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Native Strains *T. longibrachiatum* UCF17-M4 and *Trichoderma* sp. UCPF2 Reduce Cd Uptake in Cacao CCN51 Under Controlled Conditions

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Abstract: The cacao trade and export industry has been impacted by cadmium (Cd^{2+}) accumulation in soils, as the metal is absorbed by plants and transferred to the tissues. Consequently, cacao beans and their derivatives can become contaminated, sometimes exceeding permissible limits. In this study, the capacity of native *Trichoderma* strains to reduce Cd accumulation in cacao was evaluated. Twelve *Trichoderma* strains were analyzed to assess their cadmium removal capacity through in vitro assays and their ability to reduce Cd concentration in cacao plants under controlled in vivo conditions. The in vitro results showed that several *Trichoderma* strains could remove cadmium and accumulate it in their biomass. However, this process is complex as it depends on metal concentration and environmental conditions. Notably, *T. afroharzianum* UCF18-M1 and CP24-6 exhibited high removal efficiencies at 100 ppm (61.79 ± 2.98% and 57.93 ± 4.14%, respectively). In contrast, the in vivo assays revealed that, contrary to expectations, some strains—including those with the highest removal efficiency—stimulated Cd uptake in plants, even at toxic levels, such as *T. orientale* BLPF1-C1. However, *T. longibrachiatum* UCF17-M4 and *Trichoderma* sp. UCPF2-C1 significantly reduced Cd accumulation in the stem. These findings highlight the potential of these strains to mitigate Cd contamination in cacao.

Keywords: bioremediation; sustainability; cadmium; Native Cacao; *Trichoderma*

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1. Introduction

Cacao (*Theobroma cacao* L.), native to the South American Amazon region [1], is an economically important crop [2]. In Peru, cacao is mainly produced on small farms (1–5 ha) in regions such as San Martín, Pasco, Ucayali, Junín, Cusco, Ayacucho, Huánuco, and Amazonas, reaching an export volume of 142,390 tons in 2024, valued at USD 1.17 billion [3]. However, high cadmium (Cd^{2+}) levels in cacao-growing soils and international regulatory limits threaten Peruvian cacao production [4,5].

Cadmium (Cd^{2+}) is a non-essential heavy metal that can be naturally present in the soil or introduced through external sources. Although typically found in small concentrations,

its bio-availability can increase depending on soil physicochemical conditions such as pH and organic matter content [6–8]. This increased availability, combined with its well-documented toxicity [9], represents an environmental and food safety concern [10]. The latter is particularly relevant for certain crops, such as cacao, which can absorb Cd from the soil, translocate it to its vegetative and reproductive organs, and introduce it into the food chain [11,12].

Cadmium contamination in cacao-growing soils has increased over time. Several studies have reported Cd presence in soils [13–15], cacao beans, and final products [16,17]. Furthermore, recent findings indicate a direct relationship between soil–Cd levels and cacao products [18–21]. In Peru, high Cd levels have been detected in samples from the main cocoa-producing regions. A characterization analysis of leaves and beans collected from eight cacao-growing regions revealed Cd concentrations exceeding 0.96 μg/g in samples from Piura, Tumbes, and Huánuco [15]. Similarly, Cd was detected in samples of roots, leaves, testa, and cotyledons collected from various districts in Bagua Province (Amazonas region), with concentrations ranging from 0.49 μg/g to 2.53 μg/g [22], exceeding, in some cases, the maximum permissible limit (0.80 μg/g) established by Commission Regulation (EU) No. 488/2014 [4].

Some mitigation strategies have been developed to address this issue. A georeferenced analysis of Cd content in soils led to the creation of a risk map for the Amazonas region. This study identified that 39% of the territory exceeds the tolerable limit for agricultural soil (1.4 μg/g), while 19% presents levels close to but still within the permitted range (1.0–1.4 μg/g) [23]. Although this initiative aims to guide producers in selecting more suitable cultivation areas, the reduction in arable land could negatively impact cacao production. In this context, the development of complementary sustainable strategies to reduce Cd accumulation in cacao beans without compromising soil use is essential.

It is well known that several *Trichoderma* species establish beneficial interactions with various plants, including cacao [24,25]. This saprophytic fungus is commonly found colonizing soil and rhizospheric ecosystems. As described by [26], members of this genus are generally not considered pathogenic or harmful to plant development. Furthermore, some are regarded as strategic partners in agriculture and industry, mainly due to their roles as biocontrol agents, their plant growth-promoting effects, and their physiological capacity to produce industrially important metabolites [27–30]. In addition, certain *Trichoderma* strains have also been reported to exhibit heavy metal tolerance and uptake capabilities [31–35]. These properties are attributed to several physiological and biochemical mechanisms, such as adsorption, heavy metal flux across the cellular membrane, and intracellular chelation by metallothionein (MT) and specific peptides [36]. Although several studies have demonstrated the cadmium-removal capacity of *Trichoderma* strains [37,38], little is known about their effectiveness in association with cacao plants. Therefore, this study aims to determine the Cd-uptake capacity of 12 native *Trichoderma* strains isolated from cacao-growing soils in Amazonas under in vitro conditions and to evaluate their effectiveness in reducing Cd levels in cacao plants.

