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Field scale remediation of Cd and Pb contaminated paddy soil using three mulberry (*Morus alba* L.) cultivars

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ABSTRACT

Mulberry tree (*Morus alba* L.) is an effective material available for the remediation and utilization of heavy metal contaminated soil. A three-year experiment was carried out to assess the effects of three mulberry varieties under three planting densities on the removal of Cadmium (Cd) and lead (Pb). Meanwhile, the pollution levels of the farmland by Cd and Pb were also evaluated. Results showed that the soils were strongly to extremely polluted by Cd and moderately to strongly contaminated by Pb. The biomasses of mulberry trees were increased with the increasing planting densities both in 2014 and 2015, except the biomasses of Nongsang 14 in the planting density 30,000 plants/hectare (ha) in 2014. The total biomasses of Yuesang 11 were the highest among the test three cultivars. In addition, the concentrations of Cd and Pb in the root were much higher than those in the stem or branch or leaf. One hectare mulberry shoots could extract 1.92–7.89 g Cd and 9.70–83.58 g Pb every year, respectively. The three mulberry varieties were all not hyperaccumulators for Cd or Pb because both the bioconcentration factors (BCFs) and translocation factors (TFs) were less than 1. These results indicated that mulberry trees can be used as an alternative plant to make use of heavy metal polluted paddy soils and Yuesang 11 may be regarded as a candidate species for phytostabilization of Cd and Pb, with the planting density of 30,000 plants/ha.

1. Introduction

Heavy metals contaminated soil has turned into a worldwide environmental issue, leading to a concern in innovating effective and reasonable treatment methods (Zhong et al., 2015). Cadmium (Cd) and lead (Pb) are non-essential elements of the human body and are highly toxic environmental chemical pollutants which can be discharged into the environment mainly from the metallurgy, ceramics, electroplating industry and chemical industry (Abdus-Salam and Bello, 2015). Cd and Pb are extremely toxic to plants and animals, and they are the two most common pollutants in soils of China (Liu et al., 2015). Phytoremediation, that takes advantage of plants to extract and detoxify heavy metals, is a prospective technology (Jiang et al., 2017). It is a green and relatively novel technique and is considered as a cost-efficient, eco-friendly technology with better public recognition (Ali et al., 2013).

A lot of researchers have pointed out that the utilization of some woody plants could be an alternative method for the extraction or detoxification of heavy metals from polluted soils (Chen et al., 2015; Nada et al., 2007; Peng et al., 2012; Shukla et al., 2011). Mulberry trees (*Morus alba* L.) also have the potential to remediate heavy metals contaminated soils (Jiang et al., 2017; Zhou et al., 2015). Mulberry is a perennial woody tree with the characteristics of deep root systems, rapid growth, and high biomass. Recently, many researchers have proved that there are a few differences in the growth and development of silkworms (*Bombyx mori* L.), the production and quality of cocoons when the silkworms were fed on leaves from the mulberry tree cultivated in heavy metals polluted field areas. And developing sericulture may be a safe, economical, eco-efficient utilization of heavy metals contaminated areas (Huang et al., 2018; Jiang et al., 2017; Zhou et al., 2015).

The entire mulberry-silkworm food chain could be used as a biomodel to monitor the transportation of heavy metals in terrestrial ecosystems because of heavy metals tolerance of mulberry and the larger size and ease of culture of silkworms (Ashfaq et al., 2009; Zhou et al., 2015). Prince et al. (2001) assessed the food chain mobility of Cd and Cu in natural ecosystem by the pot experiment. It was found that lots of Cd and Cu were accumulated by mulberry roots, with a limited transport to the leaves. Both in the case of Cd and Cu treatments, higher mobility was observed from soil to root, followed by leaf to larva, larvae to faecal, root to leaf. Despite this, mulberry is tolerant towards soil Cd pollution to different degrees. The leaf yield showed an initial increase only up to 10 μg/g Cd treatment. The number of branches and

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individual leaf area/plant showed initially a slight increase up to 20 μg/g and decreased afterward. Shoot development was inhibited at 640 μg/g of Cd treatment (Prince et al., 2002). Basically, soil Cd pollution has no obvious effects on the products of mulberry. Moreover, there was scarcely any Cd entering the human food chain through mulberry. Therefore, mulberry trees would be a good replacement for those sensitive crops in the polluted region (Wang, 2002). A three-year micro-plot experiment of mulberry cultivation with Cd contaminated soil and silkworms breeding experiments by feeding with exogenous or endogenous Cd polluted mulberry leaves were made to assess the toxic effects of Cd on mulberry and silkworms. There was no apparent harmful effect on mulberry growth at a soil Cd content of 8.49 mg/kg. At a soil Cd content of 75.8 mg/kg, a yield reduction in the leaves became apparent. Whereas at 145 mg/kg Cd in the soil, the plants exhibited marginal growth (Wang et al., 2004). Similarly, the transportation of Pb in the soil-mulberry tree-silkworm food chain was also investigated by the pot experiment. Most of Pb was accumulated in mulberry roots when the Pb concentrations in the soil were 200, 400, 800 mg/kg. And over 92% of the Pb in leaves was located in the cell wall, reducing the bioavailability of Pb (Zhou et al., 2015).

