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Hyperaccumulators' Diversity Enhances Cd-Contaminated Soil Restoration and Reduces Rice Cd Uptake under an Intercropping System

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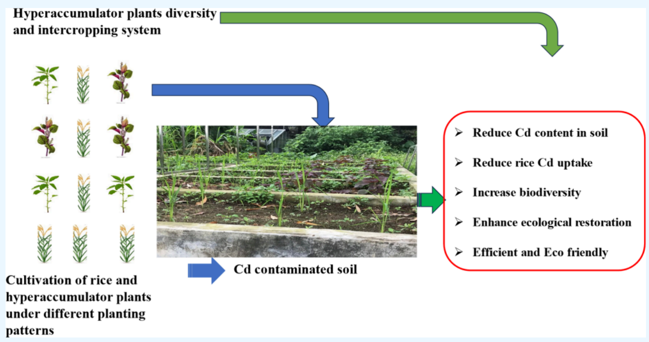

ABSTRACT: Cd accumulation in rice-cultivated soils across China is a major problem that needs to be tackled. A plot experiment was carried out using heavy metal (HM) hyperaccumulators *Amaranthus hypochondriacus* L. and *Perilla frutescens* (L.) Britt. intercropped with low-accumulation rice to obtain safe edible rice while reducing the soil Cd concentration. It was found that Cd concentration in soil was decreased by 7.43 and 2.86% under rice intercropped with *Amaranthus hypochondriacus* L. and *Perilla frutescens* (L.) Britt., respectively, compared to single cropped rice. In addition, enhanced effects were noted under the combination of *Amaranthus hypochondriacus* L., *Perilla frutescens* (L.) Britt, and rice in which a 20.35% decrease in soil Cd content was recorded compared to single-cultivated rice soil. In addition, the available Cd in soil was reduced by 4.00 and 5.00% under rice/*Amaranthus* and rice/*Perilla*, respectively, and 12.00% under rice/*Amaranthus*/*Perilla* mixed culture. Moreover, the concentration of Cd in various parts of rice was under permissible limits. However, rice biomass was decreased by the presence of hyperaccumulators. This study suggests that combining HM hyperaccumulator plants and low-accumulation rice provides efficient Cd extraction results and could be a crucial option for restoring Cd-contaminated soil without reducing rice production.

1. INTRODUCTION

The recent estimations of the Ministry of Agriculture and Environmental Protection of China indicate that more than 120000 ha of cultivated land are polluted in China.[1] Thus, finding economical, reasonable, and feasible technical strategies to control contaminated arable land has recently been one of the hottest topics. The current soil restoration technology can be roughly divided into physical restoration technology, chemical restoration technology, and biological restoration technology.[2−4] However, it has recently been discovered that plant barrier technology based on low-accumulation crops and plant extraction technology based on hyperaccumulator plants effectively restore arable land contaminated by HMs.[2−4] However, further studies are still needed concerning the impacts of hyperaccumulators' identity and diversity and the planting patterns on HM-contaminated soil restoration.

Intercropping is a cultivation method that increases farmland productivity.[5−8] For instance, studies have shown that intercropping between legumes and cereals can significantly increase the plant's leaf area index and the total amount of dry matter accumulation per unit area of each crop.[7,8] In addition, it has been demonstrated that intercropping between cotton and peanuts improves economic benefits.[9] Moreover, intercropping simultaneously enhances plant biomass and disease and insect resistance.[9] For instance, it was found that the intercropping system improves the microclimate of the broad bean canopy while controlling the development of wide bean scarlet spot disease.[10,11] However, the effects of rice/hyperaccumulator plants on Cd-contaminated soil restoration still need further studies.

