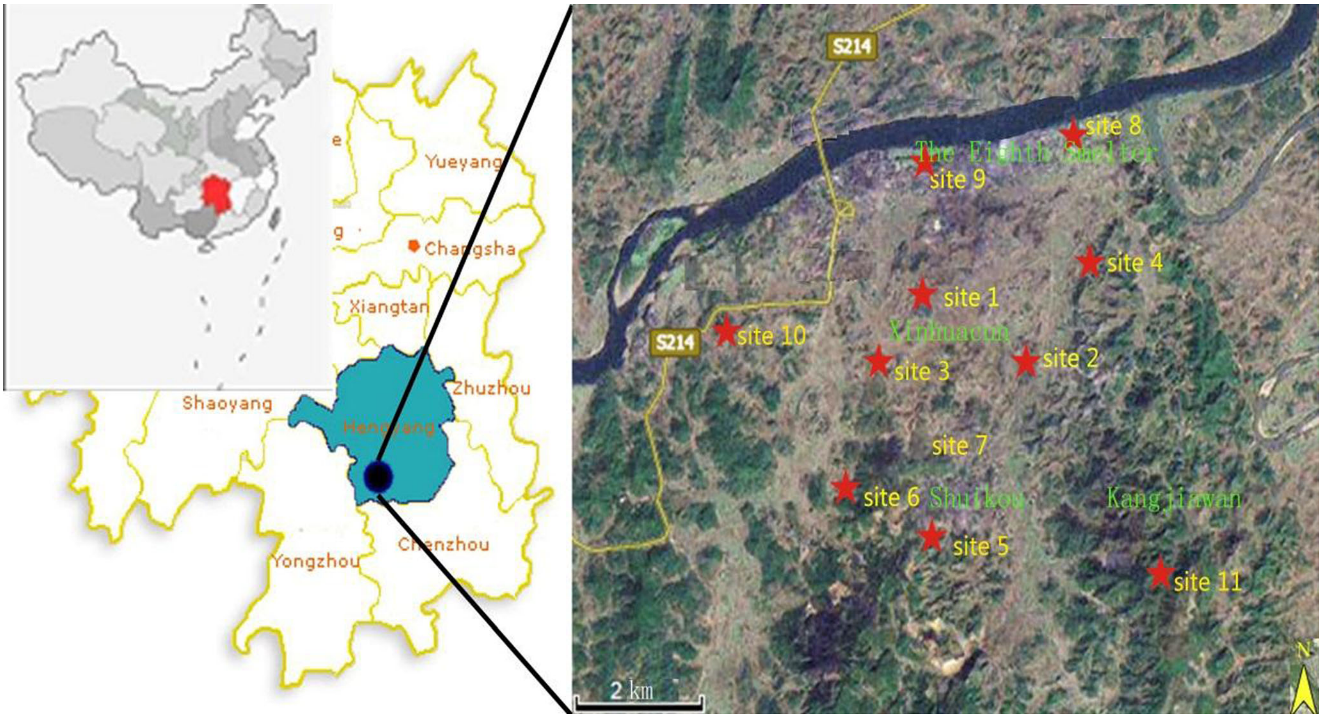

Fig. 1 Location of contaminated sites investigated. The Shuikou area is a former tailings dam. Kangjiawan indicates the Kangjiawan mining and milling area. Xinhuacun is sewage irrigation farmland. The Eighth Smelter is a main smelter of Shuikoushan Pb/Zn mining area. Detailed composition of soils is given in Table 1

from 0, 10, and 20-cm depth and homogenized to a composite sample at each point. The five samples from the five points in the belt transect were the five replicate at each site.

Leaves, branches, trunks, and roots were taken from selected specimens of *Broussonetia papyrifera* (L.) Vent. at site 8 and *Paulownia fortunei* (Seem.) Hemsl. at site 4 in October 2012. Three samples of about 80 g (DW) from each tissue of each tree species were collected. Trunk samples were taken at a height of between 0.5 and 1.5 m using pruning shears. Branch samples were taken randomly from the whole tree canopy using a tree

Table 1 The main characteristics of the soil from each site selected for the investigation

