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The mobility of cadmium and lead in the soil-mulberry-silkworm system

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HIGHLIGHTS

- Bioconcentration factors for Cd and Pb were lower than 1 for all the three mulberry cultivars.
- The Cd and Pb contents in pupa and cocoon both met corresponding standards.
- Cd and Pb showed no significant influences on the rates of ingestion and digestion.
- Cd and Pb had significant effects on the ratios of cocoon shell with Yuesang 11.
- Mulberry trees can be used as an alternative plant to make use of heavy metals polluted paddy soils.

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ABSTRACT

Evaluation of the transportation of heavy metals in food chain has received a great deal of attention. In this study, the mobility of cadmium (Cd) and lead (Pb) in soil-mulberry-silkworm system was assessed. The results showed that bioconcentration factors for Cd and Pb were lower than 1 for all the three mulberry cultivars. Higher translocation factors (TFs) were observed in the levels from branch to leaf, larvae to excrement. The BCFs of Pb in root and silkworm excrement were higher than those in the other parts. Meanwhile, most of Cd accumulated from soils located in the root (48.00–54.40%) and only about 10% was in the leaf. But the Cd and Pb had significant effects on the ratios of cocoon shell with Yuesang 11 under different planting densities. For Yuesang 11 and Qiangsang 1, the Pb percentages were roots > branches > leaves > stems. The rates of ingestion (IR) and digestion (DR) were a little higher than those in the control at first and then decreased gradually with time. The IR reached the lowest values on 8th day while the DR arrived at the highest. Planting mulberry and raising silkworm could be a reasonable method for the utilization of heavy metal contaminated paddy soils.

1. Introduction

The paddy soil contaminated by heavy metals has been getting more and more attention in recent years, resulting in a concern in innovating effective and reasonable treatment methods (Zhou et al., 2015; Jiang et al., 2019). Cadmium (Cd) and lead (Pb), the two most common pollutants in soils of China, could be discharged into the environment mainly from the metallurgy, ceramics, electroplating industry and chemical industry (Abdus-Salam and Bello, 2015; Liu et al., 2015) and they are highly toxic environmental chemical pollutants and non-essential elements for the human body. Phytoremediation is a green and relatively novel technique, which is considered as a cost-efficient, eco-friendly technology with better public recognition (Jiang et al., 2018).

Some woody plants could be used as an available way for the extraction or detoxification of metals from polluted soils, such as willow (Pilipović et al., 2019), poplar (Ancona et al. 2019) and apple tree (Wang et al., 2016). Mulberry trees (*Morus alba* L.) also have the potential to reuse heavy metals contaminated paddy soils for its deep root systems, rapid growth, and high biomass (Zhou et al., 2015; Jiang et al., 2017). The surveys conducted in the arid and semiarid areas of the North China showed that the total root length

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of one year old mulberry could reach 100 m and that of 10 years old could achieve 10,000 m (Jiang et al., 2017). Furthermore, it has been proved that there are a few differences in the growth and development of silkworms (*Bombyx mori*), the production and quality of cocoons, when the silkworms were fed on leaves from the heavy metals polluted areas (Zhou et al., 2015; Jiang et al., 2017). And developing sericulture may be a safe, economical, eco-efficient utilization of the heavy metals contaminated paddy soils (Zhou et al., 2015; Jiang et al., 2017).

Basically, soil Cd pollution has no unfavorable effects on mulberry tree. The leaf biomass and number of branches showed initially a slight increase when the Cd concentration in soil was up to 20 mg/kg, and decreased afterward (Prince et al., 2002). When the Cd content in soil was 8.49 mg/kg, there was no apparent harmful effect on mulberry growth. A yield reduction in the leaves became distinct at the soil Cd content of 75.8 mg/kg. Whereas at 145 mg/kg Cd in soil, the plants exhibited marginal growth (Wang et al., 2004). In our previous study, it was found that 53.3–70.2% of Cd the mulberry root accumulated from soil was stored in the root and only about 10% was in the leaf. Lots of the Cd was located in the cell wall of the mulberry root and in soluble fraction of the mulberry leaf (Huang et al., 2018). Therefore, there was a very small amount Cd entering the human body through the soil-mulberry-silkworm food chain and mulberry trees would be a good replacement for those sensitive crops in the Cd polluted region (Wang, 2002). Similarly, the transportation of Pb in the soil-mulberry-silkworm food chain was also evaluated by pot experiment. Plenty of Pb was stored in root, reducing its migration to stem and leaf. Over 92% of the Pb in the leaves was deposited in the cell wall, and 95.3–95.6% of the Pb in the mulberry leaves was integrated with oxalic acid, pectates and protein, which had low bioavailability and reduced the damage to organelle (Zhou et al., 2015).

Price et al. (Prince et al., 2001) studied the food chain mobility of Cd and Cu in natural ecosystem by pot experiment and found that mulberry root located more amounts of Cd and Cu, with a limited transport to the leaf. Both for Cd and Cu treatments, higher mobility was observed from soil to root, followed by leaf to larva, larvae to faecal, root to leaf. The entire mulberry-silkworm food chain could be used as a bio-model to monitor the movement of trace elements in terrestrial ecosystems because of the tolerance of mulberry to heavy metals and the large size and easy culture of silkworm (Zhou et al., 2015).

