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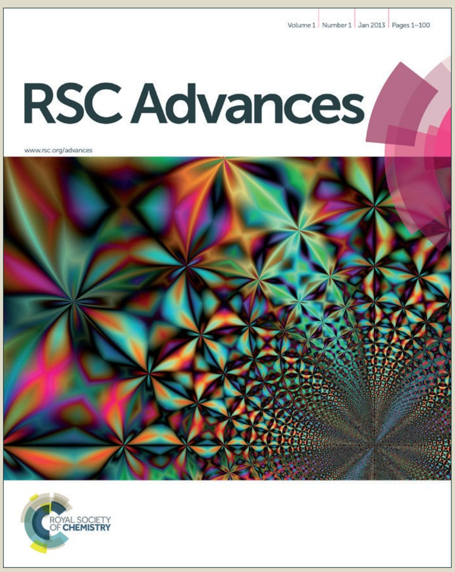

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Cadmium and Lead Accumulation and Low-molecular-weight Organic Acids Secreted by Roots in an Intercropping of a Cadmium Accumulator *Sonchus asper* L. with *Vicia faba* L.

Fang-dong Zhan, Qin Li, Xian-hua Guo, Jian-bo Tan, Ning-ning Liu, Yan-qun Zu and Yuan Li*

Sonchus asper L. and *Vicia faba* L. are a local cadmium (Cd) accumulator and a main winter crop, respectively, found in the Huize lead-zinc mining area in Yunnan Province, Southwest China. The biomass and low-molecular-weight organic acids (LMWOAs) secreted by the roots of these plants, Cd and lead (Pb) contents and their accumulation in a *S. asper* monoculture, *V. faba* monoculture and *S. asper*/*V. faba* intercrop were investigated in a field experiment at 35, 80 and 180 d after planting. The results showed that (1) intercropping had no notable influences on plant biomass and grain yields of *V. faba* but led to a significant increase in the amount of stem and leaf biomass of *S. asper* at 180 d after planting. (2) The major LMWOAs secreted by the roots of both *V. faba* and *S. asper* were oxalic acid, tartaric acid and citric acid. Intercropping resulted in an increase and decrease of the LMWOA contents secreted by *V. faba* and *S. asper* roots, respectively. (3) Along with plant growth, the available Cd content decreased, and the available Pb contents did not exhibit obvious changes in the soil samples of a *V. faba* monoculture. The amount of available Cd and Pb both increased in the soil of the *S. asper* monoculture but decreased in that of the *S. asper*/*V. faba* intercrop. (4) Intercropping resulted in a decrease in the contents and accumulation of Cd and Pb in *V. faba* plants, but an increase in both the contents and accumulation of Cd and Pb in *S. asper* plants. Moreover, intercropping enhanced the enrichment and translation coefficients of Cd for *S. asper*. The remediation efficiency was the highest at 180 d after planting. (5) There were significant negative correlations between the contents of citric acid, malic acid (secreted by *V. faba* roots), oxalic acid and tartaric acid (secreted by *S. asper* roots) and the available Cd content in the soil samples. In addition, there was a significant positive correlation between the available Cd content in the soil and the Cd contents in the roots and grains of *V. faba*. Intercropping reduced the Cd contents in the plants and grains of *V. faba* and was closely related to the decrease in the available Cd content in the soil samples, which was mediated by plant roots that secreted LMWOAs.

1. Introduction

Heavy metal pollution in farmlands is a major environmental problem that is a cause for global concern. Production activities, such as wastewater irrigation, sludge for agricultural utilization, exploitation and smelting of mineral resources and pile-up harmful waste, result in a diffusion of heavy metals such as cadmium (Cd), lead (Pb), Zinc (Zn) and arsenic (As) into farmland soils.[1] The heavy metals released into farmlands are absorbed by the crops, subsequently hindering the growth of crops raising the heavy metals contents of the edible parts of the crops. Together, heavy metals seriously threaten food safety and human health through the food chain.[2, 3] Hence, the remediation of heavy metal-polluted farmlands and the reduction of the heavy metal contents in agricultural products grown in polluted farmlands have drawn a much attention.

Among the remediation technologies available for treating heavy metal-polluted soils, phytoremediation has advantages such as ease of implementation in the field, relatively low cost, soil improvement and lack of secondary pollution; thus, it has become one of the major methods for the remediation of heavy metal-polluted farmlands.[4] However, it also has deficiencies such as slow growth of the accumulators, small biomass, and restriction by climatic factors, which makes phytoremediation a long-term and low efficiency process. These deficiencies have limited the wide application of phytoremediation in heavy metal-polluted farmlands.[5] In addition, phytoremediation requires agricultural production to stop, which does not conform to the national conditions of China (i.e., “an enormous population but with less per capita cultivated land”); this makes popularization and application of this technology difficult.[6]

Intercropping is a significant agricultural planting measure that not only promotes effective use of agricultural resources, such as soil nutrients, water and light, but also has prominent ecological and environmental benefits.[7, 8] The intercropping of an accumulator (or hyperaccumulator) with a crop was used to remediate heavy metal-polluted soils and was found to have many advantages. Intercropping allows people to carry out agricultural activities and apply accumulators or hyperaccumulators to remediate polluted soil at the same time. Some intercropping patterns of accumulators

