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Prevention of Cadmium Contamination by Microbial Inoculant and Its Potential Mechanism

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Abstract: Cadmium is the main heavy metal contaminant of food in the world. The extent of cadmium pollution in peanut in China remains unclear. To determine the cadmium pollution level in peanut, samples from the main producing regions in China were assessed. The findings revealed that the cadmium pollution level in Chinese peanuts was relatively low. Moreover, the Aflatoxin Rhizobia Couple *B. amyloliquefaciens*, *B. laterosporu*, *B. mucilaginosus*, *E. ludwiggi* (ARC-BBBE) microbial inoculants on cadmium contamination in peanut were evaluated. The fertilization methods were categorized into conventional fertilization and conventional fertilization supplemented with 60 kg/hectare of microbial inoculant ARC-BBBE as the base fertilizer. The cadmium contents in the soil and peanut plant parts were detected and analyzed. The results demonstrated that the microbial inoculant ARC-BBBE significantly reduced the total cadmium content in peanut, as well as the available cadmium and exchangeable cadmium in soil. Furthermore, the pH and urease and alkaline phosphatase activities in soil were significantly enhanced, suggesting that the microbial inoculant ARC-BBBE decreased cadmium content in soil and reduced the cadmium uptake by plants through a combination of the action of the bacteria itself and the secretion of extracellular substances. This ultimately achieves the goal of reducing the cadmium content in peanut seeds.

Keywords: ARC microbial inoculant; peanut; cadmium; morphological distribution

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1. Introduction

According to the published "National Soil Pollution Survey Communique" [1], the soil pollution situation in China is extremely serious, particularly in arable land, where the over-standard rate reaches as high as 19.4%. Cadmium (Cd), as the most widespread pollutant [2], poses a significant threat due to its high fluidity [3] and ease of accumulation. Once it reaches a certain concentration, Cd becomes toxic to most organisms [4]. Presently, Cd pollution in paddy soil has led to excessive Cd content in rice, garnering widespread concern from various sectors [5]. In addition, the long-term consumption of crops and products grown in Cd-contaminated soil poses a grave threat to human health [6,7]. Consequently, reducing the level of Cd pollution in soil and crops has emerged as a pressing issue for the agricultural industry. In recent years, an increasing number of scholars have centered their research on enhancing crop quality [8] and mitigating Cd pollution [9,10] in farmland soil. Researchers have developed relevant technical methods, including physical [11–13], chemical [14], and biological [15–17] approaches, to reduce soil Cd pollution, increase crop yields, and improve quality. For example, a remediation device was designed for heavy metal-contaminated soil based on electrodynamic remediation technology [18]. According to the results, the device fully reacts the electrolyte with heavy metal ions in soil, has the advantage of resource reuse and a good removal effect on heavy metal in soil, and is conducive to improving soil remediation efficiency. The effects of amendments, including lime, diatomaceous earth, sodium phosphate, steel slag, and charcoal, were investigated on the immobilization of Cd and found that the lime–diatomaceous earth–charcoal complex exhibited the most favorable repair performance [19]. However, the aforementioned repair approaches possess numerous drawbacks, such as the high cost of physical repair technology, the limited application scope of chemical repair technology, and secondary pollution. Furthermore, previous research indicated that microbial inoculants could change the soil bacterial community composition, thereby reducing Cd accumulation and enhancing soil quality [20,21]. Microbial remediation refers to a remediation technique that employs specific microorganisms to mitigate the activity of detrimental pollutants in soil or to decompose pollutants into innocuous substances. Boasting advantages such as low cost, straightforward operation, and no resulting harm to the soil structure, this method is regarded as one of the most promising soil remediation technologies. Considering the economic cost and practical application effect, low-cost and safe biological management methods have become extensively employed as effective solutions.

Peanut is one of the main oil crops in China. The annual peanut planting area in China exceeds 4.8 million hectares, and the output reaches 18.3 million tons. At present, research on peanut quality primarily revolves around aspects such as oil content, protein, and aflatoxins. The extent of Cd pollution in peanuts in China and its influencing mechanisms remain unclear. To promote the healthy development of China's peanut industry, ensure the safety of peanut consumption, and more effectively practice the national strategy of food safety production, this experiment tested the Cd content of peanuts from four major production areas in China and initially assessed the Cd pollution level of peanuts in China. On this basis, Quanzhou county, which has a high risk of Cd pollution, was selected as the test site. The Aflatoxin Rhizobia Couple *B. amyloliquefaciens*, *B. laterosporu*, *B. mucilaginosus*, *E. ludwiggi* (ARC-BBBE) microbial inoculant independently developed by our team [22] was used to evaluate its effectiveness in inhibiting Cd pollution in peanuts, and the mechanism of Cd reduction was preliminarily explored.

