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Morphophysiological and Biochemical Responses of *Zea mays* L. under Cadmium and Drought Stresses Integrated with Fungal and Bacterial Inoculation

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Abstract: Cadmium and drought stress are the most destructive of the abiotic stresses with negative effects on both metabolism and photosynthesis. The present experiment aimed to analyze the impact of inoculation with *Bacillus paralicheniformis* and *Trichoderma asperellum* on the growth and antioxidant response modulation of maize (*Zea mays* L.), under drought and Cadmium (Cd) stresses. Regarding plant biomass analysis, fungi inoculation increased leaf dry biomass significantly (11.92%) towards uninoculated ones. The leaf area was affected significantly by bacterial application, 12.15% more than the control. A significant trend (drought+ Cd stress) was observed between fungi-inoculated maize leaves (15.07 µmol/g FW) and bacterial-inoculated leaves (18.71 µmol/g FW) regarding the malondialdehyde quantity. Furthermore, the activities of superoxide dismutase were notably higher (9.63–40.88%) in microorganism-inoculated roots. Similarly, under drought + Cd stress, peroxidase demonstrated a higher activity under bacterial inoculation than fungal ones (92.11% more). The maximum translocation factor was observed in the uninoculated group (under Cd stress), while the bioconcentration factor under drought stress showed a significant increase by microorganisms. The maximum relative water content under bacterial inoculation (82.66%) was achieved. The fungi and bacterial inoculation minimized Cd accumulation in the leaf significantly under drought and drought + Cd stress. Generally, the microorganism inoculation positively and partially maintained the plant's performance, despite the presence of drought and Cd stress.

Keywords: *Bacillus paralicheniformis*; abiotic stress; drought tolerance; heavy metals; plants growth; *Tricoderma asperellum*

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1. Introduction

Maize is a plant from the cereal group that plays a major role in the world's agricultural production and ranks third in the world among crops in terms of the cultivated area. The rapid population growth in countries, along with the increasing need for food determine the necessity of increasing agricultural production. Food security and human health are threatened by drought, which presents a serious problem for corn plants [1]. The antioxidant stress relief provided by indigenous microorganism inoculation has been the subject of numerous reviews and research papers. They may lead to the development of biotechnologies for plant growth promotion in arid and semiarid regions, owing to their ability to withstand severe environments and mitigate drought stress [2].

Cadmium (Cd) has disastrous impacts on human health, crop growth, and production, and it is also exceedingly poisonous and carcinogenic. Plants use their roots to absorb Cd from the soil, and they use a variety of particular and non-specific transporters of other vital nutrients to move Cd from the roots to various organs [3]. It has been established that several transporter families, including the ATP-binding cassette G subfamily protein, natural resistance-associated macrophage protein, zinc/iron-regulated transporter-like protein, and heavy metal ATPase, are involved in the movement of Cd in plants. Plants that are exposed to Cd stress go through several chemical changes that affect signaling pathways, osmoregulation, and secondary metabolites [4]. Heavy metals disturb organelles and cell membranes, which results in electrolyte leakage and reactive oxygen species (ROS) in plants. The chain reaction of ROS disrupts cellular metabolism and denatures signaling protein molecules, rendering them worthless [5]. Moreover, ROS damages phospholipids, which are a crucial component of the plasma membrane, resulting in the lysis of cells [6]. Plants establish a defense mechanism that includes an antioxidant response to deal with ROS and counteract their negative effects. Nevertheless, several plants are able to tolerate and accumulate heavy metals at high concentrations [7]. Moreover, in heavy metal-polluted semiarid and arid areas, drought significantly alters a series of physiological, biochemical, and molecular processes which limits plant growth and establishment as a result [8]. Plants grow best when contaminant levels are low since high concentrations can limit growth and take a long time to eliminate. Accordingly, drought leads to a reduction in the overall plant growth by lowering hormone production, photosynthetic activity, and membrane stability [9]. The lack of water in plants leads to a decrease in CO_2 accessibility to the chloroplasts, eventually inhibiting the photosynthetic rate and increasing the photosystem's vulnerability [10]. Variations in the functioning of enzymes, like superoxide dismutase, catalase, hydrogen peroxide, the amount of malondialdehyde, and electrolyte leakage can all be caused by drought-induced circumstances [11]. Since free radicals are thought to cause membrane damage via lipid peroxidation, several enzymes are tasked with preventing their formation. The hydraulic conductivity of roots is typically decreased under drought stress, while it usually returns to pre-drought levels if the plant is re-watered. While the degree of recovery is largely determined by the severity of the stress, there is typically a leak of solutes from the intracellular space into the medium after the plant tissues have been rehydrated [12]. If plants are drought-tolerant, they can retain the solutes and return to their initial levels with a reasonable efficiency, whereas drought-sensitive plants may not be able to do so and may sustain irreparable damage to their tissues as a consequence [13]. A variety of environmental and human health problems can result from improper fertilizer and pesticide inputs; moreover, these agricultural crop production approaches are both expensive and non-renewable. Sustainable and environmentally friendly agriculture practices can be achieved using beneficial microbes as stress-protecting agents for plants. The development of plant growth-promoting bacteria (PGPB) for plant growth and tolerance represents an emerging and preferred approach for combating multiple stresses [14]. To increase the agricultural productivity of metal-contaminated soils, plant growth-promoting bacteria have been widely introduced into plants [15].

Various mechanisms have been proposed to increase the plant resistance to these environmental stresses using beneficial microorganisms, including fungi and plant growth-promoting bacteria through their effects on rhizosphere biochemistry and the physiological and molecular processes of plants [16]. This can lead to the improved growth and nutritional status of plants through the production of siderophores, phytohormones (such as gibberellins, auxins, and ethylene), enzymes (such as _ ACC (1-aminocyclopropane-1-carboxylate) deaminase), and microbial extracellular polymers, such as exopolysaccharides (EPS), which include cellulose, dextran, motan, alteran, pullulan, and curdlan [17]. Indole acetic acid (IAA) produced by microorganisms can also increase root and lateral root growth, thereby helping plants to increase their water and nutrient absorption and reduce drought stress as a result [18]. Additionally, exopolysaccharides, which are long-chain

polysaccharides composed of sugar units, including glucose, galactose, and rhamnose in various ratios, are released during various microbial growth stages [19]. The chemical composition of EPS is such that they can combine with metal ions and protect the host microbial and plant cells from heavy metal stress [20].

Bacillus sp. confers resistance against both biotic and abiotic stresses in a variety of plants. The Gram-positive, spore-forming *Bacillus,* such as *paralicheniformis* is attracting growing interest as one of the most favorable Plant Growth-Promoting Rhizobacteria (PGPRs), primarily due to its innate durability and prolonged storage capacity [21]. *Bacillus* sp. have been shown to increase nutrient acquisition using phosphate-solubilizing enzymes, produce phytohormones, and fix nitrogen [22]. Furthermore, it promotes the development and expansion of the root system of various plants, thereby increasing the absorption surface for metals and subsequently reducing their transfer from the root to the aerial parts [23]. In addition, by secreting siderophores, it binds not only ferric iron (Fe_{3+}) but also other heavy metal ions, thereby reducing the concentration of toxic metals in the environment [24].

Moreover, in addition to PGPB, fungi are also helpful. *Trichoderma asperellum* is one of the most important plant growth-promoting fungi (PGPF), which promotes plant resistance to drought stress and Cd stress through several mechanisms, including: inducing the production of PGP hormones, enhancing nutrient uptake, improving antioxidant defense system, helping to reduce water loss in plants by inducing the closure of stomata, and bind and immobilize Cd ions, which reduces their availability to plants and subsequently decreases the toxicity of Cd stress to protect plant cells from Cd-induced damage [25].

Nowadays, the utilization of beneficial soil microorganisms and their relationships with various plants to reduce the effects of non-biological stresses on host plants has been reported across various studies. However, the potential of *Bacillus paralicheniformis* and *Tricoderma asperellum* to influence maize seedling establishment and water relations during well-watered, drought stress conditions, and Cd stress at early developmental stages, especially at the formation of shoots stage still remains unknown. The main and specific goal of the present study was to determine whether the examined bacteria and fungi are able to reduce the stress sensitivity of maize in water-stressed or Cd-contaminated areas, as well as under double-stress conditions. In addition, the question to be examined was what physiological effects these microbial treatments cause, and which may play a role in possible protective processes, with special regard top lant growth parameters and antioxidant processes. In light of the aforementioned issues, the present study aimed to assess the mechanisms of *B. paralicheniformis* and *T. asperellum* inoculation on the morphology, growth, membrane integrity, and enzymatic defenses of a drought-sensitive plant, *Zea mays* L., under integration with water deficit and Cd toxicity stresses.

2. Materials and Methods

2.1. Soil Preparation and Analysis

To conduct this research, a sufficient amount of soil was collected from the surface horizon (0 to 30 cm) of the Kuye-Asatid soil series (loamy-skeletal over fragmental, carbonatic, mesic, and Fluventic Xerorthents) in the agricultural station of Bajgah region, Fars, Iran (29°43′5″ N, 52°35′28″ E, and 1810 m) from the School of Agriculture, Shiraz University, Fars, Iran. Several physical and chemical characteristics of the soil were evaluated after drying in the air and being passed through a 2 mm sieve. A hydrometer was used to determine the soil texture [26], while organic matter was determined using the wet oxidation method [27]. The pH meter and electrical conductivity (EC) of the saturated extract were measured using an electrical conductivity meter [28]. The cation exchange capacity of the soil was measured by replacing cations with $C_2H_3NaO_2$ as the equivalent of $CaCO_3$ using the method of neutralization by HCl [29]. The extractable P was assessed by extracting with Na_2CO_3 [30]. The total N was determined by Kjeldahl [31], and K was determined by extracting with $C_2H_7NO_2$ and was read using a flame photometer (410 00 009-UK) [32]. The low-consumption elements of cations were extracted using the diethylene triamine

pentaacetic acid (DTPA) extraction method, and their concentration was determined by an atomic absorption device (Shimadzu AA-670- Japan). The characteristics of the soil used are presented in Table 1. Soil EC and pH at the end of the experiment are presented in the supplementary material (see Supplementary Figures S1 and S2).

Table 1. Physicochemical characteristics of the soil used in the present experiment.

Feature	The Amount
Sand	32%
Silt	37%
Clay	31%
Texture class	Clay loam
pH saturated dough	7.8
Electrical conductivity of the saturated extract	0.9 dS/m
Cation exchange capacity	15 $cmol^+/kg$
$CaCO_3$ equivalent	2.25%
Organic C	0.81%
Organic matter	1.04%
Total N	0.09%
P extractable by $NaHCO_3$	25 mg/kg
K extractable by $C_2H_7NO_2$	386 mg/kg
Extractable Fe with DTPA	1.43 mg/kg
Extractable Cu with DTPA	21.2 mg/kg
Extractable Mn with DTPA	47.3 mg/kg
Extractable Zn with DTPA	1.02 mg/kg
Extractable Cd with DTPA	0.8 mg/kg

2.2. Microbial Inoculation

The corn seeds were disinfected with 96% alcohol (30 s) and a dilute solution of sodium hypochlorite (5%) (3–5 min). Following that, sterile distilled water was used to wash the seeds several times to remove any remaining sodium hypochlorite. Following several days of incubation at 25 °C, the sterilized corn seeds were spread uniformly on sterile filter paper in glass Petri dishes for inoculation with microbial cultures. For one hour, germinated maize seeds were immersed in bacterial *B. paralicheniformis* and fungus *T. asperellum* inoculum in two separate Petri dishes and were then placed on a shaker. Plants were also inoculated with 1 mL of bacterial and fungal inoculum when planted in the culture bed [33]. The bacterium and fungus were provided from the microbial collection of Iran's Soil and Water research institute and the Soil Biology Laboratory from Shiraz University, respectively.

2.3. Experimental Design and Growth Condition

A factorial, completely randomized greenhouse experiment, was conducted in four replications. There were 2 main factors:

1. stress [blank (without drought and cadmium), drought (50% of field capacity) [34], Cadmium (200 mg/kg) [35], and drought + Cadmium (50% of field capacity +200 mg/kg Cadmium];
2. microorganism [without microbial inoculation (uninoculated), bacterial inoculation, and fungal inoculation].

In total, there were 12 treatments with 4 repetitions for each treatment (48 pots) (Table 2).

Table 2. Experimental work plan. Each treatment had four replications.

Treatments
Blank without microbial inoculation
Blank with bacterial inoculation
Blank with fungal inoculation
Drought without microbial inoculation
Drought with bacterial inoculation
Drought with fungal inoculation
Cadmium without microbial inoculation
Cadmium with bacterial inoculation
Cadmium with fungal inoculation
Drought + Cadmium without microbial inoculation
Drought + Cadmium with bacterial inoculation
Drought + Cadmium with fungal inoculation

A 2 mm sieve was used to pass the soil treated with Cd three times to ensure a uniform mixture. Following the application of Cd, the samples of treated soil and control soil were moistened until they reached field capacity and were kept at a temperature of 20 °C for 120 days. These conditions induced reactions between Cd and the soil, resulting in the generation of pollution conditions that are closer to those found in nature. After filling the pots with soil contaminated with Cd, two maize seeds were planted in each pot. Soil moisture was maintained within the field capacity during the experiment. A dry treatment was also applied after the plant had been established. During the growing season (after three weeks of planting and at the time of stress onset), irrigation was performed daily by bringing the moisture to the values mentioned in the soil moisture treatment so that the weight of all the pots was measured every day with a digital scale. By comparing the initial weight of each pot (watering day weight) with its daily weight, the available moisture was calculated, and irrigation was performed according to the treatment.