Intercropping between hyperaccumulator plants and low-accumulated crop varieties can stabilize and reduce crop HM uptake while increasing the restoration efficiency of the contaminated soil.[2,12,13] Intercropping between hyperaccumulator plants and ordinary crops has been used to improve the biomass of hyperaccumulator plants and their HM absorption without compromising the growth and yield of the intercropped crop.[2,6,8] However, the impacts of hyper-

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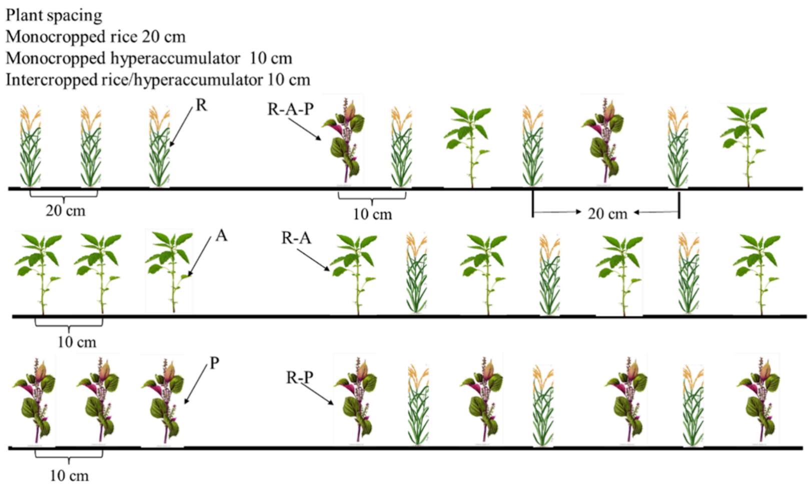

Figure 1. Scheme illustrating the planting patterns. Note: R refers to monocropped rice, A refers to monocropped *Amaranthus*, P refers to monocropped *Perilla*, R-A refers to intercropped rice/*Amaranthus*, R-P refers to intercropped rice/*Perilla*, and R-A-p refers to intercropped rice/*Amaranthus*/*Perilla*.

accumulator identity and diversity under a rice/hyperaccumulator plant intercropping system on Cd concentration in soil and rice uptake still need further investigations.

The main HM hyperaccumulator plants are terrestrial, and the rice fields are aquatic and wet ecological environments where these hyperaccumulator plants cannot grow normally.[14] In addition, less reports have been focused on the effects of hyperaccumulator identity and diversity and the interaction between hyperaccumulator and low-accumulation rice variety under different planting patterns on Cd-contaminated soil restoration in rice fields.[2,15,16] Thus, a plot experiment was carried out to study the impact of intercropped low-accumulation rice variety and HM hyperaccumulator plants *Amaranthus hypochondriacus* L. and *Perilla frutescens* (L.) Britt. on Cd removal in soil and rice Cd uptake.

2. MATERIALS AND METHODS

2.1. Materials. *2.1.1. Soil Collection and Preparation.* The soil utilized in this study was obtained from the ecological farm of South China Agricultural University. The concentration of Cd, which exceeds the national standard (GB15618−2018), was 1.89 mg kg^{-1}, while the available Cd was 0.270 mg kg^{-1}, and the soil pH was 4.11.

2.1.2. Test Plants. The hyperaccumulators *Amaranthus* (*Amaranthus hypochondriacus* L.), *Perilla* (*Perilla frutescens* L. Britt.), and rice crops were used as test plants. *Amaranthus hypochondriacus* seeds were obtained from the Long Xinxian Research Group, Department of Environmental Science and Engineering, School of Resources and Environment, South China Agricultural University, while *Perilla frutescens* L. Britt. Seeds were purchased from the Guangdong Academy of Agricultural Sciences. The rice variety used (*Oryza sativa*), known as Futyou 918, is a low-accumulating type of rice concerning Cd.[15]

2.2. Experimental Design. This study was conducted in cement pools with a surface area of 1.5 m × 0.8 m each.

The compound fertilizer $N-P_2O_5-K_2O$ (nutrient ≥45%) was applied in each pool at a 50 kg per acre rate before transplantation. The experiment included six treatments in a randomized complete block design (RCBD), and each treatment was replicated three times. The experimental treatments were as follows: monocropped rice (R), monocropped *Amaranthus* (A), monocropped *Perilla* (P), intercropped rice/*Amaranthus* (R-A), intercropped rice/*Perilla* (R-P), and intercropped rice/*Amaranthus*/*Perilla* (R-A-P). Rice, *Amaranthus*, and *Perilla* seeds were sown in seedbeds, and same-sized and healthy seedlings were transplanted after 2 weeks of germination. The number of rows in each pool under the monocropping system was 3 for rice and 6 for hyperaccumulators, while the ratios of R-P, R-A, and R-A-P were 2:3, 2:3, and 3:2:2, respectively. The spaces applied between rows in monocultured rice and hyperaccumulators were 20 and 10 cm, respectively. Figure 1 displays a comprehensive layout that includes both row and plant spacings, providing a detailed configuration of the intercropping pattern. Plants were irrigated every 2 days with tap water from the experimental station.