However, to our knowledge, there were few studies that reported the accumulation and translocation efficiency both on Cd and Pb by various mulberry trees under different planting densities in field. In order to evaluate the translocation rule of Cd and Pb in soil-mulberry-silkworm system under filed and natural agro-climatic conditions, mulberry planting and silkworm rearing experiments were conducted to investigate the connection between the Cd and Pb concentrations in soil, mulberry trees and silkworms. The effects of Cd and Pb on the survival rate of flarva-pupa and the ratio of cocoon shell were studied in the same time. The effects of planting density and mulberry varieties on the accumulation rule, BCFs and TFs of Cd and Pb in mulberry were also evaluated.

2. Materials and methods

2.1. Experimental design

The experimental field and design were partly the same as the previous paper (Jiang et al., 2019). The one-year-old seedlings of the three mulberry varieties (Nongsang 14, Yuesang 11 and Qiangsang 1) were cultured in the designed field plot in April, 2013 with three densities of 15,000, 30,000 and 45,000 plants/hectare (ha). Each mulberry species was planted in three columns and two rows of mulberry trees were planted around the test area to prevent the marginal effects. These trees grew naturally under natural agro-climatic conditions with moderate fertilization and pesticide spraying. The mulberry trees turned into mulberry forest in a year by professional management.

2.2. Experiment of silkworm rearing

The silkworm variety Hu·Bin × Ming·Guang, bred by the Sericultural Research Institute of Hunan Province, was chose as a representative of silkworm varieties for its easy raising and high silk production. The ants were collected in late August in 2014 and reared to the fifth instar in a demonstration base of our institute, which was located in Lixian, Changde City, Hunan province. Then the silkworms were transported to a farmer's house near the experimental farmland. These silkworms were divided into 30 districts (3 mulberry varieties × 3 densities × 3 repetitive tests and 3 control tests) and each district had 200 silkworms. The mulberry leaves from the experimental field polluted by Cd and Pb were used to raise silkworms. The silkworms in control team were raised by the mulberry leaves which were not from Cd and Pb contaminated farmland.

2.3. Sample collection and treatment

The surface soil (0–20 cm) was collected by the five point crossing method at the beginning of this experiment to reflect the pollution level of the farmland by Cd and Pb. In October of 2014, the soil and plant samples were obtained, respectively. For each density and each mulberry variety, three plants were selected randomly. Whole plant samples of 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. From the first day of the fifth instar, recorded the weight of mulberry leaf before raising the silkworm and remained after nibbling. Also, the silkworm excrement in the next day before providing the leaf was also weighted.

The soil samples were air-dried at room temperature. The selected basic physio-chemical properties of paddy soil were detected by the routine analytical methods (Lu, 1999). While the plant samples (root, stem, branch and leaf) were pre-dried at 105° in an oven for 30 min, and then the root and stem were cut into slices, respectively. The whole plant samples and animal materials (silkworm, silkworm excrement, cocoon and pupa) were dried to a constant weight at 70° for the determination of the dry matter, and then the whole samples were ground to powder and sieved (100 meshes), kept in clean polythene bags for further analysis. One gram of both of the soil and plant-animal 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–$HClO_4$ (9:1, v/v) on a hot plate in a fume cupboard until a clear solution was obtained, respectively. When the hot clear solution cooled, it was added into a 50 mL standard volumetric flask and then made up to the mark with distilled water. For quality control, certified reference materials GBW07401(GSS-1) and GBW07603 (GSV-2) from the China National Standard Materials Center for soils and plants, respectively, were used to give a comparison with experimental samples. The concentrations of Cd and Pb in the plant, animal and soil samples were analyzed by atomic absorption spectrometry (AAS) (Thermo Fisher ICE-3400, America). The recovery rates of Cd were 95–99% and 95–105% in soil and plant-animal samples, respectively. The recovery rates of Pb were 85–97% and 91–115% in soil and plant-animal samples, respectively.

2.4. *Statistical analysis and calculation*

Analysis of variance (ANOVA) was used for statistically significant differences by SPSS software (version 17.0). All data are the means ± SD (n = 3) of three replicates. Duncan's multiple range test ($P < 0.05$) was utilized to compare any significant differences between means of different treatments. 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 to estimate the potential of a plant for phytoremediation or phytostabilization (Zhou et al., 2015; Jiang et al., 2019).

$$BCF = \frac{\text{Metal concentration in the receiving level}}{\text{Metal concentraion in soil}} \quad (1)$$

$$TF = \frac{\text{Metal concentration in receiving level}}{\text{Metal concentration in the source level}} \quad (2)$$

The rates of ingestion (IR) and digestion (DR) (Wang et al., 2004) are defined as:

$$IR = 1 - \frac{\text{Leaves remained after nibbling}}{\text{Leaves fed to the worms}} \times 100\% \quad (3)$$

$$DR = 1 - \frac{\text{Worm excrement}}{\text{Leaves fed to the worms} - \text{Leaves remained after nibbling}} \times 100\% \quad (4)$$

$$\text{Survival rate of larva} - \text{pupa} = \frac{\text{Number of cocoon with living pupa}}{\text{Number of silkworm reared}} \times 100\% \quad (5)$$

$$\text{Ratio of cocoon shell} = \frac{\text{Cocoon shell weight}}{\text{Whole cocoon weight}} \times 100\% \quad (6)$$

The cocoon shell weight is the sum weight of cocoon layer, cocoon floss and cocoon grip. The whole cocoon weight is the weight of one complete cocoon.

