



Full Length Article

Inoculation of AM Fungi: An Effective Tool to Reduce Cd Accumulation in Peanut Kernel

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Abstract

China is an important global peanut producer and exporter. Nevertheless, excessive Cd in peanut kernels has become an important constraint of peanut exportation. Concerns over the heavy metal contamination in food crops have prompted scientists to find ways to solve these problems, including applications of microbiology. In the present research, a greenhouse pot culture experiment was conducted to investigate the effect of arbuscular mycorrhizal fungi (AMF) inoculation on cadmium (Cd) uptake and translocation in peanut plants at different Cd levels. The peanut seeds were sown in pots, where culture substrate was previously mixed with a given amount of *Glomus intraradices* and 0, 2 and 10 mg kg⁻¹ Cd. In the control plot, *G. intraradices* was inactivated before mixed into culture substrate. Symbiotic relationships were successfully established between AMF and peanut root at all Cd levels with an average colonization rate of 65.0%. Compared with control plants, AMF inoculation significantly improved phosphorus nutrition supply to peanut plants, increased chlorophyll by 7.5%, photosynthesis by 11.8%, transpiration by 13.9% and root dry weight by 27.0%. In AMF inoculated peanut plants, the concentration and accumulation of Cd were 45.9 and 87.4% higher, respectively, in root system but 31.1 and 31.8% lower, respectively in the aboveground part than in the control plants. At the Cd level of 10 mg kg⁻¹, the translocation rate of Cd in AMF inoculated peanut plants was 51.8% lower than in AMF non-inoculated plants. In summary, AMF inoculation could improve peanut plant growth, result in Cd immobilisation in the peanut root system and inhibit Cd translocation in peanut plants. In conclusion, AMF inoculation may be an effective way to reduce Cd accumulation in peanut kernels. © 2017 Friends Science Publishers

Keywords: Arbuscular mycorrhizal fungi; Cadmium; Colonization rate; Heavy metal; Peanut; Translocation

Introduction

Heavy metals are mobilized into the environment through extensive use of fertilisers and pesticides and rapid industrial development. Soil erosion problems, such as exceed of overland flow and soil losses are enhancing in conventional agricultural plots this problematic issue (Rodrigo Comino *et al.*, 2016a, b; García-Díaz *et al.*, 2017). This phenomenon leads to global concern on heavy metal pollution of soils (Marques *et al.*, 2011; Hassan *et al.*, 2013). According to a soil pollution survey in China, the cadmium (Cd) concentration in cultivated lands is 7.0% higher than the threshold (Zhang and Zhang, 2015). This indicates the poor soil quality of the cultivated lands in China. Cd in soil is absorbed by root system of plants. Cd can also be translocated into plants and accumulate in edible parts. These properties of Cd increase its risks of uptake by human through food chains, thus, threatening human health (Ren *et al.*, 2003; Qiu *et al.*, 2011). As an oil crop with high protein

content in the seeds, peanut (*Arachis hypogaea* L.) plants can easily accumulate Cd in the kernels compared with other crops (Wang, 2002). Previous research showed that Cd concentration in peanut kernels was much higher than the limitation of FAO/WHO food standards of Cd ≤ 0.1 mg kg⁻¹ in spite of the unpolluted peanut producing areas (Wang *et al.*, 2007a, b). Concerns over the heavy metal contamination in food crops have prompted scientists to find ways to solve this problem. Application of microbial inoculants including arbuscular mycorrhizal fungi (AMF) is one of the options.

AMF can form symbiotic mycorrhizal structures with the root system in more than 90% terrestrial vascular plants and such structures can facilitate soil mineral (e.g., P, N and K) uptake of host plants, improve nutrition to plants and consequently promote plant growing (Smith and Read, 2008; Grunwald *et al.*, 2009). It is reported that AMF can reduce heavy metal concentration in host plants (Lins *et al.*, 2006; Lin *et al.*, 2007; Wong *et al.*, 2007; Słomka *et al.*, 2011). Chen *et al.* (2007) and Dong *et al.* (2008) have