However, in our knowledge, there have been few studies that reported the accumulation efficiency both on Cd and Pb by mulberry trees in the field. In order to evaluate the phytoextraction potentials under filed and natural agro-climatic conditions, three consecutive years of experiments were conducted to investigate the connection between the heavy metals concentration in soil and their bioaccumulation in the mulberry trees. The effects of planting density and mulberry species on the mulberry biomass and the accumulation of Cd and Pb in mulberry were studied at the same time. The pollution levels of farmland by Cd and Pb were also evaluated.

2. Materials and methods

2.1. Experimental design

The field experiment was carried out near the Taolin lead-zinc mine from May of 2013 to November of 2015. A farmland (about 600 m^2) of Zhongfang town, Linxiang city (located in 113°18′ E, 29°24′ N), which belongs to Yueyang city of Hunan province, China (Fig. 1), was chosen as for experimental region. Linxiang city is located in the north subtropical and warm temperate regions. The annual average temperature is about 16.4℃, frost free period of 259 days, sunshine rate 41% and the annual precipitation is about 1469.1 mm. The agricultural soil was contaminated by Cd, Pb, and Zn, etc. by agricultural irrigation. The one-year seedlings of the three mulberry trees (Nongsang 14, Yuesang 11 and Qiangsang 1) were planted in the designated field plot in April 2013 with different densities of 15,000, 30,000 and 45,000 plants/ha. Each mulberry variety was planted in three columns and two rows of mulberry trees were planted around the test area to prevent the marginal effect. The plants were allowed to grow naturally under natural agro-climatic conditions with moderate fertilization and pesticide spraying. The mulberry trees planted was turned into the mulberry forest in a year by means of the professional management.

2.2. Sample collection

In order to reflect the pollution level of the farmland by Cd and Pb, the surface soil (0–20 cm) was sampling by the five point crossing method at the beginning of this experiment (Fig. 1). In October of 2014 and 2015, the soil and plant samples were obtained, respectively. For each density of mulberry varieties, three plants were selected randomly. Whole plant samples of one mulberry were harvested and separated into root, stem, branch and leaf. While surface soil samples (about 500 g) were collected around each selected plant root zone using hand trowel.

2.3. Sample treatment

The collected soil samples were air-dried at room temperature. The selected basic physio-chemical properties of paddy soil were detected by the routine analytical methods of agricultural chemistry in soil (Lu, 1999). The available Cd and Pb in soil were extracted by diethylene-triamene pentaacetate (DTPA)-$CaCl_2$-triethanolamine (TEA) (Li et al., 2018). The mixture of DTPA (0.005 mol/L), $CaCl_2$ (0.01 mol/L) and TEA (0.1 mol/L) at pH 7.30 was added into soil samples with a soil-to-solution ratio (w/v) of 1:5. After shaking at 25 °C, 180 rpm on a reciprocal water-bath shaker for 2 h, the suspensions were processed by centrifugation and filtrated by a 0.45 μm membrane before analysis. While the plant samples (root, stem, branch and leaf) were firstly cleaned with tap water and then rinsed with deionized water and then pre-dried at 105 °C in an oven for 30 min, and then the root and stem were cut into slices, respectively. The whole plant samples were dried

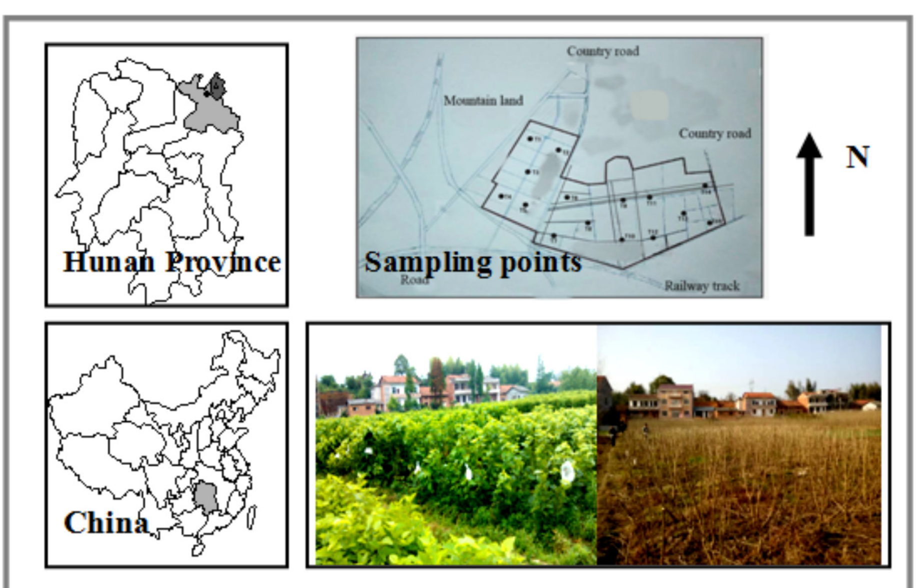

Fig. 1. The geographic locations of heavy metals contaminated farmland.

Table 1
Heavy metal concentrations (mg/kg) and I_{geo} of paddy soil in Linxiang city.