2. Materials and Methods

2.1. Materials

Four hundred and ninty-six samples were collected from 15 provinces in four principal production areas in China. Quanzhou county in Guangxi served as a testing ground for examining the potential influence mechanism of microbial inoculants on the Cd content in peanuts.

Excellent-grade pure hydrofluoric acid was purchased from Sinopod Chemical Reagent Co., Ltd. (Shanghai, China). Analytical-grade pure acetic acid and analytical-grade pure triethanolamine analysis were purchased from Aladdin reagent. Analytical-grade pure anhydrous calcium chloride was purchased from Xilong Chemical Co.(Shantou, China), Ltd. The Solarbio soil urease activity detection kit, Solarbio soil phosphatase activity detection kit, and Solarbio soil alkaline phosphatase activity test kit were purchased from Solarbio (Beijing, China).

2.2. Tested Microbial Agent

The tested microbial inoculant, ARC-BBBE, was prepared from four strains of exogenous and endophytic bacteria isolated from peanut pods and rhizosphere soils in China, including *Bacillus amyloliquefaciens*, *Brevibacillus laterosporus*, *Bacillus mucilaginosus*, and *Enterobacter ludwigii*. The main technical indicators are as follows: the number of viable bacteria of *Bacillus amyloliquefaciens* ≥ 200 million/g; the effective viable bacteria count of *Brevibacillus laterosporus* ≥ 200 million/g; the number of viable bacteria of *Bacillus mucilaginosus* ≥ 1 billion/g; the number of viable bacteria of *Enterobacter ludwigii* ≥ 1 billion/g. The ARC-BBBE microbial inoculant was provided by the Oil Crop Research Institute, Chinese Academy of Agricultural Sciences.

2.3. Experimental Design

A piece of land was divided into a control group and treatment group in Quanzhou county, Guangxi. The peanut variety was Yihua 668. The control group adopted local conventional sowing methods, and conventional manual management measures such as weeding, pest control, and growth control were adopted. In the treatment group, 60 kg/hectare of the microbial inoculant, ARC-BBBE, was added. The microbial inoculant was mixed mechanically and applied to the soil as a base fertilizer. Other field production and management methods were the same as those used in the control group. The sampled peanut seeds were stored in a cool and dry place after harvest and then shelled, sliced, crushed, and bottled before use. The sampling of roots, stems, leaves, shells, and seeds was conducted during the harvest period, in which 30 g per piece was prepared and stored in a cool and dry place.

2.4. Experimental Methods

The Cd content of peanut seeds was determined using an automatic Cd detector (Changsha Kaiyuan Instrument Co., Ltd., Changsha, China). The organic matter in each sample was decomposed using the electrothermal evaporation–atomic absorption method through high-temperature combustion (pyrolysis), and the volatiles were further decomposed by high-temperature filler. The high-temperature filler (catalyst) selectively captured the trace Cd that escaped during combustion. Cadmium was released from the combustion ash and fillers through the gentle switching of the atmosphere, and the result was measured using a micro-flame atomic absorption spectrometer through an interface device. The specific parameters of the measurement method are shown in Table 1. The total Cd content was detected by using the national standard method (GB 5009.15-2014) [23]. The soil's pH value was detected by the pH meter (Shanghai Yizheng Scientific Instrument Co., Ltd., Shanghai, China). Available Cd was detected following the national standard method (GB/T 23739-2009) [24]. Soil urease activity, soil alkaline phosphatase activity, and soil sucrase activity were detected using detection kits (Beijing Suolaibao Science & Technology Co., Ltd., Beijing, China).

Table 1. Parameters of automatic cadmium detector.