concluded that the aboveground part of plants inoculated with AMF showed lower concentrations of Cd, As and Cu. As reported by Bissonnette *et al.* (2010), inoculation with *G. intraradices* reduces Cd and Cu concentrations in the aboveground part of *Salix viminalis*. By contrast, other studies have stated that AMF inoculation cannot reduce the heavy metal uptake of plants, because they increase their tolerance to heavy metals, such as Cd (de Andrade *et al.*, 2008), Cu (Andrade *et al.*, 2010) and Zn (Hidebrandt *et al.*, 2006). Nowadays, more studies have reported the effect of AMF on the tolerance of host plants to heavy metals. In general, AMF does not only resist heavy metal toxicity, but also directly or indirectly influences the growth and heavy metal uptake and translocation in host plants, thus improving their heavy metal tolerance (Audet and Charest, 2006; Göhre and Paszkowski, 2006; Hildebrandt *et al.*, 2007; Feddermann *et al.*, 2010; Miransari, 2011). Although reports have confirmed benefits of several crops from AMF inoculation in reduction of heavy metals, there has been almost no document that demonstrates details of effects of AMF inoculation on Cd uptake and translocation in peanut crops.

Peanut is a major oil-bearing crop in the world and important resource of plant protein directly consumed or used for food processing (Liu *et al.*, 2012). The heavy metal Cd easily accumulates in peanut kernels and mainly combined with proteins and carbohydrates (Wang, 2002). Although Cd in peanut kernels does not influence oil products, it threatens the quality and safety of directly edible products (Wang and Zhang, 2008). China is an important global peanut producer and exporter. Peanut exports account for more than 40% of the world's total peanut trade (Wan, 2009). Nevertheless, excessive Cd in peanut kernels has become an important constraint of peanut exportation (Wang and Zhang, 2008). Peanut is usually grown on sandy soil with poor fertilization in China (Zuo *et al.*, 2000; Wan, 2003). Poor soils are beneficial for AMF colonization to host plants and increases mycorrhizal effect (Schwab *et al.*, 1983; Abbott *et al.*, 1984). Therefore, in this study, Cd pollution was simulated through a greenhouse pot culture experiment. The main aims of this study were to: (1) elucidate whether AMF could form a good symbiosis with peanut root under Cd pollution, (2) study the effect of AMF on peanut growth and P uptake at different Cd levels; and (3) analyze Cd uptake and distribution in peanut plants. Research results are expected to provide scientific references to prevention of Cd pollution and improvement of hygienic quality of peanut.

Materials and Methods

Biological Materials and Soil

The culture substrate was a mixture of soil and river sands (2:1). The used soil was a typical fluvo-aquic soil with the following physico-chemical properties: pH (water/soil in

volume: 2.5) 8.37, 16.35 g kg⁻¹ organic matter, 0.04% total nitrogen, 6.70 mg kg⁻¹ available phosphorus, 60.64 mg kg⁻¹ available potassium and 0.46 mg kg⁻¹ total Cd. The river sand sample was washed by water for 5 times. The soil and river sand sample was air dried, sieved (2 mm) and dry-heat sterilised at 121°C for 2 h.

The mycorrhizal inoculum of *G. intraradices* was obtained from the Beijing Academy of Agriculture and Forestry Sciences. The inoculum material contained spores, mycelium, sandy soil and host plant root fragments.

Experimental Design

The experiment was a 2×3 factorial design with mycorrhizal colonization (inoculated or non-inoculated) combined with three levels of Cd (0, 2, 10 mg kg⁻¹, as 3CdSO₄ 8H₂O in aqueous solution) with 4 replicates. Cylindrical plastic pots of 20.5 cm (upper calibre) × 13 cm (lower calibre) × 14 cm (height) were used as planting containers. The pots were immersed in 0.5% KMnO₄ solution overnight, cleaned and then dried. Each pot was filled with 3 kg of culture substrate. In all treatments, basal nutrients were added to the culture substrate at the rates of 150 mg kg⁻¹ N (Ca(NO₃)₂), 15 mg kg⁻¹ P (KH₂PO₄) and 100 mg kg⁻¹ K (K₂SO₄). The culture substrate was equilibrated for one week after the nutrients were thoroughly mixed. Plants of peanut (*Arachis hypogaea* L. cv. Huayu) were grown in plastic pots containing 50 g of the mycorrhizal inoculum, covered with 1.8 kg culture substrate. In the control plot, each pot contained 50 g of dry-heat sterilised mycorrhizal inoculum and 25 mL of the mycorrhizal inoculum filtrate. Full and even-sized peanut seeds were surface sterilised with 10% (v/v) hydrogen peroxide for 10 min and washed with deionised water. The seeds were then incubated at 28°C in RDN-1000D-3 Artificial Climate Chest (Ningbo Southeast Instrument Company, China) for accelerated germination. Four seeds with similar size were sown per pot. All peanut plants were cultured in a glass greenhouse with natural illumination. The seedlings were trimmed to three plants with similar growth per pot 4 d after emergence.