Site	pH	HC (%)	OC (%)	T N (g/kg)	T Pb (mg/kg)	T Zn (mg/kg)	T Cd (mg/kg)	T Cu (mg/kg)
1	6.14 (0.25)a	75.35 (5.58)a	21.98 (1.96)a	1.72 (0.08)af	529 (83.4)a	1,063 (141)a	12.53 (0.79)ac	85.1 (5.66)ab
2	6.65 (0.43)ab	64.83 (2.74)abd	18.25 (1.80)ab	1.81 (0.29)ac	830 (29.3)ac	2,452 (243)bc	10.76 (1.33)ad	67.7 (8.3)ab
3	5.73 (0.59)a	73.24 (5.64)a	34.76 (3.73)c	2.45 (0.42)cf	1,221 (108)c	1,583 (178)ab	22.43 (3.08)ce	116.8 (13.2)cdf
4	6.35 (0.50)ab	71.68 (4.36)ac	22.51 (2.79)a	0.74 (0.12)bde	3,783 (168)b	5,784 (470)d	19.73 (1.55)cd	268.3 (40.2)e
5	7.62 (0.85)bcd	53.85 (4.03)bc	8.02 (1.63)d	0.14 (0.03)b	1,213 (163)ce	4,916 (366)de	8.35 (0.82)a	53.5 (12.4)ab
6	8.06 (0.48)c	49.33 (3.89)c	7.35 (0.61)de	0.35 (0.06)b	1,478 (74.9)ef	4,729 (587)e	32.43 (3.35)be	104.7 (6.5)cd
7	7.53 (0.51)bcd	58.73 (4.04)cd	9.60 (0.85)df	0.15 (0.08)b	950 (94.3)c	1,676 (342)abc	13.25 (0.62)ac	44.4 (5.9)a
8	6.77 (0.37)ad	73.45 (5.85)a	23.33 (1.96)a	1.81 (0.05)ac	1,259 (21.9)e	2,962 (215)c	30.83 (3.55)be	215.1 (2.7)f
9	6.75 (0.56)ad	79.67 (8.35)ae	32.45 (2.25)c	2.50 (0.49)ce	2,816 (331)d	7,413 (520)f	79.33 (9.47)f	415.8 (41.2)g
10	5.93 (0.39)a	65.37 (3.89)abd	15.24 (2.93)bf	1.65 (0.32)a	1,725 (54.1)f	2,996 (445)c	15.16 (0.60)ac	90.4 (9.1)cb
11	5.69 (0.31)a	68.73 (5.23)ad	17.39 (0.62)af	1.22 (0.30)ad	459 (76.4)a	1,934 (217)abc	17.72 (0.75)ac	97.1 (8.4)cb
B.Hn.	–	–	–	–	29.7	94.4	0.126	27.3
B.CN.	–	–	–	–	26.0	74.2	0.097	22.6

Herbaceous coverage (*HC*), organic carbon (*OC*), total N (*TN*), total Pb (*T Pb*), total Zn (*T Zn*), total Cd (*T Cd*), total Cu (*T Cu*), background content of Hunan province (*B.Hn.*), background content of China (*B.CN.*) (Wei et al. 1991). Values in blocks are standard deviation ($n=5$). Values with the same letter were not significantly different according to the Tukey HSD test ($p<0.05$)

Table 2 Concentrations (mean and range, mg/kg, DW) of heavy metals in leaves of the woody species collected from each site

Species collected	Site	Pb	Zn	Cd	Cu
Paulownia fortunei (Seem.) Hemsl.	4	935.42 (716.61–1,179.03)	1,462 (,1023–1,719)	19.58 (14.73–26.26)	88.83 (68.76–113.69)
	5	298.46 (267.95–298.72)	1,347 (1,336–1,355)	12.75 (11.58–12.86)	32.55 (30.49–34.16)
Sapium sebiferum (L.) Roxb.	2	246.71 (222.59–250.14)	964 (955–976)	15.82 (15.15–16.36)	26.53 (26.15–27.27)
Cunninghamia lanceolata (Lamb.) H.	6	98.35 (93.78–104.10)	441 (350–528)	5.18 (4.03–6.25)	17.35 (13.43–20.01)
Symplocos paniculata (Thunb.) Miq.	11	158.43 (121.59–180.67)	822 (679–973)	13.15 (11.29–14.53)	40.76 (22.98–53.82)
Amorpha fruticosa L.	1	205.94 (122.98–311.23)	680 (336–892)	25.49 (20.58–44.16)	37.43 (34.84–39.02)
	7	217.93 (184.57–235.25)	707 (496–716)	13.91 (11.56–15.15)	16.75 (13.53–18.56)
Glochidion puberum (L.) Hutch.	2	123.47 (121.64–125.49)	594 (593–598)	8.42 (8.32–8.55)	24.97 (23.18–26.76)
Melia azedarach L.	11	52.17 (46.47–58.54)	1,174 (1,079–1,256)	18.47 (16.95–21.09)	16.54 (15.23–18.17)
	9	202.67 (158.78–253.94)	1,228 (1,007–1,383)	32.11 (27.85–36.79)	53.69 (37.52–64.15)
Quercus fabri Hance	11	179.53 (162.86–184.69)	791 (698–824)	20.54 (18.42–22.61)	64.67 (59.75–69.37)
Vitex negundo var. *heterophylla*	8	87.47 (76.22–91.83)	1,411 (1,211–1,520)	13.21 (12.41–14.38)	29.44 (26.79–31.22)
	6	108.73 (103.24–113.76)	1,003 (895–1,108)	9.92 (9.72–11.08)	15.43 (13.24–16.30)
Broussonetia papyrifera (L.) Vent.	8	767.88 (669.13–973.38)	1,537 (1,217–1,884)	34.21 (26.87–43.63)	144.96 (122.05–160.21)
	7	513.46 (497.24–592.15)	693 (682–703)	14.18 (13.90–14.43)	19.07 (24.08–26.45)
Robinia pseudoacacia L.	5	103.23 (97.31–103.84)	692 (534–701)	16.73 (15.84–18.22)	12.11 (10.11–13.43)
Rhus chinensis Mill	7	247.33 (132.78–474.95)	493 (388–505)	19.16 (10.54–36.66)	14.95 (13.5–17.1)
Liquidambar formosana Hance	3	225.06 (201.77–241.30)	1,171 (1,150–1,198)	24.68 (20.97–26.87)	33.04 (31.12–37.03)
Pinus massoniana Lamb	10	266.33 (204.55–306.36)	753 (638–761)	18.13 (17.52–18.63)	20.14 (19.27–21.49)
Zenia insignis Chun	3	296.23 (239.26–350.59)	1,830 (1,558–1,968)	39.15 (36.12–44.40)	36.43 (33.94–38.12)
Lagerstroemia indica L.	2	153.37 (147.20–156.78)	952 (863–1,119)	23.17 (16.21–26.65)	61.21 (53.92–64.95)
Camellia oleifera Abel	6	104.35 (83.12–146.86)	357 (173–727)	6.26 (3.64–11.41)	17.94 (15.89–19.21)
Cinnamomum camphora (L.) Presl.	10	126.75 (97.47–183.35)	293 (256–367)	10.34 (9.30–11.38)	24.365 (20.07–30.23)