2.4. Sample Collection and Parameter Measurement

Following plant harvest and the separation of the aerial parts and roots, all samples were washed with distilled water and were examined for the following items, including antioxidant enzymes (Cat, SOD, APX, and POD), total protein (TP), and malondialdehyde (MDA), which were all measured in the leaves at 4 stages: stage (T1) before drought stress application, stage (T2) at 3 weeks after stress, stage (T3) at 6 weeks after stress, and stage (T4) at the time of harvest.

2.4.1. Morphological Parameters

After the trial, total chlorophyll (chl) content was measured using a chlorophyll meter (SPAD-502 Minolta, Osaka, Japan). The SPAD meter calculated a three-digit SPAD value by comparing the transmission of red (650 nm) and infrared (940 nm) light through the leaf two times before stress (T1) and after harvest (T2) [36], and the leaf surface was assessed using a leaf area meter device model (CRLA1-Baher Kimia Rahavard, Tehran, Iran). The plants were then completely removed from the pots. Many factors were checked and measured after the roots had been cleaned, and soon after the plants were harvested, the root fresh weight (FW) was calculated using a digital scale (0.001 g). The roots were then oven-dried at 80 °C for 24 h before their dry biomass (DW) was measured. Using a four-digit scale, the roots' fresh and dried weights were calculated using a digital scale (0.001 g) [37]. When the experiment was complete, the plant height was measured using a scientific ruler, and the stem diameter was measured using a caliper (0.01 mm accuracy) [37].

2.4.2. Electrolyte Leakage (EL)

Each 0.5 g sample of fresh leaves was collected from recently grown, comparable-sized branches. The samples were maintained in falcon tubes with 10 mL of deionized water after being washed in deionized water. After that, the sample was incubated on a shaker

for 24 h at 25 °C. After the samples reached room temperature and were autoclaved at 121 °C for 20 min, both the electrical conductivity (EC_1) for each solution was calculated, and the ultimate electrical conductivity (EC_2) was calculated [38].

2.4.3. Relative Water Content (RWC)

Each plant's fully developed leaves were used to measure the RWC. Fresh leaf samples were weighed, and then put into test tubes with distilled water to saturate them. They were placed on a shaking machine and kept at room temperature for 24 h. The turgid weight was then determined by reweighing it 24 h later. Once the samples had been dried in an oven for 24 h at 60 °C, their dry weights were determined [39].

$$\text{RWC} = (\text{fresh weight} - \text{dry weight})/(\text{turgid weight} - \text{dry weight}) \times 100$$

2.4.4. Malondialdehyde and Total Protein

To measure the malondialdehyde (MDA) concentration, fresh leaf tissue (0.5 g) was homogenized in trichloroacetic acid (2 mL, 1%) (TCA) and then centrifuged at 10,000× *g* for 10 min. A mixture of the supernatant (250 µL) and 0.5% thiobarbituric acid (TBA) in 1 mL of 20% TCA was combined. The combination was submerged in 90 °C hot water for 30 min before being rapidly chilled in an ice bath and centrifuged. A microplate reader spectrophotometer was used to measure the absorbance at 532, 600, and 450 nm, respectively (Epoch Biotek, Winooski, VT, USA) [40].

Bradford's assay was used to calculate the amount of total protein in the roots and leaves, and measurements of absorbance at 595 nm were then obtained [41].

2.4.5. Enzyme Extraction

To prepare the sample for enzyme extraction, fresh leaf tissue (0.5 g) was removed and pulverized in liquid nitrogen with a mortar and pestle. The sample was homogenized with 2 mL extraction buffer (50 mmol/L potassium phosphate buffer, pH = 7, containing 2 mmol/L ethylene diamine tetraacetic acid and 1% polyvinylpyrrolidone). The homogenization was conducted in an Eppendorf tube (2 mL) before being centrifuged at 13,000× *g* (4 °C) for 10 min. The total protein content and all enzyme activities were measured based on the supernatant. Enzyme activities were read using spectrophotometry (Dynamica, Livingston, NJ, USA) and the specific activity was calculated as U/mg protein (Habibi et al., 2020) [42].

2.4.6. Superoxide Dismutase Activity

Three mL of reaction mixture consisted of 50 µL of enzyme extract, 50 mL of potassium phosphate (pH = 7.8), 13 mg of methionine, 75 µg of nitro tetrazolium chloride (NBT), 0.1 mg of EDTA, and 4 µg of riboflavin. The mixtures were placed in a light chamber with four fluorescent lamps of 20 v for 15 min to accelerate the reaction. To terminate the reaction, the lamps were turned off, and the samples were then placed in the dark. Zero percent solution (device control) was a reaction mixture without enzyme extract that was not exposed to light, while a control solution was a reaction mixture exposed to light and generating maximum color. Each sample was then read using a spectrophotometer (7315 model, JENWAY Company, London, UK) at a 560 nm wavelength to determine its absorbance value. A unit of enzyme activity per mg of fresh sample weight was reported [42].

2.4.7. Catalase Activity

As a measure of catalase enzyme (CAT) enzyme activity, a spectrophotometer at a 240 nm wavelength was used to measure the reduction of light absorption due to the decomposition of hydrogen peroxide over intervals of 10 s for 1 min. Three mL of the reaction mixture contained 50 µL of enzyme extract, 50 mL of potassium phosphate buffer (pH = 7), and 10 mL of hydrogen peroxide. Using the extinction coefficient (ε = 39.4 $mM^{-1}cm^{-1}$), the

enzyme activity was measured in µmol of hydrogen peroxide decomposed in 1 min per g of FW [42].

2.4.8. Peroxidase Activity

As a measure of peroxidase (POD) enzyme activity, a spectrophotometer at a 470 nm wavelength was used to measure the reduction in the light absorption due to the decomposition of guaiacol oxidation over intervals of 10 s for 1 min. Three ml of the reaction mixture contained 50 µL of enzyme extract, 2.9 mL of potassium phosphate buffer (pH = 7), and 0.05 mL of guaiacol. The reaction began with the addition of 20 mL of 40 molar hydrogen peroxide. For one gram of fresh sample weight in 1 min, the enzyme activity was calculated and reported using the silent coefficient (26.6 $mm^{-1}cm^{-1}$) [42].

2.4.9. Ascorbate Peroxidase Activity

As a measure of ascorbate peroxidase (APX) enzyme activity, a spectrophotometer at a 290 nm wavelength was used to measure the reduction in the light absorption due to the decomposition of ascorbate oxidation over them in intervals of 10 s during 1 min. One mL of the reaction mixture contained 50 µL of enzyme extract, 50 mmol of potassium phosphate buffer (pH = 7), 0.5 mmol of ascorbate, 1 mmol of EDTA-Na, and 0.1 mmol of hydrogen peroxide. The reaction began with the addition of 20 mL of 40 molar hydrogen peroxide. For a g of FW in a minute, the enzyme activity was calculated and reported using the silent coefficient (2.8 $mM^{-1}cm^{-1}$) [42].

2.4.10. Translocation Factor (TF) and Bioconcentration Factor (BF)

The translocation factor was calculated to estimate the transfer of heavy metals from the roots to the shoots ($TF = (C_{shoot}/C_{root})$; C stands for metal concentration) [43].

The bioconcentration factors was also calculated in this way:

$$BF = C_{root}/C_{soil}$$

2.5. Statistical Analysis

Every treatment consisted of 4 parallel repetitions. Due to the two-factorial design, in comparisons demonstrating the effects of stress, mean values included all the data regardless of the inoculation treatments, having mean values of 12 individual data. Similarly, mean values demonstrating the effects of inoculations also included the all the data irrespective of stress treatments, having mean values of 16 individual data. A general linear model was employed for statistical analysis and in dealing with the entire experimental data using R software (Version 4.3.0). Then graphs were created with Microsoft Excel software (Version 2016). The mean comparisons between the main and interaction effects among the experimental factors, including stress (four levels) and microorganisms (three levels) were performed using the LSD test at p-value ≤ 0.05. Principal component analysis (PCA) was also performed based on a correlation matrix generated from Minitab software (Version 19).

3. Results

3.1. Plant Height, Stem Diameter, and Leaf Area

Stress groups produced significant effects on the plant height of the *Zea mays* plant (Table 3). However, microorganism treatments and their interaction effects were not found to be significant (Figure 1). Mean comparisons in the control group (plants without either Cd or drought stress) revealed that fungal inoculation caused a significant increase in plant height (9.42%) in comparison with the uninoculated group. Evaluation of stress treatment impacts revealed that the minimum plant height at drought + Cd (62.25% reduction towards control) and the maximum plant height was under drought stress after the control treatment (Figure 1(A1)).

Table 3. Analysis of variance (ANOVA) for plant height (PH), stem diameter (SD), leaf area (LA), leaf fresh weight (LFW), leaf dry weight (LDW), root fresh weight (RFW), root dry weight (RDW), relative water content (RWC), electrolyte leakage (EL), translocation factor (TF), bioconcentration factor (BCF), spad value-T1 (SVT1), and spad value-T2 (SVT2), as affected by the independent variables: cadmium, drought stresses (S), *B. paralicheniformis* and *T. asperellum* microorganisms (M), and stresses and microorganisms (S × M).

	PH (cm)	SD (mm)	LA (cm^2)	LFW (g)	LDW (g)	RFW (g)	RDW (g)	RWC (%)	EL (%)	TF	BF	SVT1	SVT2
S	**	**	**	**	**	**	**	**	**	**	**	**	**
M	ns	ns	*	**	**	*	**	**	ns	**	ns	**	**
S × M	ns	ns	ns	ns	**	ns	ns	ns	ns	**	**	**	**

* and ** are significant at 5% and 1%, respectively, ns are not statistically significant.

The results of the analysis of variance (Table 3) showed that the stem diameter was significantly affected by various stress groups, but microorganism treatments and their interaction effects were not significant (Figure 1(B2,B3)). The results of these mean comparisons showed that the stem diameter under drought stress was 9.17%, under Cd stress was 24.33%, and under drought + Cd stress, 29.49%, respectively, which decreased significantly compared with the control group (Figure 1(B1)).

The leaf area was significantly influenced by both the stress groups and microorganism treatments, but their interaction effect was not found to be significant (Table 3). Under drought stress alone, bacterial inoculation increased the leaf area significantly compared with the uninoculated group (21.89%). Under Cd stress alone, fungal inoculation caused a significant enhancement towards the uninoculated (12.01%). Evaluation of the stress effects showed that the maximum leaf area value was achieved under Cd stress, which was higher than the drought + Cd (25.02%), control (11.33%), and drought (8.64%), respectively. Bacterial inoculation revealed a 12.15% increase compared with the uninoculated, while fungal inoculation did not show a significant difference with the two other treatments (Figure 1(C2,C3)).