College of Resources and Environment, Yunnan Agricultural University, Kunming 650201, PR China, E-mail: liyuan03@ynau.edu.cn; Fax: +86 871 65227550; Tel: +86 871 65227651

with crops have been applied to heavy metal-polluted soils, such as *Solanum nigrum*/onion,[9] *Sedum alfredii*/maize,[10] *S. alfredii*/upland kangkong,[11] *Brassica juncea*/alfalfa,[12] and *Pteris vittata*/*Panax notoginseng* intercropping.[13] These intercropping patterns do not require the suspension of agricultural production while the accumulator is used to remediate the heavy metal-polluted soils, reduce the heavy metal contents in crops products to meet safety and quality requirements, and to execute "agricultural production accompanied by remediation". Therefore, this method exerts a positive influence on both the remediation of polluted farmlands and the safety of agricultural products.[14, 15] People used this method to simultaneously facilitate remediation and agricultural production, and was considered to be more suitable for polluted farmlands in China [16, 17] However, the mechanisms on how intercropping influences heavy metal accumulation in the accumulators and crops remain unclear.

Under heavy metals stress, the secretion of low-molecular-weight organic acids (LMWOAs) by plant roots is commonly increased.[18] The LMWOAs enter the soil, which obviously alters the bioavailability of heavy metals in soils and influences their absorption and accumulation by plants.[19-21] Hence, LMWOAs play an important role in the heavy metal accumulation process in plants.[22-24] For example, citric acid increased the contents of available heavy metals in soil samples and enhanced the accumulation of heavy metals in plants.[25-27] The presence of citric acid, malic acid and acetic acid alleviated the bio-toxicity of Cd on plants, resulting in an increase in the Cd accumulation of plants.[23, 28] Some studies found that oxalic acid caused a mobilization of Cu and Zn in soil, while others found that citric acid led to the immobilization of Pb in soil and reduced the absorption and accumulation of heavy metals in plants.[22, 29] However, the role of LMWOAs secreted by plant roots in the intercropping of accumulators and crops remains unclear.

Yunnan Province is an important Pb-Zn mine production base in southwest China. Long-term exploitation and smelting of the Pb-zinc mine has resulted in serious Cd and Pb pollution in the farmlands around the mining area.[30-32] In addition, there are large numbers of mining wastelands that feature high heavy metal contents and poor soil fertility, and only a minority of plant species is able to grow in these wastelands. Among these plants, some are classified as accumulators and have the capability of enriching a large amount of heavy metals, which provides local plant resources for the remediation of heavy metal-polluted soils.[33] *Vicia faba* L. is a major winter-crop that is found in the farmlands around the Pb-zinc mine area in Yunnan Province. Due to the great variations in climate across China, agricultural production modes and plant species also vary across the different regions of China. Consequently, each type of accumulator and intercropping program for soil remediation can only be applied to a specific region and not to the whole country. Furthermore, there are problems associated with the introduction of exotic accumulators to local areas, such as a lack of adaptation to local soil and climatic conditions and the invasion of alien species.[15] Hence, it's necessary to adopt the local accumulator resources to establish local accumulators and crops intercropping pattern for remediation on the heavy metal-polluted farmlands.

According to natural plant resources and the agricultural production mode in the Huize Pb-Zn mine area, Yunnan Province, we established an intercropping pattern during the summer that consisted of a local Cd accumulator, *Sonchus asper* L., and maize (*Zea mays*). The *S. asper*/maize intercropping pattern obviously reduced the amount of Cd and Pb in the maize plants and grains and enhanced Cd and Pb accumulation in *S. asper* for both pot and field experiments.[34, 35] We then established a similar intercropping pattern that consisted of *S. asper* and *V. faba* during the winter and conducted a field experiment. Taking both *S. asper* and *V. faba* monocultures as controls, the effects of *S. asper*/*V. faba* intercropping on the plant biomass, LMWOA secretion by plant roots, contents of available Cd and Pb in soils, and contents and accumulation of Cd and Pb in plants at 35, 80 and 180 d after planting were investigated. We assumed that (1) intercropping had advantages on promoting the remediation capacity of the accumulator, *S. asper*, reducing the Cd and Pb contents in crops and enhancing the quality and nutritional safety of the *V. faba* grains; and (2) the LMWOAs secreted by the intercropping plant roots played an important role in influencing the bioavailability of Cd and Pb in the soil during the intercropping remediation process.

2. Materials and methods

2.1 Experimental Field

The experimental field was located at Maseka Village (E 103°38′12. 9″, N 26°34′21.1″, and altitude 2130 m), Huize County, Yunnan Province in Southwest China. The annual average temperature was 12.6 °C, and the annual precipitation was 840 mm. The soil type was red soil. Its physiochemical properties included the following: a pH of 6.11, organic matter content of 21.8 $g \cdot kg^{-1}$, total N, P and K contents of 1.54, 1.75 and 7.44 $g \cdot kg^{-1}$, respectively, available N, P and K contents of 38.1, 71.4 and 614.3 $mg \cdot kg^{-1}$, respectively, and total Cd and Pb contents of 4.59 and 392 $mg \cdot kg^{-1}$, respectively.

2.2 Experimental Design

A local Cd accumulator, *S. asper*, and a main winter-crop, *V. faba* (variety name: Manila), were planted in the experimental farmland. Seeds of *S. asper* were collected from the Pb-Zn mining area in Huize County, Yunnan Province. The seeds were disinfected with 10% H_2O_2 for 30 min and were then sowed into a floating plate filled with a flue-cured tobacco-type matrix. After the *S. asper* seedlings grew to 5~6 cm, the seedlings and the *V. faba* seeds were simultaneously transplanted into the farmlands on October 5th. Both the *S. asper* and *V. faba* plants were grown from October of 2014 to April of 2015.

