Intercropping *Sedum Alfredii* and *Cicer Arietinum* L. Does Not Present a Suitable Land Use Pattern For Multi-Metal-Polluted Soil

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Abstract

Background and aims Intercropping of hyperaccumulators with commercial crops is widely accepted in single-metal-polluted farmland. However, the ecological risks of non-hyperaccumulated metals in intercropping systems have not been revealed.

Methods To evaluate Pb and Cu activation and absorption in an intercropped system of *Sedum alfredii* and *Cicer arietinum* L., sequential extraction and dissolved organic matter (DOM) characterization were used to describe the migration of metals in the intercropping system.

Results This study found that the concentrations of DOM in the *S. alfredii* monoculture and intercropping system were significantly higher than those in the *C. arietinum* L. monoculture, and DOM from the former two cultivation strategies had significantly higher Cd, Pb, and Cu extraction capacity than those from the latter. Compared with the *C. arietinum* L. monoculture, *C. arietinum* L. intercropping had significantly lower Cd content owing to the depletion of Cd by *S. alfredii*. However, Cu and Pb concentrations in the former were significantly higher than those in the latter because *S. alfredii* mobilized these metals but did not hyperaccumulate them.

Conclusion The results indicated that intercropping efficiently decreased the potential risk of Cd, but deteriorated the ecological risk of Pb and Cu. Therefore, intercropping does not present a suitable land-use pattern for multi-metal-polluted soil.

Introduction

Cadmium contamination is a severe threat to the ecological system and food safety owing to its high activity and toxicity (Chen et al., 2021; Farooq et al., 2020; Rafiq et al., 2014). Soil pollution with Cd has taken millions of hectares of agricultural land out of crop production. For instance, Hu et al. (2016) reported that Cd in paddy soils is efficiently taken up by rice plants and accumulates in the grains, threatening the quantity and quality of food supply in China. Therefore, decontaminating Cd-polluted soils has attracted worldwide attention.

Green plant-based decontamination technologies, including phytostabilization, phytoextraction, phytofiltration, and phytoexcretion, are regarded as environmentally friendly, practical, and economical methods for metal decontamination in polluted soils (Farahat and Galal, 2018; Luo et al., 2019a). High biomass-generating plant species, such as *Salix planifolia*, *Populus nigra*, and *Jatropha curcas* (Baldantoni et al., 2014), and accumulators, such as *Celosia argentea* for Mn, and *Picris divaricata* for Zn, *Pteris fauriei* for As, and *Potentilla griffithii* for Cd (Li et al., 2018), have been evaluated to reveal the mobilization, absorption, translocation, and resistance of various metals. Among these plants, the Cd hyperaccumulator *S. alfredii* has been widely used for cleaning Cd-polluted soil owing to its superior Cd-concentrating capacity and relatively high biomass-generation ability (Hu et al., 2019; Xiao et al., 2017). However, when using phytoremediation methods for metal-polluted soil, hyperaccumulators need to be planted, replacing the crops and preventing the agricultural land from making income. To safeguard the

economic benefits of landowners during the phytoremediation process, different intercropping strategies have been used as supplementary means for phytoremediation in moderately polluted fields.

Intercropping, which can improve the utilization efficiency of light, nutrients, space, and ecological niche, has been used by farmers for more than 20 centuries in China (Zeng et al., 2019a). Previous studies have reported that when vegetable crops such as *Solanum melongena*, *Vitis vinifera*, and *Vicia faba* are intercropped with a specific hyperaccumulator, interlacing of the root systems not only stimulates the decontamination of metal-polluted soil but also decreases the absorption of pollutants by the vegetables (De Conti et al., 2019; Tang et al., 2017; Zu et al., 2020).

It has been reported that the superiority of intercropping in metal-polluted fields is due to the effective metal absorption capacity of specific hyperaccumulators, which reduces metal bioavailability in the rhizosphere soils, thereby decreasing the metal extraction and accumulation of the intercropped agricultural products (Wan et al., 2017; Yang et al., 2017). Therefore, a hyperaccumulator can remove specific metals from the field, and qualified crops can be obtained. However, previous studies have found that the metal activation capacity of hyperaccumulators might increase the leaching risk of heavy metals. For instance, Li et al. (2018) studied the variations in Cd mobility evaluated by EDTA kinetic extraction in contaminated soils after repeated phytoextraction using *Sedum plumbizincicola*, and found that when the Cd extraction rate of the species was lower than the Cd replenishment rate, the superfluous active Cd would be taken up by other plants. Gove et al. (2002) revealed the uptake of Cd and Zn by *Hordeum vulgare* sharing a rhizosphere with a Cd hyperaccumulator *Noccaea caerulescens* and found that the Cd content in *H. vulgare* was increased by 2.4 times when it was intercropped with *N. caerulescens*. Conversely, Wan et al. (2017) found that *Pteris vittata* resulted in an apparent As valley around its rhizosphere, decreasing the concentration of As in the intercropped *Morus alba*. Therefore, the mechanism of the intercropping system on phytoremediation efficiency and safe utilization of metal-polluted soils requires further research.

