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Effects of Intercropping with Tolerant Plants on Cadmium Accumulation of *Brassica chinensis*

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Abstract: The pot experiment was conducted to study the effects of intercropping with cadmium (Cd) tolerant plants (*Arenaria serpyllifolia*, *Clinopodium confine*, *Ranunculus sieboldii* and *Plantago asiatica*) on growth and Cd accumulation of *Brassica chinensis*. Compared with the monoculture, only intercropping with *C. confine* increased the root biomass, shoot biomass, and photosynthetic pigment contents of *B. chinensis*, and intercropping with *A. serpyllifolia*, *R. sieboldii* and *P. asiatica* decreased that. Intercropping with tolerant plants increased the Cd contents in roots and shoots of *B. chinensis*, and the orders of that were ranked as intercropping with *C. confine* > intercropping with *P. asiatica* > intercropping with *A. serpyllifolia* > intercropping with *R. sieboldii* > monoculture. Intercropping with *C. confine* increased the Cd contents in roots and shoots of *B. chinensis* by 107.67% and 72.00% respectively compared with the monoculture Therefore, intercropping with Cd tolerant plants could increase the Cd uptake of *B. chinensis*, which should be carefully considered in Cd-contaminated soil.

Introduction

With the increase of the heavy metals pollution in soil, China's cadmium (Cd) contaminated arable land area reache 1.3×10^4 ha, involving 11 provinces and cities 25 areas [1]. In addition, the Cd content in vegetable is also exceeded, about 24.1% of the vegetable samples exceeded in China [2]. Therefore, for arable land or garden of heavy metal pollution control is imminent. The adjustment of planting pattern is a relatively new remediation method for remedying the soil heavy metal by agricultural ecology. It can reduce the heavy metal contents in agricultural products and improve the edible safety of vegetables, including low-enrichment plant rotation, different enrichment ability of vegetable intercropping, hupeyaccumulator plants intercropping with crop, etc. [3]. When the vegetables intercropping with hupeyaccumulator plants, the toxic effects of heavy metals on vegetables may be able to reduce, and reduces the heavy metal enrichment of vegetables [4]. Some studies show that intercropping with hyperaccumulator plant *Sedum alfredii* significantly reduces the Cd and zinc contents in maize and ryegrass [5]. In terms of vegetables, the absorption capacity of Cd in different vegetables is related to the type of vegetables, and the absorption capacity of leaf vegetables is usually greater than that of fruit vegetables [6].

Brassica chinensis is a cruciferous vegetable with adaptability, fast growth and high yield [7]. In this study, the seedlings of *B. chinensis* were intercropped with the Cd tolerant plants *Arenaria serpyllifolia*, *Clinopodium confine*, *Ranunculus sieboldii* and *Plantago asiatica* [8] under Cd-contaminated soil. The aim of the study was to determine whichtolerant plant could reduce the Cd accumulated in *B. chinensis* seedlings, and to provide a reference for phytoremediation of soils contaminated with heavy metals.

Materials and Methods

Materials. In April 2015, the seedlings of *A. serpyllifolia*, *C. confine*, *R. sieboldii* and *P. asiatica* with two or three euphyllas expanded were collected from the Ya'an campus farm of the Sichuan Agricultural University (29°59′N, 102°59′E), China, from uncontaminated soil areas. The seeds of *B. chinensis* were purchased in the market.

The inceptisol soil samples (purple soil in the Genetic Soil Classification of China) were collected from the Ya'an campus farm in August 2013. The basic properties of the soil are described in Lin et al. (2014) [9].

Experimental Design. The experiment was conducted in the greenhouse of the Ya'an campus farm from February to June 2015. The soil samples were air dried and passed through a 5-mm sieve. Three kilograms of the air-dried soil was weighed into each polyethylene pot (18 cm high and 21 cm in diameter). Cd was added to soils as $CdCl_2{\cdot}2.5H_2O$ at 10 mg/kg [10] in February 2015. The soil moisture was maintained at 80 % of field capacity for 2 months. The seeds of *B. chinensis* were sown in farmland of the Ya'an campus farm in March 2015. The five experimental treatments in the experiment were *B. chinensis* monoculture (control), and *B. chinensis* intercropping with each of the four plant species (*A. serpyllifolia*, *C. confine*, *R. sieboldii* and *P. asiatica*). The uniform seedlings of *B. chinensis* (two euphyllas expanded) were transplanted into each pot in April 2015. The monoculture planted four seedlings of *B. chinensis* in each pot, and the intercropping treatment planted three seedlings of *B. chinensis* and one tolerant plant seedling in each pot. Each treatment was replicated five times using a completely randomized design with 10-cm spacing between pots, and the soil moisture content was maintained at 80 % of field capacity from the time the plants were transplanted into the pots until the time the plants were harvested. 2 months later (June 2015), the upper mature leaves of *B. chinensis* were collected to determine the photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid) contents [11]. The plants were then gently removed from the soil, and the roots, stems, and leaves were washed with deionized water and dried at 80 °C to constant weight for dry weight and Cd content determination [12].

Statistical analyses. Statistical analyses were conducted using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with least significant difference (LSD) at the p = 0.05 confidence level. The following calculated were used: translocation factor (TF) = Cd content in shoots/ Cd content in roots [13].