3. Results and discussion

3.1. *Cd and Pb concentrations in soil-mulberry-silkworm system*

The basic physiochemical properties of paddy soil were showed in one of our previous studies (Jiang et al., 2019).The total Cd and Pb concentrations in the soil were 3.22 mg/kg and 181.23 mg/kg, respectively (Table 1). The soils were strongly to extremely polluted by Cd and moderately to strongly contaminated by Pb (Jiang et al., 2019). The concentrations of Cd and Pb in different parts of the three mulberry cultivars, silkworm, silkworm excrement, cocoon and pupa were shown in Table 1. 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. As a whole, the concentrations of Cd in stems, leaves and branches were close to each other (Table 1). The highest Cd concentration in the leaf of Qiangsang 1 was only 0.23 mg/kg and the Cd concentrations in root were about 5 times of those in the stems or branches or leaves (Table 1). The Pb concentrations, however, in mulberry trees were in the order: root > leaf > stem > branch, indicating that mulberry root has a

Table 1
Cd and Pb concentrations in soil, mulberry, larvae and silkworm excrement, cocoon and pupa.

Heavymetal	Mulberry variety	Planting density (plants/ha.)	Metal content in soil (mg/kg, dry)	Heavy metal content in mulberry (mg/kg, dry)				Heavy metal content in mulberry (mg/kg, dry)							
				root	stem	branch	leaf	larvae in 5th instar			silkworm excrement			silkworm cocoon	pupa
								1st day	4th day	8th day	1st day	4th day	8th day		
Cd	Xiang 7920	10,000	/	/	/	/	/	0.04 ± 0.01	0.03 ± 0.02	0.01 ± 0.00	0.05 ± 0.01	0.08 ± 0.03	0.14 ± 0.00	0.02 ± 0.01	0.03 ± 0.01
	Nongsang 14	15,000	3.22 ± 0.92	0.68 ± 0.08	0.15 ± 0.02	0.14 ± 0.05	0.14 ± 0.05	0.07 ± 0.01	0.06 ± 0.01	0.34 ± 0.03	0.05 ± 0.01	0.16 ± 0.08	0.17 ± 0.04	0.02 ± 0.01	0.02 ± 0.01
		30,000		0.59 ± 0.05	0.14 ± 0.07	0.14 ± 0.08	0.15 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.43 ± 0.03	0.08 ± 0.01	0.18 ± 0.01	0.19 ± 0.04	0.02 ± 0.01	0.04 ± 0.01
		45,000		1.03 ± 0.09	0.23 ± 0.01	0.18 ± 0.04	0.22 ± 0.03	0.07 ± 0.01	0.05 ± 0.02	0.42 ± 0.02	0.10 ± 0.02	0.20 ± 0.01	0.16 ± 0.05	0.02 ± 0.01	0.04 ± 0.02
	Qiangsang 1	15,000		0.61 ± 0.17	0.17 ± 0.04	0.15 ± 0.03	0.19 ± 0.02	0.04 ± 0.01	0.06 ± 0.02	0.10 ± 0.04	0.04 ± 0.02	0.31 ± 0.03	0.11 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
		30,000		0.72 ± 0.08	0.19 ± 0.06	0.20 ± 0.07	0.23 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.16 ± 0.03	0.06 ± 0.02	0.20 ± 0.04	0.17 ± 0.03	0.02 ± 0.01	0.04 ± 0.01
		45,000		0.86 ± 0.22	0.22 ± 0.10	0.22 ± 0.11	0.14 ± 0.04	0.06 ± 0.01	0.06 ± 0.01	0.25 ± 0.03	0.08 ± 0.01	0.23 ± 0.04	0.17 ± 0.01	0.01 ± 0.01	0.04 ± 0.01
	Yuesang 11	15,000		0.98 ± 0.17	0.29 ± 0.11	0.26 ± 0.09	0.20 ± 0.08	0.05 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.08 ± 0.02	0.21 ± 0.03	0.19 ± 0.02	0.01 ± 0.01	0.05 ± 0.01
		30,000		0.87 ± 0.14	0.23 ± 0.03	0.19 ± 0.05	0.14 ± 0.04	0.07 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.07 ± 0.02	0.20 ± 0.02	0.17 ± 0.01	0.02 ± 0.00	0.04 ± 0.03
		45,000		0.83 ± 0.06	0.29 ± 0.04	0.17 ± 0.04	0.14 ± 0.02	0.05 ± 0.01	0.07 ± 0.01	0.01 ± 0.00	0.07 ± 0.02	0.17 ± 0.02	0.19 ± 0.02	0.02 ± 0.00	0.03 ± 0.01
Pb	Xiang 7920	10,000	/	/	/	/	/	0.05 ± 0.01	0.07 ± 0.01	0.08 ± 0.03	0.10 ± 0.02	0.12 ± 0.05	0.16 ± 0.04	0.07 ± 0.03	0.21 ± 0.13
	Nongsang 14	15,000	181.23 ± 40.07	4.53 ± 0.68	1.75 ± 0.23	2.28 ± 0.56	4.48 ± 2.32	0.22 ± 0.02	0.23 ± 0.03	0.15 ± 0.02	2.48 ± 0.46	5.65 ± 0.57	5.43 ± 1.30	0.07 ± 0.01	0.37 ± 0.04
		30,000		5.18 ± 1.27	1.72 ± 0.25	1.28 ± 0.26	4.08 ± 1.37	0.27 ± 0.04	0.23 ± 0.01	0.17 ± 0.01	3.05 ± 0.26	5.48 ± 0.40	6.04 ± 0.15	0.07 ± 0.01	0.33 ± 0.11
		45,000		7.13 ± 3.65	1.79 ± 0.14	0.71 ± 0.08	3.57 ± 0.36	0.24 ± 0.04	0.24 ± 0.05	0.16 ± 0.01	3.09 ± 0.06	5.37 ± 0.62	6.13 ± 0.17	0.05 ± 0.03	0.41 ± 0.16
	Qiangsang 1	15,000		5.91 ± 1.78	1.62 ± 0.49	1.42 ± 0.69	3.05 ± 1.28	0.12 ± 0.01	0.16 ± 0.03	0.09 ± 0.05	2.10 ± 0.42	2.97 ± 0.52	4.50 ± 1.44	0.07 ± 0.01	0.49 ± 0.08
		30,000		4.68 ± 0.40	1.92 ± 0.59	2.57 ± 0.44	3.07 ± 1.05	0.12 ± 0.01	0.18 ± 0.01	0.12 ± 0.01	1.90 ± 0.19	3.62 ± 0.51	3.14 ± 0.45	0.08 ± 0.02	0.39 ± 0.12
		45,000		6.21 ± 1.85	2.26 ± 0.72	1.74 ± 0.16	1.45 ± 0.29	0.19 ± 0.05	0.15 ± 0.00	0.12 ± 0.01	1.69 ± 0.50	2.96 ± 0.26	3.58 ± 0.57	0.07 ± 0.01	0.27 ± 0.01
	Yuesang 11	15,000		4.98 ± 0.57	2.64 ± 0.15	2.28 ± 0.56	4.37 ± 1.19	0.24 ± 0.02	0.25 ± 0.03	0.54 ± 0.10	3.12 ± 0.35	7.34 ± 0.80	7.09 ± 0.51	0.05 ± 0.01	0.47 ± 0.08
		30,000		4.87 ± 0.34	2.60 ± 0.43	1.78 ± 0.48	2.46 ± 1.21	0.25 ± 0.02	0.19 ± 0.01	0.40 ± 0.03	3.39 ± 0.11	7.25 ± 0.36	7.02 ± 0.15	0.04 ± 0.01	0.23 ± 0.06
		45,000		5.95 ± 1.06	2.49 ± 0.58	1.97 ± 0.27	3.76 ± 0.54	0.24 ± 0.01	0.23 ± 0.05	0.36 ± 0.10	3.08 ± 0.06	6.11 ± 0.12	6.87 ± 0.25	0.03 ± 0.01	0.28 ± 0.11