Hyperaccumulators can change the chemical forms of heavy metals in soils by varying the soil pH, dissolved organic matter (DOM) content, and microstructure (Zu et al., 2020), but only hyperaccumulate specific metal species. Therefore, some mobilized non-hyperaccumulated metals may be absorbed by intercropped agricultural products. In fact, multi-metal pollution is a common phenomenon in the real world, and therefore, the advantages and disadvantages of intercropping in the phytoremediation of multi-metal-polluted fields should be fully revealed before extrapolating the results obtained from greenhouse experiments to the real world.

The DOM concentration and fractionation were examined in this study, because DOM, which can enhance metal bioavailability by generating metal-DOM complexes or releasing metal ions from soil particles with competing adsorption points, was changed significantly in the intercropping system (Zu et al., 2020). In addition, hyperaccumulators tend to acidify soils (Gonzaga et al., 2009; Martínez-Alcalá et al., 2016), and Christensen and Christensen (2000) reported that the chemical forms of metals are primarily controlled

by the DOM characteristics when the soil pH is higher than 5.5. Therefore, the variations in soil DOM and pH in the intercropping system containing hyperaccumulators must be evaluated.

Chickpeas (*C. arietinum* L.) are one of the most important leguminous food crops grown in more than 10 million ha in Asia, the Mediterranean basin, East Africa, the Middle East, and Oceania (Vashisth and Nagarajan, 2008). The species can draw water from soil depths of more than 1.5 m, and its water consumption is highest in the top 30 cm of soil layer where most of the active roots are present (Mridha et al., 2016). Because metal pollution generally occurs in the surface layer, the edible security of this species in intercropping systems containing hyperaccumulators needs to be estimated.

This study aims to (1) assess the influences of intercropping on biomass yield and Cd uptake capacity of *C. arietinum* L.; (2) estimate the variations in content, fractionations, and metal extraction capacity of DOM in soils under different cultivation strategies; and (3) evaluate the impacts of intercropping systems on the edible security of *C. arietinum* L. and the safe utilization pattern for multi-metal-polluted soils.

Materials And Methods

Soil and plant seedlings

The multi-metal-contaminated soil samples used in the current experiment were sampled from the surface soil layer (0–20 cm) of an electronic waste (e-waste) recycling center in Guiyu. According to the results of a previous soil survey, soil in the e-waste dismantling site had the worst contamination, followed by residential quarters, farmland, and marginal regions (Zhao et al., 2015). Compared with the barren e-waste dismantling site, weeds can develop spontaneously in residential quarters, which means that phytoextraction might be performed in this area. Some of the soils in residential quarters have been seriously polluted by e-waste dismantling activities, resulting in detrimental effects on the health of the indigenous population, especially vulnerable people (Kim et al., 2019).

200 surface soil samples were gathered near homes and workshops. The soils were sieved using 2-mm meshes and blended completely to obtain a preprocessed substrate. The preprocessed sample was wetted to saturation after it was dried under ambient conditions to equilibrate its chemical components. The drying and saturating procedure was repeated several times, resulting in a homogeneous distribution of metals in the composite substrate. After each drying and saturating procedure, 20 soils were sampled randomly for metal determination to evaluate the distribution of metals by calculating their variable coefficients. Even distributions of metals were assumed if their variable coefficients were lower than 15%.

The soil pH was measured using a pH meter in the suspension (soil/water, 1:2.5, wt.: vol.). The composite soil was separated into 6 kg aliquots and padded to sterilized pots for *S. alfredii* and *C. arietinum* L. cultivation. The soil pH was determined to be 6.1 ± 0.2 before transplanting the plant seedlings. The initial soil Pb, Cu, and Cd concentrations were 83.6 ± 9.2 , 95.7 ± 10.2 , and $4.2 \pm 0.5 \text{ mg kg}^{-1}$, respectively. The contents of Pb, Cu, and Cd exceeded their safe limits of 50, 50, and 0.3 by 0.7, 0.4, 0.9, and 13.0 times, respectively.

S. alfredii seedlings were gathered from a multi-metal mine in Zhejiang Province, considering the distribution of the species. *S. alfredii* was cultivated in soil that has been clean for three generations to minimize its internal metal content. Healthy seedlings with similar morphological characteristics were selected and grown for two weeks under greenhouse conditions with a 16/8 day/night photoperiod (25/20°C and 80/70% humidity) according to Li et al., (2013b). The *C. arietinum* L. seeds were provided by the Guyue Landscape Company. Seeds with no visible defects were germinated in clean soil. During germination, a basic nutrient solution (Conesa et al., 2009) was used to maintain a 60% soil water-holding capacity.