Sample Analysis

After 9 weeks of cultivation, chlorophyll concentration in leaves was estimated by the soil and plant analyzer development (SPAD) value using SPAD-502 Chlorophyll Meter (Konica, Japan), leaf photosynthetic rate (Pn) and transpiration rate (Tr) were determined by using CIRAS-3 Portable Photosynthesis System (PP Systems, USA). Afterwards, the plants were harvested, washed and dried. The plants were divided at the basal part of the stem into the aboveground part and the root system. Firstly, root length was measured using a scanner (EPSON PERFECTION V700 PHOTO, Japan) and root system analysis software (WinRHIZO Pro, Version 2004a, Canada). Then, the roots were cut into 1 cm segments. About 0.5 g of the fresh root was randomly weighed and then stained with trypan blue to

determine the mycorrhizal colonization rate through the root segment observation method (Phillips and Hayman, 1970). The remaining root system and aboveground part were subjected to enzyme deactivation for 30 min at 105°C and then dried to a constant weight at 78°C. The biomass of the remaining root system and aboveground part were then tested. Finally, the dried samples under different treatments were ground and digested to determine Cd and P concentration.

The Cd concentration in the plant and soil were processed using HNO₃-H₂O₂ and HNO₃-HF-H₂O₂ digestive system, respectively, and then sealed in high-pressure digestion tank. Cd concentration was evaluated through graphite furnace atomic absorption spectrometry (AA-7000 SHIMADZU, JAPAN). The translocation rate of Cd in peanut plants was calculated based on the translocation rate definition by Suzuki *et al.* (2008) through the following formula:

$$\text{Cd translocation rate (\%)} = \frac{\text{Cd accumulation in the aboveground part}}{\text{Total Cd accumulation of peanut plant}} \times 100$$

The P concentration in the plant was determined after the samples were digested with H₂SO₄-H₂O₂. The soil physico-chemical properties were determined using conventional methods (Lu, 2000).

Data Analysis

Microsoft Office Excel software was adopted for data processing and diagramming. Results are presented as means ± standard deviation (n = 4), and all data were subjected to multi-comparisons by the least significant difference (LSD) at the 5% level using Statistical Product and Service Solutions software.

Results

Mycorrhizal Colonization Rate and Peanut Plants Growth

The mycorrhizal colonization rate, biomass, root length and leaf SPAD value of peanut plants under different treatments were presented in Table 1. No mycorrhizal colonization was found in the root of non-inoculated peanut plants. The mycorrhizal colonization rate in the AMF inoculated peanut root system was maintained at a high level with soil Cd concentration increasing.

The biomass of AMF inoculated and non-inoculated peanut plants significantly decreased with increasing Cd addition level (Table 1). Under a similar Cd addition level, AMF inoculation did not obviously accelerate the growth of the aboveground part. However, AMF inoculated plants presented leaf SPAD value, root biomass and root length significantly higher than AMF non-inoculated plants. At Cd addition level of 10 mg kg⁻¹, the leaf SPAD value, root biomass and root length of AMF inoculated plants were 5.8%, 20.1% and 16.3%, respectively higher than those of the control group.

Phosphorus Nutrition of Peanut Plants

The concentration and accumulation of P in all peanut plants declined with increasing Cd addition to the soil (Table 2). At Cd addition levels of 2 and 10 mg kg⁻¹, AMF non-inoculated peanut plants exhibited P deficiency. Under a similar Cd addition level, AMF inoculated plants presented higher P concentration and accumulation than non-inoculated plants. At Cd addition level of 10 mg kg⁻¹, the P concentration in the shoots and roots of AMF inoculated plants increased by 75.8% and 68.4%, respectively. At Cd addition level of 10 mg kg⁻¹, the P accumulation in the shoots and roots of AMF inoculated plants increased by 41.3% and 52.9%, respectively.