good accumulation ability for Pb (Table 1). Which worked as a defensive mechanism to protect the mulberry species against the harmful effects of toxic levels on photosynthetic processes (Bonanno et al., 2018; Huang et al., 2018). The Cd and Pb concentrations in the larvae fed with polluted leaf were higher than that in control. And the Cd concentrations in the larvae at the 1st day and 4th day were only minor changes. At 8th day, the Cd concentrations in larvae raised by Nongsang 14 and Qiangsang 1 were higher than those of at 4th day. Only the Cd contents in larvae fed by Yuesang 11 were decreased at 8th day. The Cd concentrations in larvae fed Nongsang 14 and Qiangsang 1 at 8th day are about 35 and 5 times of that on Yuesang 11, respectively. However, the Cd concentrations in silkworm excrement, cocoons and pupae at different days under various planting densities and mulberry varieties showed no significant differences (Table 1). So it was probable that urine secreted by the larvae raised by Yuesang 11 at 8th day had lots of Cd. Silkworm will excrete silkworm excrement and urine before cocooning and the urine was difficult to collect. And it was not clear that what resulted in this phenomenon only for Yuesang 11. Though the mean concentration of Pb in the Yuesang 11 was higher than that in Nongsang 14 and Qiangsang1, the Pb concentrations in cocoon and pupa in Yuesang 11 were the lowest among the three mulberry varieties. Because most of the Pb accumulated by the larvae fed on the Yuesang 11 were in the silkworm excrement (Table 1). The maximum concentrations of Cd and Pb in the pupae were 0.05 and 0.49 mg/kg, respectively. They were lower than the corresponding values (0.1 mg/kg for Cd and 0.50 mg/kg for Pb) in the standard of Contaminant Limit in Food (GB2762-2017, China). Similarity, the highest Cd and Pb contents in cocoon also met the Technical Specifications of Ecological Textiles (GB/T 18,885-2009, China). In order to make the pupa more safe to eat, some soil additives (e.g. biochar (Tan et al., 2015), hollow mesoporous carbon nanospheres (Shao et al., 2019), rhamnolipid (Liu et al., 2018)) should be used to decrease the mobility of heavy metals in soils.