trimmer. Leaves were distinguished into tender, functional, and older (yellow but still attached), and roots were distinguished into coarse (>2 cm), intermediate (2 mm–2 cm), and fine (<2 mm).

Analysis of plants and soil

Leaves, branches, and wood from the trunks were gently washed three times with deionized water to remove soil particles. Root samples were first washed three times with tap water, followed by deionized water to remove any soil or tailing particles attached to the root surfaces. Finally, roots were exposed to a 0.05 M $CaCl_2$ solution to remove metals from the apparent free space of the root as completely as possible. All plant samples were dried at 65 °C to constant weight before they were ground.

Soil samples were air-dried and ground and then passed through a 2-mm stainless-steel mesh for pH analysis (Sartorius PB-10, Germany). The soil/distilled water was 1:2.5 (v/v). Samples were then passed through a 0.15-mm stainless-steel mesh for organic carbon and total nitrogen analysis (GB 7857-87, GB 7173-87), and for total metal (Pb, Zn, Cd, Cu) analysis.

Plant samples (0.1 g) were digested using a mixture of 6 ml HNO_3 and 2 ml H_2O_2 in a closed microwave system (CEM MARS6, USA), and soil samples (0.15 g) were digested using a mixture of 5 ml HNO_3, 4 ml HF, and 2 ml $HClO_4$. Measurement of Pb, Zn, Cd, and Cu concentrations was then performed by ICP-MS (Agilent 7700×, USA). Replicate samples, certified reference materials (GBW 07604, GBW 07405, China) and blanks were included for quality assurance.

Application of fuzzy synthetic evaluation and proposal of comprehensive bio-concentration index

In our study, we applied the membership function of fuzzy synthetic evaluation to assess the trees' comprehensive accumulation ability of multi-metals through several pollution influence factors such as Pb, Zn, Cd, Cu, etc. The CBCI was proposed to stand for comprehensive metal accumulation ability as follows:

1. The fuzzy set or the factor set U: U=(u_1, u_2, u_3 …… u_i), here U is the trees' comprehensive accumulation ability level, and u_i are those various metal pollution factors.
2. Bio-concentration factors (BCF), also known as accumulation factors or enrichment factors, which are defined as the ratio between total metal concentration in leaves and soil, have been previously used to estimate the ability of

plants to accumulate a certain heavy metal (Harada et al. 2011; Liu et al. 2008; Shi et al. 2011). The value of fuzzy membership function for trees' accumulation ability of each metal pollution factor (BCF) can be calculated by the formula as follows:

$$\mu(x) = \begin{cases} 0 & x = x\min \\ \dfrac{x - x\min}{x\max - x\min} & x\min < x < x\max \\ 1 & x = x\max \end{cases}$$

Where x is the BCF of a certain metal pollution factor, $x_{\min}$ is the minimum value of the BCF of the metal among the woody species investigated, and $x_{\max}$ is the maximum value of the BCF of the metal among the woody species investigated. The maximum μ(x) is denoted as the fuzzy membership value 1, which has contributed most to the trees' comprehensive accumulation ability of various pollution factors. The minimum is denoted as the fuzzy membership value 0, which has contributed the least to the trees' comprehensive accumulation ability of various pollution factors, or low provision level.