The three planting patterns included a *S. asper* monoculture, *V. faba* monoculture, and a *S. asper*/*V. faba* intercrop. For the *S. asper* monoculture, both the between-plant and between-row spaces were 10 cm. For the *V. faba* monoculture, the between-plant and between-row spacings were 20 and 30 cm, respectively. The intercropping pattern consisted of *V. faba* rows with intervals of two *S. asper* rows. For the *S. asper*/*V. faba* intercrop, the between-plant space for *V. faba* was 20 cm; both the between-plant and between-row spacings for *S. asper* were 10 cm, and the row space between *S. asper* and *V. faba* was 10 cm. Each planting pattern had 3 plots, and there were a total of 9 plots. The size of each plot was 3.0 m × 2 m, which were randomly arranged in the field.

2.3 Sample Collection and Biomass Measurement

Sampling was done at 35 (seedling), 80 (flowering) and 180 d (maturation) after transplantation of *V. Faba*. Then, 3 plants planted in the soil of each monoculture plot, and 3 plants of *V. faba* and *S. asper* planted in the intercropping plot were randomly chosen and removed from the field. Then, the plants with soil attached to the roots were brought back to the laboratory.

The *S. asper* plants were divided into underground (roots) and aboveground (stems and leaves) sections. The *V. faba* plants were divided into three parts (roots, stems and leaves) at both the seedling and flowering stages; the plants were divided into five parts (roots, stems, leaves, pods and grains) when they were in the maturation stage. All plant parts were washed with tap water and deionized water 3 times. The plant parts were placed in a drying oven at 105 °C for 30 min to deactivate the enzymes. Then, the samples were dried at 75 °C for 72 h to obtain consistent masses, and the biomass of the different plant parts was measured.

2.4 Measurement of Cd and Pb Contents in Soil and Plant Samples

The dried plant samples were grinded with a pulverizer and separated by a sieve with a pore size of 0.25 mm. The roots were shaken to obtain the attached soil, and then the soil was kept out of the sun and dried naturally by indoor air. After fully mixing the soil samples, one part of the soil sample was sized using a sieve with a pore size of 0.25 mm to measure the total contents of Cd and Pb; the remaining soil was seized by a sieve with a pore size of 2 mm to measure the available contents of Cd and Pb.

Both the soil and plant samples were digested through wet digestion according to the method published by Bao (2000).[36] For the soil samples, 5.0 g portions of the air-dried soil samples that had been subjected to a 0.25 mm nylon sieve were placed in a 150 mL conical flask. A small quantity of water was used to moisturize the soil samples. Then, 10 mL of aqua regia (V (concentrated nitric acid):V (concentrated hydrochloric acid) =1:3) was added into the conical flask, and the sample was heated at a low-temperature to a slight boiling state (140-160 °C) using an electric heating plate. After the brown nitric oxides almost dried up, the sample was removed from the heating plate and cooled. The addition of perchloric acid (5-10 mL) along the flask wall continued to heat and digest the samples until they were turned into a grey white past. The flask was removed for cooling, and distilled water was used to filter the samples into a volumetric flask and to obtain a final volume of 50 mL. For the plant samples, 0.5 g portions of the plants were weighed, digested using the same method as the wet digestion of the soil samples, and the volume was fixed to 50 mL using the distilled water.

The available Cd and Pb content in the soil samples were determined using a method reported by Bao (2000).[36] Air-dried soil samples (25.0 g) that had been filtered through a 0.25 mm sieve were transferred into a conical flask (150 mL), and 20 mL of diethylene triamine penta-acetic acid – tris (2-hydroxyethyl) amine (DTPA–TEA) was added as an extracting agent. The flasks were shaken at 180 rpm for 2 h to extract the available Cd and Pb from the samples. Then, the extract was filtered into a volumetric flask and fixed to a volume of 50 mL with the distilled water.

The Pb concentrations of the solutions in the 50 mL volumetric flasks were determined using flame atomic absorption spectrometry; the Cd concentrations of the samples were measured using graphite furnace atomic absorption spectrometry. Finally, all the Cd and Pb contents in the plants and the total and available contents of Cd and Pb in soils were calculated using a formula.

2.5 Measurement of LMWOA contents secreted by plant roots

After washing off the attached soil with tap water, the plant roots of both *V. faba* and *S. asper* were rinsed 4 times with distilled water. The clean roots were soaked in a 5 mg·L^{-1} methyl propyl phenol solution for 5 min and were then transferred to a collection vessel filled with 300 mL of a $CaCl_2$ solution (0.5 mmol·L^{-1}). The plant roots were placed in the vessel filled with the $CaCl_2$ solution, and the vessel was covered with black plastic to prevent the roots from being exposed to light. So the stems and leaves were on the top of the vessel and were kept undisturbed under natural light conditions for 2 h. After removal of the plant roots, the solution was filtered through a 0.45-μm filter to remove the root debris and collect the root exudates. The collected exudates were concentrated to a volume of 3 mL by rotary evaporation at 40 °C. Finally, the concentrations of LMWOAs (oxalic acid, tartaric acid, citric acid, malic acid and lactic acid) in the exudates were determined according the method published by Cawthray (2003) with minor modifications.[37] The amounts of LMWOAs were measured by high performance liquid chromatography (HPLC) with an Agilent 20RBAX SB-C18 (250×4.6 mm ID) column. The mobile phase was 2% methanol at a flow rate of 0.6 mL per min. A 15 μL sample volume was loaded, the peaks were detected at 210 nm, and the analysis time was 40 min.