2. Methods

2.1. Biological Material and Strain Reactivation

Twelve native *Trichoderma* strains (Table 1) from cacao agroecosystems in five provinces of the Amazonas region were used in this study. These strains were obtained from rhizospheric soil samples, using the serial dilution method on Potato Dextrose Agar (PDA) medium. The selection was based on previously reported positive effects on cacao plants [39,40]. All strains are stored in the fungal collection of the Laboratorio de Investigación en Sanidad Vegetal (LABISANV) at the Instituto de Investigación para el Desarrollo

Sustentable de Ceja de Selva of the Universidad Nacional Toribio Rodríguez de Mendoza (UNTRM). For each biological assay, the strains were reactivated using PDA medium by puncture inoculation, and incubated at 28 °C for seven days until visible mycelial growth was observed.

Table 1. Native *Trichoderma* strains.

Genus	Specie	Strain	Reference
Thichoderma	*afroharzianum*	CP24-6	[39]
		UCF18-M1	Unpublished
		UCF12-M3	[40]
	longibrachiatum	UCF10A-C1	[40]
		UCF5A-C1	
		UCF17-M4	
	orientale	CRSF1-C1	[40]
		BM18-C1	
		BLPF1-C1	Unpublished
	reesei	CNF20-C1	[40]
		BIF3-C1	
	sp.	UCPF2-C1	[40]

2.2. In Vitro Cadmium Removal and Biomass Accumulation Assay

The capacity of each *Trichoderma* strain to remove cadmium was evaluated under in vitro conditions. For this, Potato Dextrose Broth (PDB) was supplemented with 100, 200, and 300 ppm of cadmium chloride ($CdCl_2$), prepared from a previously sterilized 1000 ppm stock solution. A control treatment consisting of cadmium-free culture medium was included. Each treatment was inoculated with ten 7 mm PDA disks, containing uniform mycelial growth, and incubated at 28 °C and 210 rpm for 15 days. After the incubation period, the cultures were transferred to sterile 50 mL Falcon tubes and centrifuged at 4000 rpm for 15 min. The supernatants were filtered using 2.5 µm Whatman filter paper. The filtered cultures were diluted in sterile ultrapure water at a 1:10 ratio. Cadmium quantification was performed using atomic absorption spectrometry (Agilent Technologies, USA), following the validated protocol of the Laboratorio de Investigación en Suelos y Aguas at UNTRM. Cadmium removal efficiency was calculated using the formula $R = ((Ci - Cf)/Ci) \times 100$ [41], where

R = Cadmium removal percentage (%);
Ci = Initial cadmium concentration (ppm);
Cf = Final cadmium concentration (ppm).

Following the previous step, the cadmium uptake capacity of microbial biomass was determined. The previously obtained fungal pellet (biomass) was dried in an oven at 70 °C for 24 h. The dry mycelial weight was measured using an analytical precision balance.

Cadmium uptake capacity was calculated using the formula $q = ((Ci - Cf)/m) \times V$ [41], where

q = Cadmium absorption (mg g^{-1});
Ci = Initial cadmium concentration (ppm);
Cf = Final cadmium concentration (ppm);
m = Dry fungal biomass weight (g);
V = Volumen (L).

2.3. Inoculum Preparation

To evaluate the capacity of *Trichoderma* strains to reduce cadmium concentration in cacao plants, inoculations were performed in Cd-contaminated soil where CCN51 cacao seedlings were cultivated. The *Trichoderma* strains were first formulated using rice as a substrate. A mixture of boiled rice and water (5:1 ratio) was prepared and left to rest for 30 min. The excess water was then drained, and the rice was distributed into 400 g polypropylene bags. These bags were sterilized at 121 °C and 1 atm for 15 min, then inoculated with 10 mL of a *Trichoderma* spore suspension after seven days of growth. The inoculated bags were incubated at 27 °C for ten days. After incubation, spores were harvested by washing the 400 g of colonized rice with 2 L of sterile distilled water. The resulting solution was supplemented with 2 mL of 5% chloramphenicol and sedimented for 24 h. The sediment was then dried at 30 °C for 48 h. The dried *Trichoderma* spores were used as active compounds for plant inoculation. For this, the spores were resuspended in 250 mL of sterile distilled water and the concentration was determined using a Neubauer chamber. Finally all the inocula concentrations were adjusted to a 1×10^6 spores mL^{-1}.