2.3. Plant and Soil Samplings. Plants and soil samples were collected after 8 weeks of transplantation. The soil adhering to the monocropped plant roots was collected as a soil sample in monoculture. The soil adhering to the intersected intercropped plant roots was collected as a soil sample under the intercropping system. Plant samples were rinsed with tap water, then washed with deionized water to remove impurities attached to the sample's surface, and divided into three parts: roots, stems, and leaves. Plant and soil samples were placed in plastic bags after being dried in an oven at 65 °C for 72 h. The plant's dry weight was then determined before crushing and sieving with a 2 mm sieve plant and soil samples for chemical analysis. The concentration of Cd in plants was determined using standard methods.[10]

2.4. Soil Analysis. Soil chemical properties were determined at the Key Laboratory of Agro-Environment in the Tropics, Ministry of Agriculture, College of Natural Resources and Environment, South China Agricultural University, Guangzhou, using standard methods.[2,10,17] Concerning the Cd concentration in soil, 0.2 g of soil was weighed and placed in a digestion tube. The process described in ref 10 was followed to determine the concentration of Cd in soil. The available Cd in soil was determined by weighing a 5 g air-dried soil sample previously sieved through a 2 mm sieve in a 100 mL plastic tube. Twenty-five mL of 0.1 mol·L^{-1} $CaCl_2$ extractant was then added, and the mixture was allowed to oscillate at a constant temperature of 25 ± 1 °C. The vibration

Table 1. Effects of Hyperaccumulators and Planting Patterns on Soil Basic Chemical Properties[a]

treatments	pH	EC (μS/cm)	OM (g kg^{-1})	TN (g kg^{-1})
R	5.48 ± 0.07a	261.60 ± 4.48d	23.56 ± 0.12e	1.71 ± 0.06c
A	5.29 ± 0.19a	272.40 ± 3.54c	26.31 ± 0.17d	1.89 ± 0.01bc
P	4.76 ± 0.25b	257.20 ± 4.27e	35.15 ± 0.09b	2.26 ± 0.17a
R-A	5.42 ± 0.05a	277.20 ± 2.56c	33.71 ± 0.06c	2.20 ± 0.08ab
R-P	5.47 ± 0.02a	288.40 ± 4.14b	23.61 ± 0.12e	1.72 ± 0.12c
R-A-P	4.57 ± 0.25b	294.50 ± 3.17a	36.52 ± 0.07a	1.98 ± 0.21c

[a]R: monocultured rice, A: monocultured *Amaranthus*, P: monocultured *Perilla*, R-A: intercropped rice/*Amaranthus*, R-P: intercropped rice/*Perilla*, and R-A-P: intercropped rice/*Amaranthus*/*Perilla*. Data are presented as means ± standard errors (n = 3). Lowercase letters in the same column indicate significant treatment differences ($p < 0.05$).

Table 2. Effects of Hyperaccumulators and Planting Patterns on Rice Growth[a]

treatments	root dry weight (g)	shoot dry weight (g)	R/S	total dry weight (g)
R	18.28 ± 1.13a	32.66 ± 0.62a	0.55 ± 0.01ab	51 ± 1.31a
R-A	14.48 ± 1.12b	20.11 ± 0.96b	0.72 ± 0.01a	34.48 ± 1.11b
R-P	10.09 ± 0.86c	20.58 ± 1.35b	0.51 ± 0.01ab	30.09 ± 0.75b
R-A-P	6.90 ± 1.15d	18.06 ± 1.64c	0.38 ± 0.01b	24.9 ± 1.02c

[a]R: monocultured rice, A: monocultured *Amaranthus*, P: monocultured *Perilla*, R-A: intercropped rice/*Amaranthus*, R-P: intercropped rice/*Perilla*, and R-A-P: intercropped rice/*Amaranthus*/*Perilla*. Data are presented as means ± standard errors (n = 3). Lowercase letters in the same column indicate significant treatment differences ($p < 0.05$).

speed was 200 r min^{-1}. The mixture was filtered after 5 min. The filtrate was placed in a dry plastic bottle, and the available Cd concentration in the extract was measured by an atomic absorption spectrophotometer.