2.6 Measurement of Accumulation Features of Cd and Pb

The accumulation amount of Cd and Pb in the plants was the sum of the Cd and Pb contents multiplied by the plants' biomass. Accumulation features of Cd and Pb were expressed through an enrichment coefficient (EC) and a translation coefficient (TC). EC = the content of Cd and Pb in the aboveground parts / their contents in the soil. TC = the content of Cd and Pb in the aboveground parts / their contents in the underground parts.[28]

2.7 Data and Statistical Analyses

Preliminary data processing was performed in Excel 2010. Significant differences between treatments were determined using the independent samples T-test. The correlations between the contents of LMWOAs secreted by plant roots, the contents of available Cd and Pb in soils, between the contents of available Cd and Pb in soils and the Cd and Pb contents in each part of plants were determined using the respective functions in SPSS 22.0.

3. Results and discussion

3.1 Plant Biomass

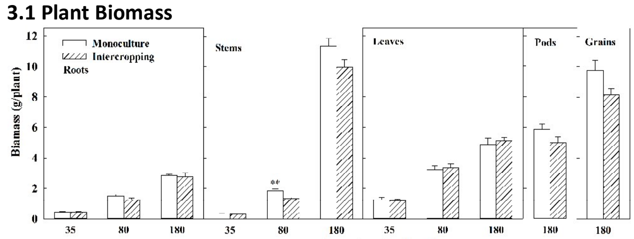

Fig. 1 Biomass of *V. faba* in the monoculture and intercropping. All values represent the mean ± standard error (SE), n=9. "**" means very significant difference (P＜0.01).

The plant biomass of *V. faba* and *S. asper* increased with the progress of plant growth. For *V. faba*, no significant difference was found on the biomass of different plant parts (roots, stems, leaves, pods and grains) between those grown in monocultures and intercrops; however, the stem biomass of the plants grown in a monoculture was significantly higher than those grown in intercrops at 80 d after planting (Fig. 1).

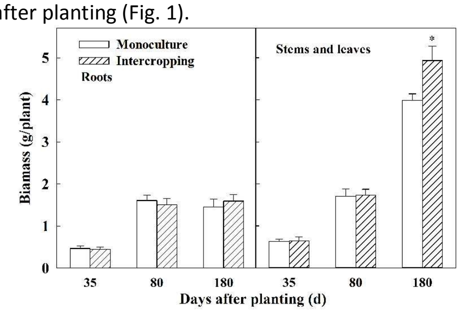

Fig. 2 Biomass of *S. asper* in the monoculture and intercropping. All values represent the mean ± standard error (SE), n=9. "*" means significant difference (P＜0.05).

For *S. asper*, there were no significant differences between plants grown in a monoculture and intercrop on the roots biomass at all the three stages or on the stem and leaf biomass at 30 and 80 d after planting. However, the stem and leaf biomass of plants grown in an intercrop were significantly higher than those grown in a monoculture at 180 d after planting (Fig. 2).

However, the effects of intercropping different accumulators (or hyperaccumulators) and crops on the contents and accumulation of heavy metals by crops were different. Some studies found that intercropping reduced the contents of heavy metals in plants and grains and increased the amount of biomass and production of crops. For example, intercropping of plants such as *Thlaspi arvense* (a Zn hyperaccumulator)/barley,[38] *T. arvense*/Chinese cabbage,[39] *Sedum alfredii* (a Zn hyperaccumulator)/maize,[10,15] *Conyza canadensis* (a Cd hyperaccumulator)/cherry seedling, *Solanum nigrum* (a Cd hyperaccumulator) /cherry seedling, *Digitaria sanguinalis* (a Cd and Pb hyperaccumulator)/cherry seedling,[40] *Pteris vittata* (a As hyperaccumulator)/*Panax notoginseng*,[13] *Brassica juncea* (a Cd hyperaccumulator)/alfalfa,[12] and *Thalia dealbata*/rice,[41] reduced heavy metal (Zn, Pb, Cd, Cu and As) contents in the plants and gains of crops and increased the biomass of crops. Similar to these research results, this study found that *S. asper*/*V. faba* intercropping resulted in a decrease in the Cd and Pb contents in *V. faba* and an increase in both the contents and accumulation of Cd and Pb in *S. asper*.

Some other studies have reported that intercropping of accumulators (or hyperaccumulators) and crops did not have a notable influence and even demonstrated that it increased the contents of heavy metals in the plants and grains of crops. For instance, *S. nigrum*/onion intercropping was shown to have no influence on the Cd contents in onion plants under field conditions.[9] *B. juncea*/oilseed rape intercropping caused an increase in the Cd contents in the plant, and a decrease on the yield of oilseed rape.[42] *Pteris cretica* (a As hyperaccumulator)/maize intercropping even resulted an increase in the contents of As, Pb and Cd in the roots, stems and leaves of maize.[43] *Sedum plumbizincicola* (a Cd hyperaccumulator)/wheat intercropping also increased the Zn and Cd contents in the aboveground parts of wheat.[44]