Process Control Parameters			
Sample pretreatment parameters	Drying	Combustion furnace drying temperature	450 °C
		Combustion furnace drying time	70 s
		Combustion furnace drying flow (air)	500 mL/min
	Pyrolysis	Combustion furnace pyrolysis temperature	750 °C
		Combustion furnace pyrolysis time	40 s
		Combustion furnace pyrolysis flow (air)	500 mL/min
Detection parameter	Detection	Detection time	80 s
		Detection flow (air)	300 mL/min
		Detection flow (hydrogen)	300 mL/min
		Catalytic furnace temperature	750 °C
		Peak adjustment time	0 s
		Peak starting time	2.0 s
		Cut-off time	60.0 s

2.5. Data Processing

Excel 2016 was used to organize the data, Graph pad prism10.1.2 was utilized for plotting, SPSS 27.0 was used for the statistical analysis of the data, Duncan's method was applied for significance tests with a confidence interval of 95%, and Pearson's method was employed for correlation analysis. All samples were tested in triplicate, and the test results were expressed as the mean ± standard error.

The enrichment coefficient (BCF) and transfer coefficient (TF) of Cd in peanut [25] were calculated according to the following formula:

$$BCF_{peanutseeds} = C_{peanut\ seeds}/C_{Soil} \quad (1)$$

$$TF_{i/j} = C_i/C_j \quad (2)$$

In the formula, $BCF_{peanut\ seeds}$ represents the enrichment coefficient of Cd in peanut, $C_{panut\ seeds}$ represents the Cd content in peanut seeds (mg/kg), and C_{Soil} represents the total Cd content in soil (mg/kg). $TF_{i/j}$ represents the transfer coefficient of Cd from the j part of the peanut plant to the i part of the peanut plant: C_i is the Cd content of the i part of the peanut plant (mg/kg), and C_j is the Cd content of the peanut plant (mg/kg).

3. Results

3.1. Assessment of Cd Pollution in Major Peanut-Producing Provinces

Heavy-metal Cd pollution poses a significant threat to the quality, safety, and health of agricultural products and the ecosystems they support, particularly in peanut-producing regions [26]. Establishing the degree and risk of Cd pollution in peanut is a crucial prerequisite for guiding the management and restoration of Cd-contaminated farmland. Peanut planting in China is primarily concentrated in the southern production area, the Yangtze River basin production area, the Yellow River basin production area, and the northeast production area. Based on the peanut planting area and output in 2022, the main peanut production regions in China include 15 provinces, including Henan, Shandong, Hebei, Guangdong, and Liaoning. Consequently, 496 samples were strategically collected from 15 major peanut-producing provinces during the peanut-harvesting period. After drying, shelling, slicing, and crushing, the Cd content of peanut seeds was analyzed. The findings revealed that the Cd contents of peanuts from 15 provinces were under the national safety limit (0.5 mg/kg), indicating a low level of Cd contamination in peanuts. However, according to the European Union standard (≤0.2 mg/kg), the Cd contents in peanuts from Guangxi were relatively high, with an average Cd content of 0.30 mg/kg. It reported mild Cd pollution in soil in Guangxi as early as 2006 [27]. Recent years have seen the intensification of human production activities, sewage irrigation, and improper fertilizer use, leading to progressive soil acidification in China. Consequently, the sampling point in Shaoshui Town of Quanzhou county (E110.87, N25.89) might be at high risk of Cd exceeding the standard for peanuts.

According to the National Soil Pollution Survey Bulletin, the spatial distribution of Cd content in agricultural soil primarily indicates that the Cd content in southern China is higher than that in northern China. As depicted in Table 2, the Cd contents in southern production areas and the Yangtze River Basin production area surpassed those in the Yellow River Basin production area, corroborating previous reports. The potential explanation for this phenomenon is that the soil in the south is predominantly acidic, while the soil in the north is alkaline [28,29]. Many heavy metals are much more bioavailable under acidic conditions than alkaline conditions; thus, the toxic effect of the same concentration of heavy metals in the south is likely to be more potent than in the north. In particular, the Cd contents of peanuts from some regions in Guanxi, Jiangxi, and Liaoning are comparatively higher. The main reason for this is that these regions are rich in mineral resources, which possess high risks of cadmium contamination [30]. Upon investigation, this study found a limestone mining area in Shaoshui Town, Quanzhou county, Guangxi, where the risk of Cd pollution was greatest. Consequently, peanut samples from this area were selected to study the impact of the ARC microbial agent on the Cd content of peanuts.