Experimental design

Three cultivation strategies with five replications, including *C. arietinum* L. monoculture (two per pot), *S. alfredii* monoculture (four per pot), and intercropping (one *C. arietinum* L. and two *S. alfredii* per pot) were used in this experiment. All the cultivation strategies were carried out under greenhouse conditions with a 16/8 day/night photoperiod (25/20°C and 80/70% humidity). All the plant species were harvested 13 weeks after transplantation, considering the physiological period and Cd uptake efficiency of *S. alfredii*. The rhizosphere soils were sampled according to Liu et al. (2004). In brief, the two plant species were carefully removed from the pot with minimal injury to their below-ground tissues, and the roots were shaken until the soils that were not tightly attaching to these tissues were released. The soil that was tightly adhering to the plant roots as rhizosphere soil was gathered using tweezers.

The plant species were separated into belowground and aerial parts. The belowground parts were rinsed using tap water to clear away the suspended particles, and then immersed in SrCl_2 (0.1 mmol L^{-1}). The aerial parts were washed with running water and immersed in EDTA (10 mM) to eliminate the ions. The treated plant tissues were oven-dried to a constant weight for metal analysis.

DOM analysis

For the DOM measurement, the dried soil samples were pulverized and sieved through 0.15 mm meshes to eliminate fibrous roots. The DOM was extracted according to Jones and Willett (2006). In Brief, 2.5 g of the sieved substrate was shaken in 25 mL of deionized water for 2 h at a constant speed (200 rpm). After centrifugation ($10,000 \times g$, 20 min), the suspension was filtered through a 0.45- μm membrane, and the concentration of the dissolved organic carbon in the filtrate was analyzed using a Shimadzu organic carbon analyzer (TOC-5000, Japan). Six DOM fractionations, such as hydrophobic acid (HOA), hydrophobic base (HOB), hydrophobic neutral (HON), hydrophilic acid (HIA), hydrophilic base (HIB), and hydrophilic neutral (HIN), were fractionated according to the procedure suggested by Zhan et al. (2020). The concentrations of the extracted fractions were measured using a Shimadzu organic carbon analyzer (TOC-5000, Japan).

Metal extraction capacity of DOM

After the elimination of cations in the DOM using a cation-exchange resin (Amberlite IR120, Na form, Sigma-Aldrich, USA) according to our previous study (Niu et al., 2021), the solution was diluted to 100 mg C L⁻¹ and mixed with 2 g of the substrate, and then centrifuged at 8000 × g for 30 min. After filtration using a membrane (0.45 μm), the concentrations of Pb, Cu, and Cd in the filtrate was analyzed by ICP-MS (Agilent 7700, USA). In addition, metals in the substrate were extracted using deionized water following the same procedure as that used for the control.

Metal analysis

The prepared soil samples and plant tissues were ground into a powder and sieved with 74-μm meshes. The pulverized sample was dissolved using aqua regia dissolution (Ok et al., 2011). After cooling, the digestion was filtered through a 0.45-μm membrane, and the concentrations of Pb, Cu, and Cd in the recovered filtrate were analyzed using ICP-MS (Agilent 7700, USA).

The fractions of Pb, Cu, and Cd in the rhizosphere soil, including the water-soluble, exchangeable, carbonate, Fe-Mn oxide, organic matter, and residual fractions were determined following the sequential extraction procedure described by Li and Thornton, 2001; Niu et al., 2021). Briefly, 1.2 g of the substrate was shaken with 10 mM CaCl₂ at a constant speed (200 rpm) for 30 min. After centrifugation (4000 × g, 15 min), the suspension was passed through a 0.45-μm membrane, and the concentrations of water-soluble Pb, Cu, and Cd were analyzed using ICP-MS. The recovered material was blended with 1 mM NH₄NO₃ for the extraction of the exchangeable form. The extraction procedure was the same as that described in the previous step. Subsequently, 1 M NaOAc, 25% HOAc containing 0.04 M NH₂·OH·HCl, and 30% H₂O₂ containing 0.02 M HNO₃ were used to extract the carbonate, Fe-Mn oxide, and organic matter fractions in sequence. After extracting the organic matter fraction, aqua regia was used to digest the residual form.

Blank and standard reference materials for the plant (GBW10010) and soil (GBW07410) were used to assess the quality control of the sample analysis.

Statistical analysis

The influence of different cultivation strategies on the biomass yield of the two plant species, DOM concentration and fractionation in the rhizosphere, metal fractions, and metal extraction capacity of DOM were evaluated using the one-way analysis of variance. Statistical analyses were performed using the SPSS 15.0.