3.2 LMWOAs Secreted by Plant Roots

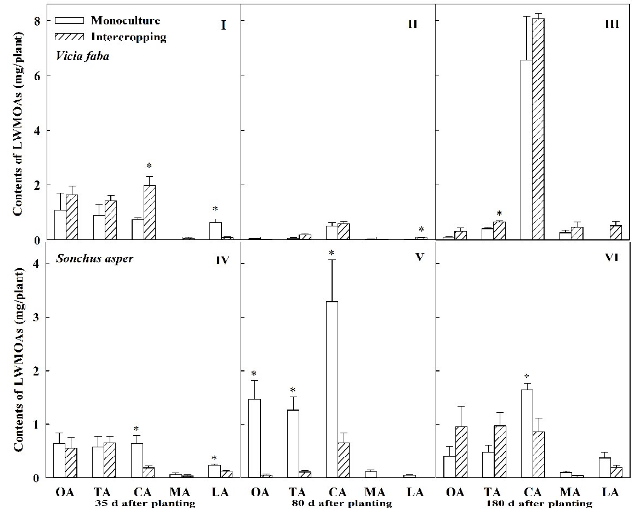

Fig. 3 Exudation of LMWOAs secreted by *V. faba* and *S. asper* roots in the monoculture and intercropping. Oxalic acid (OA), tartaric acid (TA), citric acid (CA), malic acid (MA) and lactic acid (LA). All values represent the mean ± standard error (SE), n=3. "*" means significant difference (P＜0.05).

The main LMWOAs secreted by *V. faba* roots systems were oxalic acid, tartaric acid and citric acid at 35 d after planting; citric acid was detected with the progress of plant growth. However, the main LMWOAs secreted by *S. asper* roots were oxalic acid, tartaric acid and citric acid at the three growth stages.

Intercropping altered the secretion of LMWOAs by *V. faba* and *S. asper* roots. For *V. faba*, intercropping led to a significant increase in the contents of citric acid at 35 d, lactic acid at 80 d, and tartaric acid at 180 d; meanwhile, a significant decrease in lactic acid was observed at 35 d after planting. For *S. asper*, intercropping caused a significant decrease in the contents of citric acid and lactic acid at 35 d, oxalic acid, tartaric acid and citric acid at 80 d, and citric acid at 180 d after planting. Overall, compared to plants grown in monocultures, intercropping resulted in an increase and decrease in the LMWOAs contents secreted by *V. faba* and *S. asper* roots, respectively (Fig. 3).

In fact, the intercropping influenced the roots LMWOAs exudation as reported by some studies.[45, 46] Such as the intercropping of faba bean with maize resulted in the amount of malic acid exuded by intercropped faba bean was higher than with monocropped plants.[45] However, the mechanisms on changes of the LMWOAs exudation induced by the intercropping were still unclear.

3.3 Contents of available Cd and Pb in Soil

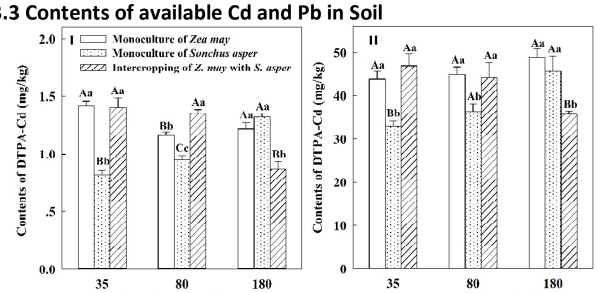

Fig. 4 Availbale Cd contents of the soils in the *V. faba* monoculture, *S. asper* monoculture and *S. asper/V. faba* intercropping. All values represent the mean ± standard error (SE), n=9. Different capital letters mean very significant difference (P＜0.01), different lowercase letters mean significant difference (P＜0.05).

In present study, the data refer to the rhizosphere soil adherent to the roots surface and is about 1-2 mm thick. The rhizosphere soil is significantly influenced by the root exudates secreted into the rhizosphere. And the effects of root exudates on the soils chemistry declined in the bulk soil.

Both at 35 and 80 d after planting, the available Cd and Pb contents were less in the soils of the *S. asper* monoculture, and were higher in the soils of the *V. faba* monoculture and the *S. asper/V. faba* intercrop. However, at 180 d after planting, the available Cd and Pb contents were smallest in the soils of the *S. asper/V. faba* intercrop, which was very significantly less than samples from both the *V. faba* and *S. asper* monocultures (Fig.4).

With the plants growth progress, both the available Cd and Pb contents decreased in the soils for the *S. asper/V. faba* intercrop, and increased for the *S. asper* monoculture. For the *V. faba* monoculture, there was a decrease in the available Cd contents and an unnotable change in the available Pb contents in the soils (Fig.4). Therefore, the *S. asper/V. faba* intercrop resulted in a decrease in the availability of both the Cd and Pb in the soils.

3.4 Accumulation Features of Cd and Pb in Plants

Compared with the *V. faba* monoculture, the intercropping led to a significant decrease on the Cd contents in the roots and grains at 180 d, and a significant decrease in the stems at 80 d after planting. Additionally, the intercropping resulted in significant decreases on the Pb contents in the roots at 80 d, the stems at 35 and 180 d after planting, respectively (Table 1). Hence, intercropping resulted in a decrease in the Cd and Pb contents in the plants and grains of *V. faba*.