Table 2. Cadmium content of peanuts in different provinces.

District	Provinces	Cadmium Content (mg/kg)
Southern production area	Fujian (n = 54)	0.107 ± 0.027
	Guangdong (n = 52)	0.097 ± 0.016
	Guangxi (n = 38)	0.296 ± 0.059
	Yunnan (n = 5)	0.132 ± 0.105
Yangtze River Basin production area	Hubei (n = 34)	0.167 ± 0.029
	Hunan (n = 5)	0.152 ± 0.008
	Jiangxi (n = 42)	0.235 ± 0.020
	Jiangsu (n = 10)	0.072 ± 0.002
	Anhui (n = 43)	0.155 ± 0.034
	Sichuan (n = 42)	0.172 ± 0.040
Yellow River Basin production area	Henan (n = 75)	0.124 ± 0.027
	Shandong (n = 42)	0.103 ± 0.016
	Hebei (n = 28)	0.077 ± 0.005
Northeast production area	Jilin (n = 6)	0.056 ± 0.007
	Liaoning (n = 20)	0.212 ± 0.024

3.2. Impact of the ARC-BBBE Microbial Inoculant on Cd Content in Various Components of the Peanut Plant

The Cd content in various organs of peanut plants is influenced by the pH value, soil heavy-metal form, and other factors [31], which can mirror the route of Cd absorption by plants. Based on the outcomes of the previous step, peanuts from Quanzhou county were selected for further research. The findings demonstrated that the accumulation of the heavy metal Cd in all parts of peanut plants exhibited a pattern of root > stem > leaves > grains > shells. It has been demonstrated that roots are the main plant tissue that accumulate Cd, which agrees with previous studies [32]. Likewise, this increases Cd accumulation in plant roots and even promotes Cd translocation from the roots to aerial tissues, which is important for hyperaccumulator plants used in phytoremediation. However, Cd in edible parts is undesirable because the presence of Cd could generate toxic effects in consumers. Therefore, it is important to characterize microorganisms that prevent Cd translocation from the root to the aerial parts. Therefore, the microbial inoculant, ARC-BBBE, was used to notably decrease the Cd content in peanut stems, leaves, shells, and grains. Based on the trend of Cd accumulation, the root tissue is the first to be contaminated by heavy metals. Plant roots will secrete organic acids, sugars, and nucleic acids to form coordination compounds with heavy metals and regulate the rhizosphere's pH environment, resulting in a certain level of activation of heavy metals and promoting the absorption of heavy metals by plants [33], resulting in a much higher concentration of heavy metals in the roots compared to other parts of the plant. As shown in Figure 1, in comparison to the control group, the total Cd content in other parts of plants in Quanzhou was significantly reduced. The total Cd content in stems exhibited the most significant decrease (35.95%), and the total Cd content in grains was significantly reduced by 21.34% ($p < 0.01$). This suggests that Cd in peanuts is absorbed by the root system, is subsequently transported to the stem and leaves [34], and ultimately reaches the kernel. Consequently, the crux of reducing Cd absorption in peanut lies in preventing the absorption of Cd in soil by peanut roots. It is inferred that the impact of the ARC-BBBE microbial inoculant on the Cd content of peanuts could be due to the reduction in the total Cd content in soil, thereby lessening the absorption of plant roots, and thereby reducing the Cd content in peanut kernels.

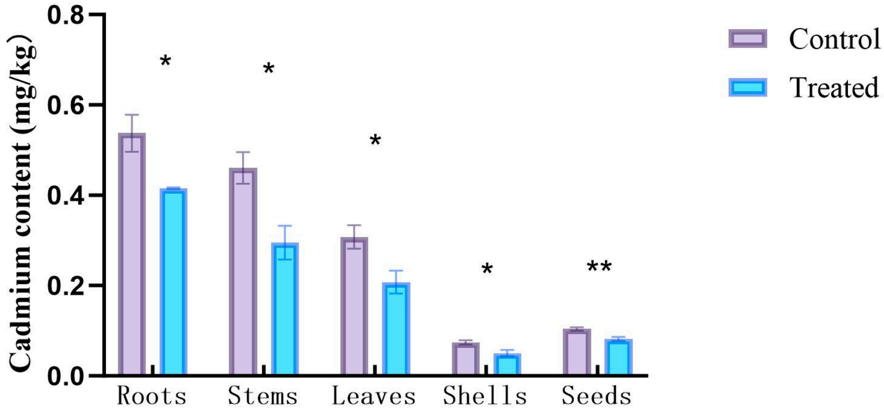

Figure 1. Effect of ARC treatment on cadmium content in different organs of peanut. * indicates significant difference at $p < 0.05$ and ** indicates significant difference at $p < 0.01$ between control group and treated group.