2.4. In Vivo Cd Reduction Assay in CCN51 Cacao Plants

The in vivo experiment was conducted in the experimental greenhouse of INDESCES (UNTRM), located in Bagua Province (5°44′02.65″ S, 78°25′00.91″ W). Polyethylene nursery bags (1 Kg capacity) were filled with a prepared substrate composed of agricultural soil (Cd < 0.0001 ppm) from cacao-growing fields (pH = 7.82 ± 0.34; CE = 1.20 ± 0.20), sand, and rice-husk (as an organic matter source) at a 2:1:1 rate. The substrate was previously sterilized through solarization by covering it with plastic and exposing it to direct sunlight for seven consecutive days. During this period, daily temperatures ranged between 15.50 and 30.03 °C, according to data from the Bagua Chica Meteorological Station [42]. The prepared bags were irrigated with 250 mL of a 5 ppm Cd solution and left to rest for 15 days to ensure cadmium adsorption to the soil. Three days before planting, CCN51 cacao seeds were germinated. This clone was selected for its widespread cultivation, economic significance, and genetic uniformity, which make it a suitable model for evaluating plant responses under controlled experimental conditions. To this, the cacao pods were washed with clean water and disinfected using a sodium hypochlorite (NaClO) solution (5%) for 5 min. The seeds, previously separated from the mucilage, were planted in each bag and inoculated with 250 mL of *Trichoderma* spore suspension at 1×10^6 spores mL^{-1}. This was applied directly to the soil around each seed to ensure close contact between the inoculum and the emerging radicle.

2.5. Growth Variables

Twelve weeks after *Trichoderma* inoculation, the cacao plants were harvested. The plants were carefully removed from the substrate, and the plant height and number of leaves were measured. The stem was separated and weighed on an analytical balance to determine the fresh weight. The samples were dried in an oven at 37 °C for 48 h. After drying, the final dry weight was recorded.

2.6. Cadmium Determination in Cacao Plants

Cadmium quantification was performed using stem tissue samples. To obtain ash from plant material, 1 g of a previously pulverized sample was incinerated in a muffle furnace at 450 °C for 8 h. The resulting ash was mixed with 1 mL of hydrochloric acid (HCl) and left to rest for 1 h. After this period, 1 mL of water and 1 mL of HCl were added, and the mixture was left to rest for another hour. The final solution was filtered and brought to a

total volume of 25 mL. Cadmium concentrations were determined using atomic absorption spectrometry, following the procedure described by [43].

2.7. Data Analysis

Data processing and analysis were performed using R-Studio (v. 2024.12.1). When normality and homoscedasticity assumptions were met, the variables were analyzed using parametric ANOVA followed by Dunnett's post hoc test, using the packages car, rstatix, and multcomp. Non-parametric factorial ANOVA analyses were performed using the ARTool package (v 0.11.2), followed by the "art.con" function with Holm corrections for multiple comparisons. Graphs were generated using the ggplot2 package (v. 3.5.2), with color, typography, and label alignment adjustments made using Illustrator (v.23.0.05), while preserving the original graphical data.

3. Results

3.1. Cadmium Removal Efficiency and Biomass Accumulation Under In Vitro Conditions

In this study, we evaluated Cd removal efficiency and its accumulation in biomass for 12 native *Trichoderma* strains isolated from the Amazonian cacao agroecosystem. The analysis was conducted using three biological replicates, cultivated under different metal concentrations under in vitro conditions. The results are presented in Table 2, where the data represent the percentage of Cd removal relative to the initial concentration and the Cd content (mg g^{-1}) accumulated in the biomass.

Table 2. Cadmium removal efficiency and biomass accumulation of *Trichoderma* strains under in vitro conditions.

Genus/Specie	Strain	Removal Efficiency (%)				Biomass Accumulation (mg/g)			
		0 ppm	100 ppm	200 ppm	300 ppm	0 ppm	100 ppm	200 ppm	300 ppm
T. afroharzianum	CP24-6	0	57.93 ± 4.14	33.35 ± 3.22	52.02 ± 1.61	0	15.17±0.62	19.23±0.66	60.15±1.42
	UCF18-M1	0	61.79 ± 2.98	33.93 ± 1.28	51.20 ± 6.95	0	14.25 ± 1.51	13.31 ± 0.53	33.90 ± 5.60
	UCF12-M3	0	10.20 ± 5.12	50.18 ± 17.49	13.55 ± 3.29	0	5.71 ± 0.57	44.26 ± 9.60	31.97 ± 3.29
T. longibrachiatum	UCF10A-C1	0	8.26 ± 5.06	2.96 ± 1.32	3.69 ± 1.01	0	6.63 ± 2.78	2.85 ± 0.78	6.55 ± 1.33
	UCF5A-C1	0	7.27 ± 0.87	nd	3.30 ± 2.36	0	5.09 ± 0.59	nd	5.41 ± 1.78
	UCF17-M4	0	0.88 ± 0.87	nd	6.75 ± 4.53	0	2.44 ± 2.02	nd	15.45 ± 5.75
T. orientale	CRSF1-C1	0	20.21 ± 12.17	18.97 ± 5.61	16.13 ± 4.52	0	5.85 ± 2.10	21.31 ± 2.07	36.62 ± 7.45
	BM18-C1	0	12.69 ± 2.29	16.09 ± 2.67	19.15 ± 4.27	0	9.24 ± 1.70	25.78 ± 3.09	76.74 ± 10.87
	BLPF1-C1	0	17.40 ± 2.62	11.41 ± 1.14	20.97 ± 6.96	0	10.78 ± 2.14	82.17 ± 8.23	111.65 ± 37.58
T. reesei	CNF20-C1	0	nd	nd	nd	0	nd	nd	nd
	BIF3-C1	0	7.30 ± 1.56	6.70 ± 4.35	10.24 ± 5.70	0	3.86 ± 0.19	6.72 ± 1.82	19.28 ± 5.68
Trichoderma sp.	UCPF2-C1	0	nd	nd	nd	0	nd	nd	nd

The results represent the mean of three replicates ± SD, grown at different cadmium concentrations (0, 100, 200, and 300 ppm). When unreliable spectrophotometric readings were obtained, they were represented as "nd" to indicate undetermined values.