2.5. Statistical Analysis and Calculations. Data were processed with Excel 2016 and analyzed using SPSS software, version 21.0. One-way ANOVA was conducted to compare the effects of different treatments on growth parameters, soil chemical properties, and Cd concentrations in soil and plants. Significant difference test (Tukey) was used for pairwise comparisons.

$$\text{bioaccumulation factor (BAF)} = \frac{\text{concentration of Cd in roots}}{\text{concentration of Cd in corresponding soil}} \quad (1)$$

$$\text{translocation factor (TF)} = \frac{\text{concentration of Cd in aerial parts}}{\text{concentration of Cd in roots}} \quad (2)$$

The transfer coefficient (TC) represents the proportion of a metal concentration in plants to the total concentration of that metal in the soil. A high TC indicates poor retention of the metal in the soil or the plant's greater ability to absorb the metal. On the other hand, a low TC reflects the strong binding of the metal to the soil colloid.[18]

$$\text{transfer coefficient (TC)} = \frac{\text{concentration of Cd in plants}}{\text{concentration of Cd in corresponding soil}} \quad (3)$$

3. RESULTS

3.1. Variations in Soil Chemical Properties. An increase in soil pH was noted in all treatments compared with the original soil pH (Table 1). Similar trends were observed in soil organic matter (OM). For instance, the soil OM was higher under mixed culture than under monoculture (Table 1). Concerning, soil electrical conductivity (EC) and total nitrogen (TN), significant increases were noted under an intercropping system compared with the monocropping system. Soil EC was ranged between 257.20 and 294.50 μS/cm, with the highest value observed under intercropped rice/*Amaranthus*/*Perilla* (Table 1). This study shows that plant—soil interactions improve soil health with enhanced effects under an intercropping system and hyperaccumulator plant diversity.

3.2. Variations in Plant Growth Parameters. Significant decreases in rice root and shoot dry weight were observed under rice/*Amaranthus*/*Perilla* mixed culture compared with that of single-cultured rice (Table 2). For instance, a greater total biomass was observed under monocultured rice compared to other treatments. Moreover, rice total biomass was smaller under its coevolution with the hyperaccumulators. The root/shoot was also decreased under mixed culture compared to that under single culture (Table 1). The planning patterns had significant impacts on rice growth parameters. This study shows that the presence of a hyperaccumulator significantly reduced rice biomass.

3.3. Variation in the Total Cd and the Available Cd in the Soil. The concentrations of total Cd and available Cd in soil are shown in Figure 2. The results showed a significant decrease of Cd in cultivated soil compared to the original soil.

Concerning the total Cd in soil, which ranged between 0.17 and 0.19 mg kg^{-1}, the lowest concentration was observed under rice/*Amaranthus*/*Perilla* mixed culture followed by the rice and *Amaranthus* intercropping. The total Cd under rice/*Amaranthus* was 0.08 mg kg^{-1} lower than under rice/*Perilla* intercropping. The concentration of available Cd was also decreased under rice/*Amaranthus*/*Perilla*, rice/*Amaranthus*, and rice/*Perilla* compared with the original and monocultured rice soil. This study shows that a rice/hyperaccumulator intercropping system reduces the level of Cd in soil, with enhanced effects under hyperaccumulator diversity.