Results

Changes in pH under different cultivation strategies

Compared with the initial soil pH (6.1 ± 0.2), the pH in the *S. alfredii* monoculture (5.8 ± 0.3) and *C. arietinum* L. (6.3 ± 0.2) monoculture did not vary significantly, while the intercropping treatment (5.5 ± 0.2)

had a significantly lower soil pH than the initial value. The change in the pH of 0.6 units in the intercropping system could mobilize soil metals, especially Cd (Burges et al., 2017; Strobel et al., 2005). The influence of soil pH on Cd desorption was evaluated by Strobel et al. (2005), who suggested that the distribution coefficients of Cd had log-linear relationships with pH, which can predict the solubility of Cd during soil acidification. Based on Eq. 4 in that study, K_d (Cd) was reduced from 3,419.79 kg⁻¹ at the beginning of the experiment to 2,523.48 and 1,862.09 in the *S. alfredii* monoculture and intercropping systems, respectively, indicating that the change in soil pH was a crucial factor for Cd and Cu mobilization, particularly in the intercropping system.

DOM characterization

The soil DOM level in the soil before transplantation was 136.1 ± 10.2 kg⁻¹. The *S. alfredii* monoculture, *C. arietinum* L. monoculture, and intercropping system obviously enhanced the content of DOM in the soil by 27.3%, 16.9%, and 62.9%, respectively (Fig. 1). Under different cultivation strategies, the *S. alfredii* monoculture and intercropping system resulted in significantly higher levels of DOM than the *C. arietinum* L. monoculture (Fig. 1).

The characteristics of DOM fractionations under different planting strategies are provided in Fig. 2. The highest levels of the hydrophilic fractions, including HIA, HIB, and HIN, were found in the intercropping system, which were significantly higher than those in the two monocultures. In addition, the concentrations of these three hydrophilic fractions in the *S. alfredii* monoculture were greater than those in the *C. arietinum* L. monoculture. Conversely, the levels of the hydrophobic fractions, such as HOA, HOB, and HON, in the *C. arietinum* L. monoculture were significantly higher than those in the *S. alfredii* monoculture and intercropping system, although the total DOM content in the former was significantly lower.

Moreover, the levels of the acid fractions, such as HIA and HOA, in the intercropping system were significantly higher than those in the two monocultures. However, the concentrations of the acid fractions in the *S. alfredii* monoculture were not significantly different from those in the *C. arietinum* L. monoculture (Fig. 2). The DOM was dominated by acid fractions, which constituted 79.3%, 83.2%, and 76.0% of the total DOM in the *S. alfredii* monoculture, *C. arietinum* L. monoculture, and intercropping system, respectively.

Metal extraction capacity of DOM

The metal (Cd, Cu, and Pb) extraction capacity of DOM was significantly stronger than that of deionized water, regardless of the planting strategy. The DOM from the intercropping system had the strongest Cd extraction capacity, followed by that from the *S. alfredii* monoculture and *C. arietinum* L. monoculture (Fig. 3). For Cu and Pb, the DOM from the intercropping system and *S. alfredii* monoculture had a similar extraction capacity, which was significantly higher than that from the *C. arietinum* L. monoculture.

Metal concentrations and fractions

As shown in Table 1, all the treatments did not vary the concentrations of Cd, Cu, and Pb in the rhizosphere significantly, compared with their initial concentrations. This result agrees with Martínez-Alcalá et al. (2016) who reported that *N. caerulea* failed to change the content of Cd in soil during a 132-day phytoextraction.

Table 1
metal concentrations and fractions in the soils under different planting strategies

		water soluble	Exchangeable	Carbonate	Fe–Mn oxide	organic matter	Residual	Total
Cd	before	0.26 ± 0.03a	0.37 ± 0.05a	0.82 ± 0.12a	0.67 ± 0.09a	0.49 ± 0.05c	1.62 ± 0.18a	4.23 ± 0.52a
	S	0.17 ± 0.03b	0.26 ± 0.03b	0.75 ± 0.13a	0.61 ± 0.12a	0.72 ± 0.06b	1.53 ± 0.21a	4.04 ± 0.58a
	C	0.29 ± 0.04a	0.34 ± 0.05a	0.79 ± 0.09a	0.71 ± 0.13a	0.42 ± 0.07c	1.66 ± 0.26a	4.21 ± 0.64a
	S + C	0.13 ± 0.03b	0.19 ± 0.02c	0.77 ± 0.12a	0.56 ± 0.09a	0.87 ± 0.09a	1.59 ± 0.22a	4.11 ± 0.57a
Cu	before	0.65 ± 0.09c	1.17 ± 0.13c	3.97 ± 0.21a	14.49 ± 1.81a	24.55 ± 2.29b	50.87 ± 5.69ab	95.70 ± 10.22a
	S	2.39 ± 0.25a	2.87 ± 0.36a	3.21 ± 0.33b	10.02 ± 1.87b	35.81 ± 3.32a	40.36 ± 5.28b	94.66 ± 11.41a
	C	0.60 ± 0.05c	1.29 ± 0.19c	4.16 ± 0.37a	12.96 ± 1.62ab	26.07 ± 2.72b	52.19 ± 6.07a	97.27 ± 11.02a
	S + C	1.62 ± 0.17b	1.87 ± 0.27b	3.62 ± 0.33ab	10.12 ± 1.26b	37.67 ± 3.91a	40.38 ± 4.62b	95.28 ± 10.56a
Pb	before	1.85 ± 0.11c	3.29 ± 0.51c	12.71 ± 1.63a	21.65 ± 2.71a	8.28 ± 0.59c	35.78 ± 2.87a	83.56 ± 8.81a
	S	2.41 ± 0.16b	7.32 ± 1.03a	10.81 ± 1.39a	17.53 ± 1.79ab	12.56 ± 1.69a	34.36 ± 2.61a	84.99 ± 8.73a
	C	2.03 ± 0.19c	4.96 ± 0.52b	11.92 ± 1.09a	19.76 ± 1.66ab	9.72 ± 0.61b	35.31 ± 2.92a	83.70 ± 6.99a
	S + C	3.12 ± 0.29a	8.31 ± 0.92a	11.26 ± 1.56a	15.65 ± 1.79b	15.22 ± 1.63a	31.56 ± 2.28a	85.12 ± 8.47a