Results and Discussion

Biomass. Under Cd stress, intercropping with *C. confine* increased the root, stem and leaf and shoot biomasses of *B. chinensis* compared with the monoculture, but intercropping with *A. serpyllifolia*, *R. sieboldii* and *P. asiatica* decreased that (Table 1). The biomass of *B. chinensis* was ranked as: intercropping with *C. confine* > monoculture > intercropping with *P. asiatica* > intercropping with *A. serpyllifolia* > intercropping with *R. sieboldii*. Compared with the monoculture, intercropping with *C. confine* increased the root, stem and leaf and shoot biomasses of *B. chinensis* by 2.73% ($p > 0.05$), 7.20% ($p < 0.05$), 16.92% ($p < 0.05$) and 13.92% ($p < 0.05$), respectively. Intercropping with *R. sieboldii* and *P. asiatica* improved the root/ shoot ratio of *B. chinensis*, but intercropping with *A. serpyllifolia* and *C. confine* had no obvious effect or reduced that (Table 1).

Photosynthetic Pigment Content. Like the biomass, only intercropping with *C. confine* increased the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents in *B. chinensis* compared with the monoculture, which increased by 8.96% ($p < 0.05$), 19.26% ($p < 0.05$), 10.66% ($p < 0.05$) and 17.52% ($p < 0.05$), respectively (Table 2). Intercropping with *A. serpyllifolia*, *R. sieboldii* and *P. asiatica* decreased the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents in *B. chinensis* compared with the monoculture. Intercropping with *R. sieboldii* and *P. asiatica* improved the chlorophyll a/b of *B. chinensis*, and intercropping with *A. serpyllifolia* and *C. confine* reduced that (Table 2). The order of chlorophyll a/b was intercropping with *P. asiatica* > intercropping with *R.*

sieboldii > monoculture > intercropping with *A. serpyllifolia* > intercropping with *C. confine*.

Table 1 Biomass of *Brassica chinensis*

Treatments	Roots (g/plant)	Stems (g/plant)	leaves (g/plant)	Shoots (g/plant)	Root/ shoot ratio
Monoculture	0.293±0.003a	0.542±0.008b	1.218±0.010b	1.760±0.018b	0.166
A. serpyllifolia	0.266±0.006b	0.468±0.006d	1.138±0.013c	1.606±0.018d	0.166
C. confine	0.301±0.013a	0.581±0.013a	1.424±0.011a	2.005±0.024a	0.150
R. sieboldii	0.261±0.010b	0.432±0.007e	1.064±0.020d	1.496±0.027e	0.174
P. asiatica	0.291±0.006a	0.493±0.010 c	1.184±0.010b	1.677±0.024c	0.174

Table 2 Photosynthetic pigment content in *Brassica chinensis*

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b	Carotenoid (mg/g)
Monoculture	0.681±0.009b	0.135±0.006b	0.816±0.003b	5.044	0.234±0.002b
A. serpyllifolia	0.492±0.007d	0.099±0.008c	0.591±0.015d	4.970	0.172±0.005d
C. confine	0.742±0.010a	0.161±0.009a	0.903±0.002a	4.633	0.275±0.004a
R. sieboldii	0.462±0.010e	0.091±0.005c	0.553±0.015e	5.087	0.161±0.003e
P. asiatica	0.619±0.006c	0.121±0.010b	0.740±0.004c	5.156	0.220±0.002c

Cadmium Content. Intercropping with tolerant plants increased the Cd contents in roots, stems, leaves and shoots of *B. chinensis* compared with the monoculture (Table 3). The Cd contents in roots, stems, leaves and shoots of *B. chinensis* were ranked as intercropping with *C. confine* > intercropping with *P. asiatica* > intercropping with *A. serpyllifolia* > intercropping with *R. sieboldii* > monoculture. Intercropping with *A. serpyllifolia* increased the Cd contents in roots and shoots of *B. chinensis* by 66.87% ($p < 0.05$) and 54.22% ($p < 0.05$) respectively compared with the monoculture, intercropping with *C. confine* increased by 107.67% ($p < 0.05$) and 72.00% ($p < 0.05$) respectively compared with the monoculture, intercropping with *R. sieboldii* increased by 41.71% ($p < 0.05$) and 34.67% ($p < 0.05$) respectively compared with the monoculture, and intercropping with *P. asiatica* increased by 84.05% ($p < 0.05$) and 60.00% ($p < 0.05$) respectively compared with the monoculture. Intercropping with tolerant plants reduced the TF of *B. chinensis*, and the order of TF was monoculture > intercropping with *R. sieboldii* > intercropping with *A. serpyllifolia* > intercropping with *P. asiatica* > intercropping with *C. confine*. (Table 3).

Table 3 Cadmium content in *Brassica chinensis*

Treatments	Roots (mg/kg)	Stems (mg/kg)	Leaves (mg/kg)	Shoots (mg/kg)	TF
Monoculture	3.26±0.113e	0.29±0.010e	3.12±0.057d	2.25±0.042d	0.690
A. serpyllifolia	5.44±0.127c	0.39±0.016c	4.73±0.184b	3.47±0.134b	0.638
C. confine	6.77±0.297a	0.53±0.013a	5.23±0.170a	3.87±0.141a	0.572
R. sieboldii	4.61±0.099d	0.33±0.007d	4.12±0.099c	3.03±0.071c	0.657
P. asiatica	6.00±0.156b	0.43±0.014b	4.92±0.156ab	3.60±0.127ab	0.600

Conclusions

Under Cd stress, only intercropping with *C. confine* increased the root biomass, shoot biomass, and photosynthetic pigment contents of *B. chinensis* compared with the monoculture, and intercropping with *A. serpyllifolia*, *R. sieboldii* and *P. asiatica* decreased that. Intercropping with tolerant plants increased the Cd contents in roots and shoots of *B. chinensis*, and the orders of that were ranked as intercropping with *C. confine* > intercropping with *P. asiatica* > intercropping with *A. serpyllifolia* > intercropping with *R. sieboldii* > monoculture. Therefore, intercropping with tolerant plants could increase the Cd uptake of *B. chinensis*, which should be carefully considered in Cd-contaminated soil.

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