Net Photosynthetic Rate and Transpiration Rate of Peanut Leaves

The Pn and Tr in all peanut leaves declined with increasing Cd addition levels (Fig. 1). Under a similar Cd addition level, the inoculation of AM fungi significantly increased the Pn and Tr in peanut leaves. At Cd addition level of 0, 2, 10 mg kg⁻¹, compared with those of non-inoculated plants, Pn and Tr of AMF inoculated peanut leaves increased by 10.3, 10.8, 14.3% and 22.0, 11.0 and 8.6%, respectively.

Cd Concentration and Accumulation in Peanut Plants

The Cd concentration and accumulation in the aboveground part and root system under different treatments are shown in Table 3. Cd concentration and accumulation differed between AMF inoculated and non-inoculated peanut plants in treatments without Cd, indicating the strong dependence of peanut on the mycorrhizae. In treatments with Cd, the Cd concentration and accumulation in the aboveground part and root system of all peanut plants were proportional to the Cd concentration in the soil.

At the same Cd addition level, compared with non-inoculated plants, the inoculation of AM fungi significantly decreased the Cd concentration and accumulation in the aboveground part but increased those in the root system. At Cd addition level of 2 and 10 mg kg⁻¹, AMF inoculated peanut plants demonstrated 22.8 and 31.0% lower Cd concentration, respectively, in the aboveground part and 32.4 and 29.0% higher Cd concentration, respectively, in the root system. The Cd accumulation in the aboveground part of AMF inoculated peanut plants decreased by 22.6-32.0%. The Cd accumulation in the root system of AMF inoculated plants was significantly higher (about 1.6 times) than those of the control group.

The Translocation Rate of Cd in Peanut Plants

As shown in Fig. 2, the translocation rate of Cd in AMF inoculated peanut plants was significantly lower than that in the corresponding control group under a similar soil Cd

Table 1: Peanut plants growth and mycorrhizal colonization rate

Cd level (mg kg ⁻¹)	Treatment	Shoot dry weight (g)	Root dry weight (g)	Root length (cm)	SPAD value	Colonization rate (%)
0	-AM	7.16±0.27a	1.89±0.09bc	1688.03±5.03c	45.18±0.94c	0
	+AM	7.29±0.18a	2.70±0.06a	2170.20±8.50a	47.75±0.63a	65±4a
2	-AM	6.62±0.33b	1.67±0.13d	1560.25±7.09e	42.19±1.03d	0
	+AM	6.63±0.26b	1.97±0.11b	1738.48±6.66b	46.83±0.72ab	66±3a
10	-AM	5.66±0.19c	1.44±0.07e	1380.00±6.03f	43.00±0.47d	0
	+AM	5.75±0.05c	1.73±0.03cd	1604.27±7.02d	45.50±0.37bc	65±3a

-AMF and +AMF represent non-inoculation treatment and inoculation treatment with *G. intraradices*, respectively. Data are the means of four replicates ± standard deviation. Different letters in the same column indicate significant differences by LSD multi-comparison at 5% level.

Table 2: P nutrition of peanut plants under different treatments

Cd level (mg·kg ⁻¹)	Treatment	P concentration (mg g ⁻¹)		P accumulation (mg g ⁻¹)	
		Shoot	Root	Shoot	Root
0	-AM	0.68±0.02b	1.50±0.08b	4.95±0.17b	2.82±0.08b
	+AM	0.85±0.03a	1.66±0.04a	6.08±0.12a	4.48±0.07a
2	-AM	0.42±0.03d	1.03±0.04d	2.76±0.23e	1.72±0.14e
	+AM	0.59±0.04c	1.34±0.03c	3.90±0.16c	2.63±0.10c
10	-AM	0.33±0.01e	0.79±0.06e	1.88±0.10f	1.15±0.06f
	+AM	0.58±0.03c	1.33±0.03c	3.26±0.06d	2.30±0.03d

-AMF and +AMF represent non-inoculation treatment and inoculation treatment with *G. intraradices*, respectively. Data are the means of four replicates ± standard deviation. Different letters in the same column indicate significant differences by LSD multi-comparison at 5% level

Table 3: Cd concentration and accumulation in peanut shoots and roots under different treatments