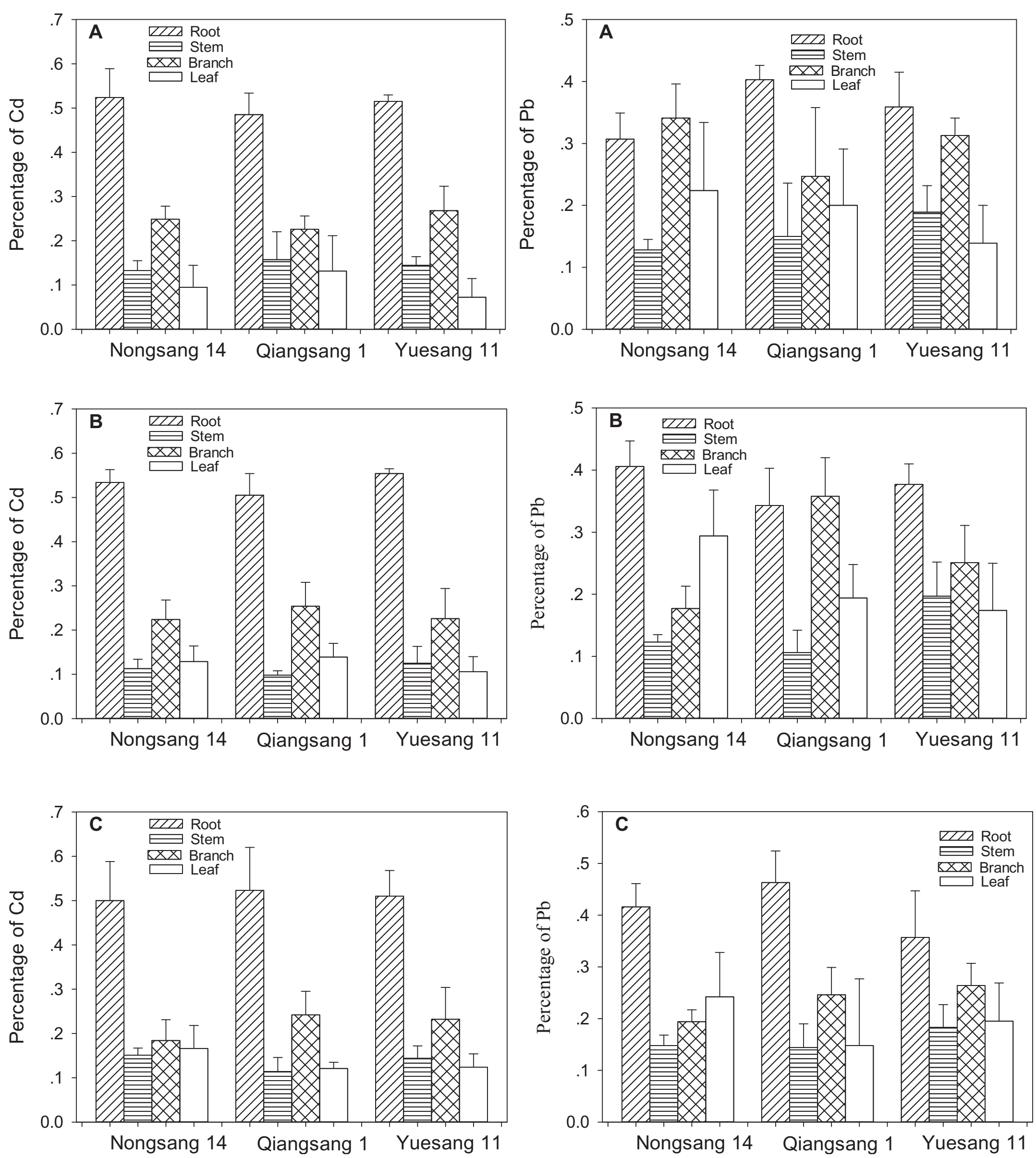

Fig. 1. Percentages of Cd and Pb in different parts of mulberry trees in various planting densities. (A, 15,000 plants/ha. B, 30,000 plants/ha. C, 45,000 plants/ha.)

3.2. Percentages of Cd and Pb in different parts of mulberry

The percentages of Cd and Pb in different parts of mulberry trees in various planting densities were showed in Fig. 1. For the three mulberry trees in different planting densities, the Cd percentages were in the order of roots > branches > leaves (Fig. 1). Only for Yuesang 11, the Cd percentages were all showed the following order: roots > branches > stems > leaves and the percentage of Cd in leaf was the lowest among the three mulberry cultivars in the same planting density (Fig. 1). And a lot of Cd (48.00–54.40%) was stored in the root and only about 10% was in the mulberry leaves. Which were consistent with the results in our previous study (Huang et al., 2018). The spatial distribution of Pb in mulberry trees did not show obvious regularity. For Yuesang 11, the Pb percentages were in the order: root > branch > stem > leaf (Fig. 1). And the average percentage of Pb in the leaf of Yuesang 11 was lower than that of Qiangsang 1. Zhou et al. (2015) found that the Pb distributions in the different parts of mulberry were in the order: roots > stems > leaves. More importantly, over 92% of Pb in the leaves was stored in the cell wall, and 95.29–95.57% of Pb in the leaves was connected with oxalic acid, pectates and protein, which had low bioavailability (Zhou et al., 2015). But for Nongsang 14, the percentage of Pb in branch is the highest, followed by the root, leaf and stem. With the increase of planting density, the Pb percentages were in the order: root > leaf > branch > stem (Fig. 1). The Pb percentages in the leaf were in the range of 13.90%–34.20%. Less heavy metal was in leaves, less injury to silkworms, less heavy metal in silk and pupa.