Regarding removal efficiency, the data suggest that strains of the same species tend to exhibit similar behavior, while variations exist among species. For instance, the *T. longibrachiatum* group, including strains UCF10A-C1, UCF5A-C1, and UCF17-M4, achieved the lowest efficiencies (8.26 ± 5.06%, 7.27 ± 0.87%, and 6.75 ± 4.53%, respectively). Conversely, the *T. afroharzianum* group, including strains CP24-6 and UCF18-M1 (except UCF12-M3), exhibited the highest efficiencies, exceeding 33.35 ± 3.22% and 33.93 ± 1.28%, respectively. The *T. orientale* group, represented by strains CRSF1-C1, BM18-C1, and BLPF1-C1, showed moderate efficiencies ranging from 11.41 ± 1.14% to 20.97 ± 6.96%. On the other hand, in some cases, such as strains CNF20-C1 and UCPF2-C1,

efficiency values could not be determined due to unreliable readings, and these cases were indicated as "not determined" (nd) in Table 2.

Additionally, we observed that, for almost all evaluated strains, the highest removal efficiency was achieved at 100 ppm compared to 200 and 300 ppm $CdCl_2$, except for *T. reesei* BIF3-C1, which reached maximum efficiency at 300 ppm (10.24 ± 5.70%). Notably, the strains *T. afroharzianum* CP24-6 and UCF18-M1 exhibited specific variations in removal efficiency at different Cd concentrations, suggesting the influence of multiple factors on this process. These effects were confirmed through a non-parametric factorial analysis of variance (Aligned Rank Transformed Data), which demonstrated a significant interaction effect between strain and concentration ($p < 2.22 \times 10^{-16}$). This pattern is illustrated in Figure S1, where the convergence of continuous lines indicates the interaction between both variables. Furthermore, post hoc analysis revealed significant differences in 636 out of 1128 interactions (Figure S2). Moreover, as shown in Figure 1, the multiple comparisons showed significant differences in 254 of 630 comparisons, with purple highlights indicating the most significant differences ($p < 0.05$, indicated by asterisk). These findings suggest that removal efficiency variability depends not only on the strain but also on the metal concentration in the culture medium.

On the other hand, cadmium accumulation in cellular biomass increased progressively with higher metal concentrations in the culture medium. These effects were particularly evident in the *T. afroharzianum* and *T. orientale* groups, which exhibited high and moderate removal efficiencies, respectively (Table 2). Notably, *T. orientale* BLPF1-C1 displayed the highest Cd accumulation in biomass, with values of 10.78 ± 2.14, 82.17 ± 8.23, and 111.65 ± 37.58 mg g^{-1} for 100, 200, and 300 ppm conditions, respectively.

Similarly to removal efficiency, the non-parametric factorial variance test revealed significant strain–concentration interaction effects ($p = 2.22 \times 10^{-16}$) on Cd accumulation in biomass. Additionally, post hoc analysis identified significant differences in 828 out of 1128 interactions (Figure S2) and 372 of 630 multiples comparisons (Figure 1), further suggesting that this response depends on both the *Trichoderma* strain and Cd concentration in the culture medium. A Pearson correlation analysis was also conducted to determine the relationship between removal efficiency and biomass accumulation. The results indicated a moderate, significant positive correlation for strains BIF3-C1, BLPF1-C1, BM18-C1, CRSF1-C1, UCF10A-C1, UCF18-M1, and UF12-M3, while strain CP24-6 showed no significant correlation. This suggests that, for these strains, higher Cd concentrations in the culture medium lead to greater accumulation in biomass.

3.2. In Vivo Determination of Cadmium-Reducing Capacity in CCN51 Cacao Plants

Prior to the plant assay, *Trichoderma* strains were formulated on a rice substrate and inoculated at a concentration of 10^6 spores mL^{-1}. Spore germination was performed and the results indicated germination percentages above 69.99 ± 5.26% (Table S1). After normalizing the spore concentrations, the strains were inoculated into the seedlings immediately after sowing. Twelve weeks post-inoculation, the cacao plants were harvested and transported to the laboratory. Cadmium quantification in the stem revealed a significant reduction effect ($p < 2 \times 10^{-16}$ ***) in cadmium content for treatments inoculated with *T. longibrachiatum* UCF17-M4 and *Trichoderma* sp. UCPF2-C1 compared to the positive control inoculated with Cd (Figure 2). These results suggest that these strains could contribute to reducing cadmium accumulation in plant tissues.