Cd level (mg kg ⁻¹)	Treatment	Cd concentration (mg kg ⁻¹)		Cd accumulation (µg g ⁻¹)	
		Shoot	Root	Shoot	Root
0	-AM	1.34±0.14e	1.93±0.16f	9.75±0.69e	3.65±0.48f
	+AM	0.81±0.14f	3.41±0.93e	5.78±0.35f	9.18±0.58e
2	-AM	5.65±0.76c	13.41±0.65d	37.39±0.75c	22.43±0.95d
	+AM	4.36±0.11d	17.76±0.72c	28.93±0.66d	34.99±0.92c
10	-AM	18.88±0.61a	51.61±0.53b	108.56±1.14a	74.36±1.03b
	+AM	13.04±0.96b	66.58±0.98a	73.72±1.16b	114.87±0.96a

-AMF and +AMF represent non-inoculation treatment and inoculation treatment with *G. intraradices*, respectively. Data are the means of four replicates ± standard deviation. Different letters in the same column indicate significant differences by LSD multi-comparison at 5% level

concentration. At Cd addition level of 10 mg kg⁻¹, the translocation rate of Cd in AMF inoculated peanut plants was only 39.1% and that in AMF non-inoculated plants reaches as high as 59.4%.

Discussion

AMF's colonization may affect plants differently under heavy metal pollution. The different responses of plants are closely correlated with AMF species, physiological and biochemical properties of host plants and the environmental conditions (e.g., physicochemical properties and heavy metal species). In this study, the mycorrhizal colonization rate in the peanut root system inoculated with *G. intraradices* was maintained at a high level. Soil Cd concentration increasing did not affect the mycorrhizal colonization rate. As reported by Leyval *et al.* (1997), the effect of heavy metal is different among AMF species and this has reflected the different resistance among AMF. The results obtained showed that *G. intraradices* exhibited high tolerance to Cd toxicity. In the present study, AMF inoculation significantly improved peanut growth. The root system biomass and root length significantly increased.

These increases indicated that mycorrhizae alleviated Cd toxicity and accelerated the root growth of peanut plants, thus maintaining root growth and nutrient uptake under Cd pollution. Punamiya *et al.* (2010) revealed that *Chrysopogon zizanioides* planted in soils polluted with Pb considerably increased the biomass after inoculating *Glomus mosseae*. Solís-Domínguez *et al.* (2011) found that the root length of AMF inoculated mesquite plants grown in acidic lead/zinc mine tailings increased by 27%-47%.

As reported by Madejón *et al.* (2010) with a native Australian grass, AMF inoculation promotes the uptake of nutrients and water, increases the leaf chlorophyll concentration, and as a consequence improves leaf photosynthesis and results in promotion of plant growth and increase in plant biomass of the host plant. Usually, mycorrhizal inoculation accelerates plant growth by increasing P uptake of host plants (Wang *et al.*, 2005). Cd²⁺, Cu²⁺, Zn²⁺ and Pd²⁺ can react and reduce the availability of phosphate in soils, causing difficulty of P uptake by plants. Nevertheless, mycorrhizal plants can improve P uptake by exploiting the vast mycelium network of the underground part (Luo *et al.*, 2013). In this study, AMF inoculation significantly increased the P

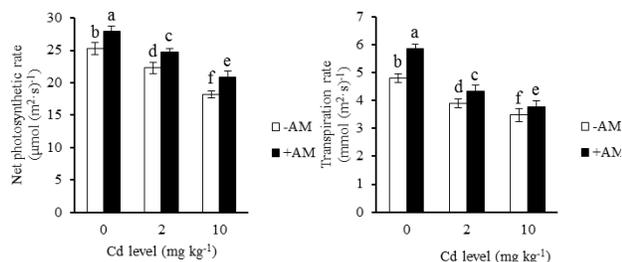


Fig. 1: Effect of different treatments on net photosynthetic rate and transpiration rate of peanut leaves

-AMF and +AMF represent non-inoculated treatment and inoculation treatment with *G. intraradices*, respectively. Bars represent means \pm standard deviation (n = 4). Different letters above the in the bars indicate significant differences by LSD multi-comparison at the 5% level