Elements	No. of samples	Concentration (mg/kg)			Std. dev.	Background values	I_{geo}		
		Min.	Mean	Max.			Min.	Mean	Max.
Cd	15	1.15	3.22	5.75	0.92	0.126	2.61	4.10	4.93
Pb	15	112.10	181.23	301.40	40.07	29.70	1.34	2.03	2.77

to a constant weight at 70 °C for the determination of the dry matter, and then the mulberry samples were ground to powder and sieved. The soil samples were ground and sieved (100 meshes) and kept in clean polythene bags for further analysis. One gram of each of the soil and plant samples was digested with 10 mL of aqua regia (a mixture of 3 parts concentrated HCl to 1 part concentrated HNO_3) and HNO_3–$HClO_4$ (3:1, v/v) respectively, on a hot plate in a fume cupboard, until a clear solution was obtained. Distilled water was added periodically to avoid drying up of the digest. The hot solution was then added into a 50 mL standard volumetric flask and then made up to the mark with distilled water. The concentrations of Cd and Pb in the extract and digest were analyzed by atomic absorption spectrometry (AAS) (Thermo Fisher ICE-3400, America).

2.4. Statistical calculations and analyses

Geoaccumulation index (I_{geo}) (Hu et al., 2013) is a classical assessment model to evaluate the heavy metal pollution in soils by comparing current concentrations with background levels. The geoaccumulation index is defined by the following equation:

$$I_{geo} = \log_2\left(\frac{C_i}{1.5 \times B_i}\right) \quad (1)$$

where C_i is the measured concentration of the examined heavy metal (i) in the soil and B_i is the geochemical background concentration of the metal (i). In this study, B_i is the background content of metal i in Chinese soil (Lu et al., 2009). The constant of 1.5 is introduced to minimize the variation of background values due to lithogenic origins, and I_{geo} is a quantitative index of metal enrichment or contamination levels.

Heavy metal extraction amount is a good indicator for assessing the phytoremediation efficiency in phytoextraction technology (Zhan et al., 2013; Zhang et al., 2014).

$$\text{Metal extraction amount} = \text{Metal concentration in plant tissue} \times \text{tissue biomass} \quad (2)$$

The bioconcentration factor (BCF) and translocation factor (TF) are used to evaluate the ability of mulberry trees to tolerate and accumulate heavy metals (Rafati et al., 2011). These factors are also key values that are needed to estimate the potential of a plant for phytoremediation or phytostabilization (Rafati et al., 2011; Zhang et al., 2014).

$$\text{BCF} = \frac{\text{Metal concentration in plant shoot}}{\text{Metal concentration in soil}} \quad (3)$$

$$\text{TF} = \frac{\text{Metal concentration in plant shoot}}{\text{Metal concentration in plant root}} \quad (4)$$

Data were presented as the mean with standard error of three replicates. Statistical analyses were performed with SPSS software (version 17.0). The total biomass, the ratio of root to shoot biomass (R/S), the extraction amount of Cd and Pb, BCF and TF were analyzed by a two-way analysis of variance (ANOVA) to examine the main effects and interactions between mulberry species and planting density. All data are the means ± SD (n = 3) of three replicates. The biomass data from different parts of mulberry and other data were analyzed by the Duncan's multiple range test ($P < 0.05$) to compare any significant differences between means of different treatments. The original biomass data, which does not meet the normal distribution, was normalized by the data transformation (Levene test).

3. Results and discussion

3.1. Contamination levels of Cd and Pb

The pH of the soil in the investigated area was 6.88. The soil organic matter was 2.09%, the cation exchange capacity (CEC) was 3.85 cmol (+)/kg, alkali-hydrolyzed nitrogen was 58.04 mg/kg, available phosphorus was 13.26 mg/kg, available potassium was 106.17 mg/kg. The soil mechanical compositions were as follows: clay 18.22%, silt 37.26%, sand 44.52%. The available Cd and Pb content in soil were 0.61 mg/kg and 2.35 mg/kg, respectively. The total Cd and Pb concentrations in the soil were 3.22 mg/kg and 181.23 mg/kg, respectively (Table 1). A seven level classification of I_{geo} is defined as: unpolluted ($I_{geo} \le 0$), unpolluted to moderately polluted ($0 < I_{geo} \le 1$), moderately polluted ($1 < I_{geo} \le 2$), moderately to strongly polluted ($2 < I_{geo} \le 3$), strongly polluted ($3 < I_{geo} \le 4$), strongly to extremely polluted ($4 < I_{geo} \le 5$), and extremely polluted ($I_{geo} > 5$) (Hu et al., 2013; Muller, 1981). The I_{geo} values of soil from the experimental region are 2.61 to 4.93 (average 4.10) for Cd, 1.34 to 2.77 (average 2.03) for Pb, respectively (Table 1). The averaged pollution degree of these metals decreased in the following order: Cd > Pb. The I_{geo} values of Cd and Pb indicated that the study area were strongly to extremely polluted by Cd and moderately to strongly contaminated by Pb.