3. Calculation of the evaluation index:

$$\text{CBCI} = (1/\text{N})\sum_{i=1}^{N} \mu_i$$

where N is the total number of metal analyzed, and μ_i is μ(x) of metal i.

Results

Soil characteristics

The main characteristics of the soil from each site are given in Table 1. The pH ranged from 5.07 to 8.08. Soils collected from sites 5, 6, and 7 had significantly higher pH ($p<0.05$) (Table 1). The reason for this difference may be the tailings dam nearby. The higher pH may have been influenced by the disposal of the mill tailings. The mean herbaceous coverage ranged from 49.33 to 79.67 %. Soils from sites 5, 6, and 7 had significantly lower herbaceous coverage, and correspondingly their organic carbon and total N were lower (Table 1).

Soils from each site contained Pb, Zn, Cd, and Cu at levels that far exceeded pollution levels, according to The Environmental Quality Standards for Soils (GB 15618-1995, Table 1). The main metal pollutants were Pb, Zn, and Cd.

Metal concentrations in leaves

Heavy metal concentrations in leaves varied considerably among the species. Values of total Pb, Zn, Cd, and Cu of each species are given in Table 2. The highest Pb concentration (1,179.03 mg/kg) was found in leaves of a *P. fortunei* specimen at site 4, followed by *B. papyrifera* at site 8 (973.38 mg/kg). Notably, the Pb BCF of *B. papyrifera* was much higher (Fig. 2), reaching 0.701 elevated Pb concentration and BCF were also found in *Amorpha fruticosa* L., *Quercus fabri* Hance, and *Symplocos paniculata* (Thunb.) Miq. (Fig. 2).

Zn concentration in leaves was species-dependent. The highest value (1,968 mg/kg) was found in a *Zenia insignis* Chun specimen at site 3. *P. fortunei* at site 4, *B. papyrifera* and *Vitex negundo* var. *heterophylla* at site 8 also had significantly higher Zn concentrations in their leaves, ranging from 1,023 to 1,884 mg/kg (Table 2). These species showed relatively higher Zn BCF (Fig. 2) ranging from 0.503 to 0.946, which suggested that they are Zn accumulators. Notably, both the highest leaf Zn concentration and the highest BCF value were found in the same endemic species, *Z. insignis*. This species is nationally protected and is characterized by its root nodules and its rapid growth in poor-quality soils.

Leaf Cd concentration was highest in *Z. insignis* at site 3, ranging from 36.12 to 44.40 mg/kg (Table 2). Its mean BCF was 1.74, slightly lower than *A. fruticosa* at site 1 (12.58–44.16 mg/kg; BCF 2.04), *Lagerstroemia indica* L. at site 2 (16.21–26.65 mg/kg; BCF 2.05), and *Robinia pseudoacacia* L. at site 5 (15.84–18.22 mg/kg; BCF 2.02) (Table 2, Fig. 2). This suggested that this endemic species may also be a good Cd accumulator. The concentration of Cu was also analyzed. Compared with Pb and Zn, its concentration was substantially lower in the woody species investigated. The highest value (144 mg/kg) was found in a specimen of *B. papyrifera* (Table 2).

Comprehensive metal accumulation ability

By applying the CBCI to data for each species investigated, we found that *B. papyrifera* had the highest CBCI value (2.93), which was calculated as follows:

$$\text{CBCI} = (\mu_{\text{Pb}} + \mu_{\text{Zn}} + \mu_{\text{Cd}} + \mu_{\text{Cu}})/4 = [(0.61–0.07)/(0.61–0.07) + (0.52–0.08)/(0.95–0.08) + (1.11–0.16)/(2.15–0.16) + (0.67–0.13)/(0.70–0.13]/4 = 2.93$$

Where 0.07, 0.08, 0.16, and 0.13 are the minimum mean values of the BCF of Pb, Zn, Cd, and Cu, respectively, among the woody species investigated, and 0.61, 0.95, 2.15, and 0.70 are the maximum values of the BCF of Pb, Zn, Cd, and Cu, respectively. The *B. papyrifera*' mean BCF of Pb, Zn, Cd, and Cu are 0.61, 0.52, 1.11, and 0.67 (Table 2). Higher CBCI values are also found in the following woody species: *A. fruticosa* (2.72), *L. indica* (2.53), *Z. insignis* (2.44),