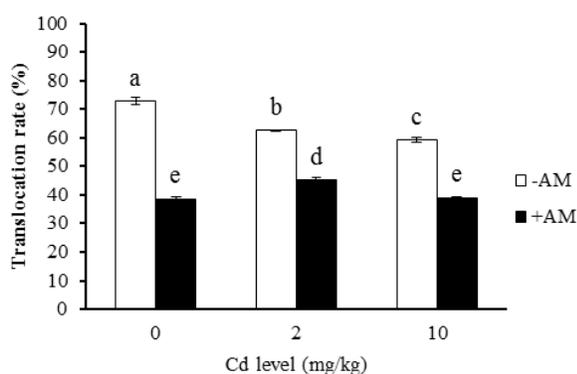


Fig. 2: Cd translocation rate in peanut plants

-AMF and +AMF represent non-inoculated treatment and inoculation treatment with *G. intraradices*, respectively. Bars represent means \pm standard deviation (n = 4). Different letters above the in the bars indicate significant differences by LSD multi-comparison at the 5% level

concentration and accumulation in the peanut plants. Moreover, the SPAD value, Tr and Pn in AMF inoculated peanut leaves increased significantly. As reported by Andrade *et al.* (2010), AMF significantly improves P uptake of *Coffea Arabica* seedlings in soils polluted by heavy metals. Thus, *G. intraradices* inoculation could accelerate peanut plants growth via improving P uptake, resulting in the peanut plants tolerance to Cd increased.

AMF can store abundant heavy metals in the root system through the vast mycelium network (Hildebrandt *et al.*, 2007; Garg and Chandel 2012; Wang *et al.*, 2012). AMF can also increase metals chelate in the host plant root (Leyval *et al.*, 1997). Heavy metals chelated in the root are not easily translocated to other plant parts. Heavy metals may also be stored in the fungal structure (e.g., intraradical hyphae, arbuscules and vesicles) in the AMF root system, thus reducing the translocation of heavy metals to plant cells. This process is called “compartmentation”. Chen *et al.* (2007) has discovered that AMF colonization changes bio-adsorption characteristics and results in heavy metal retention by the root system to a certain extent. In the present experiment, mycorrhizal peanut significantly

increased Cd concentration and accumulation in the root system but decreased those in the aboveground plant parts. This finding indicated that AMF inoculation could adjust the distribution of Cd in peanut plants, reduce the translocation of Cd from root system to the aboveground part, and thus alleviate Cd toxicity in the aboveground part of peanut plants. Similar to our results, Garg and Aggarwal (2012) found that *G. mosseae* inoculation increased the Cd concentration in the root system of pigeon pea but reduced Cd translocation to the aboveground parts. Chen *et al.* (2005) reported that AMF inoculation increased Cd accumulation in the root of *Medicago sativa* but reduced Cd accumulation in the aboveground part. In summary, AMF could store Cd within the AMF-plant root symbiont by accelerating root growth and inhibiting Cd translocation to the aboveground parts.

The present research confirmed that peanut and AMF could form a positive symbiotic relationship under Cd pollution. AMF inoculation could significantly improve P nutrition, increase leaf chlorophyll concentration and photosynthesis, thus accelerating root growth of peanut plants. AMF inoculation could also change the Cd uptake and distribution in peanut plants, and inhibit Cd translocation to the aboveground parts. As a preliminary understanding, AMF inoculation alleviates Cd contamination in peanut seeds. Previous studies have revealed that Cd in peanut kernel mainly come from the uptake by roots and the transport pathway through “root - stem (leaf) - kernel” (Popelka *et al.*, 1996; McLaughlin *et al.*, 2000). Theoretically, AMF inoculation reduces Cd accumulation in peanut kernels. However, the present study focuses on the early growth stage of peanut. Further research is required to determine whether the positive effect of AMF inoculation changes as peanut plant grows. Moreover, greenhouse pot experiments are the cultivation experiments under controlled conditions. Due to the strict control of water, nutrient, temperature and light conditions, pot experimental results are more accurate than the field experiments.

Conclusion

AMF inoculation resulted in successful establishment of symbiotic relationships between the AMF and peanut root under Cd pollutions irrespective of soil Cd concentrations. AMF inoculation significantly improved phosphorus nutrition supply and facilitated P uptake by peanut plants, increased leaf chlorophyll, photosynthesis, and consequently resulted in promotion of peanut plant growth and increase in plants biomass. Moreover, mycorrhizal peanut significantly inhibited the translocation of Cd from root system to the aboveground parts and reduced the Cd concentration and accumulation in the aboveground part. In conclusion, AMF inoculation may be an effective way to reduce Cd accumulation in peanut kernels.

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