3.2. Effect of planting density on mulberry biomass

In this study, variations of root, stem, branch and leaf dry weights of one mulberry were observed under different planting densities in the year of 2014 (Fig. 2) and 2015 (Fig. 3). In 2014, for a mulberry tree, the root, stem, branch and leaf biomasses of the three tested mulberry varieties were all decreased with the increasing planting density. The root, stem, branch and leaf biomasses of the three mulberry cultivars tested in the planting density of 45,000 plants/hectare showed a significant decrease compared with those of 15,000 plants/hectare, except the leaf biomasses of Qiangsang 1, which showed no significant differences among the planting density of 15,000, 30,000 and 45,000 plants/hectare (Fig. 2D). The root, stem, branch and leaf biomasses of the three tested mulberry varieties under planting density of 15,000, 30,000 and 45,000 plants/hectare in 2015 were shown in Fig. 3A, Fig. 3B, Fig. 3C, Fig. 3D, respectively. According to agriculture require of cutting off mulberry, the all branches and parts of the stems were cut off in the summer. So in 2015, the root biomasses of the three tested mulberry trees were significantly increased compared with those in 2014, respectively (Fig. 3A). The leaf biomasses of the three tested mulberry varieties in different planting densities were no significant difference ($P > 0.05$) (Fig. 3D). Generally speaking, a plant with the high tolerant capability can grow in heavy metals contaminated soil normally and act with no significant decrease in biomass due to the stress of heavy metals (Liu et al., 2010; Sun et al., 2009). So the heavy metal tolerance of plants can be evaluated by their variation of shoot biomasses compared with the control (Liu et al., 2011). It seemed that the three tested mulberry trees had a considerable tolerance to Cd and Pb. In the present study, the stem and root biomasses of one mulberry

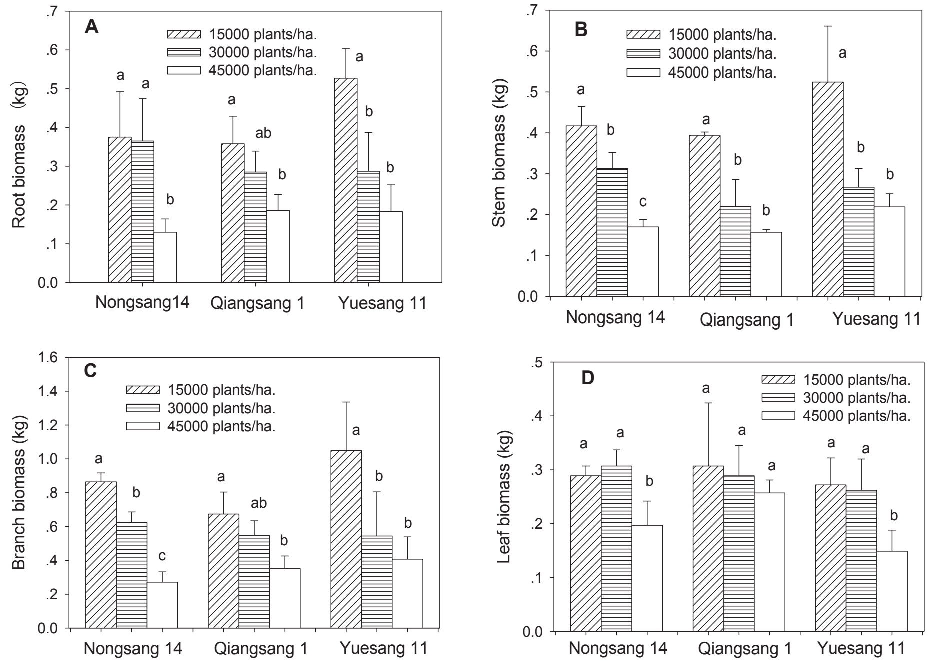

Fig. 2. Effects of planting density on the biomass of different parts in mulberry trees in 2014. Error bars represent ± standard errors (n = 3). Means with the same letter in the same mulberry specie did not differ significantly (Duncan's test, $P < 0.05$).

among the three mulberry varieties in 2015 were higher than that in 2014, respectively. The total biomasses of one hectare mulberry trees in 2015 were higher than those in 2014 (Table 2). Also, the total biomasses of the three mulberry cultivars were increased with the increasing planting density, except the biomass of Nongsang 14 with planting density 30,000 plants/ha in 2014 and the total biomasses of Yuesang 11 were the highest among the test three cultivars both in 2014 and 2015 (Table 2).

Biomass is the major indicator of energy accumulation in plants. Changes in the ratio of root to shoot biomass often reflect the optimal allocation of biomass that plants adapt in order to maximize their access to resources (Zhang et al., 2014). In this study, the ratio of root to shoot biomass of Yuesang 11 was higher that of Nongsang 14 and Qiangsang 1 in 2014 without considering the planting density (Table 2). It indicated that Yuesang 11 might allocate more energy to roots for the absorption of Cd and Pb. It also might be that under the pressures of Cd and Pb, Yuesang 11 need lots of energy to keep the normal functions such as alleviating the shortage of water caused by the excessive heavy metals effects (Zhang et al., 2014). In 2015, however, the ratio of root to shoot biomass was in the following order, Yuesang