3.3. Influence of the ARC-BBBE Microbial Inoculant on Cd Content and pH Value in Peanut Planting Soil

The total soil Cd content serves as an evaluation metric for soil Cd pollution, reflecting the degree of environmental contamination. To validate the previous inference and elucidate the influence of the ARC-BBBE microbial inoculant on soil pollution levels, the total Cd content in peanut planting soil from Shaoshui Town, Quanzhou, Guangxi was examined, with the results presented in Figure 2A. In comparison to the control group, the total Cd content in peanut planting soil from Quanzhou, Guangxi demonstrated a significant reduction from 0.35 mg/kg to 0.22 mg/kg following the application of the microbial inoculant. This finding suggests that the employment of the ARC-BBBE microbial inoculant indeed decreases the total Cd content in soil, potentially due to the adsorption and accumulation effects of the microorganisms in the inoculant on heavy metals, thereby reducing the heavy metal content in soil [35] and subsequently minimizing Cd absorption via peanut plant roots.

The pH level significantly influences the occurrence and accumulation of heavy metals in soil [36,37]. Consequently, examining the pH level of soil can be used to determine the impact of the ARC-BBBE microbial inoculant on heavy metal accumulation in peanut planting soil. As illustrated in Figure 2B, the pH level of soil treated with microbial inoculant increased by 0.21 units (from 4.65 to 4.86) in comparison to the control group in Quanzhou, Guangxi. Prior research has demonstrated that a decline in the soil pH level significantly augments the content of available Cd [38]. Concurrently, as the pH increases, an increasing number of negatively charged ligands (such as carboxyl, phosphate, imidazole, and amino) become accessible, attracting metal ions to the cell surface through bioadsorption [39], thereby diminishing the Cd content in the soil. Thus, the increment in soil pH levels might be attributed to the adsorption of soil Cd onto the surface of the bacteria, thereby mitigating soil pollution. It was determined that the implementation of the ARC-BBBE microbial inoculant induced a change in the pH level of peanut planting soil, which resulted in an alteration in the distribution of heavy metal forms in soil and minimized the total Cd content that plants could absorb and utilize, thereby reducing the Cd content in peanut seeds.