3.3. BCFs and TFs of Cd and Pb in mulberry and silkworm

BCF is an important indicator for the accumulation trend of chemicals in plants, which could reflect the difficulty of heavy metal migration into plants in some extent. TF is an important indicator for the ability of plants to transfer chemicals from root to shoot (Liu et al., 2015). . (Jiang et al., 2019). BCF>1.0 and TF > 1.0 were considered as two critical standards for the selection of hyper-accumulators (Liu et al., 2011). Table 2 shows the BCFs and TFs of Cd and Pb in soil-mulberry-silkworm system under different planting densities among the three mulberry cultivars. All the three mulberry cultivars had lower BCFs for Cd and Pb (<1.0) (Table 2), which indicated that the tested mulberry trees were not the hyperaccumulators for Cd and Pb. Meanwhile, most of Cd accumulated from soils located in the root for it's the highest BCFs, followed by silkworm excrement. The BCFs of Pb in root and silkworm excrement were higher than those in other parts. This was consistent with the result of a previous study (Zhou et al., 2015), which indicated that the root and silkworm excrement accumulated more Pb and lots of Pb passed through the silkworm alimentary canal and was enriched in silkworm excrement, except for a slight uptake because of the alkaline environment in the guts of the silkworms (Zhou et al., 2015). So the BCFs of Cd and Pb in cocoon and moth were very low, less than 0.03. The TFs of Pb from root to stem were all over 1.0, indicating that Pb translocated from root to stem easily, so did the TFs from branch to leaf, except the TF of Qiangsang 1 under 45,000 plants/ha. Especially, the TFs from larvae to silkworm excrement were in the range of 13.02–39.35, suggesting that Pb was more easily to transport to the silkworm excrement. As for Cd, the TFs from branch to leaf, larvae to silkworm excrement were bigger than those in other groups (Table 2), indicating that Cd could be easy to translocate to the mulberry leaf and silkworm excrement. So the silkworm excrement from heavy metals polluted areas should be collected and treated safely to avoid secondary pollution.

Table 2
Bioconcentration and translocation factors of Cd and Pb between the different parts of mulberry and the different stages of silkworm.

Heavy metal	Mulberry variety	Planting density (plants/ha.)	Bioconcentration factor (BCF)								Translocation factor (TF)							
			BCF1	BCF 2	BCF 3	BCF 4	BCF 5	BCF 6	BCF 7	BCF 8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
			root/soil	stem/soil	branch/soil	leaf/soil	larvae/soil	feces/soil	cocoon/soil	moth/soil	root/soil	stem/root	branch/stem	leaf/branch	larvae/leaf	feces/larvae	cocoon/larvae	moth/larvae
Cd	Nongsang 14	15,000	0.21	0.05	0.04	0.04	0.11	0.05	0.01	0.01	0.21	0.22	0.92	1.16	2.43	0.50	0.05	0.07
		30,000	0.18	0.05	0.05	0.05	0.13	0.06	0.00	0.01	0.18	0.25	0.99	1.21	2.84	0.45	0.04	0.09
		45,000	0.32	0.07	0.06	0.07	0.13	0.05	0.01	0.01	0.32	0.23	0.77	1.23	1.92	0.38	0.04	0.09
	Qiangsang 1	15,000	0.19	0.05	0.05	0.06	0.03	0.03	0.01	0.01	0.19	0.28	0.91	1.24	0.54	1.04	0.16	0.23
		30,000	0.23	0.06	0.06	0.07	0.05	0.05	0.01	0.01	0.23	0.27	1.01	1.23	0.70	1.07	0.14	0.23
		45,000	0.27	0.07	0.07	0.04	0.08	0.05	0.00	0.01	0.27	0.27	0.97	0.69	1.79	0.68	0.06	0.16
	Yuesang 11	15,000	0.31	0.09	0.08	0.08	0.01	0.06	0.00	0.02	0.31	0.29	0.96	1.05	0.15	6.54	0.45	1.68
		30,000	0.27	0.07	0.06	0.04	0.01	0.05	0.01	0.01	0.27	0.27	0.83	0.82	0.20	5.85	0.64	1.36
		45,000	0.26	0.09	0.05	0.05	0.00	0.06	0.01	0.01	0.26	0.36	0.58	0.84	0.09	14.16	1.54	2.11
Pb	Nongsang 14	15,000	0.02	0.01	0.01	0.02	0.00	0.03	0.00	0.00	0.02	4.95	1.33	1.90	0.03	36.90	0.48	2.53
		30,000	0.03	0.01	0.01	0.02	0.00	0.03	0.00	0.00	0.03	5.03	0.71	5.15	0.04	36.29	0.41	2.00
		45,000	0.04	0.01	0.00	0.02	0.00	0.03	0.00	0.00	0.04	14.45	0.40	5.02	0.04	38.81	0.30	2.56
	Qiangsang 1	15,000	0.03	0.01	0.01	0.02	0.00	0.02	0.00	0.00	0.03	4.85	1.01	2.76	0.04	39.35	0.61	4.82
		30,000	0.03	0.01	0.01	0.02	0.00	0.02	0.00	0.00	0.03	7.14	1.41	1.12	0.04	26.21	0.65	3.28
		45,000	0.03	0.01	0.01	0.01	0.00	0.02	0.00	0.00	0.03	12.76	0.81	0.84	0.08	31.06	0.61	2.33
	Yuesang 11	15,000	0.03	0.01	0.01	0.02	0.00	0.04	0.00	0.00	0.03	5.10	0.86	1.78	0.12	13.02	0.10	0.87
		30,000	0.03	0.01	0.01	0.01	0.00	0.04	0.00	0.00	0.03	10.08	0.71	1.54	0.16	17.69	0.10	0.59
		45,000	0.03	0.01	0.01	0.02	0.00	0.04	0.00	0.00	0.03	15.41	0.82	1.91	0.10	18.92	0.08	0.77

Table 3
Effects of Cd and Pb on the IR and DR of the silkworms in different dates at the fifth instar.