Table 1 Contents (mg/kg) of Cd and Pb in monoculture and intercropping plant of *V. faba*

Heavy metals	Plant parts	Planting pattern	35 d after planting	80 d after planting	180 d after planting
Cd	Roots	Monoculture	5.67±0.41	5.37±0.31	5.19±0.32*
		Intercropping	4.91±0.27	5.19±0.39	4.30±0.25
	Stems	Monoculture	3.25±0.32	4.62±0.36	3.69±0.43
		Intercropping	2.73±0.29	4.77±0.32	3.23±0.28
	Leaves	Monoculture	2.72±0.09	4.56±0.22**	3.95±0.42
		Intercropping	3.47±0.42	2.92±0.36	3.42±0.32
	Pods	Monoculture	-	-	3.60±0.24
		Intercropping	-	-	2.96±0.20
	Grains	Monoculture	-	-	1.08±0.09*
		Intercropping	-	-	0.72±0.09
Pb	Roots	Monoculture	154.5±9.3	131.0±8.3*	136.8±9.3
		Intercropping	143.6±4.2	107.7±6.8	136.9±11.8
	Stems	Monoculture	73.9±5.6*	92.2±6.2	146.9±8.9*
		Intercropping	55.8±2.9	98.5±8.3	116.2±6.0
	Leaves	Monoculture	52.6±4.4	101.1±7.5	110.2±3.9
		Intercropping	56.2±2.1	109.4±4.6	100.4±2.7
	Pods	Monoculture	-	-	98.0±5.5
		Intercropping	-	-	90.2±5.4
	Grains	Monoculture	-	-	50.2±4.3
		Intercropping	-	-	44.4±3.1

All values represent the mean ± standard error (SE), n=9. "**" means very significant difference (P＜0.01), and "*" means significant difference (P＜0.05) between monoculture and intercropping. "-" means without the plant parts at the sampling time.

Furthermore, the intercropping led to a significant decrease on the Cd accumulation in the leaves at 80 d and 180 d after planting, and a very significant decrease in the pods at 180 d after planting; and resulted in significant decreases on the Pb accumulation in the roots (at 80 d), stems (at 35 and 80 d) and pods (at 180 d); and a very significant decrease in the stems at 180 d after planting (Table 2). Therefore, the intercropping also resulted in a decrease in the Cd and Pb accumulation in the plants and grains of *V. faba*.

Table 2 Accumulation (ng/plant) of Cd and Pb in monoculture and intercropping plant of *V. faba*

Heavy metals	Plant parts	Planting pattern	35 d after planting	80 d after planting	180 d after planting
Cd	Roots	Monoculture	2.6±0.2	8.0±0.5	14.9±1.2
		Intercropping	2.2±0.2	6.7±0.8	11.9±1.4
	Stems	Monoculture	1.3±0.2	8.9±1.3	40.7±3.7
		Intercropping	0.9±0.1	6.3±0.4	32.5±3.7
	Leaves	Monoculture	3.6±0.5	14.7±1.1*	19.4±2.7*
		Intercropping	4.2±0.6	9.9±1.5	17.7±2.0
	Pods	Monoculture	-	-	20.9±1.5**
		Intercropping	-	-	15.1±1.8
	Grains	Monoculture	-	-	10.2±0.5
		Intercropping	-	-	5.9±0.8
Pb	Roots	Monoculture	72±8	195±14*	393±33
		Intercropping	66±6	138±14	384±54
	Stems	Monoculture	27±4*	168±9*	1675±138**
		Intercropping	18±1	131±12	1158±86
	Leaves	Monoculture	71±14	326±30	534±43
		Intercropping	67±5	364±32	515±28
	Pods	Monoculture	-	-	570±37*
		Intercropping	-	-	440±27
	Grains	Monoculture	-	-	496±64
		Intercropping	-	-	363±32

All values represent the mean ± standard error (SE), n=9. "**" means very significant difference (P＜0.01), "*" means significant difference (P＜0.05) between monoculture and intercropping. "-" means without the plant parts at the sampling time.

In contrast, intercropping resulted in an increase in both the contents and accumulation of Cd and Pb in the stems and leaves of *S. asper*. Compared with a *S. asper* monoculture, the Cd contents in the stems and leaves obviously increased in the three stages, and the Cd accumulation in the stems and leaves increased significantly or very significantly at 35, 80 and 180 d after planting. A very significant increase for the Pb contents at 35 d and a significant increase for the accumulation of Pb in the roots, stems and leaves at 180 d after planting were observed (Table 3 and 4).

Table 3 Contents (mg/kg) of Cd and Pb in monoculture and intercropping plant of *S. asper*

Heavy metals	Plant parts	Planting pattern	35 d after planting	80 d after planting	180 d after planting
Cd	Roots	Monoculture	4.96±0.35	4.80±0.34	9.33±1.58

		Intercropping	4.00±0.27	5.83±0.53	9.85±1.08
	Stems and leaves	Monoculture	3.40±0.26	4.15±0.62	6.63±0.52
		Intercropping	5.40±0.35**	6.57±0.37**	10.30±0.86**
Pb	Roots	Monoculture	104.1±6.9	233.8±15.9	364.1±32.9
		Intercropping	97.8±5.7	208.8±11.6	449.2±36.9
	Stems and leaves	Monoculture	41.9±1.7	184.4±16.3	386.3±31.4
		Intercropping	58.0±1.3**	215.0±19.0	424.5±30.2

All values represent the mean ± standard error (SE), n=9. "**" means very significant difference (P＜0.01) between monoculture and intercropping.