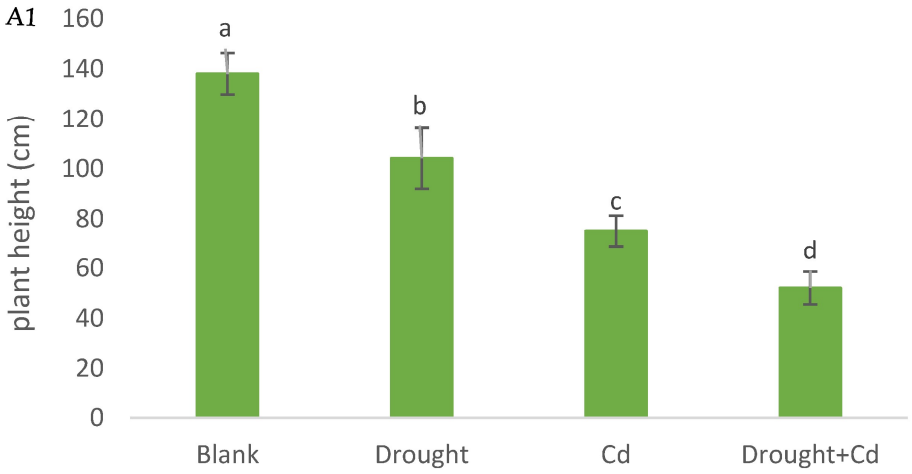
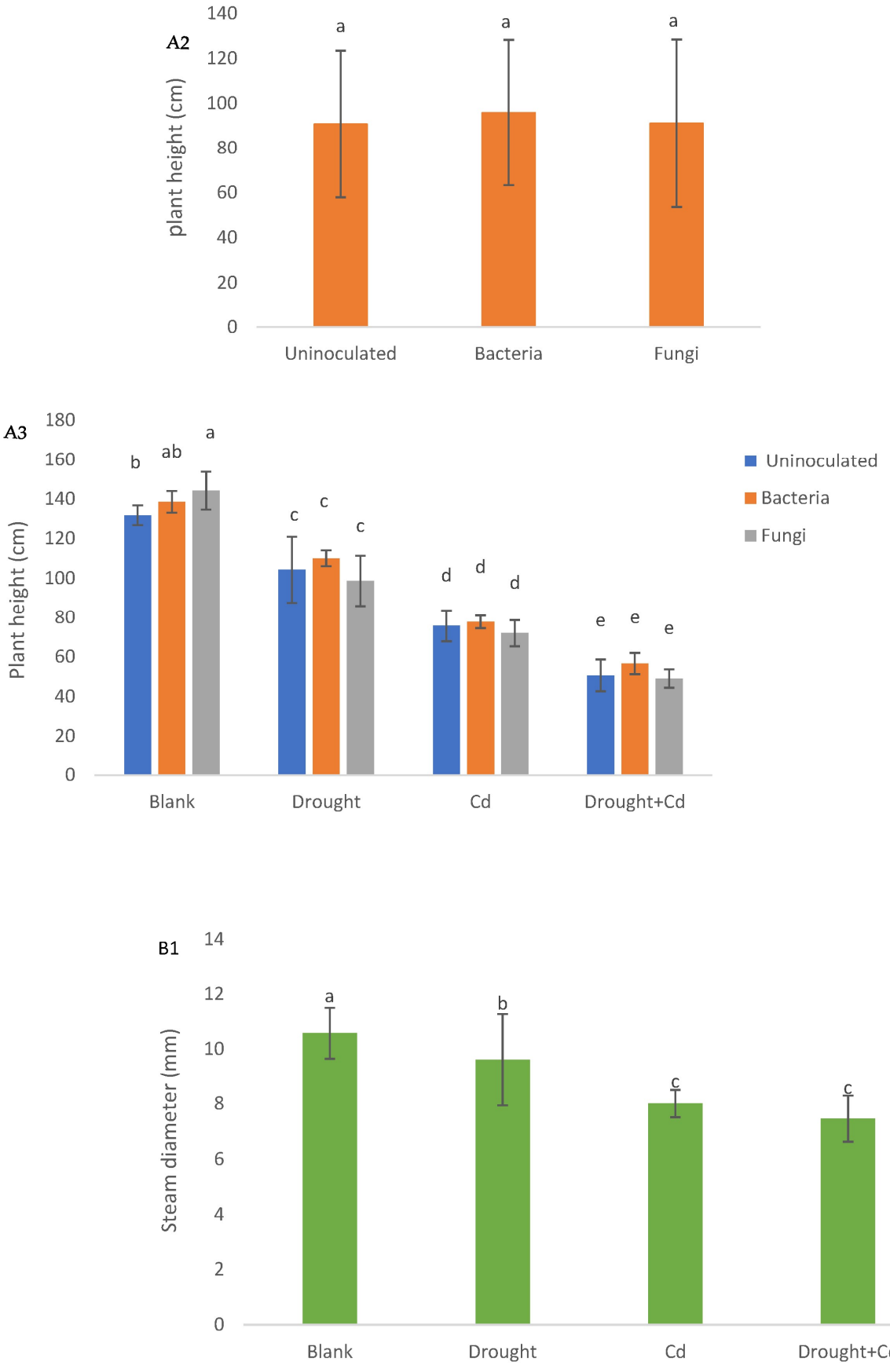


Figure 1. *Cont.*



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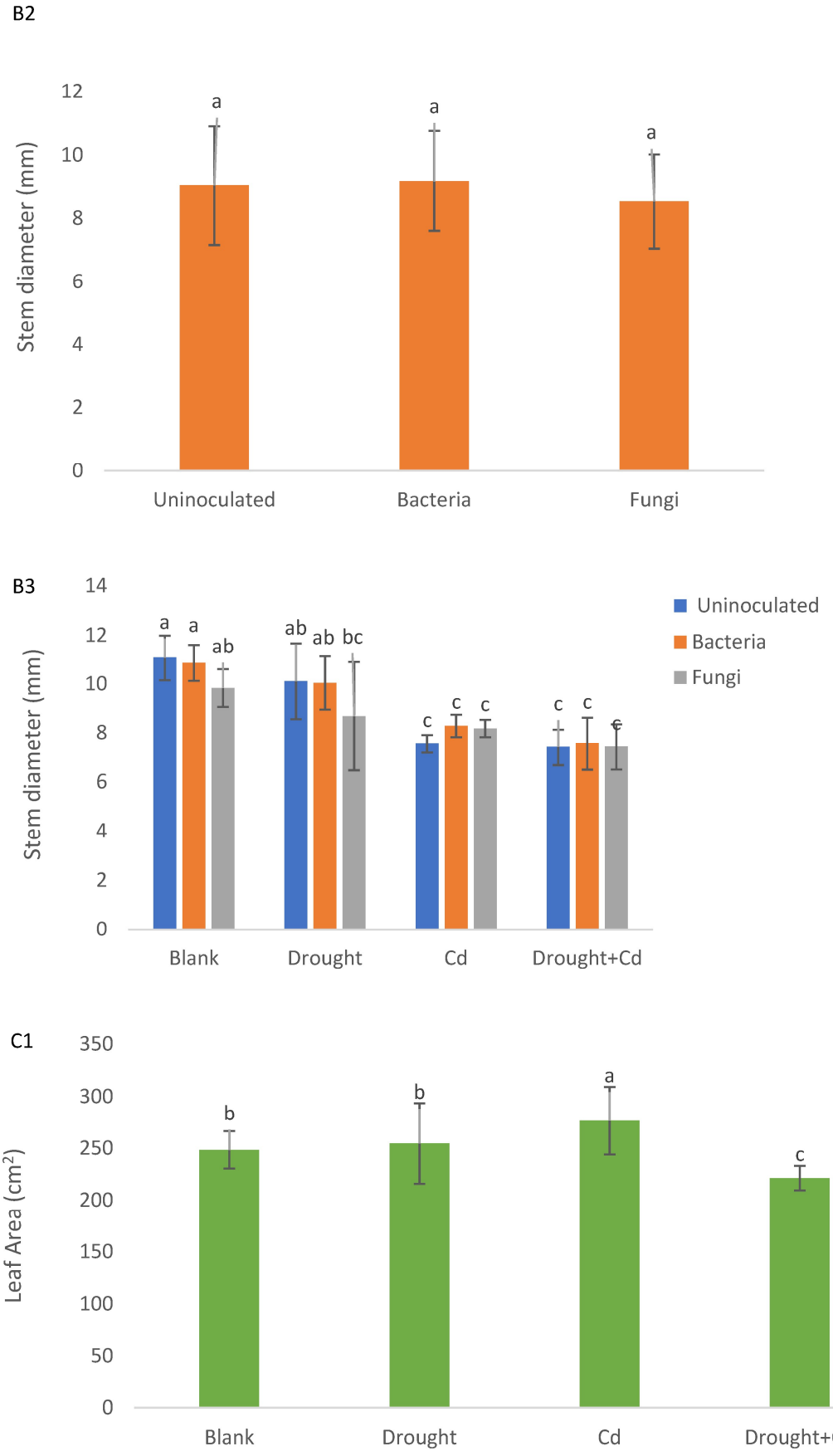

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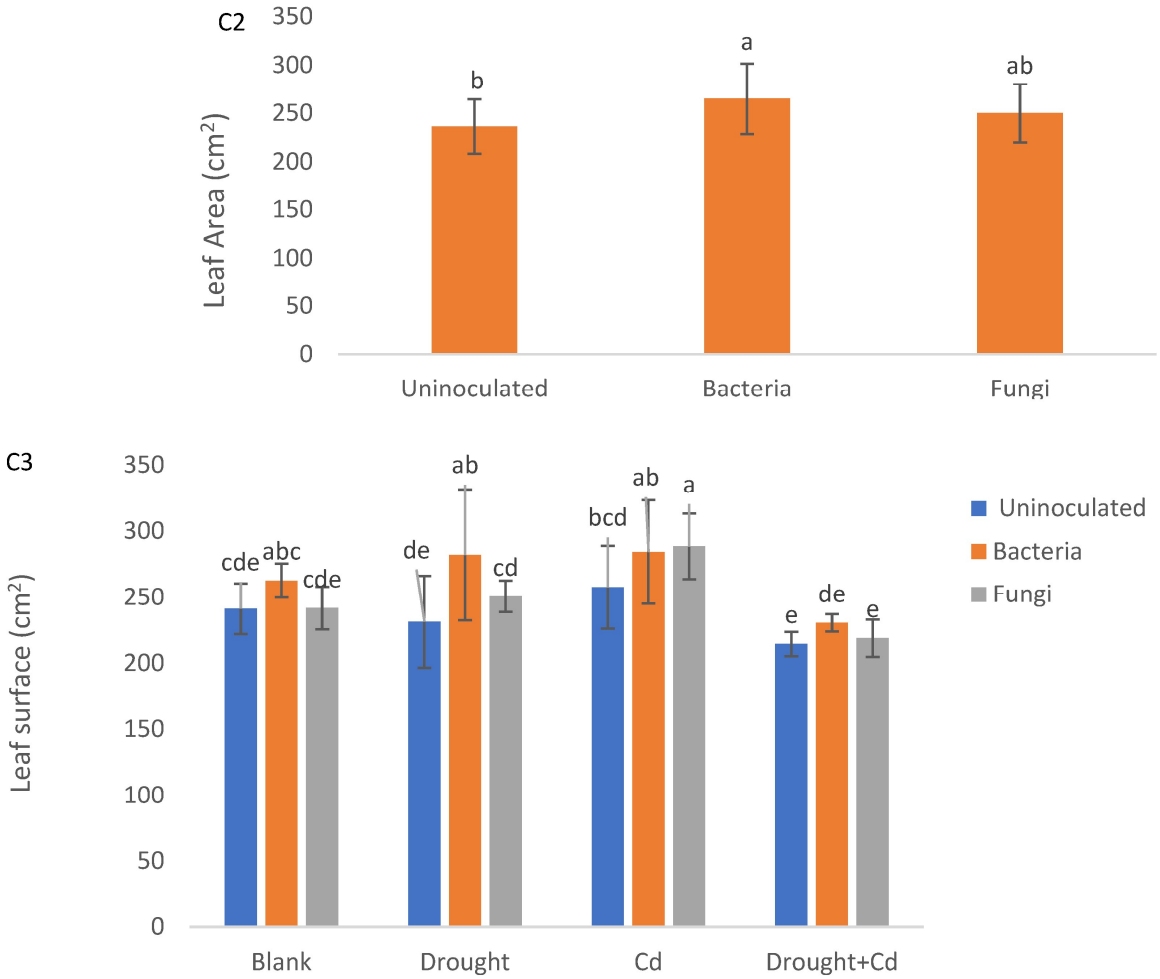
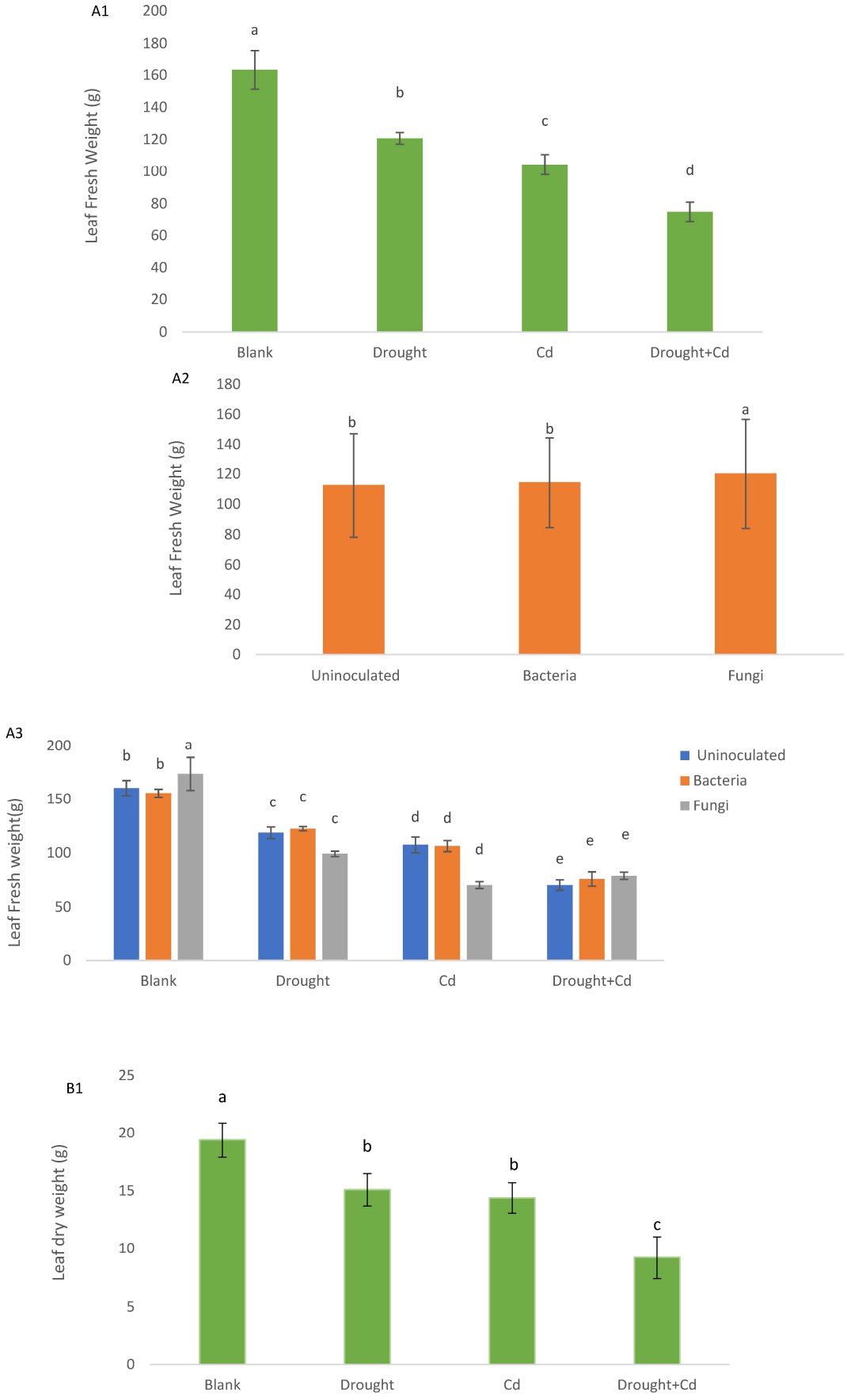