Different letters are significant differences ($p < 0.05$) in concentrations and chemical forms of Cd, Cu, and Pb in the soils under different planting strategies (mg kg^{-1}) estimated using Fisher's LSD post-hoc tests.

Compared with the initial concentrations, the levels of water-soluble and exchangeable Cd decreased significantly in the *S. alfredii* monoculture and intercropping system but did not vary significantly in the *C. arietinum* L. monoculture at the end of the experiment (Table 1). Furthermore, the intercropping system had significantly lower levels of exchangeable Cd than the *S. alfredii* monoculture.

The concentrations of water-soluble and exchangeable forms of Cu and Pb increased significantly in the intercropping system and *S. alfredii* monoculture compared with the initial values, while the levels of these two chemical forms in the *C. arietinum* L. monoculture did not vary significantly (Table 1). The *S. alfredii* monoculture and intercropping system enhanced the proportion of organic matter Cd, Cu, and Pb from 11.0%, 25.7%, and 9.9% before transplantation to 17.8% and 21.2%, 37.8% and 39.5%, and 14.8% and 17.9%, respectively, at the end of the treatments (Table 1). The *C. arietinum* L. monoculture did not vary the proportion of organic matter Cd, Cu, and Pb under any planting strategy.

These results suggest that the hyperaccumulator can mobilize the non-hyperaccumulated metals in the soil by decreasing the soil pH and forming the metal-DOM complexes, as manifested in the significantly higher soluble forms of these metals in its rhizosphere soils, but does not absorb them. This argument is supported by the result of the stronger metal extraction capacity of DOM from the intercropping system.

The concentrations and proportions of the other metal chemical fractions did not vary significantly during treatment.

Phytoextraction potential and ecological risk

Compared with the *C. arietinum* L. monoculture, the intercropping system significantly reduced the shoot Cd concentration in *C. arietinum* L. by 72.7%, which could be attributed to the efficient consumption capacity of *S. alfredii*. However, *S. alfredii* with *C. arietinum* L. significantly increased the concentrations of Cu and Pb in the aerial parts of *C. arietinum* L. by 108.3% and 287.5%, respectively (Table 2). This result, which can be explained by the variations in the chemical fractions of metals under different planting strategies, suggests that intercropped *S. alfredii* decreases the edible safety of *C. arietinum* L.

Table 2

Dry weight and metal concentrations of *S. alfredii* and *C. arietinum* L. under different planting strategies

		<i>S. alfredii</i>		<i>C. arietinum</i> L.	
		roots	shoots	roots	shoots
Biomass	S	0.67 ± 0.09b	8.56 ± 0.91b	-	-
	C	-	-	3.52 ± 0.59a	13.29 ± 1.08a
	S + C	1.16 ± 0.12a	12.17 ± 1.31a	2.89 ± 0.43a	9.87 ± 0.76b
Cd	S	24.3 ± 2.2a	75.6 ± 4.9b	-	-
	C	-	-	1.59 ± 0.12a	0.11 ± 0.02a
	S + C	27.1 ± 3.1a	109.2 ± 5.9a	0.76 ± 0.09b	0.06 ± 0.01b
Cu	S	137.1 ± 9.2b	29.1 ± 1.7b	-	-
	C	-	-	7.32 ± 1.03b	5.19 ± 0.68b
	S + C	182.5 ± 11.3a	33.5 ± 2.9a	19.65 ± 2.26a	10.81 ± 1.23a
Pb	S	276.3 ± 10.2a	68.9 ± 6.1b	-	-
	C	-	-	4.31 ± 0.62b	0.16 ± 0.02b
	S + C	249.6 ± 15.1b	59.6 ± 5.2b	6.03 ± 0.49a	0.42 ± 0.05a
Different letters are significant differences ($p < 0.05$) in dry weight (g) and metal concentrations (mg kg ⁻¹) of <i>S. alfredii</i> and <i>C. arietinum</i> L. under different planting strategies (mg kg ⁻¹) estimated using Fisher's LSD post-hoc tests.					