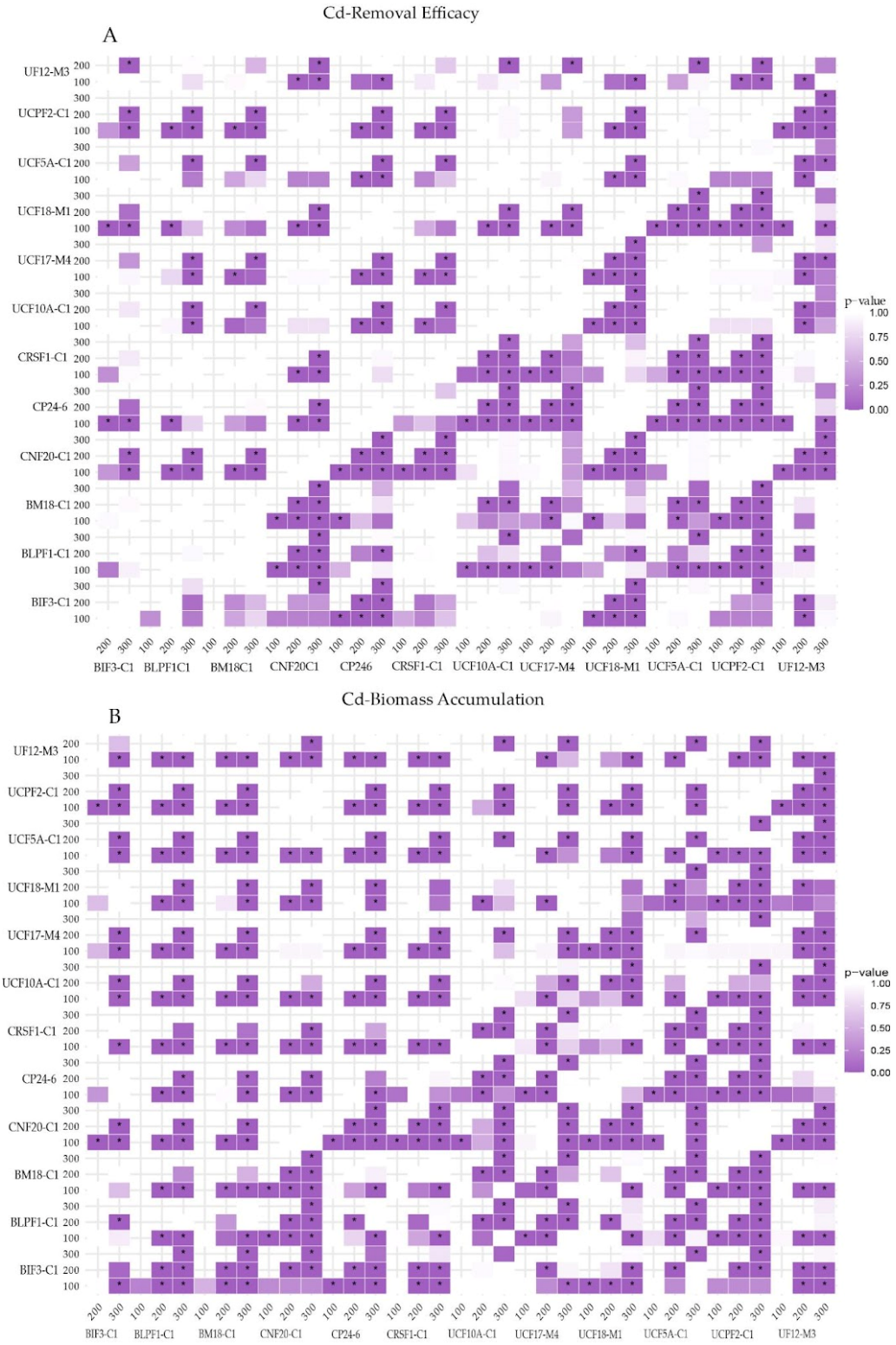

Figure 1. Significance of multiple comparisons of cadmium removal (**A**) and biomass accumulations (**B**). For both datasets, the data were analyzed using a non-parametric factorial ANOVA with the ARTool package, followed by the "art.con" function with Holm corrections. The results indicate significant differences among multiple comparisons of different combinations of strain and Cd concentrations, with purple highlights denoting the most significant differences ($p < 0.05$, indicated by asterisks). Graphs were generated using the ggplot2 package, and adjustments to color, typography, and label alignment were made in Illustrator software while preserving the integrity of the original graphical data.