Figure 1. Variation in certain maize morphological traits, such as the plant height stem diameter and the leaf area under the studied treatments. (**A1**,**A2**,**B1**,**B2**,**C1**,**C2**) represent the effects of the individual treatments. (**A3**,**B3**,**C3**) represent the effects of the interaction between two factors. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

3.2. Leaf and Root Biomass

The analysis of variance (as shown in Table 3) demonstrated that the effects of the stress groups and microorganism treatments on the leaf fresh weight were statistically significant ($p < 0.01$), but that their interaction effect was not significant. The uninoculated plants grown under various stresses showed a decrease in the leaf fresh weight compared to the control group. Evaluation of the stressed groups showed that the minimum value was observed at drought + Cd (75.06), which showed a 37.81% reduction towards drought stress, and 28.06% reduction towards Cd stress compared to the control, respectively (Figure 2(A1)). Both fungal and bacterial inoculation caused a significant increase compared to the uninoculated treatment (6.75% and 1.61% increase, respectively) (Figure 2(A2)). Results of the mean comparison showed that the maximum leaf fresh weight was observed in the fungi-inoculated treatment (without Cd and drought). Minimum values were observed under integrated Cd and drought stress compared with control (54.53 and 51.21% reduction in fungi-inoculated and bacterial-inoculated, respectively (Figure 2(A3)).



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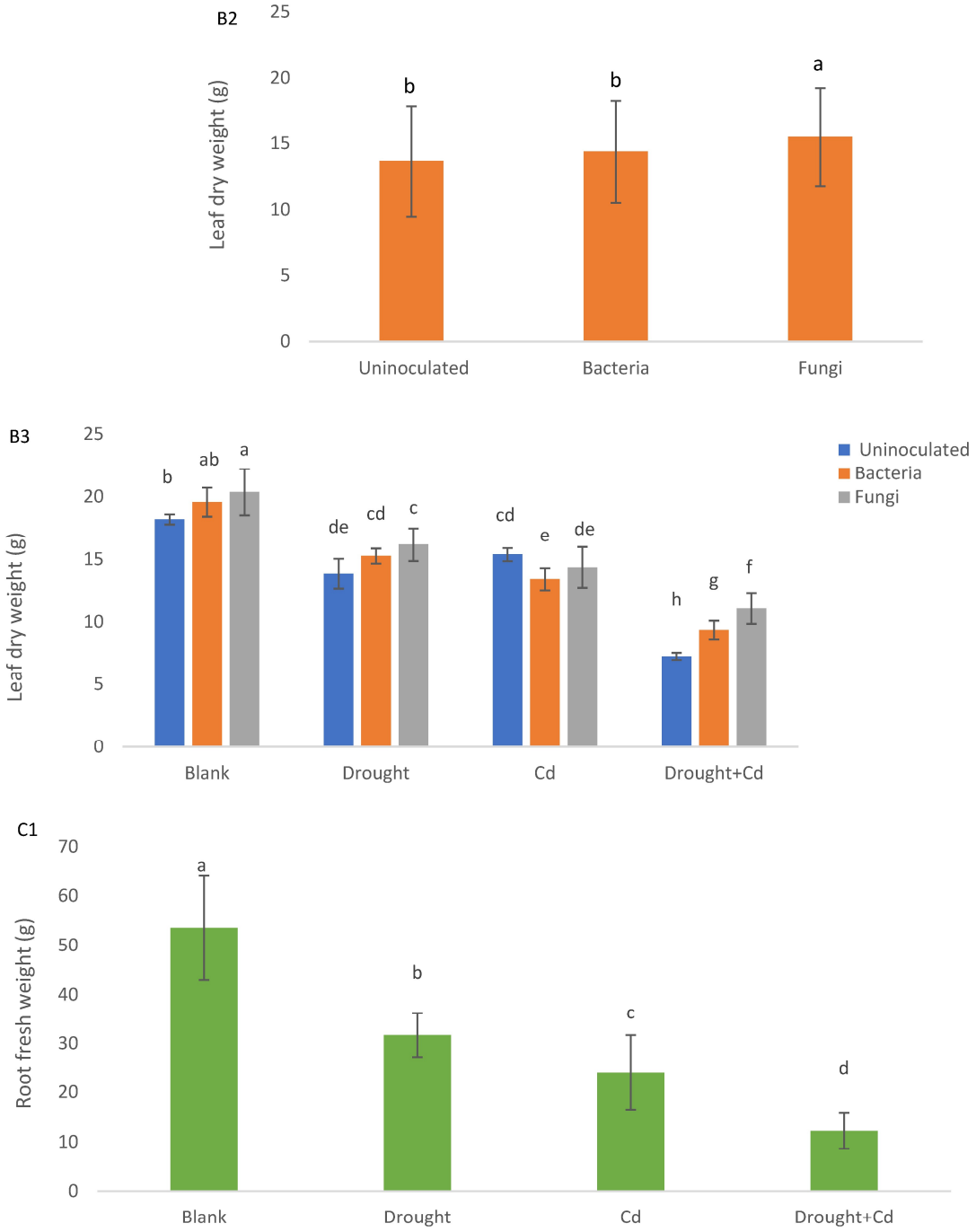

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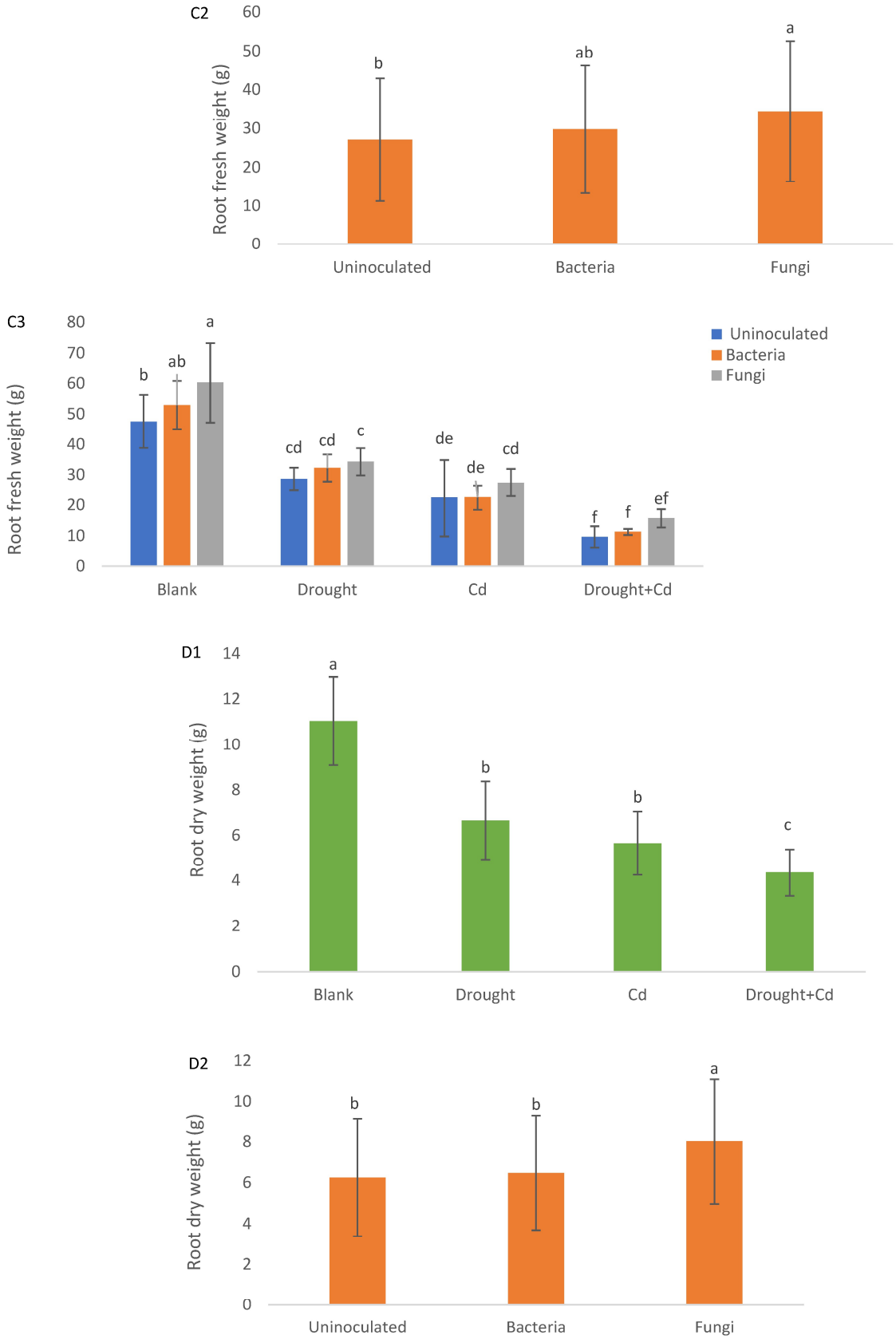

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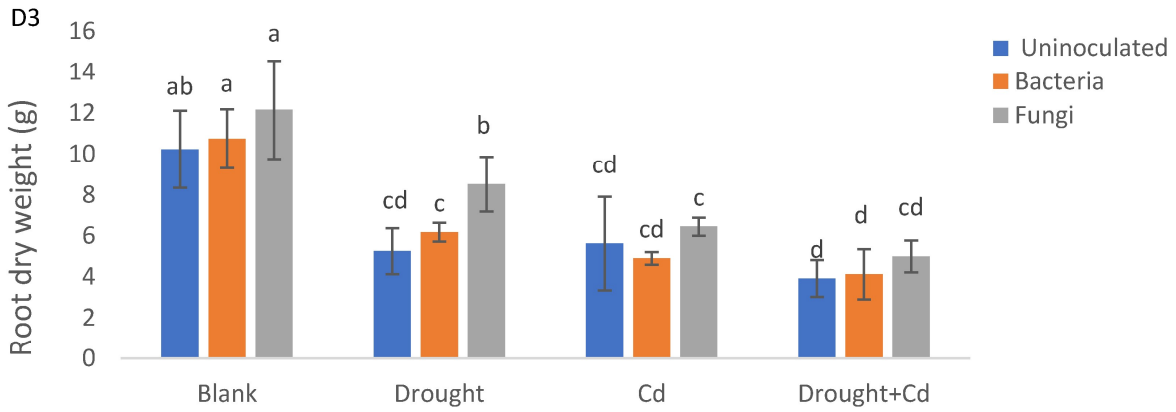


Figure 2. Variation in certain maize morphological traits, such as the leaf and root dry and fresh weights under the studied treatments. (**A1**,**A2**,**B1**,**B2**,**C1**,**C2**,**D1**,**D2**) represent the effects of the individual treatments. (**A3**,**B3**,**C3**,**D3**) represent the effects of the interaction between two factors. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

Stress groups and microorganism treatments, along with their interactions had significant effects on the leaf dry weight of the *Zea mays* plant (Table 3). In the pot experiment, the uninoculated *Z. mays* grown under Cd, drought, or Cd + drought stress displayed a significant decrease in the plant leaf dry weight. However, the maximum decrease in the leaf dry weight by 60 was observed under Cd + drought stress compared with the control (Figure 2(B3)). In contrast, the inoculation of fungi increased the plant dry weight though diminishing the negative effects of drought and Cd + drought stress. In measurements of the leaf dry weight represented of that in the control group, fungal inoculation was found to have significantly increased the leaf dry weight towards the uninoculated treatment (11.92%) (Figure 2(B2)). The minimum value was observed at integrated Cd and drought stress (Figure 2(B1)). Under integrated Cd and drought stress, fungal inoculation showed the maximum value compared with the uninoculated in the same treatment group (with a 52.69% increase) (Figure 2(B3)).