Mulberry variety	Planting density (plants/ha.)	Leaf Cd and Pb (mg/kg, dry)		Rate of ingestion (IR)									Rate of digestion (DR)								
		Cd	Pb	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Xiang 7920	10,000	/	/	0.67	0.64	0.58	0.58	0.56	0.57	0.55	0.46	0.14	0.56	0.60	0.46	0.40	0.33	0.34	0.37	0.71	0.75
Nongsang 14	15,000	0.14 ± 0.05	4.48 ± 2.32	0.69	0.70	0.62	0.57	0.49	0.44	0.28	/	/	0.56	0.61	0.50	0.41	0.41	0.35	0.66	/	/
	30,000	0.15 ± 0.02	4.08 ± 1.37	0.71	0.70	0.63	0.59	0.52	0.46	0.33	/	/	0.58	0.61	0.51	0.46	0.42	0.35	0.78	/	/
	45,000	0.22 ± 0.03	3.57 ± 0.36	0.68	0.70	0.63	0.60	0.57	0.56	0.35	/	/	0.57	0.62	0.50	0.41	0.40	0.37	0.79	/	/
Qiangsang 1	15,000	0.19 ± 0.02	3.05 ± 1.28	0.72	0.73	0.67	0.66	0.59	0.49	0.29	/	/	0.57	0.61	0.45	0.36	0.32	0.32	0.82	/	/
	30,000	0.23 ± 0.02	3.07 ± 1.05	0.74	0.74	0.69	0.66	0.63	0.64	0.43	/	/	0.61	0.64	0.47	0.32	0.39	0.29	0.71	/	/
	45,000	0.14 ± 0.04	1.45 ± 0.29	0.71	0.72	0.66	0.65	0.62	0.60	0.34	/	/	0.59	0.61	0.47	0.39	0.36	0.32	0.77	/	/
Yuesang 11	15,000	0.20 ± 0.08	4.37 ± 1.19	0.72	0.74	0.64	0.60	0.52	0.39	0.28	/	/	0.62	0.63	0.49	0.43	0.40	0.32	0.77	/	/
	30,000	0.14 ± 0.04	2.46 ± 1.21	0.72	0.73	0.65	0.63	0.56	0.49	0.32	/	/	0.62	0.65	0.52	0.37	0.41	0.38	0.77	/	/
	45,000	0.14 ± 0.02	3.76 ± 0.54	0.70	0.73	0.65	0.62	0.58	0.47	0.29	/	/	0.53	0.66	0.53	0.41	0.44	0.33	0.76	/	/

3.4. Effects of Cd and Pb on the IR and DR of the silkworms

Generally speaking, it will take 8–8.5 days for the silkworms at fifth instar to spin cocoons. The leaves for the control were transported from a mulberry garden in Changsha, Hunan province, China. They were not as fresh as in the farmland near the breeding field. So the silkworms in control took another 2 days to spin cocoons (Table 3). As showed in Table 3, from 2nd day to 4th day, the values of IR with polluted leaves were a little higher than those with the control. The growth of organs and accumulation of nutrients are the main activities in silkworm at this stage. From the start of 4th day, the values of IR were decreased gradually for the three mulberry varieties under three planting densities and reached the lowest values at the 8th day (Table 3). With the intake of heavy metals contaminated leaf, the accumulated metals may affect the palatability of leaf or hurt the digestion system of silkworm, and then influence the DR. Results from the silkworm breeding experiment by feeding mulberry leaves contaminated by endogenous Cd (Cd originating from the soil of micro-plots by mulberry uptake) showed that there were not any differences in the IR and DR of the leaves between Cd-polluted treatments and the control. Only for the leaf Cd content of 1.66 mg/kg, the IR and DR were reduced by 9.40% and 17.5% respectively, compared with the control (Wang et al., 2004). The values of DR with the polluted leaves were close to the control from 2nd day to 7th day. From 2nd day to 3rd day, the DR showed a little increase and then decreased gradually from 4th day to 7th day (Table 3). The values of DR at 8th day were the highest, because at this stage is mainly about the formation of silk material. The growth of silk is expected to be rapid and the protein metabolism is vigorous.

3.5. Effects of Cd and Pb on the survival rate of flarva-pupa and the ratio of cocoon shell

As showed in Fig. 2, the survival rates of larva-pupa with the three tested mulberry varieties under the planting densities of 15,000 plants/ha and 30,000 plants/ha were significantly higher than those with the control. However, the survival rates of larva-pupa among the three mulberry varieties under different planting densities showed no significant differences. The ratio of cocoon shell depicts the total content of shell available in the cocoons and it

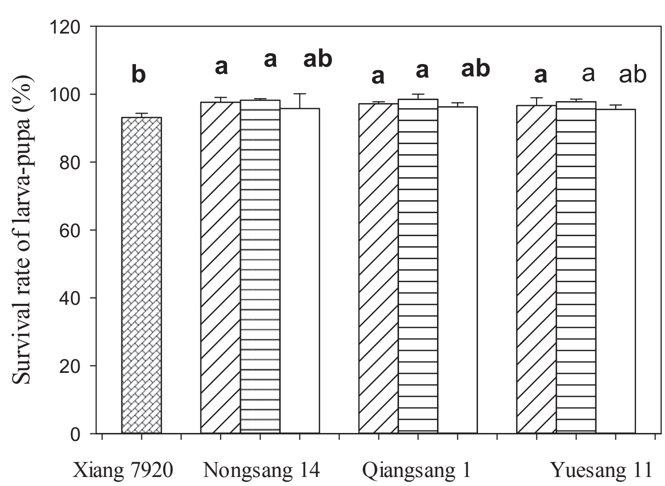

Fig. 2. Effects of Cd and Pb in mulberry leaf under different planting densities on the survival rate of larva-pupa. Means with the same letter in the different mulberry species did not differ significantly (Duncan's test, $P < 0.05$). (Control, 15,000 plants/ha. 30,000 plants/ha. 45,000 plants/ha.)