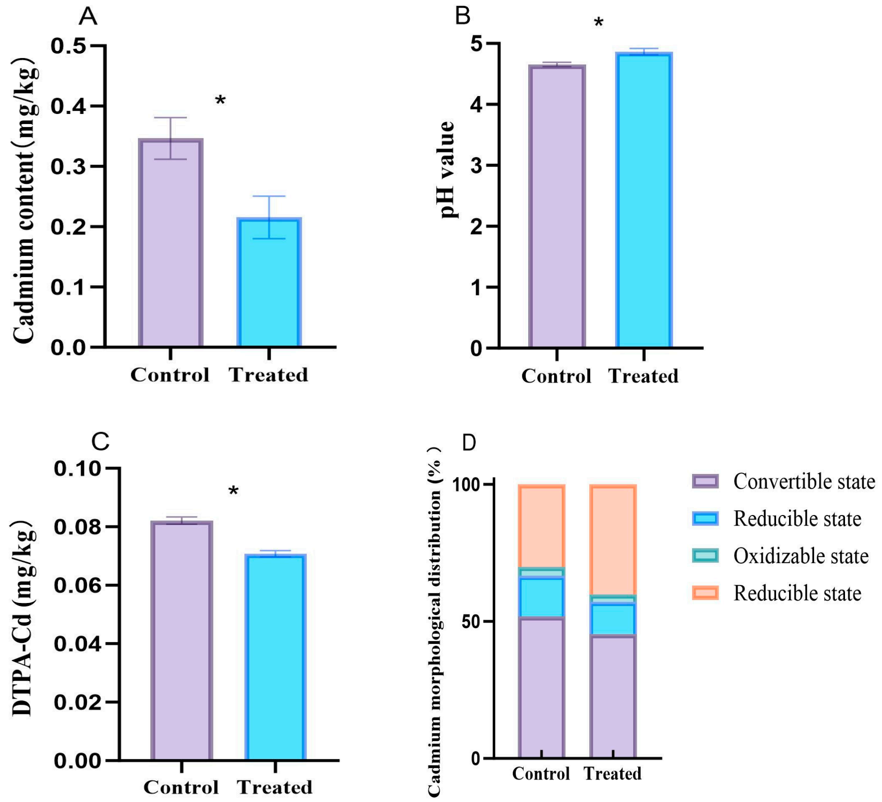

Figure 2. (**A**) Effect of ARC-BBBE microbial inoculant treatment on cadmium content of soil. (**B**) Effect of ARC-BBBE microbial inoculant treatment on pH of soil. (**C**) Effect of ARC-BBBE microbial inoculant treatment on the content of available cadmium in soil. (**D**) Effect of ARC-BBBE microbial inoculant treatment on the morphological distribution of cadmium in soil. * indicates a significant difference at $p < 0.05$ between control group and treated group.

3.4. Influence of the ARC-BBBE Microbial Inoculant on the Available State and Morphological Distribution of Cd in Peanut Planting Soil

The available Cd in soil is the portion that crops can utilize, and its concentration reflects the mobility of Cd in soil and the risk of Cd absorption and accumulation by plants. This parameter can rapidly and accurately characterize the actual level of soil pollution and its impact on plant health [40]. Figure 2C displays the test results of available Cd content in peanut planting soil. A significant reduction was observed in the available Cd content of the treatment group ($p < 0.05$) from 0.083 mg/kg to 0.071 mg/kg, amounting to a total decrease of 0.011 mg/kg. This suggests that the application of the ARC-BBBE microbial inoculant can decrease the content of available Cd in soil and mitigate the severity of soil pollution. Prior research has demonstrated that varying quantities of microbial inoculants can significantly reduce the content of available Cd in soil [41], as the strains in the microbial inoculants are capable of adsorbing Cd from soil. Furthermore, microorganisms interact with environmental factors during their growth, producing metabolites that can react with Cd or chelate to fix Cd, thereby reducing the diethylenetriaminepentaacetic acid-Cd (DTPA-Cd) content in soil. Additional studies have found that the application of microbial agents to soil can significantly elevate the pH value of crop rhizosphere soil. The content of DTPA-Cd in rhizosphere soil exhibits a downward trend as the soil pH value increases [42]. Thus, the decrease in soil available Cd content could be attributed to the production of increased metabolites (e.g., exopolysaccharides) by microorganisms to adsorb or fix Cd in soil, effectively inactivating the available Cd. Additionally, the increase in the soil pH value could cause a reduction in DTPA-Cd content, thereby reducing the total absorption of Cd by plants.

The toxicity of Cd in soil is primarily determined by its current state and its proportion [43]. The four Cd types of exchangeable Cd, reducible Cd, oxidizable Cd, and residual

Cd can be identified using the improved BCR continuous extraction method to examine the morphological distribution of Cd in soil. Heavy metals in the exchangeable state exhibit strong fluidity [44], are highly susceptible to environmental conditions, and can be readily absorbed and accumulated by plants [45]. Reducible and oxidizable Cd can transform into exchangeable Cd or residual Cd under specific conditions. However, residual heavy metals possess long-term stability in natural sediments and are challenging for plants to absorb or utilize [46]. As shown in Figure 2D, compared to the control group, the proportion of exchangeable Cd content in the soil of the Quanzhou treatment group diminished from 51.79% to 45.43%, a decline of 6.36%. The proportion of residual Cd in soil escalated from 30.14% to 40.14%, an increase of 10.00%. Relevant studies have demonstrated that the microbial inoculant itself exhibits adsorption and accumulation effects on heavy metals, which can augment the content of residual Cd in soil, reduce the content of exchangeable Cd, and facilitate the conversion of Cd in soil into insoluble Cd [47]. By altering the manner in which pollutants bind to soil or their form of occurrence in soil, the migration and bioavailability of pollutants in soil can be diminished, thereby lessening the pollution risk. Therefore, the potential explanation for this phenomenon is that the microorganisms present in the microbial inoculant alter the heavy metal Cd from the exchangeable state to the residual state through their own adsorption and accumulation of heavy metals, thereby reducing the content of exchangeable Cd in the soil and diminishing the absorption of Cd by peanuts.