Regarding the root fresh biomass analysis, while the stress groups and microorganism treatments were found to be statistically significant, their interaction effect was not significant (Table 3). According to Figure 2(C1), a minimum was observed in the integrated drought + Cd stress group that showed a 42.62–56.59% reduction towards Cd stress alone. Furthermore, in the integrated drought + Cd stress group towards drought stress alone, 54.17–66.14% reduction was observed. All three stress treatment groups showed significant reductions compared with the control. Evaluation of microorganism effects showed that fungi inoculated with bacterial inoculated, and bacterial inoculated with uninoculated treatment didn't show any significant difference. But fungi inoculated showed 27.19% increase towards uninoculated treatment (Figure 2(C2)). The results of the analysis of variance (Table 3) demonstrated that stress groups and microorganism treatments on the root dry biomass were statistically significant, but their interaction effect was not significant. The root dry biomass showed that the integrated drought + Cd stress showed a significant difference with all treatment groups (60.59% reduction towards the control, 34.59% reduction towards the drought stress group, and 23.01% reduction towards the Cd stress group, respectively) (Figure 2(D1)). Furthermore, microorganism impacts on the root dry biomass revealed that fungi inoculation showed a maximum value that represented a 28.48% increase towards the uninoculated treatment and a 23.92% increase towards bacterial inoculation, respectively (Figure 2(D2)).

3.3. Relative Water Content (RWC) and Electrolyte Leakage (EL)

The effect of the stress groups and microorganism treatments on the RWC was found to be statistically significant, but their interaction effect was not significant (Table 3). Mean

comparisons of the RWC in the stressed groups revealed that there was no significant differences among the three stressed groups (drought, Cd, and drought + Cd), but all these groups showed a significant reduction towards the control group (9.28–15.19% reduction) (Figure 3(A1)). The application of microorganisms caused positive and significant impacts on the RWC. In this way, bacterial inoculation caused a 10.57% increase (compared with the uninoculated treatment) (Figure 3(A2,A3)).

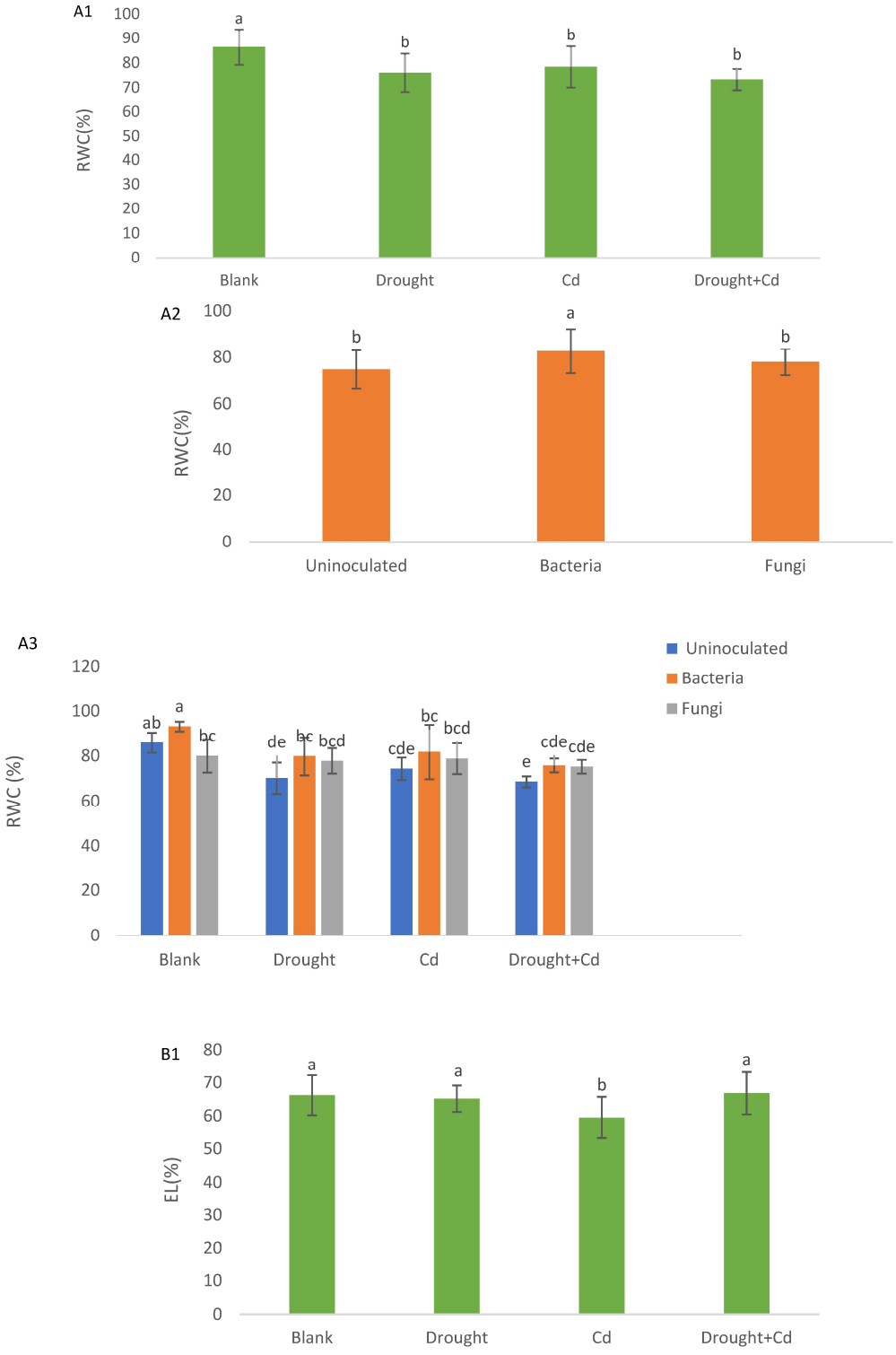


Figure 3. *Cont.*

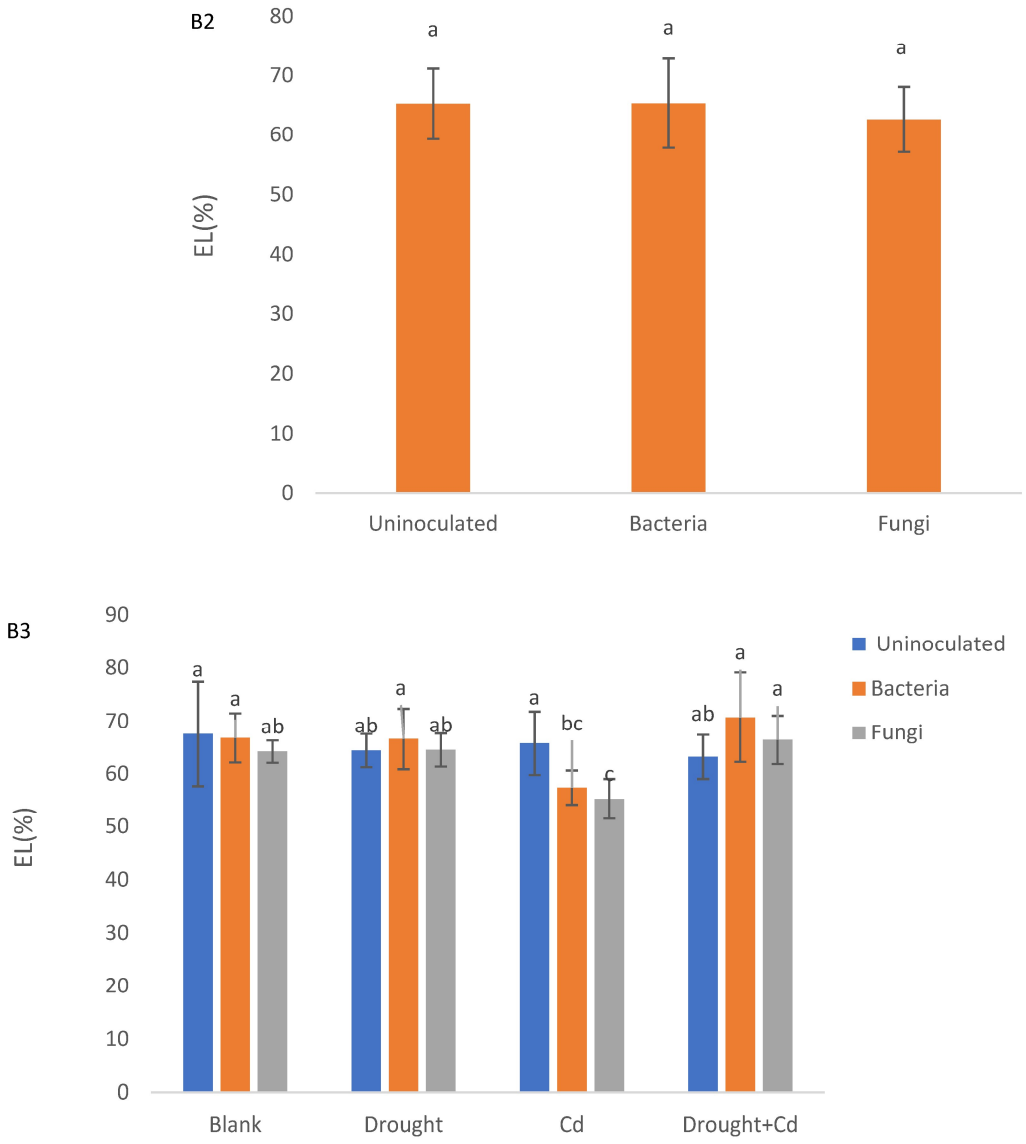


Figure 3. Variation in the maize leaf relative water content and electrolyte leakage under the studied treatments. (**A1**,**A2**,**B1**,**B2**) represent the effects of the individual treatments, (**A3**,**B3**) represent the effects of the interaction between two factors. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to LSD test.

Results of the analysis of variance (Table 3) showed that stress had a significant effect on the EL, but microorganism treatments and their interaction effects were not significant (Figure 3(B2,B3)). The minimum EL was achieved under Cd stress (10.13% reduction compared with the control treatment) (Figure 3(B1–B3)).

3.4. SPAD

Stress groups and microorganism treatments, and their interaction were all found to have significant effects on the spad value of the *Zea mays* plant (Table 3). Evaluations of the SPAD value showed that under drought and Cd stress, microorganisms were able to maintain a chlorophyll index similar to the control. Among two sampling times, T2 showed more value than T1 in most of the treatment groups (Figure 4).

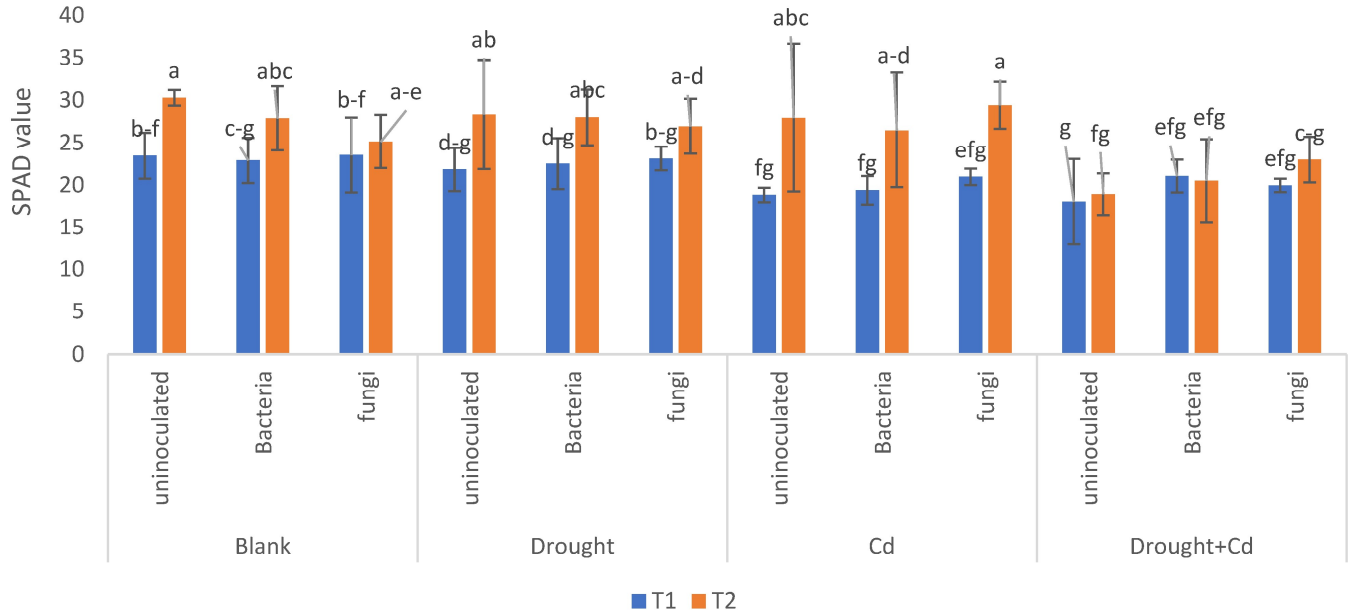


Figure 4. Variation in the maize chlorophyll index (SPAD) under the studied treatments. Figure represent the effects of the interaction between two factors at T1 (before stress) and T2 (harvest day). Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

3.5. Translocation Factor (TF) and Bioconcentration Factor (BF)

Stress groups and microorganism treatments, and their interaction were all found to have significant effects on the TF of *Zea mays* (Table 3). Results regarding the TF showed that the maximum value was observed under the uninoculated + Cd stress (0.98) (Figure 5(A3)). Regarding the stress group analysis, there was a significant increase (121.36%) observed in the Cd stress towards the control (Figure 5(A1)). Fungal and bacterial inoculation showed significant reductions compared with the uninoculated treatment (51.28 and 44.47%, respectively) (Figure 5(A3)).