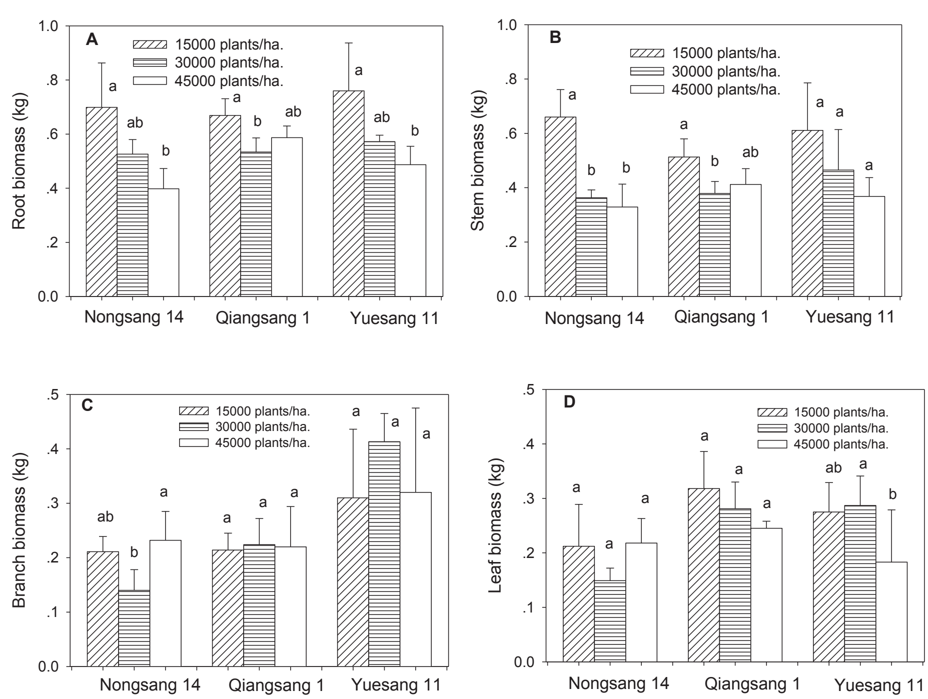

Fig. 3. Effects of planting density on the biomass of different parts in mulberry trees in 2015. Means with the same letter in the same mulberry specie did not differ significantly (Duncan's test, $P < 0.05$).

Table 2
The total biomass, R/S of biomass and heavy metal extraction amount of mulberry trees with different planting densities.

Year	Mulberry variety	Planting density (plants/ha.)	Total biomass (t/ha.)	R/S of biomass	Cd Extraction Amount (g/ha.)	Pb Extraction Amount (g/ha.)
2014	Nong sang 14	15,000	29.180 ± 3.323b	0.236 ± 0.057a	3.457 ± 0.866a	59.335 ± 12.886a
		30,000	37.970 ± 6.401a	0.284 ± 0.063a	5.799 ± 3.062a	78.495 ± 18.066a
		45,000	34.515 ± 5.301a	0.202 ± 0.037a	6.062 ± 1.641a	53.404 ± 4.500a
	Qiang sang 1	15,000	25.995 ± 4.091b	0.261 ± 0.044a	3.609 ± 1.613a	40.181 ± 16.189a
		30,000	40.180 ± 7.579a	0.270 ± 0.015a	6.371 ± 0.532a	83.575 ± 43.210a
		45,000	42.810 ± 6.510a	0.241 ± 0.022a	6.482 ± 2.012a	59.342 ± 12.097a
	Yue sang 11	15,000	35.580 ± 7.642a	0.293 ± 0.056a	7.248 ± 0.590a	73.526 ± 28.521a
		30,000	40.800 ± 13.720a	0.268 ± 0.034a	6.264 ± 2.112a	69.776 ± 27.857a
		45,000	43.140 ± 8.313a	0.231 ± 0.053a	7.271 ± 2.163a	84.922 ± 4.630a
	Analysis of variance					
	Mulberry variety (M)		$P > 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$
	Planting density (P)		$P < 0.05$	$P > 0.05$	$P < 0.05$	$P < 0.05$
	M × P		$P > 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$
2015	Nong sang 14	15,000	26.734 ± 3.085c	0.666 ± 0.224a	1.920 ± 0.688c	11.993 ± 2.725b
		30,000	35.340 ± 2.456b	0.820 ± 0.168a	4.703 ± 0.457b	19.473 ± 2.017b
		45,000	52.965 ± 4.893a	0.510 ± 0.082a	7.104 ± 0.829a	32.915 ± 7.323a
	Qiang sang 1	15,000	25.710 ± 1.454c	0.654 ± 0.152a	2.436 ± 0.507b	9.701 ± 3.872b
		30,000	42.540 ± 1.229b	0.606 ± 0.084a	5.894 ± 2.202ab	22.902 ± 1.838b
		45,000	60.880 ± 2,521a	0.674 ± 0.097a	7.611 ± 2.981a	40.244 ± 18.394a
	Yue sang 11	15,000	29.342 ± 3.084b	0.657 ± 0.210a	4.232 ± 0.906b	20.562 ± 7.383b
		30,000	52.110 ± 6.730a	0.498 ± 0.060a	6.900 ± 1.760a	33.847 ± 14.683ab
		45,000	61.110 ± 8.139a	0.564 ± 0.079a	7.884 ± 0.953a	41.915 ± 5.918a
	Analysis of variance					
	Mulberry variety (M)		$P < 0.05$	$P > 0.05$	$P < 0.05$	$P < 0.05$
	Planting density (P)		$P < 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
	M × P		$P < 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

11 < Qiangsang 1 < Nongsang 14. The ratios of root to shoot biomass of all mulberry cultivars in 2015 were 3–4 times than those in 2014, respectively (Table 2). That is because the roots biomasses of all mulberry trees have been increased in a year.