3.5. Influence of the ARC-BBBE Microbial Inoculant on Soil Enzyme Activities

The secretion of various enzymes by microorganisms in soil plays a crucial role in numerous biochemical reactions in soil ecology [48,49]. This enzyme activity serves as a vital indicator for assessing the impact of soil heavy-metal remediation. The activities of urease, alkaline phosphatase, and sucrase in soil decrease under heavy-metal stress. Consequently, the levels of Cd stress in soil treated with the ARC-BBBE microbial inoculant were determined by examining the alterations in soil enzyme activity following the application of the ARC microbial inoculant. The soil enzyme activity was assessed, and the results are presented in Figure 3. There was a significant increase in the activity of soil urease in the treatment group ($p < 0.05$), from 50.99 u/g to 60.51 u/g (Figure 3A). As shown in Figure 3B, compared to the control group, the activity of soil sucrase in the treatment group was elevated, albeit insignificantly. As illustrated in Figure 3C, there was a significant increase in soil alkaline phosphatase activity in the treatment group, accompanied by a highly significant increase of 1540.36 u/g ($p < 0.001$). These results indicate that the application of the ARC-BBBE microbial inoculant is advantageous for mitigating Cd stress on crops and ameliorating soil pollution. The microbial inoculant thereby enhances the activities of soil urease and alkaline phosphatase, fostering a favorable soil environment for peanut growth.

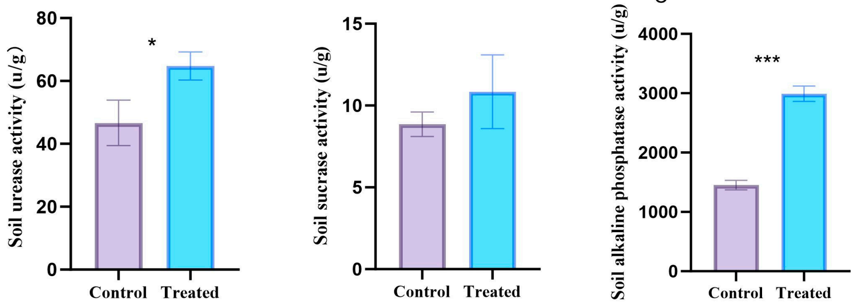

Figure 3. (**A**) Effect of ARC-BBBE microbial inoculant treatment on soil urease activity. (**B**) Effect of ARC-BBBE microbial inoculant treatment on soil sucrase activity. (**C**) Effect of ARC-BBBE microbial inoculant treatment on soil alkaline phosphatase activity. * indicates significant difference at $p < 0.05$ and *** indicates significant difference at $p < 0.001$ between control group and treated group.

3.6. Effects of ARC Microbial Agent on Cd Enrichment and Transfer in Peanut

Table 3 shows the enrichment coefficient of Cd in peanut samples and the transfer coefficient of Cd from root to stem, stem to leaf, root to seed, and stem to seed. It can be seen that the enrichment coefficient of Cd in treated peanuts, $BCF_{Peanut\ seeds}$, had no significant difference from the control group. As a result, C_{Soil} was smaller. Compared with the control group, $TF_{Stem/Root}$ decreased by 19.94% after ARC treatment, indicating that ARC treatment could inhibit the transfer of Cd from root to stem. The transfer coefficient of other parts changed little.

Table 3. Enrichment coefficient and transport coefficient.