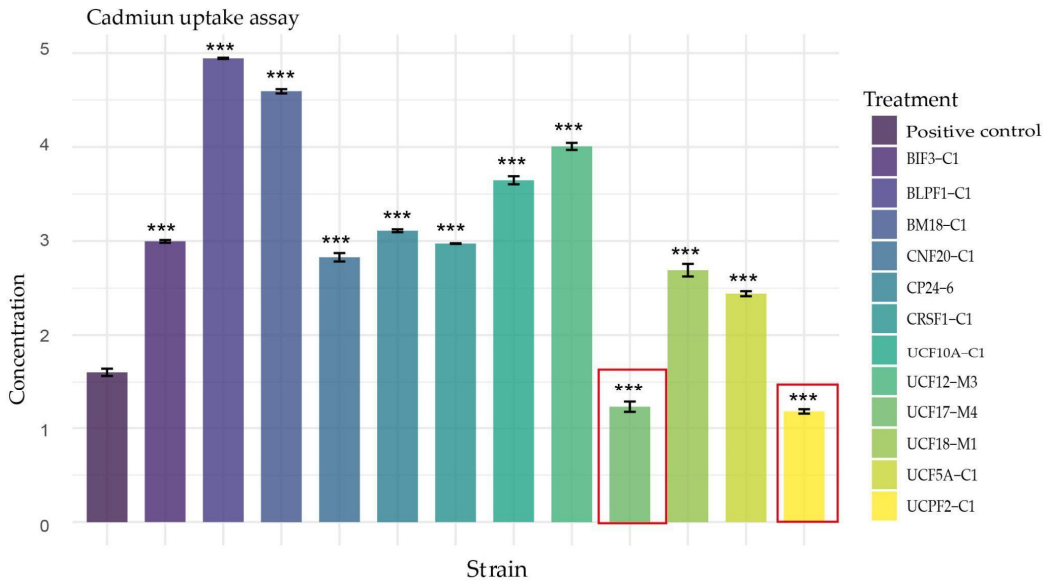

Figure 2. Cadmium-reducing capacity in CCN51 cacao plants by *Trichoderma* strains. The bars represent the means of four to five replicates ± SD. Cadmium accumulation was analyzed using a one-way ANOVA, followed by Dunnett's test for post hoc comparisons. Statistically significant differences are indicated as follows: *p*-value < 0 (***). Most *Trichoderma* strains induced cadmium accumulation, except for *Trichoderma* sp. UCPF2-C1 and *T. longibrachiatum* UCF17-M4, which significantly reduced cadmium concentration in leaves. These strains are highlighted with a red rectangle.

Similarly, significant effects were observed for strains BIF3-C1, BLPF1-C1, BM18-C1, CNF20-C1, CP24-6, CRSF1-C1, UCF10A-C1, UCF12-M3, UCF18-M1, and UCF5A-C1. However, contrary to expectations, these strains stimulated Cd uptake in the plant, reaching concentrations exceeding 2.83 ppm (Figure 2). Finally, no cadmium was detected in the uninoculated, Cd-free treatment.

3.3. Promotive Effect on Cacao Plants Under Heavy Metal Stress

Additionally, the growth-promoting effect of *Trichoderma* strains was evaluated in cacao plants grown under heavy metal stress. For this purpose, plant height, number of leaves, fresh weight, and dry weight were measured (Table 3). Statistical analyses revealed no significant differences between *Trichoderma*-inoculated treatments and the Cd control for plant height and number of leaves, suggesting that these strains do not affect these variables under metal stress conditions. Conversely, in some cases, inoculation proved detrimental; notably, the *T. orientale* strain BLPF1-C1 significantly reduced both the dry and fresh weight of the plants (Figure 2).

Table 3. Growth variables of *Theobroma cacao* CCN51.

Genus/Specie	Strain	Growth Variables			
		Height (mm)	Number of Leaves	Fresh Weight (g)	Dry Weight (mg)
T. afroharzianum	UCF18-M1	190.99 ± 16.40	8 ± 0.91	3.37 ± 0.83	1.24 ± 0.31
	UCF12-M3	192.71 ± 22.12	7.2 ± 0.74	3.17 ± 0.63	1.38 ± 0.28
	CP24-6	171.60 ± 8.59	6.40 ± 0.25	2.15 ± 0.34	0.86 ± 0.22
T. longibrachiatum	UCF17-M4	216.95 ± 7.10	10.2 ± 0.92	4.26 ± 0.13	1.62 ± 0.04
	UCF10A-C1	209.98 ± 24.08	8 ± 0.55	3.76 ± 0.55	1.45 ± 0.22
	UCF5A-C1	174.61 ± 24.75	7.50 ± 1.76	2.71 ± 0.87	1.07 ± 0.36

Table 3. *Cont.*

Genus/Specie	Strain	Growth Variables			
		Height (mm)	Number of Leaves	Fresh Weight (g)	Dry Weight (mg)
T. orientale	CRSF1-C1	193.53 ± 20.94	9.2 ± 1.14	3.48 ± 0.64	1.41 ± 0.24
	BM18-CI	182.67 ± 19.64	9.2 ± 0.58	3.82 ± 0.62	1.60 ± 0.26
	BLPF1-C1	115.70 ± 16.15	5.4 ± 0.93	1.28 ± 0.34 ***	0.42 ± 0.15 ***
T. reesei	CNF20-C1	162.90 ± 18.00	7.25 ± 0.75	3.46 ± 0.58	1.20 ± 0.20
	BIF3-C1	147.93 ± 7.28	7.40 ± 0.40	4.04 ± 0.20	1.45 ± 0.07
Trichoderma sp.	UCPF2-C1	156.03 ± 13.22	7.5 ± 0.29	2.40 ± 0.58	0.87 ± 0.17
	Control + Cd	172.1 ± 2.47	9 ± 1.35	4.31 ± 0.36	1.62 ± 0.16
	Control - Cd	179 ± 21.07	9 ± 1.53	5.55 ± 0.66	1.88 ± 0.22

The results represent the mean of four to five replicates ± SD. The data were analyzed using a one-way parametric ANOVA, followed by Dunnett's test for post hoc comparisons. Statistically significant differences compared to the control + Cd are indicated as follows: *** $p < 0.001$.