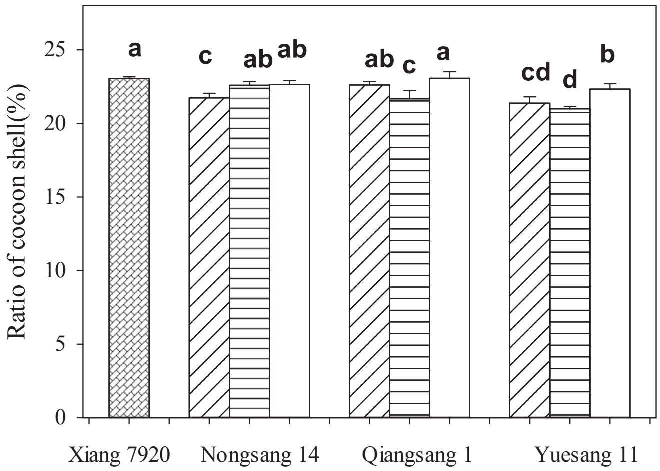

Fig. 3. Effects of Cd and Pb in mulberry leaf under different planting densities on the ratio of cocoon shell. Means with the same letter in the different mulberry species did not differ significantly (Duncan's test, P < 0.05). (control, 15,000 plants/ha., 30,000 plants/ha., 45,000 plants/ha.)

is an important index to reflect the quality of cocoon (Ramesha et al., 2009). The ratios of cocoon shell with Nongsang 14 under 30,000 and 45,000 plants/ha, Qiangsang 1 under 15,000 and 45,000 plants/ha had no significantly differences with the control (Fig. 3). It has been proved that the weight of cocoons and rate of silk reeling were significantly reduced only when the Cd concentration in leaf reached 1.66 mg/kg (Wang et al., 2004). In this study, the highest Cd concentration in leaf was only 0.23 mg/kg (Table 1). But the ratios of cocoon shell with Yuesang 11 under three planting densities were significantly lower than those with control (Fig. 3). The reason may be that the presence of Pb increased the toxicity to silkworms. The existence of Pb in Cd polluted soils improved the Cd content in the soil solution, raised the bioavailability of Cd, and promoted its absorption by plants (Zhou et al., 2015; Liu et al., 2015). However, the interactions between Cd and Pb are very complex, especially in soils. Moreover, there are many factors influencing the interactions among metals, such as plant species, culture methods, physicochemical properties of soils and the concentrations of metals (Zeng et al., 2008). So the interactions between Cd and Pb are still needed to explore deeply. Meanwhile, in our previous study, taking the total biomasses, the extraction amounts by 1 ha mulberry 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 (Jiang et al., 2019). However, in this study the ratios of cocoon shell with Yuesang 11 were the lowest among the control, Nongsang 14 and Qiangsang 1 (Fig. 3). 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 and Pb polluted soils (Jiang et al., 2019). So just considering use of polluted paddy soils and the increase of farmers' income, Nongsang 14 and Qiangsang 1 could be recommended to plant in the Cd and Pb contaminated soils with the planting density 45,000 plants/ha. Once the mulberry garden is established, they could reduce the soil erosion and phytostabilize heavy metals by changing the physicochemical properties of rhizosphere soil (Pulford and Watson, 2003; Jiang et al., 2017). Moreover, the leaf could be utilized to raise silkworms safely, which not only increases the farmers' income, but also reduces the risk of food chain pollution.

4. Conclusions

A field scale mulberry planting and silkworm rearing experiments were conducted to evaluate the mobility of Cd and Pb in the soil-mulberry-silkworm system. Results showed that the three mulberry species are not hyperaccumulators for Cd or Pb. The Pb distribution in mulberry tree did not show obvious regularity. The concentrations of Cd and Pb in the mulberry root were higher than those in stem, branch and leaf. 48.00–54.40% of Cd accumulated by mulberry was stored in root and only about 10% was in leaf. The IR and DR were a little higher than those with the control from 2nd to 4th day and then decreased gradually. The IR reached the lowest values on 8th day while the DR arrived at the highest. The survival rates of larva-pupa among the three mulberry varieties under different planting densities showed no significant differences. But the ratios of cocoon shell with Yuesang 11 under different planting densities were significantly lower than those with control. In this study, planting mulberry (Nongsang 14 and Qiangsang 1 under the planting density 45,000 plants/ha) and raising silkworms could be an effective model for use of heavy metals polluted paddy soils. It could take advantage of the heavy metals contaminated paddy soils, increase the farmers' income and reduce the injury of heavy metals to human body through the food chain.

Declaration of competing interest

This is a manuscript by "Yongbing Jiang, Shimeng Jiang, Xinpei Yan, Zhixiong Qin, Chaohua Jia, Zhangbao Li, Jun Zhang, Renzhi Huang" and entitled "The mobility of cadmium and lead in the soil-mulberry-silkworm system". The manuscript has not been published previously, and not under consideration for publication elsewhere, in whole or in part. We confirm that all the listed authors have participated actively in the study and have approved the manuscript submit to Chemosphere. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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