3.4. Cd Accumulation in Rice and Hyperaccumulators Various Organs. The concentration of Cd in rice and hyperaccumulators in different organs varies between treatments (Figure 3). The concentration of Cd was higher in roots

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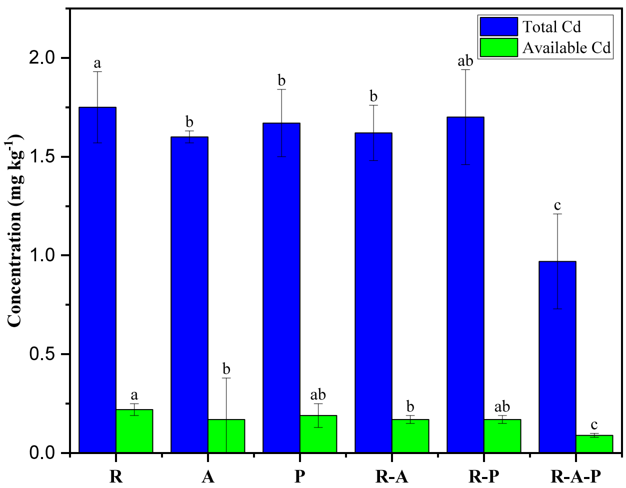

Figure 2. Total and available Cd in soil under different planting patterns. Note: R: soil under monocultured rice-soil, A: soil under monocultured *Amaranthus*, P: soil under monocultured *Perilla*, R-A: soil under intercropped rice/*Amaranthus*, R-P: soil under intercropped rice/*Perilla*, and R-A-P: soil under intercropped rice/*Amaranthus*/*Perilla*. Data are presented as means ± standard errors (n = 3). Lowercase letters in the same column indicate significant treatment differences (p < 0.05).

compared to other plant parts across all treatments. In addition, higher Cd content was noted under monoculture compared with mixed cultured plants (Figure 3). For instance, rice Cd content was higher in single culture compared to other planting patterns in all organs. Similar results were noted concerning *Amaranthus* and *Perilla*. The concentration of Cd in rice significantly decreased under rice/*Amaranthus*/*Perilla* intercropping. More importantly, it was found that the content of Cd in rice grains meets food hygiene standards (GB 13078–2001). This study suggests that hyperaccumulator diversity and density reduce rice Cd uptake and promote safe production.

3.5. Variation in Cd Accumulation and Mobility and Correlation between BAF, TF, and Growth Parameters. The bioaccumulation factor (BAF) and translocation factor (TF) of Cd are listed in Figure 4. The results showed that hyperaccumulator plants significantly affect the BAF of Cd in rice. Higher BAF was recorded under single-cultivated rice compared with other treatments (Figure 4A). This study suggests that hyperaccumulator diversity reduces the BAF of Cd in rice. Concerning the TF of Cd in rice from root to grain, no significant differences were recorded between treatments. However, compared with other treatments, a slight increase in rice TF from root to shoot was recorded under intercropped rice with *Amaranthus*. A positive correlation was also noted between BAF and TF, in contrast to BAF and rice growth parameters (Figure 4B). This study suggests that combining *Amaranthus* and *Perilla* with rice reduces the BAF of Cd in rice while reducing its content in soil.

3.6. Variation in Cd Transport Coefficient and Correlation between TF and TC. The transfer coefficient (TC) from soil to roots was slightly increased under rice/