The effect of stress groups and their interaction on the BF was found to be statistically significant, but the effects of the microorganism treatments were not found to be statistically significant (Table 3). The results showed that under drought stress, fungal and bacterial inoculation increased the BF towards the uninoculated. Meanwhile, under the control treatment, the opposite trend was observed (fungal and bacterial inoculation reduced the BF towards the uninoculated, 35.17 and 26.48%, respectively). Results of the mean comparisons between the stressed groups represented that the maximum BF was achieved under drought stress (with a 60.27% increase towards the control) (Figure 5(B1–B3)).

3.6. Leaf and Root MDA

Stress groups and microorganism treatments, and their interaction on the leaf and root malondialdehyde were all determined to be statistically significant ($p < 0.01$) (Tables 4 and 5).

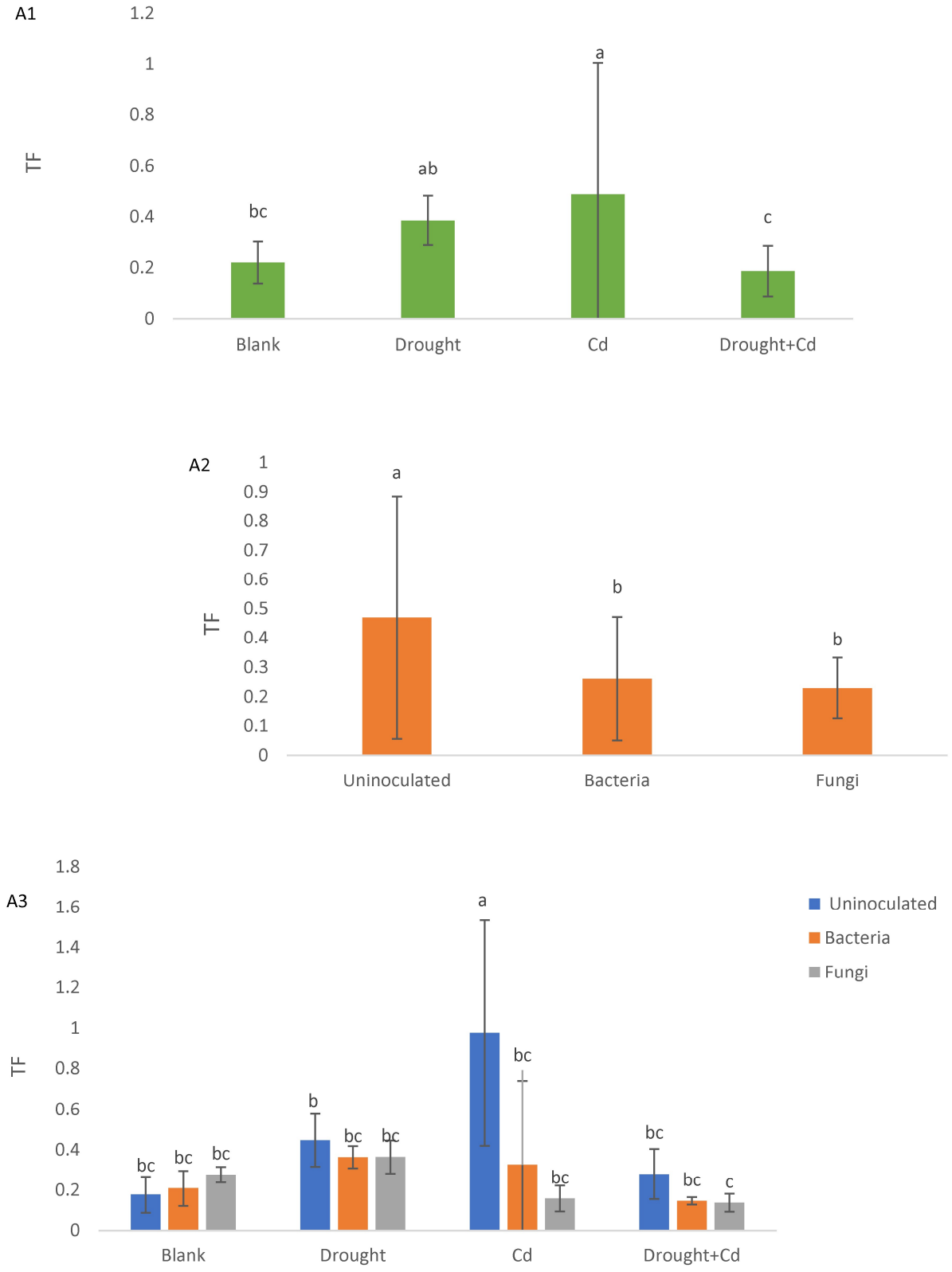


Figure 5. *Cont.*

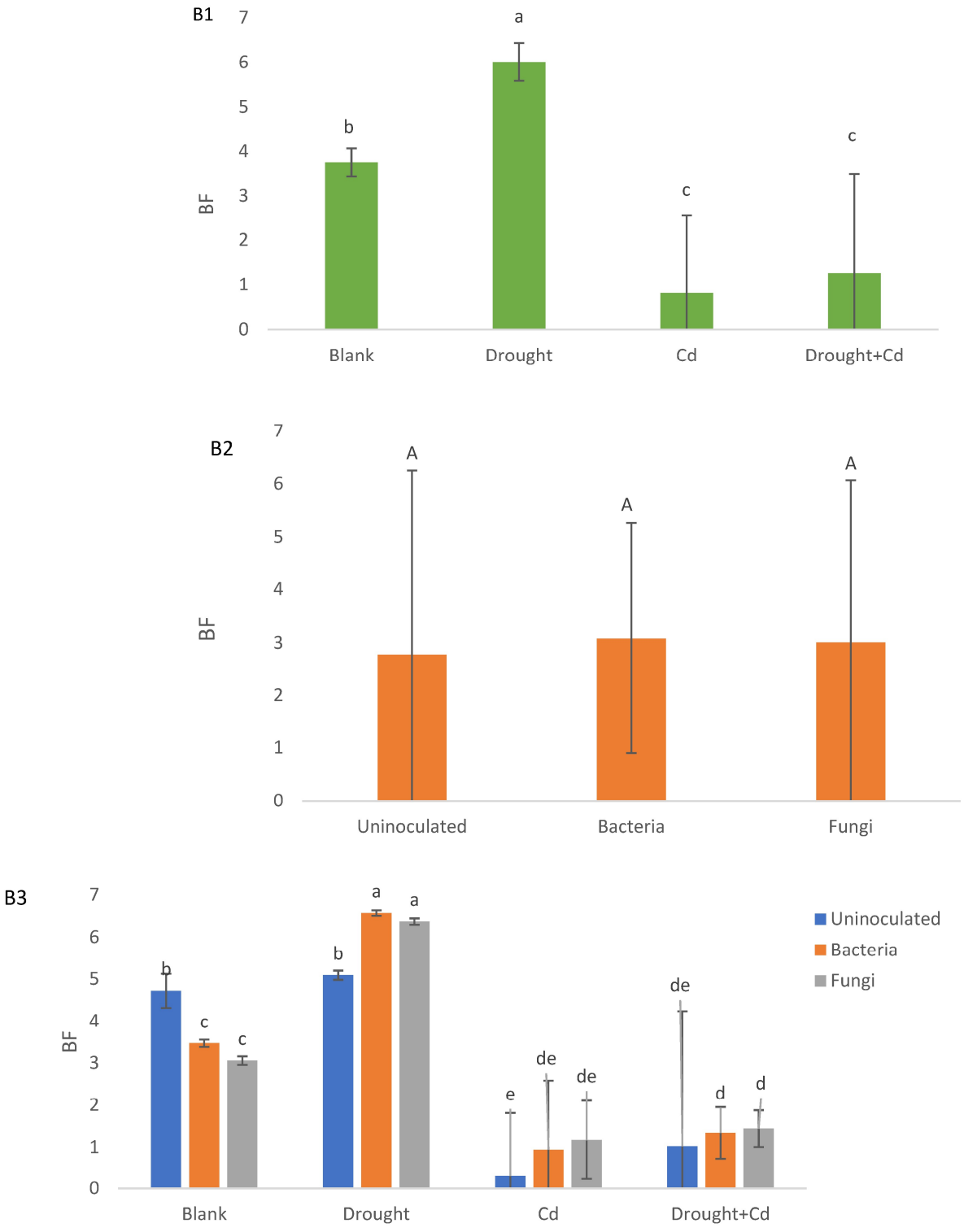


Figure 5. Variation in the maize translocation factor (TF) and the bioconcentration factor (BF) under the studied treatments (**A1**,**A2**,**B1**,**B2**) represent the effects of the individual treatments, (**A3**,**B3**) represent the effects of the interaction between two factors. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

Table 4. Analysis of variance (ANOVA) for root malondialdehyde (RMDA), root total protein (RTP), root superoxide dismutase (RSOD), root catalase (RCAT), root ascorbate peroxidase (RAPX), root peroxidase (RPOD), leaf Cd accumulation (LCA), root Cd accumulation (RCA), soil Cd accumulation (SCA), EC, and pH as affected by the independent variables: cadmium and drought stresses (S), *B. paralicheniformis* and *T. asperellum* microorganisms (M), and stresses and microorganisms (S × M).

	RMDA (µmol/g FW)	**RTP (mg/gFW)**	**RSOD (Unit/mg FW)**	**RCAT [(Mm/cm)/FW.min]**	**RAPX [(Mm/cm)/FW.min]**	**RPOD [(Mm cm)/FW.min]**	**LCA [(mg kg)/DW]**	**RCA [(mg kg)/DW]**	**SCA [(mg kg)/DW]**	**EC (ds/m)**	**pH**
S	**	**	ns	**	ns	**	**	**	**	**	**
M	**	**	**	**	ns	*	**	**	**	ns	**
S × M	**	**	ns	ns	ns	**	**	*	ns	ns	**

* and ** are significant at 5% and 1% respectively, ns are not statistically significant.

Table 5. Analysis of variance (ANOVA) for leaf malondialdehyde (LMDA), leaf total protein (LTP), leaf superoxide dismutase (LSOD), leaf catalase (LCAT), leaf ascorbate peroxidase (LAPX), leaf peroxidase (LPOD), as affected by the independent variables: stresses (S), microorganisms (M), time (T), and stresses, microorganisms and time (S × M × T).

	LMDA [(µmol/g)/FW]	**LTP [(mg/g)/FW)]**	**LSOD (Unit/mg FW)**	**LCAT [(Mm/cm)/FW.min]**	**LAPX [(Mm/cm)/FW.min]**	**LPOD [(Mm/cm)/FW.min]**
S	**	**	**	**	**	**
M	**	**	**	**	**	**
Time	**	**	**	**	**	**
S × M × T	**	**	**	**	**	**

** is significant at 1%.

Under drought stress, at T4, bacterial inoculation was found to be more effective than fungi inoculation to reduce an aerial part of MDA (7.32% lower); while under Cd stress, fungi inoculation was found to be more effective than bacterial inoculation (11.01% lower) (Figure 6A).

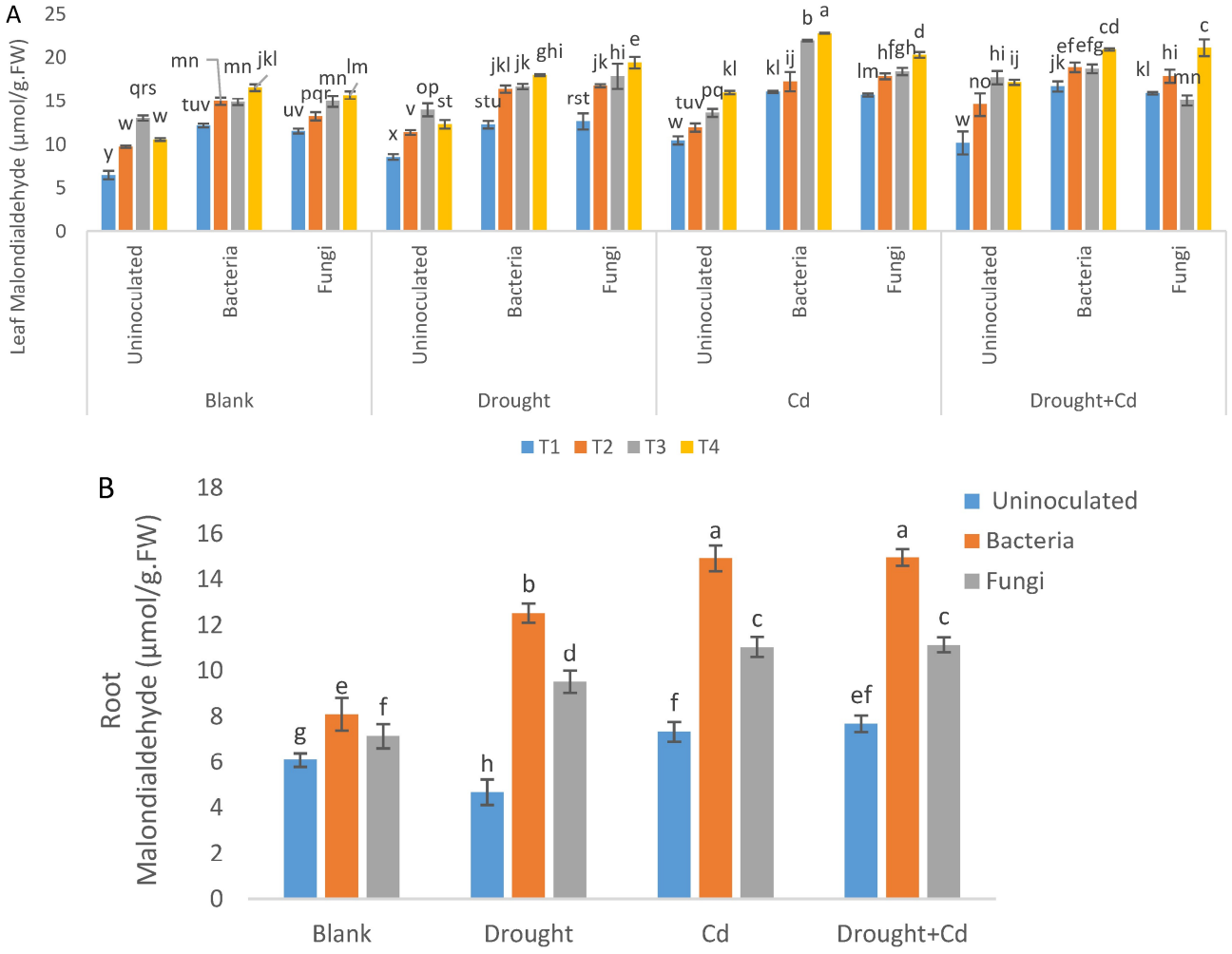


Figure 6. Variation in the maize malondialdehyde content in the leaf and root under the studied treatments. (**A**,**B**) represent the effects of the interaction between two factors. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