Treatments	$BCF_{Peanut\ seeds}$	$TF_{Stem/Root}$	$TF_{Leaf/Stem}$	$TF_{Peanut/Root}$	$TF_{Peanut/Stem}$	$TF_{Peanut/Leaf}$
Control	0.329 ± 0.05	0.947 ± 0.04 [a]	0.668 ± 0.05	0.214 ± 0.07	0.226 ± 0.20	0.339 ± 0.04
Treated	0.335 ± 0.02	0.748 ± 0.01 [b]	0.704 ± 0.08	0.208 ± 0.04	0.278 ± 0.13	0.395 ± 0.10

Data in table are means and standard deviation; different lowercase letters after the data in the same column indicate that the difference between treatments reaches a significant level of 5% (Duncan's method, α = 0.05). $BCF_{Peanut\ seeds}$—the enrichment coefficient of Cd in peanut, $TF_{Stem/Root}$—the transport coefficient of Cd from root to stem, TFLeaf/Stem—the transport coefficient of Cd from stem to leaf, $TF_{Peanut/Root}$—the transport coefficient of Cd from root to peanut, $TF_{Peanut/Stem}$—the transfer coefficient of Cd from stem to peanut, TFPeanut/Leaf—the transport coefficient of Cd from leaf to peanut.

4. Discussion

As can be seen from Figure 2, there are two main ways to reduce the Cd content in peanuts. One is to reduce the biological availability of Cd in soil, because the form of Cd in soil is closely related to its biological availability. Among them, the exchangeable state has the highest activity and is easily released into a mobile free state and absorbed by plants, which is the main form causing soil Cd pollution. Reducible and oxidizable states are the binding states Cd forms by adsorption and chelation, which are released into the environment only when the soil REDOX conditions change. However, residual Cd is stable and has low bioavailability, and it is not easily absorbed by plants [50]. The second is to prevent and control the transfer of Cd from peanut organs to grain. This is consistent with the results of Peng Ou's [51] previous research on rice.

It was found that after treatment with the ARC microbial agent, the content of available cadmium and the proportion of exchangeable Cd in soil were significantly reduced, while the proportion of residual state was increased. This is consistent with the research results of the previous study [52]. The reason for this may be that the bacteria can form chelates with Cd^{2+}, which contributes to the specific and non-specific adsorption of Cd on its surface, thus affecting the migration and bioavailability of Cd^{2+} [53]. In addition, the ARC microbial agent also contains a large number of live bacteria, which can significantly enhance soil enzyme activity after application [41], promote the formation of organic complexes of Cd^{2+} in soil, and improve the adsorption capacity of soil pollutants. By comparing the Cd-decreasing effect of different materials on alkaline soil, it is found that a strong ion exchange ability can be combined with Cd through adsorption, coordination, co-precipitation, and other methods to reduce its mobility [54]. It was also found that the cadmium content in each part of peanut plant and the transfer coefficient of cadmium from peanut root to stem and from root to kernel were decreased after the ARC-BBBE microbial inoculant treatment, which indicated that the ARC-BBBE microbial inoculant could inhibit the transfer of Cd from peanut root organs to kernels.

5. Conclusions

From the perspective of heavy-metal criteria, the total Cd contents of 496 peanut samples from 15 provinces were under the national standards. However, due to the increasing acidification of the soil, there is a risk of Cd exceedance in peanuts in Quanzhou county in future. The implementation of the ARC-BBBE microbial inoculant significantly reduced the total Cd content of stems, leaves, hulls, and peanut seeds in Guangxi, with a gradual declining trend observed in the Cd content of stems, leaves, seeds, and hulls. The reduction in stems was the most significant at 35.95%. This application also significantly lowered the total Cd content and available Cd content in soil, facilitating the conversion of exchangeable Cd to residual Cd. The exchangeable Cd content was reduced by 6.36%, while the residual Cd content increased by 10.00%. The application of the ARC-BBBE microbial inoculant significantly enhanced the soil pH value, as well as urease and alkaline phosphatase activities. Although the sucrase activity increased by only 0.21 units, the urease activity surged by 9.52 u/g, and the activity of alkaline phosphatase skyrocketed by 1540.36 u/g.

The study demonstrated that the ARC-BBBE microbial inoculant possessed the ability to reduce Cd content in peanut grains. The potential mechanism underlying this effect involves the adsorption and fixation of the heavy metal Cd in soil by either the bacteria itself or by extracellular substances secreted by the bacteria. This process results in a decrease in the efficiency of Cd absorption and transport in plants, thereby reducing the Cd content in peanut grains. Alternatively, the application of these bacteria may activate plant root cells, enhancing the fixation of soil Cd by root cells and minimizing the Cd transport in plants. Ultimately, this leads to a reduction in the Cd content in peanuts.

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