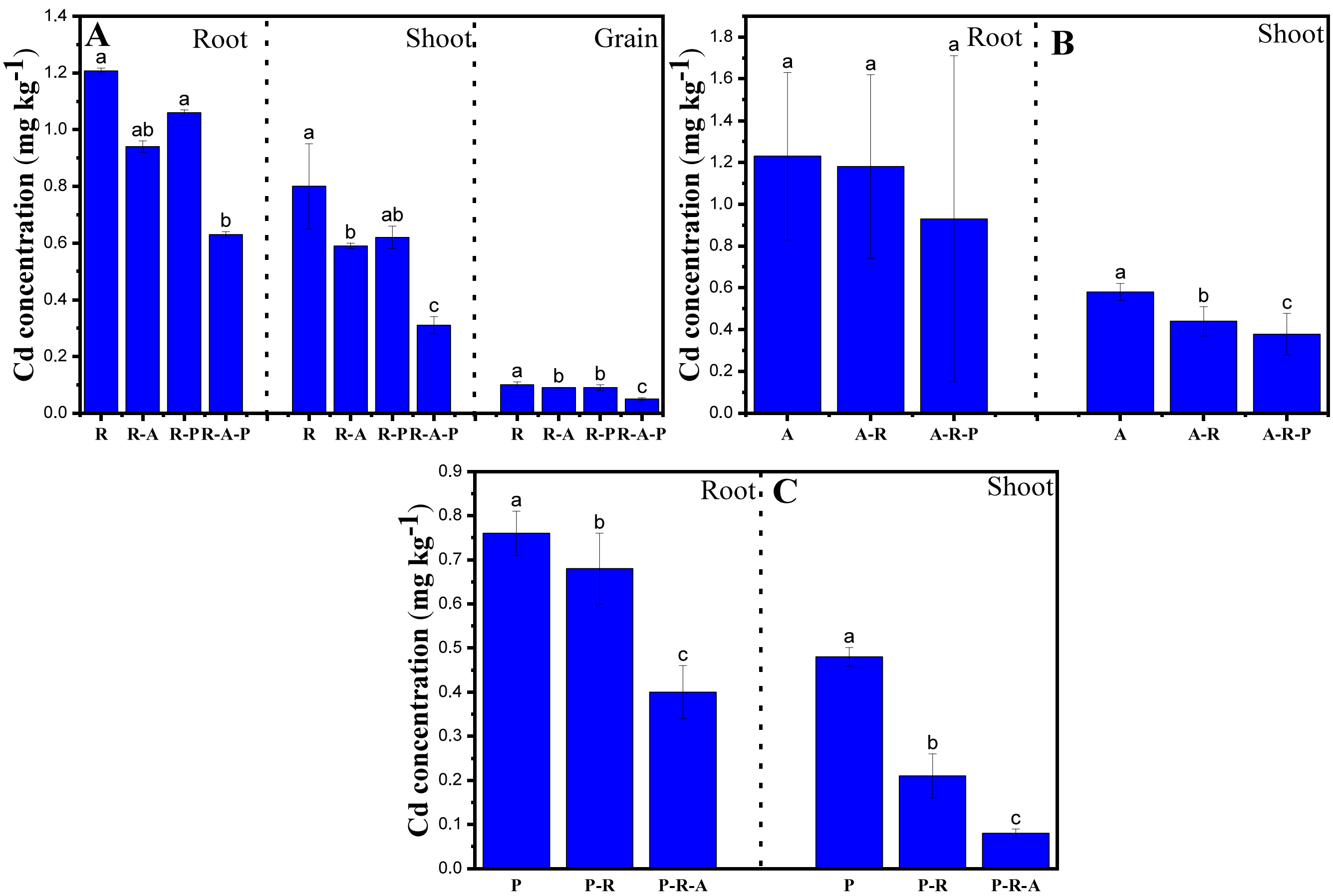

Figure 3. Cd in rice (A), *Amaranthus* (B), and *Perilla* (C) under different planting patterns. R: rice, A: *Amaranthus*, P: *Perilla*. Data are presented as means ± standard errors (n = 3). Lowercase letters in the same column indicate significant treatment differences (p < 0.05).

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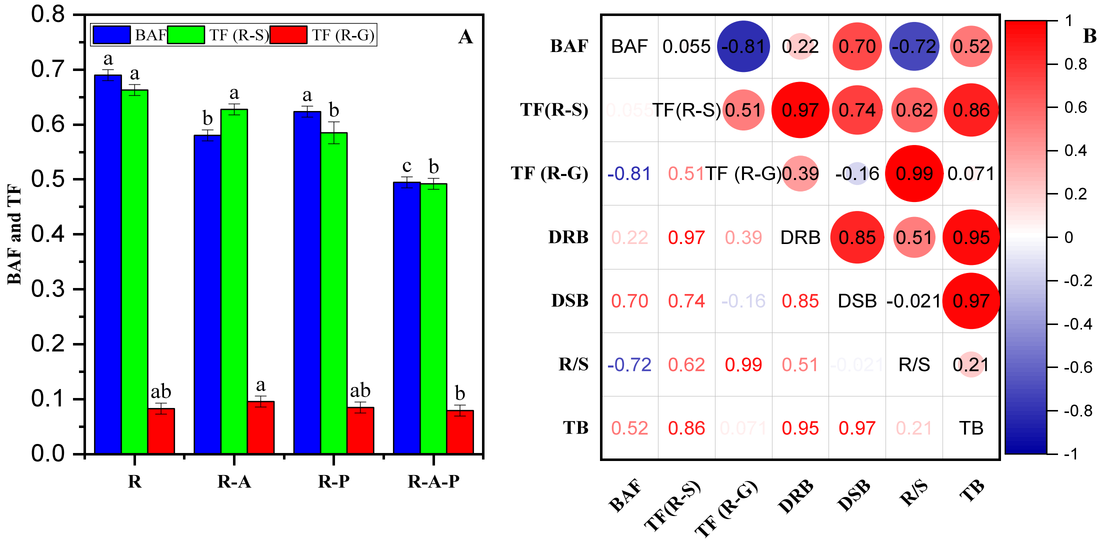