The root MDA content was found to have remarkably increased in uninoculated plants grown under Cd, drought, and Cd + drought stress conditions compared to the control (Figure 6B). Results of the mean comparison showed that fungi inoculation was more effective than bacterial inoculation in reducing MDA in the root (Figure 6B). Regarding this, 24.02% reduction in the drought stress group, 25.84% reduction in Cd stress group, and 25.37% reduction were observed in the drought + Cd stress group, respectively (Figure 6B).

3.7. Leaf and Root Protein

Stress groups and microorganism treatments, and their interaction on the leaf and root protein were found to be statistically significant ($p < 0.01$) (Tables 4 and 5). With increasing stress time, the leaf protein content showed a significant decrease in most of the treatment groups. The maximum difference between the fungal-inoculated plants was observed under drought stress, with a 31.2% decrease (between T1 and T4). Similarly, the maximum difference observed between the bacterial-inoculated plants was also observed under drought stress, with a 35.39% decrease (between T1 and T4) (Figure 7A). Evaluation

of the root protein content revealed that a maximum reduction of protein content (22%) was detected in plants grown under Cd + drought stress. In the Cd and drought stress group, fungal inoculation was found to have increased more than bacterial inoculation, corresponding to 21.52 and 16.77%, respectively (Figure 7B).

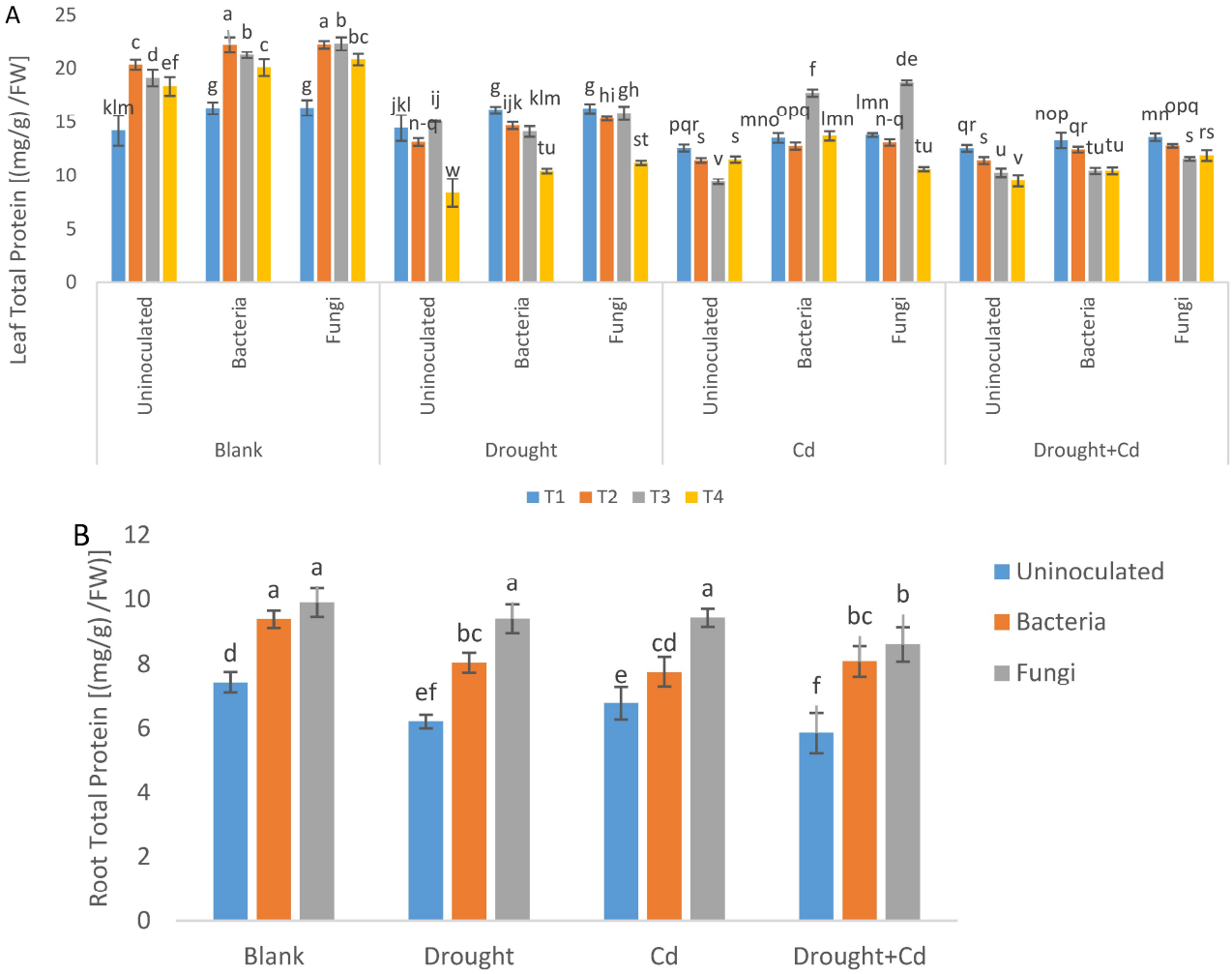
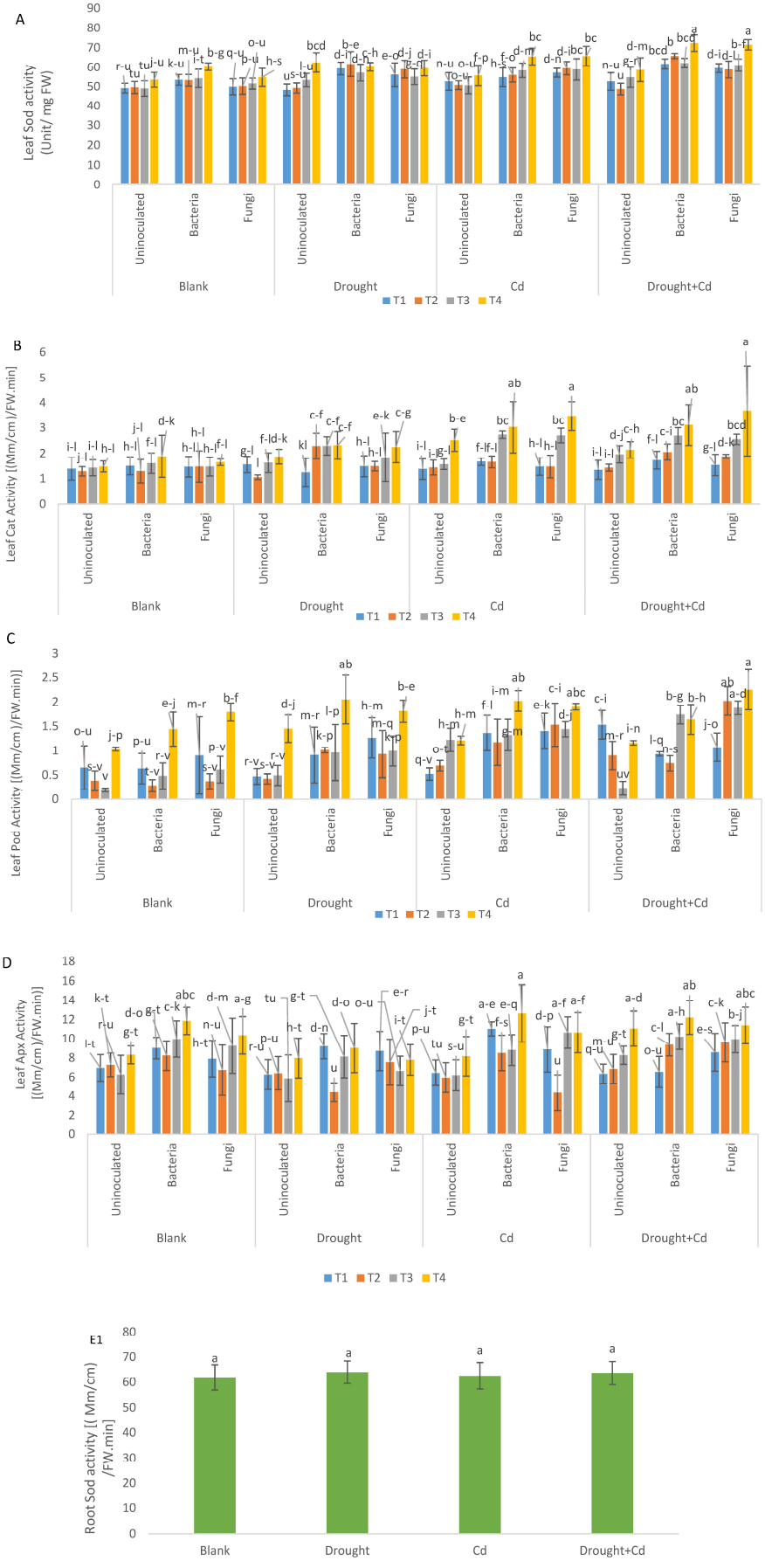


Figure 7. Variation in the maize protein content in the leaf and root under the studied treatments. (**A**,**B**) represent the interaction between two factors. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

3.8. Aerial Part and Root Enzymatic Activity

Aerial part enzymatic activity was evaluated at four stages. Stress groups and microorganism treatments, and their interaction on the leaf enzymatic activity were all determined to be statistically significant ($p < 0.01$) (Tables 4 and 5). Mean comparison results showed that under drought + Cd stress, fungi and bacterial inoculation both induced SOD activity at T4 (a day before harvest) that were both significantly different compared to T1–T3. Furthermore, the trend of the graph shows that with the increase in the stress time, the enzyme activity also increased significantly in most of the assessed groups. Under drought stress, no significant difference was observed between the plants inoculated with fungi at different sampling times, and also between the plants inoculated with bacteria at different sampling times. In contrast, under Cd stress, plants inoculated with fungi and bacteria at T4 demonstrated significant differences compared to T1–T3 (Figure 8A).