The metal accumulation in plant tissues was used to evaluate the metal removal efficiency of the plant species under different planting strategies. The concentrations of metals multiplied by the biomass yield of the plant tissues can be regarded as the metal accumulation ability of the plant. The intercropping system significantly increased the dry weights of the roots and shoots of *S. alfredii* (Table 2) because intercropped *C. arietinum* L. can fix approximately 30 kg of nitrogen per ha from the atmosphere. However, the shoot dry weight of *C. arietinum* L. declined significantly in the intercropping system because intercropped *S. alfredii* drove the species to accumulate significantly higher amounts of Cu and Pb in its shoots. The variations in the oxygen free radical generation, chlorophyll levels, and antioxidant enzyme activities in crops under different planting strategies should be analyzed in future work.

Based on the dry weight and metal concentrations, 2.65, 0.01, and 2.72 mg of Cd, 1.36, 0.19, and 1.40 mg of Cu, and 3.10, 0.03, and 2.05 mg of Pb, were eliminated in the *S. alfredii* monoculture, *C. arietinum* L. monoculture, and intercropping system, respectively. The amounts of metals in the substrate were calculated as the soil mass (6 kg) multiplied by the differences between the analyzed Cd (4.2 mg kg⁻¹), Cu (95.7 mg kg⁻¹), and Pb (83.6 mg kg⁻¹) concentrations and their corresponding safe limits (0.3, 50,

and 50 mg kg^{-1} for Cd, Cu, and Pb, respectively). The required planting cycles for remediating the substrate were the quotients of the excessive amounts of metals in the soil and the corresponding metal accumulation in the plant tissues. Therefore, 9, 2,340, and 9 planting cycles are required to eliminate the superfluous Cd in the substrate in the *S. alfredii* monoculture, *C. arietinum* L. monoculture, and intercropping system, respectively. The phytoremediation effect of *C. arietinum* L. is negligible because it is a commercial crop rather than a hyperaccumulator. Although the Cd accumulation capacity of *S. alfredii* in the intercropping system was twice that found in the monoculture (1.36 mg vs. 0.66 mg), the remediation efficiency of these two plant strategies was nearly the same, because only half the number of *S. alfredii* plants was planted in the intercropping system. A suitable planting density would enhance the remediation effect.

The results indicate that 201, 1,443, and 196 planting cycles and 65, 6,720, and 98 planting cycles are required to remove excessive Cu and Pb from the soil in the *S. alfredii* monoculture, *C. arietinum* L. monoculture, and intercropping, respectively. The required time is unacceptably long, and the intercropping method did not significantly improve the remediation effect.

Discussion

Influences of pH variations on Cd mobilization

The bioavailability of heavy metals can be classified into three categories: readily bioavailable (Cd, Zn, As, and Cu), moderately bioavailable (Co, Mn, and Fe), and least bioavailable (Pb and Cr) (Ali et al., 2013). In general, soil pH can influence the solubility and toxicity of readily bioavailable metals, which are aggravated by decreasing soil pH (Burgess et al., 2017).

Variations in the soil pH in a plant-growing substrate are partly determined by the balance of the uptake of soil cations or anions by plants during phytoremediation, and the concomitant release of hydrogen ions, hydroxyl ions, and organic acids. In addition, variations in pH can also be attributed to the respiration of plant roots and soil microorganisms, which vary significantly in the levels and forms of CO_2 in the rhizosphere, thus changing the soil pH (Kim et al., 2010). Some related studies have evaluated the changes in soil pH in non-hyperaccumulator species cultivating substrates with inconsistent findings (Kim et al., 2010; Loosemore et al., 2004). For example, *Nicotiana tabacum* L. declined the soil pH by 1.4 units after 8 d of culture (Loosemore et al., 2004), and *Brassica juncea* raised the soil solution pH by 1.3 units within 6 weeks (Kim et al., 2010). For hyperaccumulator planting soils, variations in the soil pH are also inconsistent. For example, *N. caerulescens* induced the leaching of Pb during phytoextraction by reducing the soil pH (Luo et al., 2019b), while *S. alfredii* did not significantly change the pH of the rhizosphere and bulk soil, which is in agreement with the results of this study. Therefore, the change in pH is not an exclusive explanation of metal uptake for both hyperaccumulators and non-hyperaccumulators. Christensen and Christensen (2000) estimated the influences of pH and DOM on the activity and toxicity of different metals and suggested that chemical fractions of metals were

predominantly controlled by the characterization of DOM when the soil pH was greater than or equal to 5.5. Therefore, the DOM characteristics under different cultivation strategies were evaluated in this study.