Figure 4. Variation in Cd BAF and TF (A) and correlation among BAF, TF, and rice growth parameters (B). R refers to rice; A refers to Amaranthus; P refers to Perilla; DRB refers to dry root biomass; DSB refers to dry shoot biomass; R/S refers to root/shoot ratio; TB refers to total biomass. Data are presented as means ± standard errors ($n = 3$). Lowercase letters in the same column indicate significant treatment differences ($p < 0.05$).

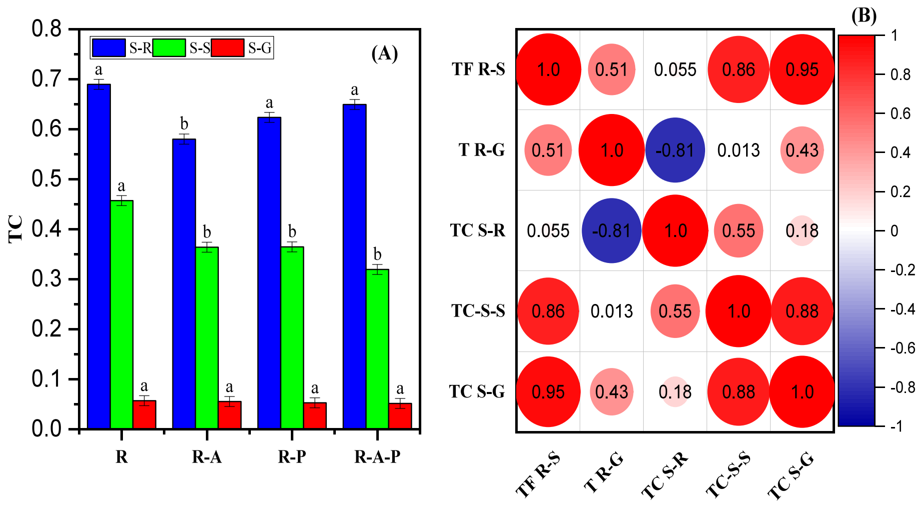

Figure 5. Cd transport coefficient (A) and correlation between TF and TC (B). Data are presented as means ± standard errors ($n = 3$). Lowercase letters in the same column indicate significant treatment differences ($p < 0.05$).

Amaranthus/*Perilla* compared with intercropped rice with a single hyperaccumulator (Figure 5a). However, a different scenario was observed regarding the soil-shoot transfer coefficient, with lower values recorded under intercropped rice than in single-cultivated rice. No significant difference was noted concerning soil-grain TC based on the planting patterns (Figure 5b). In addition, positive correlation was observed between TF and TC.

4. DISCUSSION

The study revealed that soil chemical properties vary significantly depending on the cultivated plant identity and planting pattern. For instance, the planting patterns between rice and hyperaccumulator plants had slight effects on the soil chemical properties. The findings supported prior research, indicating that plant−soil interactions increase in soil nutrient content.[19,20] Moreover, this study demonstrated that intercropping rice with hyperaccumulator plants increases the soil nutrient content, particularly under hyperaccumulator plant diversity. The study suggests that a rice/hyperaccumulator plant intercropping system can effectively enhance soil nutrient content.