3.3. The Cd and Pb content in mulberry

The concentrations of Cd and Pb in different parts of the three mulberry cultivars under different planting densities in 2014 and 2015 were shown in Table 3. For the same mulberry species, the planting density has no significant influence on the concentrations of Cd and Pb in the various parts of the mulberry in 2014 or 2015 (Table 3). In the year of 2014 and 2015, the Cd concentrations in the stem, branch and leaf of the three tested mulberry trees have a few differences (Table 3). The Cd concentrations of mulberry roots in 2015 were higher than those in 2014. These results indicated that the roots accumulated more Cd after a year of growing and the heavy metal Cd has a good transportation ability from root to shoot. Interestingly, the Cd concentrations in the leaf of the three mulberry cultivars were higher than those in 2014 (Table 3). This further indicated that the Cd has a good translocation ability and the Cd is more likely to transfer from old tissues to the new, especially the new leaves. The highest Cd concentration in the leaf of Nongsang 14 was 0.35 mg/kg in 2015 (Table 3). Wang et al. (Wang et al., 2004) have proved that there was no apparent harmful effect on mulberry growth at a soil Cd content of 8.49 mg/kg and the weight of cocoons and rate of silk reeling were significantly reduced only when the Cd contents in the mulberry leaf reached 1.66 mg/kg. As for different mulberry species, the Cd concentrations in leaves of Yuesang 11 were the lowest among the three cultivars, both in 2014 and in 2015 (Table 3). Similarly, the Pb concentrations in the roots of the three

Table 3
The Cd and Pb concentrations in different parts of mulberry with different planting densities.

Year	Mulberryvariety	Planting density (plants/ha.)	Cd(mg/kg)				Pb(mg/kg)			
			root	stem	branch	leaf	root	stem	branch	leaf
2014	Nong sang 14	15,000	0.68 ± 0.08b	0.15 ± 0.02a	0.14 ± 0.05a	0.14 ± 0.05a	4.53 ± 0.68a	1.75 ± 0.23a	2.28 ± 0.56a	4.48 ± 2.32a
		30,000	0.59 ± 0.05b	0.14 ± 0.07a	0.14 ± 0.08a	0.15 ± 0.02a	5.18 ± 1.27a	1.72 ± 0.25a	1.28 ± 0.26ab	4.08 ± 1.37a
		45,000	1.03 ± 0.09a	0.23 ± 0.01a	0.18 ± 0.04a	0.22 ± 0.03a	7.13 ± 3.65a	1.79 ± 0.14a	0.71 ± 0.08b	3.57 ± 0.36a
	Qiang sang 1	15,000	0.61 ± 0.17a	0.17 ± 0.04a	0.15 ± 0.03a	0.19 ± 0.02a	5.91 ± 1.78a	1.62 ± 0.49a	1.42 ± 0.69b	3.05 ± 1.28a
		30,000	0.72 ± 0.08a	0.19 ± 0.06a	0.20 ± 0.07a	0.23 ± 0.02a	4.68 ± 0.40a	1.92 ± 0.59a	2.57 ± 0.44a	3.07 ± 1.05a
		45,000	0.86 ± 0.22a	0.22 ± 0.10a	0.22 ± 0.11a	0.14 ± 0.04a	6.21 ± 1.85a	2.26 ± 0.72a	1.74 ± 0.16ab	1.45 ± 0.29a
	Yue sang 11	15,000	0.98 ± 0.17a	0.29 ± 0.11a	0.26 ± 0.09a	0.20 ± 0.08a	4.98 ± 0.57a	2.64 ± 0.15a	2.28 ± 0.56a	4.37 ± 1.19a
		30,000	0.87 ± 0.14a	0.23 ± 0.03a	0.19 ± 0.05a	0.14 ± 0.04a	4.87 ± 0.34a	2.60 ± 0.43a	1.78 ± 0.48a	2.46 ± 1.21a
		45,000	0.83 ± 0.06a	0.29 ± 0.04a	0.17 ± 0.04a	0.14 ± 0.02a	5.95 ± 1.06a	2.49 ± 0.58a	1.97 ± 0.27a	3.76 ± 0.54a
2015	Nong sang 14	15,000	1.48 ± 0.40a	0.12 ± 0.03b	0.16 ± 0.06a	0.18 ± 0.07b	10.53 ± 3.98a	0.82 ± 0.37a	0.58 ± 0.21a	0.72 ± 0.11a
		30,000	1.17 ± 0.24a	0.17 ± 0.04a	0.28 ± 0.09a	0.35 ± 0.03a	7.90 ± 1.72a	1.24 ± 0.21a	0.57 ± 0.22a	0.83 ± 0.05a
		45,000	1.07 ± 0.11a	0.16 ± 0.01ab	0.20 ± 0.02a	0.25 ± 0.04ab	7.79 ± 2.10a	1.27 ± 0.36a	0.83 ± 0.29a	0.62 ± 0.11a
	Qiang sang 1	15,000	1.21 ± 0.42a	0.12 ± 0.05a	0.17 ± 0.03a	0.21 ± 0.08a	9.12 ± 3.40a	0.58 ± 0.29a	0.90 ± 0.17a	0.57 ± 0.06a
		30,000	1.51 ± 0.54a	0.16 ± 0.04a	0.22 ± 0.06a	0.27 ± 0.12a	8.91 ± 3.26a	1.07 ± 0.18a	0.88 ± 0.08a	0.57 ± 0.04a
		45,000	1.48 ± 0.62a	0.20 ± 0.08a	0.20 ± 0.03a	0.26 ± 0.03a	9.94 ± 4.70a	1.78 ± 0.64a	0.77 ± 0.31a	0.47 ± 0.01a
	Yue sang 11	15,000	1.75 ± 0.40a	0.23 ± 0.01a	0.22 ± 0.03a	0.23 ± 0.04a	14.71 ± 5.53a	1.44 ± 0.53a	0.90 ± 0.26a	0.80 ± 0.21a
		30,000	1.13 ± 0.22b	0.20 ± 0.04a	0.21 ± 0.06a	0.16 ± 0.05a	6.59 ± 0.75b	1.32 ± 0.53a	0.76 ± 0.05a	0.56 ± 0.05a
		45,000	0.75 ± 0.11b	0.20 ± 0.04a	0.17 ± 0.01a	0.21 ± 0.05a	6.12 ± 1.41b	1.59 ± 0.55a	0.85 ± 0.25a	0.61 ± 0.01a