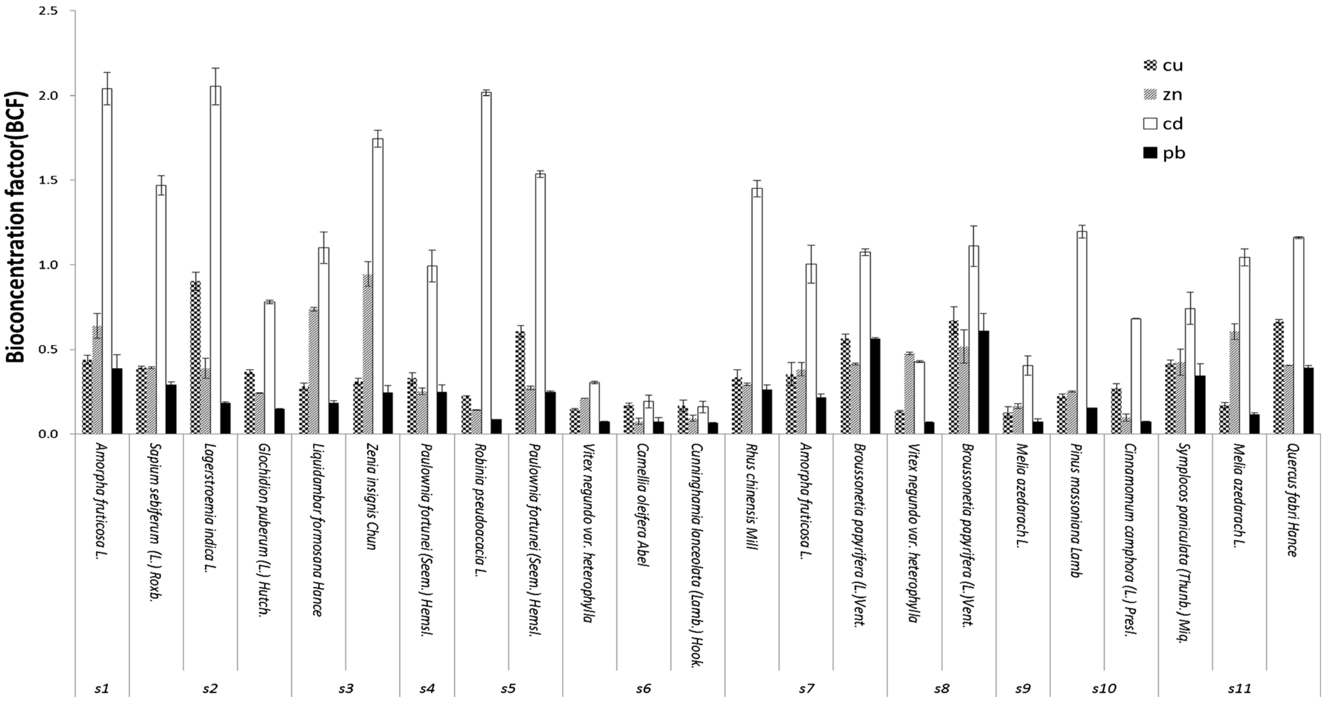

Fig. 2 Bioconcentration factor (*BCF*) of 18 native woody species we investigated. *Error bars* are standard error of the mean ($n = 3, 4, 5$)

Q. fabri (2.42), and *P. fortunei* (2.08). Their CBCI values were all >2.00 compared with other woody species we studied (Fig. 3). By comparison, the CBCI values of *V. negundo* var. *heterophylla* (0.27), *Camellia oleifera* Abel (0.10), and *Cunninghamia lanceolata* (Lamb.) H. (0.08) were much lower (Fig. 2).