Hyperaccumulator plants had significant negative effects on the rice biomass. Previous studies indicate that the legume/cereal intercropping system improves resource use efficiency without reducing biomass of either plant.[21,22] However, this study shows that rice/hyperaccumulator intercropping decreases rice growth. This situation might be explained by the fact that intercropped hyperaccumulator plants with rice are invasive plants that negatively affect the growth of their congener.[23] In addition, an enhanced decrease in the rice growth parameters was observed under two levels of hyperaccumulator plant richness. This study demonstrates that the rice/invasive hyperaccumulator plant intercropping system decreases rice growth with enhanced effects under two levels of invasive hyperaccumulator plants richness.

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The concentrations of Cd in soil and rice various organs were significantly reduced under the rice and hyperaccumulator plant intercropping system. For instance, a significant decrease in Cd content was noted under intercropped rice/hyperaccumulator cultivated soil compared to monocropped cultivated rice soil. In addition, the lowest Cd concentration in soil was recorded under a rice/*Amaranthus*/*Perilla* mixed culture. The results suggest that hyperaccumulator diversity enhances soil Cd removal in contaminated soil. In line with previous studies, this experiment demonstrated that rice/hyperaccumulators mixed culture could reduce HM concentration in contaminated soil.[6] For instance, changes in soil pH and EC can significantly influence the availability of Cd in soil.[24,25] It has been suggested that low soil pH and low EC increase the availability of Cd.[24−26] Thus, the decrease in soil Cd content can be explained by the increase in soil pH and EC. The presence of hyperaccumulator plants and changes in soil chemical properties played significant roles in reducing Cd concentration in soil and its uptake by rice.

Results showed that the intercropping system significantly reduces the concentration of Cd in all of the rice plant organs. In addition, the decrease of Cd concentration in soil and rice in various parts was more pronounced under hyperaccumulator plants diversity. However, the extent of Cd reduction varied, depending on the type and diversity of the intercropped hyperaccumulator plants. For instance, intercropping rice and *Amaranthus* resulted in lower Cd uptake by rice compared with intercropping with Perilla or single-cropped rice. This may be attributed to the higher capacity of *Amaranthus* to accumulate Cd. Moreover, this study found that intercropping rice with both *Amaranthus* and *Perilla* plants resulted in a significant decrease in rice Cd uptake, which could be due to the synergistic effect of Cd accumulation by the hyperaccumulator plants. Overall, the study suggests that increasing the hyperaccumulator plant diversity can effectively reduce rice Cd uptake.

5. CONCLUSION

HM contamination in rice cultivated soil is a crucial problem affecting rice production and product safety in China. Thus, we conducted an experiment combining low HM accumulator rice with hyperaccumulator plants well adapted to cadmium stress with a high adsorption capacity. The results showed that hyperaccumulator plant combination with rice reduces Cd concentration in soil. In addition, the concentration of Cd in various rice organs was significantly decreased under the rice/hyperaccumulator intercropping system with enhanced effects under hyperaccumulator plant diversity. This study suggests that hyperaccumulator diversity facilitates soil restoration while reducing rice Cd uptake under an intercropping system. However, further studies are still required to determine the impacts of hyperaccumulator density on rice growth and yield.

■ ASSOCIATED CONTENT

Data Availability Statement

Not applicable.

■ AUTHOR INFORMATION

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Author Contributions

Conceptualization was done by R.K., S.L., C.M., and H.L.; methodology was developed by R.K. and S.L.; software was developed by R.K.; validation was performed by H.L. and C.M.; formal analysis was done by R.K.; investigation was performed by R.K. and S.L.; resources were gathered by H.L. and C.M.; data curation was performed by R.K., S.L., P.H., and Z.L.; writing—original draft preparation was done by R.K.; writing—review and editing was performed by F.N., M.A., and S.D.; visualization was performed by H.L., C.M., and S.D.; supervision was done by H.L.; project administration was performed by H.L.; funding acquisition was performed by H.L. and C.M. All authors have read and agreed to the published version of the manuscript

Notes

The authors declare no competing financial interest.

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