Influences of DOM content and fractionations on Cd activation

The probable interpretation of the variations in the soil DOM after plant growth is exudation by the root system of DOM to activate and dissolve specific elements and compounds for nutrient uptake. For instance, Cao et al. (2018) observed that the growth of *Pennisetum americanum* significantly enhanced the levels of DOM in the rhizosphere by releasing root exudates containing organic acids and phenolic compounds. Li et al. (2013a) characterized DOM in the rhizosphere of hyperaccumulating ecotype and non-hyperaccumulating ecotype of *S. alfredii* and determined that both ecotypes increased the DOM levels in the soil, and the magnitude of enhancement in DOM for the hyperaccumulating ecotype was much higher than for the non-hyperaccumulating ecotype, which agrees with the results of the current study.

The impacts of intercropping on the yield, quality, and phytoremediation efficiency of plants has been fully revealed in previous studies. Because plants differ in the spatial patterns of nutrient acquisition, intercropping systems can capture the necessary growth resources, such as illumination, water, and nutrients, more effectively than a single species (Hong et al., 2017). As a nitrogen fixer, *C. arietinum* L. can enhance the growth of *S. alfredii* by fixing approximately 30 kg of nitrogen per ha from the atmosphere and decreasing intraspecific competition in the intercropping system by developing a canopy stratification (Luo et al., 2016).

Previous research has focused on the intercropping of hyperaccumulators with agricultural products or commercial crops on single metal-polluted soils to decrease the metal content in crops, especially in edible parts, which could allow safe agricultural production (Lin et al., 2015; Zeng et al., 2019b). Moreover, intercropped hyperaccumulators could protect neighboring crops by depleting specific metals in shared rhizospheres, enhancing the metal tolerance and edible safety of the companion crops. In contrast, researchers also found that the high metal activation capacity of hyperaccumulators could increase the risk of metal transportation to underground water, deep soil layers, or the food chain (Gove et al., 2002; Wu et al., 2007). However, these studies demonstrated the mechanisms of intercropping on phytoremediation efficiency from the perspective of the soil pH, especially in single metal-polluted fields, and available information on the influences of the DOM characteristics on the phytoremediation effect in soils polluted with multiple metals during soil remediation processes is limited.

C. arietinum L. is a leguminous plant that can vary the organic components in the soil. For instance, Zu et al. (2020) characterized the activity of Cd and the fractionation of DOM in the intercropping of *S. asper* and *V. faba*, and determined that intercropped *V. faba* secreted more citric, oxalic, acetic, and malic acid compared with the *S. asper* monoculture, because the intercropped nitrogen fixer can enhance nutrient bioavailability in the shoots of neighboring crops, promote ecological functions, and consequently alter the rhizosphere environments and root-excreted organic acid composition and proportions. This result agrees with the findings of the present experiment in that the intercropping system varied the content and

composition, especially the hydrophilic and acid fractions, in the rhizosphere soil more than in the two monocultures.

The fractionation of DOM greatly influences the activity and toxicity of soil metals. For instance, Wang et al. (2017) reported that the chemical activity and affinity of hydrophilic organic compounds are greater than those of hydrophobic compounds by comparing their bioconcentration factors. Moreover, the acid fractionations, which have a significantly higher degree of concentration in the intercropping system than those in the two monocultures in the current experiment, present a significantly greater affinity to ions compared with other DOM fractionations. Therefore, it is safe to hypothesize that DOM from the intercropping system could mobilize more metals in the soil compared with that from the two monocultures. To verify this hypothesis, the metal extraction capacities of DOM from different planting strategies were compared and discussed in this study.

Influences of planting strategies on metal extraction capacity of DOM

Metal extraction capacity of DOM from different planting strategies were consistent with the findings that the concentrations and proportions of hydrophilic fractions in the *S. alfredii* monoculture and intercropping system were significantly higher than those in the *C. arietinum* L. monoculture. Moreover, Chen et al. (2018) revealed the influence of DOM on Zn extractability in calcareous soil and found that the addition of maize straw, which supplied DOM to the soils, enhanced the concentration of extractable Zn in the central layer compared with the control. They attributed this enhanced extractable Zn to the formation of Zn-DOM compounds with the supply of DOM derived from the straw. Liu et al. (2012) determined that legumes, such as *Kummerowia striata*, *Glycine max*, and *Vigna unguiculata*, can increase the total Cd uptake of neighboring crops by secreting low molecular weight organic acids, especially acidic organic compounds, into the soils, which is in agreement with the results of the current study that the concentrations and proportions of acid fractions in the intercropping system were significantly higher than those in the two monocultures. These observations could partially explain, why DOM from the intercropping system had a significantly stronger metal-extraction capacity than that from the *C. arietinum* L. monoculture. However, the variations in metal mobilization in different planting strategies could also be explained from the perspective of metal fractions, and the changes in the metal concentrations and fractions were examined in this study.