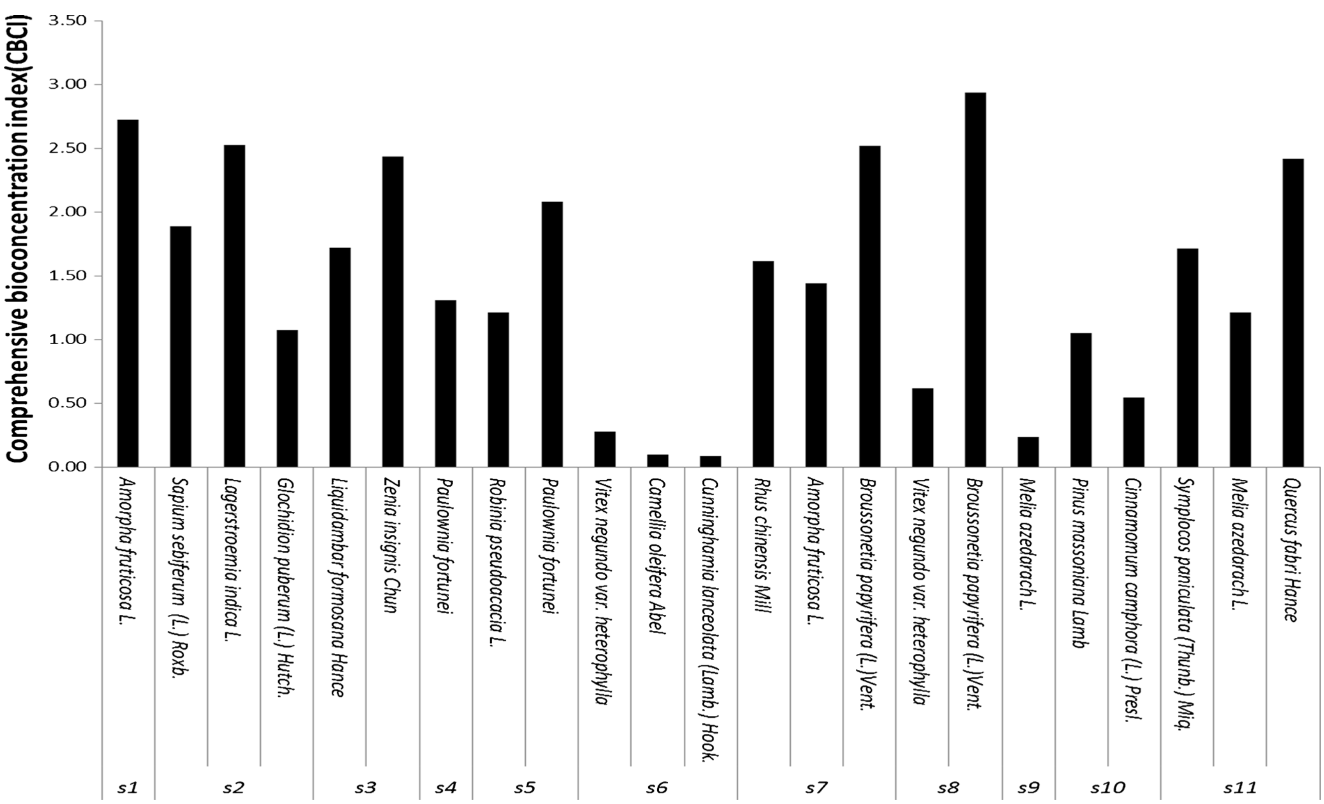

Fig. 3 Comprehensive bio-concentration index (*CBCI*) of 18 native woody species we investigated

Distribution of Pb, Zn, Cu, and Cd in tissues of *B. papyrifera* and *P. fortunei*

Metals have different distribution regularities in the tissues of trees we investigated. The highest concentration of Pb and Zn was found in older leaves of *B. papyrifera* and *P. fortunei*. By contrast, the highest concentration of Cu was found in the branches of the *P. fortunei*, relative to older leaves (Fig. 4). The functional leaves of *P. fortunei* had higher Cu levels than tender leaves and older leaves. On the contrary, the functional leaves of *B. papyrifera* had the lowest Cu concentration among the leaves of different ages (Fig. 4).

From Fig. 4, we can also see that the trunks of *B. papyrifera* and *P. fortunei* had the lowest metal concentration. A general trend was observed that Pb, Zn, Cd, and Cu concentrations increased from trunks to leaves (trunks<branches<leaves) except for Cu in *P. fortunei*. This increasing trend can also be found from trunks to fine roots (trunks<coarse roots<intermediate roots<fine roots) except for Cd in *P. fortunei*. Cd concentration in coarse roots is higher than fine and intermediate roots (trunks<intermediate roots<fine roots<coarse roots).

Discussion

Fast-growing, metal-accumulating tree species could be considered for phytoremediation practice (e.g., Pulford and Watson 2003; Puschenreiter et al. 2010). This is consistent with our results. The high heavy metal concentration in some adult trees presented in our study suggests that adult species