Table 4 Accumulation (ng/plant) of Cd and Pb in monoculture and intercropping plant of *S. asper*

Heavy metals	Plant parts	Planting pattern	35 d after planting	80 d after planting	180 d after planting
Cd	Roots	Monoculture	2.3±0.3	7.6±0.7	11.7±1.3
		Intercropping	1.8±0.3	8.6±1.0	15.6±2.2
	Stems and leaves	Monoculture	2.2±0.4	6.6±0.8	26.5±2.2
		Intercropping	3.4±0.4*	11.2±0.7**	48.9±3.1**
Pb	Roots	Monoculture	48±5	366±24	489±48
		Intercropping	43±6	308±25	696±72*
	Stems and leaves	Monoculture	27±3	299±20	1537±133
		Intercropping	38±5	359±28	2083±207*

All values represent the mean ± standard error (SE), n=9. "**" means very significant difference (P＜0.01), and "*" means significant difference (P＜0.05) between monoculture and intercropping.

As shown in Table 5, *S. asper/V. faba* intercropping obviously increased the enrichment coefficient (EC) and translation coefficient (TC) of Cd for *S. asper* at the three stages compared with the *S. asper* monoculture, and the highest remediation efficiency detected at was at 180 d after planting. In contrast, there was a very small increase in both the EC and TC of Pb. For the *V. faba*, there were no big differences on both the EC and TC of the Cd and Pb between the monoculture and intercropping. The results indicated that intercropping promoted Cd translation from the roots to the stems and leaves and enhanced Cd accumulation of *S. asper*.

Table 5 Enrichment coefficient (EC) and translation coefficient (TC) of Cd and Pb for *S. asper* and *V. faba*

Plant	Planting time (d)	Planting pattern	Cd		Pb	
			EC	TC	EC	TC
S. asper	35	Monoculture	0.74	0.69	0.11	0.40
		Intercropping	1.18	1.35	0.15	0.59
	80	Monoculture	0.90	0.86	0.47	0.79
		Intercropping	1.43	1.13	0.55	1.03
	180	Monoculture	1.44	0.71	0.99	1.06
		Intercropping	2.24	1.05	1.08	0.95
V. faba	35	Monoculture	0.62	0.50	0.15	0.37
		Intercropping	0.72	0.67	0.14	0.39
	80	Monoculture	1.00	0.85	0.25	0.75
		Intercropping	0.75	0.66	0.27	0.99
	180	Monoculture	0.63	0.56	0.26	0.75
		Intercropping	0.54	0.58	0.22	0.64

3.5 Correlation Analyses

Correlation analyses were conducted between the LMWOAs secreted by the plant roots and the available Cd and Pb contents in soils. Significant negative correlations were observed between the contents of oxalic acid and tartric acid secreted by the *S. asper* roots, citric acid and malic acid secreted by the *V. faba* roots and the contents of available Cd in soils; their correlation coefficients were -0.541 (n=18), -0.462 (n=18), -0.534 (n=18), and -0.578 (n=15), respectively. However, there was no significant correlation between the LMWOAs contents and the available Pb contents (Table 6). These results indicated that the LMWOAs secreted by the roots of *S. asper* and *V. faba* had obvious effects on reducing the availability of Cd in the soils.

Table 6 Correlation coefficient between the LMWOAs and both the available Cd and Pb contents in the soils

Heavy metals	Plant	Oxalic acid	Tartaric acid	Citric acid	Malic acid	Lactic acid
Available Cd	*S. asper*	-0.541*	-0.462*	-0.366	-0.058	0.164
	V. faba	0.240	0.193	-0.534*	-0.578*	-0.150
Available Pb	*S. asper*	-0.042	0.013	-0.163	-0.118	0.102
	V. faba	0.182	0.169	-0.083	-0.351	-0.209

"*" means significant difference (P＜0.05).

The LMWOAs excreted by intercropping plants had notable effects on the heavy metals bioavailability in soils and the uptake of plants. For example, in a barley/pea intercropping system, intercropping promoted peas to accumulate heavy metals, and this observation was related to the mobilization of heavy metals in soil by root exudates of the intercropped barley.[47] The present study found that the LMWOAs secreted by the roots of *S. asper* and *V. faba* in the intercropping system reduced the contents of available Cd in soils, and the intercropping reduced the Cd contents in the plants and grains of *V. faba*. Furthermore, a significant positive correlation was observed between the contents of available Cd in soils and the Cd contents in the roots and grains of *V. faba*. Hence, the functional mechanism of intercropping and its influence on the accumulation of heavy metals in plants was related to the LMWOAs secreted by plant roots and their effects on the bioavailability of heavy metals in soil samples.

Furthermore, a correlation analysis was conducted between the available Cd and Pb contents in soils and the Cd and Pb contents in the different parts of the plants. Significant positive correlations were observed between the contents of available Cd in soil the soil samples and the Cd contents in the roots and grains of *V. faba*; their correlation coefficients were 0.488 (n=18) and 0.835 (n=6), respectively.

Summing up the correlations of the secretion of LMWOAs, available Cd and Pb contents in the soil samples, and the Cd and Pb contents in plants, observations indicated that intercropping reduced the Cd contents in the roots and grains of *V. faba*, which was closely related to the effects of reducing the amount of LMWOAs secreted by plant roots on the availability of Cd in soil.