4. Discussion

The extensive metabolic variability of microorganisms allows them to interact with diverse substrates through multiple mechanisms, which can be leveraged for the development of increasingly efficient bioremediation strategies. Fungi, in particular, can tolerate and accumulate high concentrations of heavy metals and have been widely used for metal ion adsorption [44]. In this study, the results suggest that strains of the same species tend to exhibit similar removal efficiency, while variations exist among species. Moreover, removal efficiency depends not only on the strain but also on the metal concentration in the culture medium. This phenotypic similarity may be explained by the conservation of certain stress signaling pathways within the genomes of microorganisms from the same species [45]. However, due to their high genetic variability, these microorganisms do not always exhibit identical responses [46]. Fungal genomes, in particular, display remarkable architectural diversity, and their genetic plasticity enables adaptation to environmental changes [47]. The complexity of these interactions has been reported in a comparative study involving three *Trichoderma* species (*T. asperellum*, *T. harzianum*, and *T. tomentosum*), grown under different Cd concentrations and pH levels, which demonstrated a species-specific relationship with cadmium removal [33]. Other factors strongly influencing removal efficiency include environmental conditions such as medium pH, which affects metal solubility in aqueous solutions [36].

Various *Trichoderma* species isolated from different environments have been reported for their ability to reduce Cd and other heavy metals [31–36]. Consistent with our findings, evidence suggests that some *Trichoderma* strains exhibit high tolerance to different metal concentrations, often accompanied by high removal capacity. This behavior may be influenced by both environmental and biological factors [34–36]

In association with cacao, multiple *Trichoderma* strains have been primarily studied for their well-known antagonistic activity against phytopathogenic fungi [48–50]. However, little is known about the bioremediation potential of native *Trichoderma* strains from the cacao-agroecosystem. Although this activity has been mainly reported for bacteria [43,51–54], a recent study has demonstrated the Cd removal capacity of certain native *Trichoderma* strains [55]. That study reported in vitro removal efficiencies of 83.1%, 67.0%, and 65.8% for *T. brevicompactum* M43D, *T. harzianum* M1P, and *T. spirale* M55SM, respectively. Beyond *Trichoderma*, other Cd-tolerant fungi associated with the cacao agroecosystem include *Talaromyces santanderensis*, *Periconia igniaria*, *Metarhizium* sp., and *Annulohypoxylon* sp. [56,57].

Regarding the cadmium-reducing uptake in plants by *Trichoderma* strains, *T. longibrachiatum* UCF17-M4 and *Trichoderma* sp. UCPF2-C1 reduced the Cd concentration in cacao plants. Previous studies have shown that *T. longibrachiatum* has bioremediation potential for heavy metals. Furthermore, in addition to metal removal [58], strains of this species have been associated with enhanced plant stress tolerance and improved soil quality [59–61].

In response to cadmium toxicity, *Trichoderma* has been shown to induce the expression of genes related to reactive oxygen species (ROS) synthesis and detoxification. A recent study reported that *T. reesei* overexpressed genes associated with the MAPK signaling pathway, thereby enhancing the detoxification of ROS. Additionally, genes related to ABC transporters, viral myocarditis, and the ErbB signaling pathway were also upregulated [62]. Beyond the fungal mechanisms involved in the response to cadmium stress, it is also important to consider that environmental conditions play a critical role in the detoxification process, as they influence the bioavailability of the metal. For instance, cadmium concentrations tend to increase with higher total Cd levels and lower pH values. Moreover, in some cases, Cd concentrations also increase with decreasing organic matter content (%OM) [6]. Although soil physicochemical properties were not measured in this study, their potential influence should be considered in future experiments.