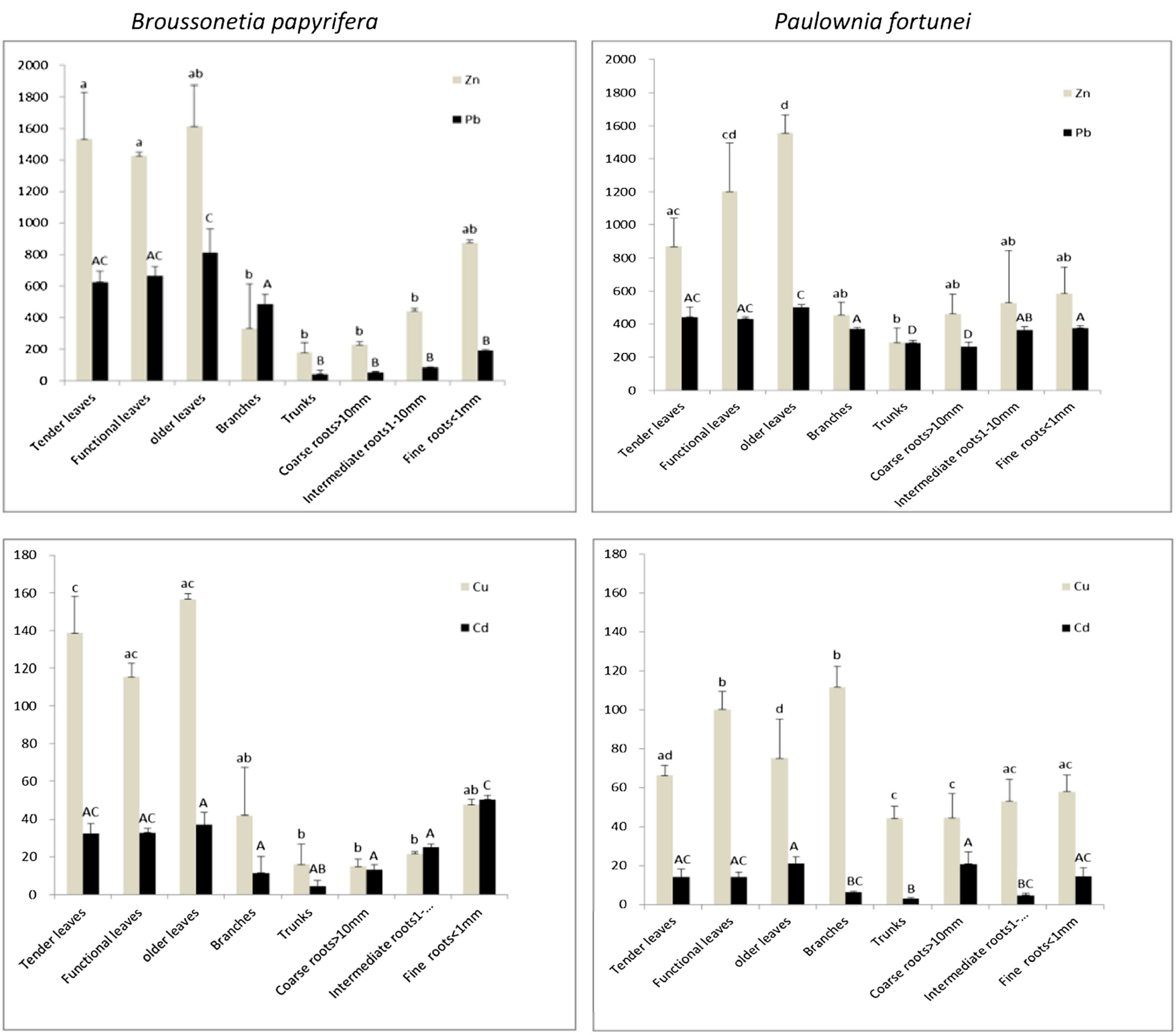

Fig. 4 Concentrations of Pb, Zn, Cu, and Cd (mg/kg, DW) in tissues of *B. papyrifera* and *P. fortunei*. *Error bars* represent standard error of the mean ($n=5$). Values with the same letter were not significantly different according to the Tukey HSD test ($p<0.05$)

such as *B. papyrifera*, *P. fortunei*, and *Z. insignis* accumulate a large amount of metals in their aerial tissues, particularly in leaves. This is an important finding for the long-term phytoremediation and further corroborates the feasibility of phytoremediation by trees, as it suggests that substantial heavy metal accumulation could be expected during a remediation period extending from seedlings to fully grown trees. The highest Pb concentration (1,179.03 mg/kg) was found in leaves of a *P. fortunei* specimen at site 4, which is more than the 1,000 mg/kg threshold of Pb hyperaccumulation plants (Baker et al. 2000). The highest Pb BCF (0.701) was found in *B. papyrifera*, a very high Pb BCF value in trees, and exceeds most public reports. The economic value of *P. fortunei* is high because of its wood and bio-fuel production due to its rapid growth and high biomass production. *B. papyrifera* is well known for its bark fibers, which are used for making paper, cloth, and rope. It is also notable for its extensive root system and tillers. *Z. insignis* belongs to a tall arbor tree species. It is characterized by its root nodules and has ecological value because of its fast growth in barren land. All these suggest that these species could be good choices for phytoextraction practice.