Table 4
The BCFs and TFs of mulberry trees for Cd and Pb with different planting densities.

Year	Mulberry variety	Planting density (plants/ha.)	Cd		Pb	
			Bioconcentration factor (BCF)	Translocation factor (TF)	Bioconcentration factor (BCF)	Translocation factor (TF)
2014	Nong sang 14	15,000	0.045 ± 0.009a	0.212 ± 0.023a	0.014 ± 0.004a	0.583 ± 0.215a
		30,000	0.047 ± 0.021a	0.253 ± 0.088a	0.012 ± 0.002a	0.431 ± 0.169a
		45,000	0.065 ± 0.010a	0.204 ± 0.051a	0.010 ± 0.001a	0.305 ± 0.130a
	Qiang sang 1	15,000	0.053 ± 0.016a	0.275 ± 0.008a	0.010 ± 0.002a	0.327 ± 0.030a
		30,000	0.065 ± 0.017a	0.284 ± 0.043a	0.014 ± 0.005a	0.538 ± 0.154a
		45,000	0.060 ± 0.026a	0.232 ± 0.088a	0.010 ± 0.003a	0.319 ± 0.194a
	Yue sang 11	15,000	0.085 ± 0.025a	0.274 ± 0.035a	0.015 ± 0.004a	0.529 ± 0.113a
		30,000	0.060 ± 0.005a	0.224 ± 0.019a	0.012 ± 0.001a	0.441 ± 0.046a
		45,000	0.064 ± 0.010a	0.250 ± 0.051a	0.014 ± 0.002a	0.422 ± 0.088a
	Analysis of variance					
	Mulberry variety (M)		P > 0.05	P > 0.05	P > 0.05	P > 0.05
	Planting density (P)		P > 0.05	P > 0.05	P > 0.05	P > 0.05
	M × P		P > 0.05	P > 0.05	P > 0.05	P > 0.05
2015	Nong sang 14	15,000	0.039 ± 0.015b	0.117 ± 0.079a	0.004 ± 0.001a	0.068 ± 0.057a
		30,000	0.079 ± 0.013a	0.210 ± 0.037a	0.006 ± 0.001a	0.129 ± 0.018a
		45,000	0.066 ± 0.005a	0.189 ± 0.009a	0.005 ± 0.002a	0.133 ± 0.073a
	Qiang sang 1	15,000	0.052 ± 0.019a	0.151 ± 0.043a	0.004 ± 0.002a	0.069 ± 0.018a
		30,000	0.071 ± 0.023a	0.155 ± 0.058a	0.005 ± 0.000a	0.111 ± 0.044a
		45,000	0.072 ± 0.018a	0.180 ± 0.103a	0.007 ± 0.002a	0.142 ± 0.070a
	Yue sang 11	15,000	0.076 ± 0.003a	0.139 ± 0.039b	0.006 ± 0.002a	0.094 ± 0.067a
		30,000	0.065 ± 0.017a	0.174 ± 0.014b	0.005 ± 0.001a	0.141 ± 0.019a
		45,000	0.066 ± 0.007a	0.271 ± 0.031a	0.006 ± 0.001a	0.189 ± 0.075a
	Analysis of variance					
	Mulberry variety (M)		P > 0.05	P > 0.05	P > 0.05	P > 0.05
	Planting density (P)		P > 0.05	P < 0.05	P > 0.05	P > 0.05
	M × P		P > 0.05	P > 0.05	P > 0.05	P > 0.05

cultivars in 2015 were bigger than those in 2014 accordingly. However, the content of Pb in the stem, branch and leaf in 2015 were less than those in 2014 (Table 3).