In addition, the interspecific root interactions between plants in an intercropping system were also shown to play a significant role in the interactive effects of intercropping plants. This included root-system spatial distribution heterogeneity caused by the recognition behavior of roots and the morphology between the roots (i.e., "root

- root"). Additionally, the biological behavior of roots in "root – root symbiont - root" systems was found to be mediated by the root symbiont.[48, 49] In heavy metal-polluted soils, intercropping led to a change in the soils' physicochemical properties, contents of available heavy metals, and on the translation of heavy metals from soils to plants. Its underground mechanism for reducing the accumulation of heavy metals by crops included the foraging and high absorption of heavy metals in soils by hyperaccumulators,[50] and the symbiotic effects of arbuscular mycorrhizal fungi in plant roots.[11] All in all, the influencing mechanisms of intercropping on the absorption and accumulation of heavy metals by hyperaccumulators and crops were still unclear and needed to be further studied.

All in all, *S. asper*/*V. faba* intercropping presented an outstanding effect on reducing the Cd and Pb contents in the plants and grains of *V. faba* and enhancing the accumulation of Cd and Pb in *S. asper* under field conditions. Notably, the remediation efficiency of *S. asper* was the highest at 180 d after planting, which was also the harvest time for *V. faba*. Therefore, both *S. asper* and *V. faba* were harvested simultaneously with significant regional advantages which included the following: (1) The local accumulator had adapted to the local soil and climate conditions, thus avoiding problems of an exotic accumulator such as environmental inadaptation and invasion threats of alien species. (2) The wild *S. asper* seeds in the Pb-zinc mining area were abundant and used to cultivate a large quantity of seedlings to meet the seedling demand for building the intercropping system in the field pattern, thus realizing continuous remediation of the polluted soil under field conditions. (3) Because this intercropping remediation did not alter the local planting modes and habits, and because the accumulator and crop were harvested at the same time, local farmers would be easily accept and apply this method to simultaneously achieve both remediation and agricultural production. However, some problems still existed, including the limited remediation efficiency, the contents of heavy metals in crop grains still exceeded the hygienic standard limits for agricultural products, and difficulties of applying intercropping remediation at field with agricultural machineries. Thus more studies need to be conducted on the remediation mechanisms and applications of intercropping methodologies.

4. Conclusions

Under field conditions, intercropping of the Cd accumulator *S. asper*/*V. faba* resulted in a decrease in the Cd and Pb contents in the plants and grains of *V. faba* and an increase in the biomass and the Cd and Pb contents in *S. asper*. Intercropping provided a new feasible way for both improving the safety of agricultural products and enhancing the remediation efficiency of accumulators on polluted farmlands. The major LMWOAs secreted by both *S. asper* and *V. faba* were oxalic acid, tartaric acid and citric acid. Intercropping resulted in an increase and decrease in the LMWOAs secreted by *V. faba* and *S. asper* roots, respectively, and a decline in the contents of available Cd and Pb in soils was observed. There were significant negative correlations between the contents of citric acid, malic acid (secreted by *V. faba* roots), oxalic acid and tartaric acid (secreted by *S. asper* roots) with the available Cd contents in soils. A significant positive correlation was observed between the available Cd content in soils and the Cd content in the roots and grains of *V. faba*. These results indicated that the mechanism of intercropping reduced the Cd contents in *V. faba* and was closely related with the bioavailability of Cd in soils mediated by LMWOAs secreted from intercropping plant roots.

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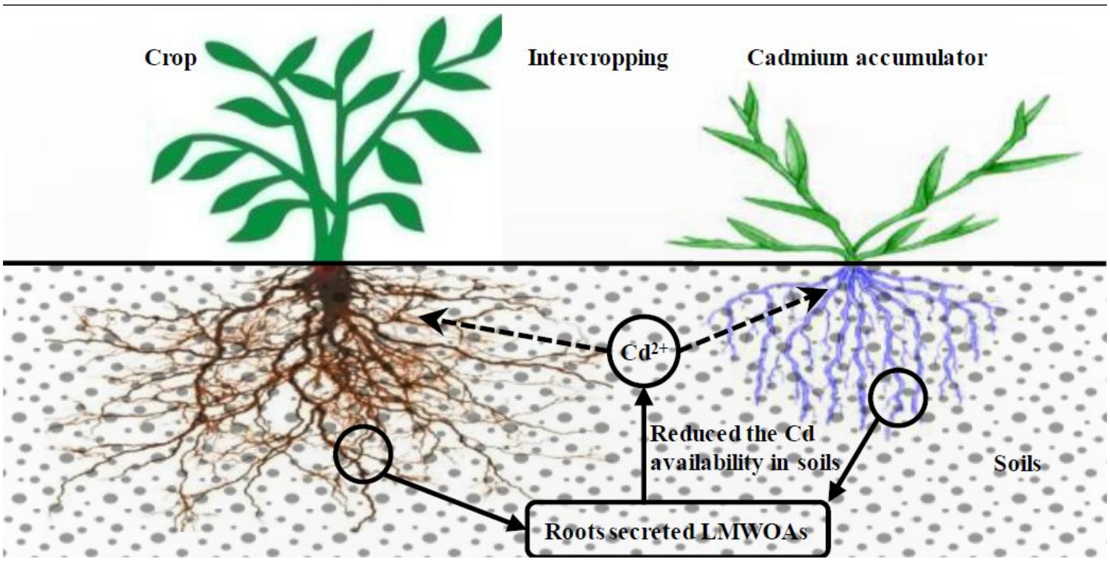

Intercropping reduced the crop Cd contents and enhanced the remediation, which was related to the roots LMWOAs exudation in soils.