The fuzzy set theory was firstly introduced by L.A. Zadeh in 1965 (Zadeh 1996) and has been used in various areas. But even now, no attempts have been made to evaluate the metal accumulation capabilities of plants by this method. Here, we applied it in our study to assess the overall performance of the woody species in terms of multi-metal accumulation and proposed a CBCI. This index could synthesize accumulation results of several metal pollution factors into one relative measurement index. By comparing the CBCI value of each tree, the multi-metal accumulation abilities would be evaluated. The CBCI values in our study show that *B. papyrifera* and *A. fruticosa* had the highest multi-metal accumulation ability. This evaluation results are consistent with the findings of Seo (2008) and Shi (2011). When compared with *R. pseudoacacia* and *Pinus densiflora* grown in gold/silver abandoned mine soils and Pb/Zn abandoned mine soils, *A. fruticosa* had the best survival rate (Seo et al. 2008). The amount of heavy metals in the shoots of *A. fruticosa* was much higher than other species grown in copper and Pb/Zn mine tailings, reported Shi in 2011 (Shi et al. 2011). Higher CBCI values were also found in *L. indica*, *Z. insignis*, *Q. fabri*, and *P. fortunei* in our study. *Paulownia* has been introduced into the USA and Europe and is used for phytoremediation in recent years due to its elevated tolerance to high concentrations of metals, strong transpiration rates, rapid growth, and high biomass production (Doumett et al. 2008; Wang et al. 2009; Azzarello et al. 2012). Other woody species with higher CBCI values such as *B. papyrifera*, *Z. insignis* etc. should be given serious consideration for phytoremediation. The proposed CBCI would allow for the selection of suitable trees for phytoremediation in the future. To further test and refine the CBCI, and to understand more about heavy metal accumulation by trees, an extensive program of soil and leaf sampling from these and additional tree species is required.

Plant species, heavy metal concentrations in the environment, and other environmental factors could collectively influence the concentration of heavy metals in plants (Xue l et al. 2013). Large variations of metal concentration and BCF within the same species at different sites (Fig. 2) confirm this viewpoint. Although the woody species we investigated grew well at their sites, most had Pb and Zn leaf concentrations higher than 0.6–28 mg/kg (Pb) and 100–300 mg/kg (Zn) (Table 2); the critical toxicity levels for Pb and Zn in plants (Fasani 2012; Krämer 2010). Their Cd and Cu concentrations were also substantially higher than critical toxicity levels (6–10 mg/kg for Cd and 20–30 mg/kg for Cu) (Maestri et al. 2010; Fasani 2012) (Table 2). This may suggest that high metal concentration in the soil might have enhanced their metal tolerance and accumulation ability. Results from previous studies have shown similar trends. Populations from metal contaminated soils had greater tolerance than populations from normal soils, and their accumulation abilities differed (Bert et al. 2002). These variations may relate to genetic status because the frequency of multi-alleles and DNA quantity were significantly higher in populations from contaminated areas (Assunção et al. 2006; Basic et al. 2006). Gene number expansion may be used in the adaptation process for metal-contaminated environments (Puschenreiter et al. 2010), and could offer opportunities for further selection of specimens with high metal accumulation capabilities.

Different metals have different distribution regularities in tissues of trees. Cu concentration in the functional leaves of *B. papyrifera* was lower than in the tender leaves and older leaves, but larger in the functional leaves of *P. fortunei* (Fig. 4) compared with the tender and older leaves. The difference between the functional leaves of *B. papyrifera* and *P. fortune* confirms that metal accumulation mechanisms are various among tree species (Fernando et al. 2006; Hall 2002). With few exceptions, a general trend was noted in *B. papyrifera* and *P. fortunei*, namely that Pb, Zn, Cd, and Cu concentrations increased from trunks to leaves (trunks<branches<leaves) and from trunks to fine roots (trunks<coarse roots<intermediate roots<fine roots). The same trends were also found in *Salix caprea* and *Populus tremula* for Cd and Zn concentration (Unterbrunner et al. 2007) and in *Holoptelia*, *Cassia*, and *Neem* for Mn concentration (Raju et al. 2008). The large amount of heavy metal accumulated in the leaves or branches compared with the trunks meets the requirement of phytoextraction practice by trees. The harvesting could mainly target leaves and possibly young branches, especially for those metals that mainly contributed in branches, such as Cu in *P. fortunei*. The trunks are maintained to produce more branches and leaves, thus creating more biomass.

Conclusion

The results presented here reveal that the native species *P. fortunei* and *B. papyrifera* have large Pb accumulation potential and the endemic species *Z. insignis* has large Zn and Cd accumulation potential. Considering the combined pollution of the most contaminated soils, the CBCI proposed here will be of a certain practical value. It would help us finding more appropriate tree species with higher multi-metal accumulation ability, and increasing the phytoremediation efficiency. Data presented here reveal that *B. papyrifera*, *A. fruticosa*, *L. indica*, *Z. insignis*, *Q. fabri*, and *P. fortunei* have comprehensive higher metal accumulation abilities. The results are consistent with previous studies and provide a valuable source for the improvement of phytoremediation technology. In addition, *A. fruticosa* and *L. indica* belong to scrub species, and when grown with tall arbor tree and low herbaceous species, the efficiency of phytoremediation would be improved. Metal concentration higher in the leaves than in branches and trunks suggest that metal pollution might be moved through pulping management and harvesting. And our date also implied that metal accumulation characteristics were quite variable between individuals, which might offer families with high accumulation abilities for phytoremediation.

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