In the present study, only considering the summer cutting and leaf for rearing silkworms, one hectare mulberry shoots could extract the amounts of Cd and Pb were 3.45–7.28 g and 40.18–83.58 g in 2014, respectively (Table 2). Which was higher than those in 2015, the shoot extraction amounts of one hectare mulberry for Cd and Pb in 2015 were 1.92–7.89 g and 9.70–41.92 g, respectively (Table 2). Both the concentrations of Cd and Pb in root were much higher than those of else parts, especially in 2015 (Table 3). Therefore, mulberry trees could be useful for phytostabilization of Cd and Pb pollution. Once the mulberry trees have become established, they could decrease the erosion of the substrate by wind and water, reduce the overall flow of water down through the soil by transpiration and phytostabilisation heavy metals by changing the metals into less bioavailable (Pulford and Watson, 2003). More importantly, the leaf could be used to rear silkworms, which not only increase farmer's income, but also reduces the risk of food chain pollution. In 2014, the Cd and Pb concentrations in the soil were 3.18 and 171.51 mg/kg, respectively. Similarly, the Cd and Pb concentrations in the soil were 2.84 and 152.95 mg/kg in 2015, respectively. These data indicated that planting mulberry trees could decrease the soil Cd and Pb concentrations 0.34 mg/kg and 18.56 mg/kg in a year, respectively, when the mulberry forest was established. If the tested soil fit the secondary level of Environmental Quality Standards for Soils (China, 1995), which is the soil limits for safeguarding agricultural production and maintaining body health, it needs about 8.6 years for Cd calculated in theory. In fact, the extraction amounts of Cd and Pb by mulberry shoots were precious few. The mulberry forest only decreased 0.01 mg/kg Cd and 0.06 mg/kg Pb in the tested paddy soil in a year. It needs hundreds of years to remediate the Cd polluted soils for the consideration of the extraction content by the mulberry shoot. So there is a lot of work to do. And how to improve the extraction efficiency of mulberry trees has become a new problem. An integrated ecological method for solving the heavy metals contamination in situ is feasible, screening the high-enrichment mulberry varieties and using mulberry trees combined with soil additives (e.g. biochar, magnesium silicate (Xiong et al., 2018a; Yuan et al., 2018), graphene oxide (Xiong et al., 2018b), fertilizer (Cheng et al., 2017), rhamnolipid (Liu et al., 2018)) to change the mobility of heavy metals in soil).

3.4. TF and BCF

Table 3 shows the BCFs and TFs of Cd and Pb in the three mulberry cultivars under different planting densities. All the three mulberry cultivars had lower TFs (< 1.0) for Cd and Pb (Table 4), which indicated that Cd and Pb were mainly distributed in the roots of mulberry rather than in shoots. These results were consistent with our previous study conducted by the pot experiment (Huang et al., 2018). Meanwhile, TFs of Nongsang 14, Yuesang 11 and Qiangsang 1 for Pb under the three planting densities were lower than 0.6, showing a lower capacity to translocate Pb from roots to shoots. Similarly to TFs of Pb, the three mulberry cultivars had lower TFs for Cd (< 0.3) (Table 4). TFs of Cd or Pb decreased in the three mulberry cultivars in 2015 when compared with those in 2014. It indicated that most of the Cd and Pb uptook by mulberry cultivars were sequestered in roots, and a small portion of Cd and Pb could be translocated to shoots. BCF > 1.0 and TF > 1.0 were considered as two critical standards for the selection of hyper-accumulators (Liu et al., 2011). So the three tested mulberry cultivars in this study are not hyper-accumulators for Cd or Pb. The BCFs of Cd were correspondingly bigger than those of Pb both in the year of 2014 and 2015 (Table 4), indicating that the Cd was easier than Pb for mulberry to uptake. The absorption of heavy metals by the plant may be associated with several factors, such as the bioavailability of heavy metals in matrix, acquisition capacities of heavy metals from matrix to roots, translocation abilities from roots to shoots, sequestration and compartmentation distribution, and plant metabolism or tolerance patterns (Huang et al., 2018; Jiang et al., 2018). It was reported that the redistribution of Cd within different parts of mulberry Yu 71-1 is highly relevant to the tolerance of Cd in the mulberry (Huang et al., 2018). Similarly, Dunbar et al. (Dunbar et al., 2003) reported that internal distribution rather than absorption resulted in the low Cd accumulation in edible parts of plants. However, whether the mulberry leaf originated from both the Cd and Pb polluted paddy soils is safe for its utilization and how the redistribution regularities of Cd and Pb in mulberry are by growing several years, are not yet understood and need further studies to gain a deeper insight. In this study, taking the total biomasses, the extraction amounts by one hectare mulberry and BCFs into account, Yuesang 11 may be regarded as a candidate species for phytostabilization of Cd and Pb pollution, with the planting density of 30,000 plants/ha (Table 2).

4. Conclusions

A field scale experiment was conduced to evaluate the extraction efficiency of Cd and Pb by three mulberry cultivars three years in a row. Results showed that the mulberry trees could grow naturally in the soils strongly polluted by Cd and Pb. The three mulberry species are not hyper accumulators for Cd and Pb. The concentrations of Cd and Pb in the mulberry root were higher than that in stem, branch or leaf. So the mulberry trees could become one of the representative substitute plants for heavy metals polluted soils. It not only makes use of the heavy metals contaminated soils, enlarges the farmer's income at the same time, but also reduces the injury of heavy metals to the human body through the food chain.

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