Influences of planting strategies on chemical forms of metals in soils

The variations in the levels of soluble Cd in different planting strategies were determined by the balance between the uptake and replenishment rate of Cd. Li et al. (2013a) found a substantial valley of Cd around the rhizosphere of *S. alfredii* owing to the high Cd consumption capacity of the hyperaccumulator. In contrast, Zhan et al. (2018) observed that the concentrations of Cd and Pb in the rhizosphere of *Athyrium wardii* were higher than those in the bulk soil. These results suggest that hyperaccumulators can assimilate the corresponding hyperaccumulated metals from their rhizosphere soils faster than the replenishment rate, whereas non-hyperaccumulators cannot.

The soluble forms of metals that can be absorbed by plant species directly under all the planting strategies accounted for less than 5% of the concentrated amount in the plant organs, demonstrating that more than 95% of the extracted metals come from other fractions. The variations in the metal fractions during plant growth under different plant strategies indicate that the *S. alfredii* monoculture and intercropping systems can mobilize the metal fractions that cannot be absorbed by plants. The characteristics of soil DOM can change the metal mobilization. It has been reported that metal-DOM complexes can also be extracted by plant species under specific conditions (Hu et al., 2018; Kim et al., 2010), and the variations in the organic matter forms of metals were consistent with the finding that the metal extraction capacities of DOM from the intercropping system and *S. alfredii* monoculture were significantly higher than those from the *C. arietinum* L. monoculture.

This study demonstrated that the intercropping method applied to soil polluted with multiple metals did not improve the phytoremediation effect of *S. alfredii* for Cd owing to the halved planting density but decreased the edible safety of *C. arietinum* L. by increasing the concentrations of Cu and Pb in its shoots. These results can be attributed to the decreased soil pH and increased concentrations of DOM, hydrophilic fractions, and acid fractions in the intercropping system. Therefore, intercropped *S. alfredii* and *C. arietinum* L. did not present a suitable land use pattern for multi-metal-polluted soil.

Conclusion

This study evaluated the effects of intercropping methods on the phytoremediation effect of a hyperaccumulator and the edible safety of a commercial crop. The results suggested that the intercropping method decreased the soil pH compared with the initial value, enhancing the release amount and rate of metals in the rhizosphere soils of the plants. Moreover, the content, hydrophilic fractions, and acid fractions of DOM were significantly higher in the intercropping system than those in the *C. arietinum* L. monoculture, which can effectively mobilize metals, including Cd, Cu, and Pb, by generating metal-DOM complexes. The concentrations of soluble Cd decreased significantly in the *S. alfredii* monoculture and intercropping system compared with the initial values owing to the strong Cd uptake capacity of the hyperaccumulator, while the variations in soluble Cu and Pb during the experiment exhibited an opposite trend, because the hyperaccumulator can transform other insoluble forms of these metals into soluble fractions. Furthermore, the metal extraction capacities of DOM from the intercropping system were significantly stronger than those from the *C. arietinum* L. monoculture, thereby increasing Cu and Pb concentrations in *C. arietinum* L. in the intercropping system. The results of this experiment indicate that the intercropping method not only failed to enhance the phytoremediation efficiency of *S. alfredii*, but also decreased the edible safety of *C. arietinum* L. Therefore, intercropped *S. alfredii* and *C. arietinum* L. did not present a suitable land use pattern for multi-metal-polluted soil.

Declarations

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Authors' contributions:

Hong Niu: formal analysis, investigation, writing - Original Draft;

Yifan Jia: formal analysis, writing - Original Draft;

Jie Sun: investigation, writing - Original Draft;

Pan Yang: formal analysis

Min Cao: writing - Review & Editing;

Jie Luo: conceptualization, methodology, validation, writing - Review & Editing, supervision, funding acquisition.

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Figures

Figure 1

Concentrations of DOM under different planting strategies Different letters are significant differences ($p < 0.05$) in DOM levels (mg kg⁻¹) evaluated using Fisher's LSD post-hoc tests.

Figure 2

Hydrophilic and hydrophobic fractionations under different planting strategies Different letters are significant differences ($p < 0.05$) in fractionations (mg kg^{-1}) evaluated using Fisher's LSD post-hoc tests. HIA, HIB, HIN, HOA, HOB, and HON are hydrophilic acid, hydrophilic base, hydrophilic neutral, hydrophobic acid, hydrophobic base, and hydrophobic neutral, respectively.

Figure 3

Metal extraction abilities of DOM from different planting strategies Different letters are significant differences ($p < 0.05$) in concentrations of Cd, Cu, and Pb extracted by DOM from different planting strategies (mg kg^{-1} soil) evaluated using Fisher's LSD post-hoc tests.