Though not extensively studied in cacao, *Trichoderma* inoculation has demonstrated cadmium-reducing effects in other plant models. For example, in *Vigna radiata*, inoculation with the hyper-tolerant strain *Trichoderma* sp. TF-13 reduced lead (Pb) and Cd uptake in root and aerial tissues by 34/39% and 47/38%, respectively, while also improving plant growth and physiological parameters [37]. Similar effects were observed in *Cicer arietinum* plants inoculated with a consortium of *Pseudomonas fluorescens* PGPR-7 and *Trichoderma* sp. T-4, where joint inoculation reduced Cd uptake in roots by 38% in plants exposed to 25 μg kg^{-1} Cd in soil [38]. In addition to a reduction in Cd accumulation, the improvement of plant growth and physiological parameters was also reported [37,63]. Furthermore, a recent metabolomic analysis identified the production of 43 key metabolites, including nicotinic acid, succinic acid, and fumaric acid, involved in Cd detoxification in *Nicotiana* plants inoculated with *T. nigricans* T32781 [63]. These findings suggest that the use of *Trichoderma*, either alone or in consortium, is a promising strategy for reducing cadmium uptake and enhancing plant growth in Cd-contaminated environments. In this context, further experimentation with *Trichoderma* strains UCF17-M4 and UCPF2-C1 must be performed. Particularly as part of a fungal consortium considering the natural complexity of soil environments and the potential for synergistic interactions among microorganisms.

While Cd reduction in plants has been documented in some studies, the opposite effect—the stimulation of metal accumulation—has also been widely studied due to its potential for phytoremediation and the effective removal of specific contaminants. Among the most recent evidence, inoculation of *T. harzianum* in combination with biochar significantly increased Cd accumulation by 187.49–308.92% and arsenic (As) by 125.74–221.43% in *Brassica juncea*, reducing the bioavailability of both metals in the soil [64]. Similarly, *T. harzianum* enhanced Cd bioavailability for *Arachis hypogaea*, significantly increasing its bioaccumulation in both roots and leaves, consequently improving the plant's phytoextraction capacity [65].

The heavy metal phytoremediation effect mediated by *Trichoderma* can be explained by different mechanisms. *Trichoderma* inoculation has been shown to stimulate soil enzymatic activity, primarily related to soil fertility, allowing for the establishment of metal-accumulating plants [66]. It can also enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase [67]. These findings align with the results of this study, where *Trichoderma* BIF3-C1,

BLPF1-C1, BM18-C1, CNF20-C1, CP24-6, CRSF1-C1, UCF10A-C1, UCF12-M3, UCF18-M1, and UCF5A-C1 strains stimulated Cd accumulation in the cacao stem, suggesting a potential role in promoting Cd uptake. Among these, BLPF1-C1 exhibited the highest Cd uptake, which corresponded with a significant reduction in plant biomass. While numerous studies have reported beneficial effects of *Trichoderma* on plant growth, its capacity to enhance heavy metal uptake, which may lead to toxicity, has also been documented. Although this capacity is desirable in phytoremediation strategies, excessive accumulation of metals in plants can result in adverse effects. The toxicity of cadmium (Cd) in plants is well documented. According to [68], Cd toxicity can cause growth retardation, alterations in photosynthetic activity, changes in stomatal movement, modifications in enzymatic activity and protein metabolism, and disruptions in cell membrane function. In cacao, morphological, molecular, and physiological changes have been reported in response to Cd toxicity, in addition to reduced absorption of essential micronutrients such as Zn and Fe [69].

Finally, whether used independently or in association with other organisms (e.g., legumes), the study and application of fungi with bioremediation potential present several advantages, including their great diversity, adaptability, rapid growth, and high biomass production [70]. Moreover, as discussed throughout this work, some of these organisms have demonstrated excellent capacities for capturing and bio-removing Cd [71,72]; however, the bioremediation effect depends on a variety of factors and therefore requires detailed study and characterization.

5. Conclusions

The results indicate that Cd removal efficiency tends to be similar within the same species but varies between species. Additionally, higher cadmium concentrations in the medium lead to increased accumulation in fungal biomass. Furthermore, Cd removal efficiency and biomass accumulation are directly correlated.

The in vitro results do not always correspond to in vivo conditions. While some strains promoted Cd accumulation in the stem, *Trichoderma* sp. UCPF2-C1 and *T. longibrachiatum* UCF17-M4 significantly reduced cadmium levels compared to other *Trichoderma* strains, suggesting their potential for mitigating heavy metal uptake.

Finally, these *Trichoderma* strains did not enhance plant growth under cadmium-stress soil conditions. Moreover, in some cases, such as with strain BLPF1-C1, inoculation had adverse effects, reducing both the dry and fresh weight of the plants.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microbiolres16060130/s1: Figure S1: The interaction results of cadmium removal and biomass accumulations. The results represent the means of four to five replicates ± SD. The variables were analyzed using a non-parametric factorial analysis of variance (Aligned Rank Transform). The lines indicate the cadmium removal efficiency (A) and biomass accumulation (B), where the convergence of continuous lines denotes the interaction for both the factors Strains and Concentrations; Figure S2: The significance of the interaction results of cadmium removal (A) and biomass accumulations (B). For both datasets, the data were analyzed using a non-parametric factorial ANOVA with the ARTool package in the R environment, followed by the "art.con" function with Holm corrections. The results indicate significant interactions among treatments and strains, with purple denoting the most significant differences (p-value < 0.05, indicated by asterisks) and white representing the least significant ones. Graphs were generated using the ggplot2 package, and adjustments to color, typography, and label alignment were made in Illustrator software while preserving the integrity of the original graphical data; Table S1: Inoculum quality evaluation.

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