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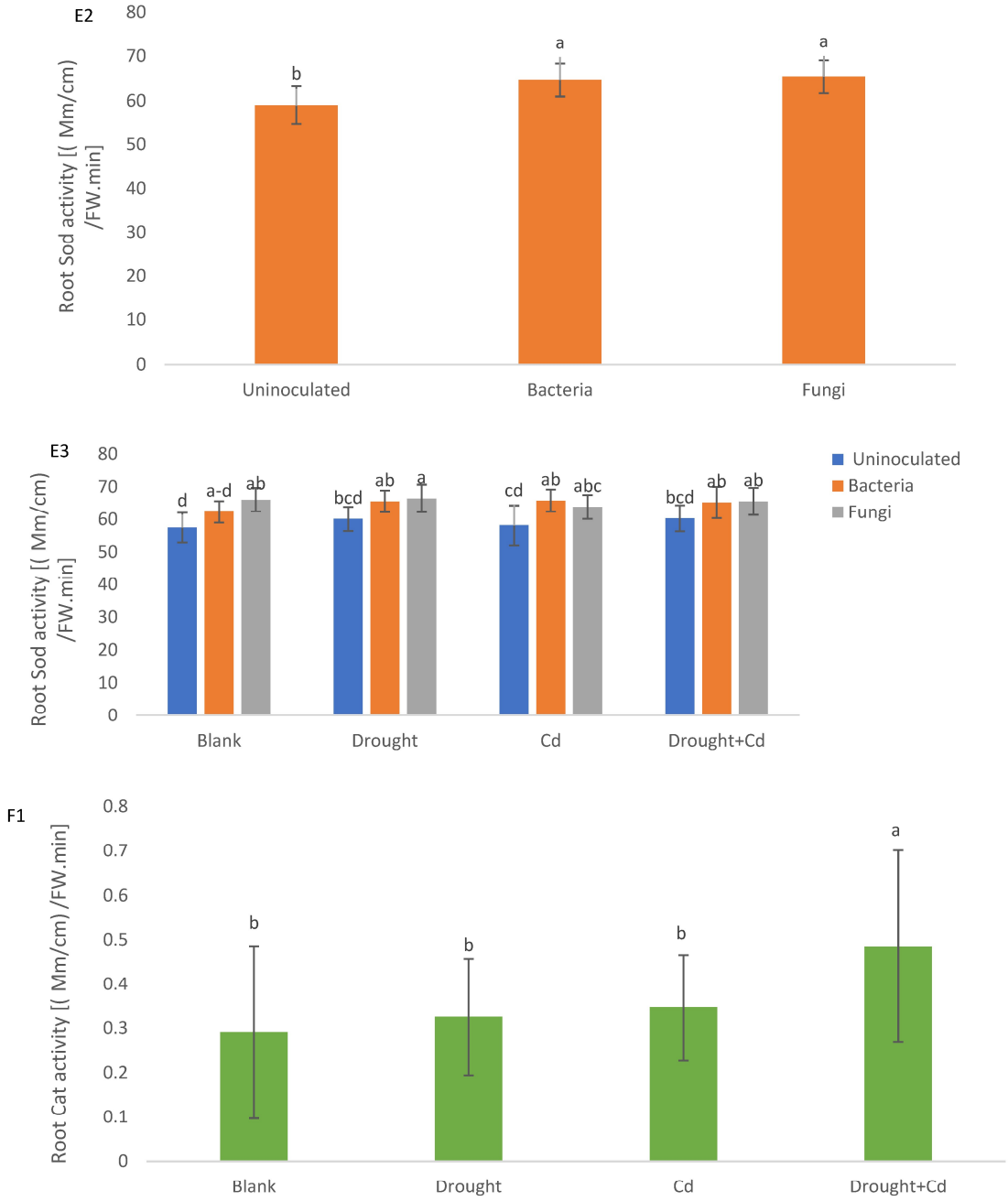

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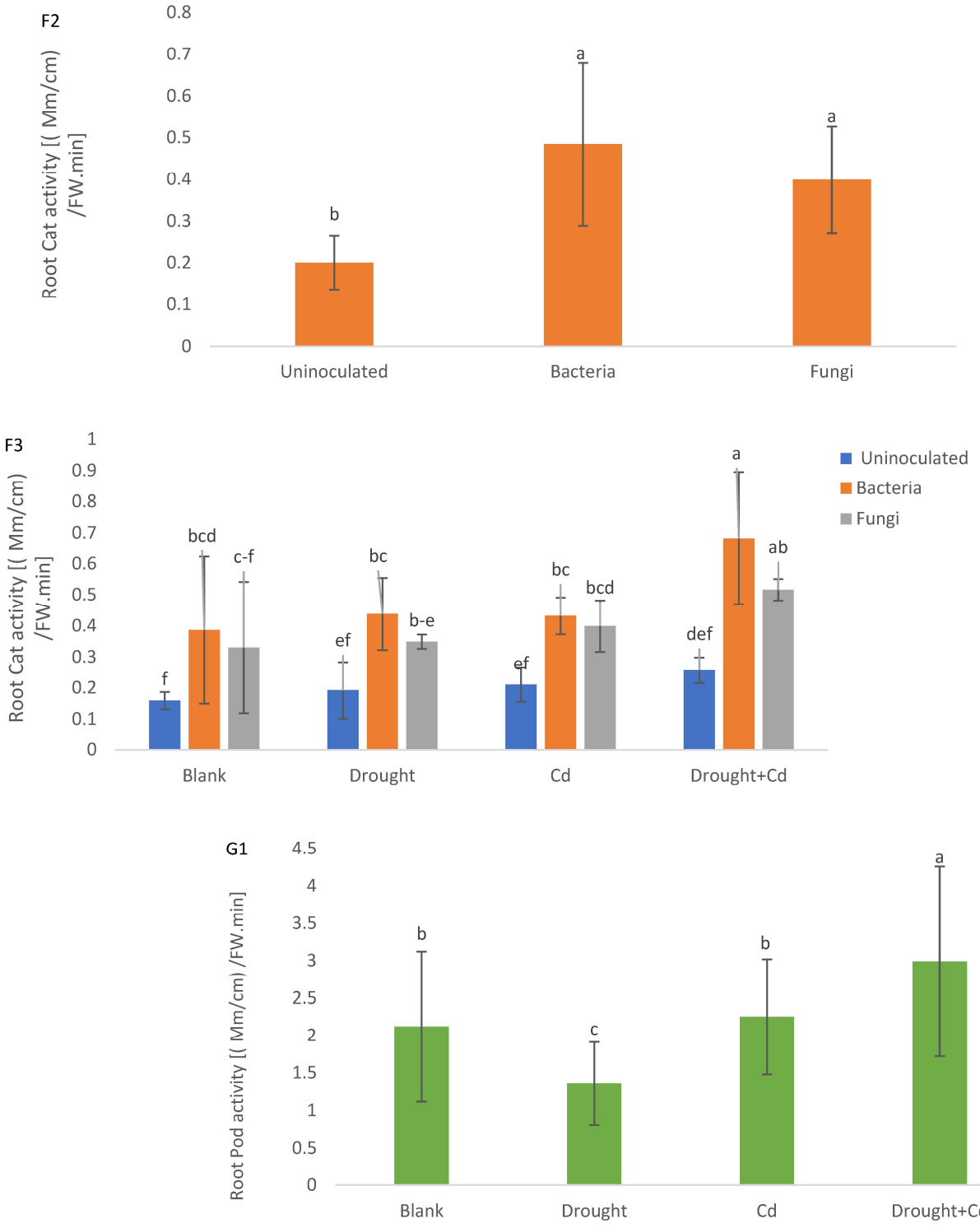

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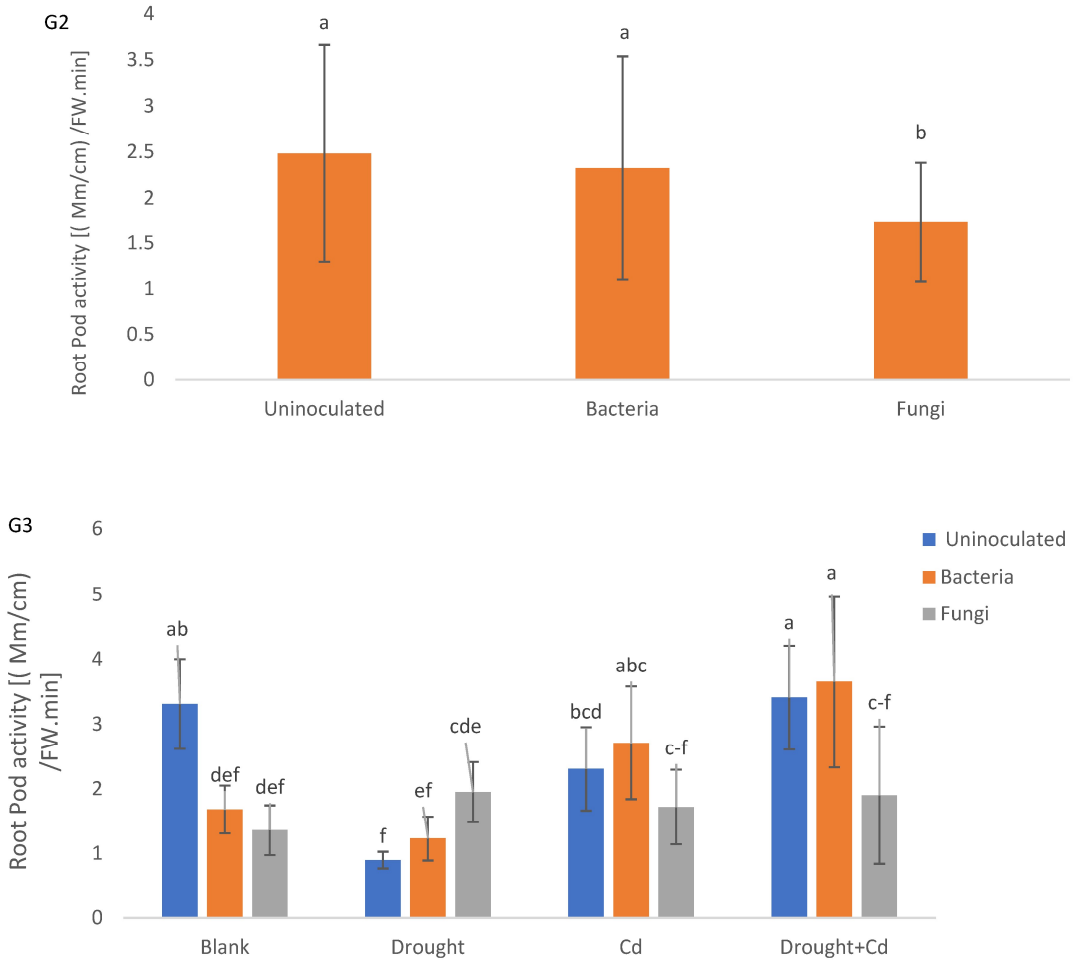


Figure 8. Variation in the maize leaf and root enzyme activity under the studied treatments. (**A–D**,**E3**,**F3**,**G3**) represent the effects of the interaction between two factors (n = 4). (**E1**,**E2**,**F1**,**F2**,**G1**,**G2**) represent the effects of the individual treatments. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

Analysis results of the CAT activity variations showed that fungi inoculation under Cd stress caused a 134.46% increase, and under drought + Cd stress a 138.96% increase was observed compared to T1, respectively. The same trend was also observed with bacterial inoculation (81.44 and 79.77%, respectively) (Figure 8B). Mean comparison results of POD showed that under T4 in some groups, a significant increase towards T1 was observed. Under drought + Cd stress fungi inoculation caused significant enhancements in T2, T3, and T4 towards T1 (88.78, 75.71, and 110.28%, respectively). Furthermore, under drought stress at T4, bacterial inoculation (102.97–125.27%) and fungal inoculation (43.65–94.62%) both caused a notable increase in POD activity compared with T1–T3. At T4, under Cd stress, bacterial (48.53–72.65%) and fungal (24.18–35.7%) inoculation were found to be able to enhance POD activity compared with T1–T3 (Figure 8C).

Results of APX activity showed that bacterial inoculation under drought and Cd stress at T4 did not show a significant difference with T1, while under drought + Cd stress, an 86.98% significant increase was revealed at T4 compared with T1. In fungi-inoculated plants under drought and Cd stress, there was no significant difference observed between T1 and T4, while under drought + Cd stress, a significant difference was observed (with a 32.63% increase) (Figure 8D).

Root enzyme activity was also assessed in the present experiment. The effect of microorganism treatments on SOD activity was found to be statistically significant, but the effects of stress groups and their interaction on SOD activity were not deemed to be

statistically significant (Table 4). Results of SOD activity showed that fungi inoculation and bacterial inoculation were able to increase SOD activity significantly towards the control, with 10.88 and 9.63%, respectively (Figure 8(E2)). The results of the analysis of variance (Table 4) demonstrated that the effects of stress groups and microorganism treatments on CAT activity was statistically significant ($p < 0.01$), but that their interaction effect was not significant. Mean comparison results showed that the maximum CAT activity was observed in the drought + Cd stress group (being 66.67% higher than the control group) (Figure 8(F1)). Furthermore, microorganisms were also found to have affected the CAT activity significantly. In this way, bacterial and fungal inoculation showed higher CAT activities compared to the control, by 142% and 99.5%, respectively (Figure 8(F2)).

The activity of POD enzymes was influenced by the stress groups and microorganism treatments, and their interaction was found to be statistically significant ($p < 0.01$) (Table 4). The results of the POD activity showed that in the drought stress group, fungi inoculation caused maximum POD activity, with a 117.63% increase compared with the control in the same group (Figure 8(G1)). However, in the drought + Cd stress group, bacterial inoculation activated the POD enzymatic pathway more than fungal inoculation (92.11% higher) (Figure 8(G3)). The activity of the APX enzyme was found to be not significant across all treatments ($p < 0.01$) (Table 4).

3.9. Cd Accumulation in the Plant Material and Soil

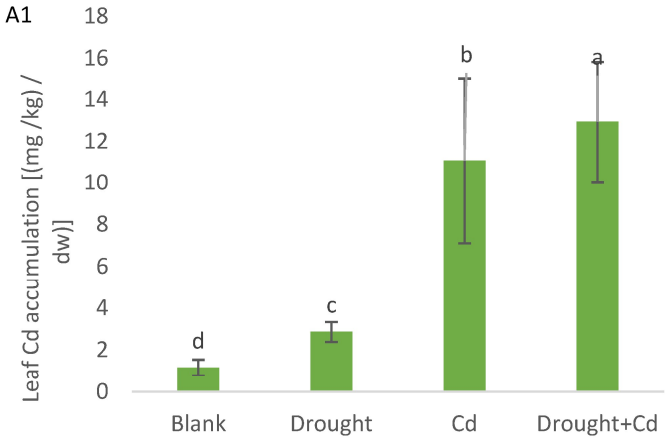
The accumulation of Cd in the shoot, root and soil of *Z. mays* under Cd, drought, and Cd + drought stress conditions are presented in Figure 9(A1–C2).

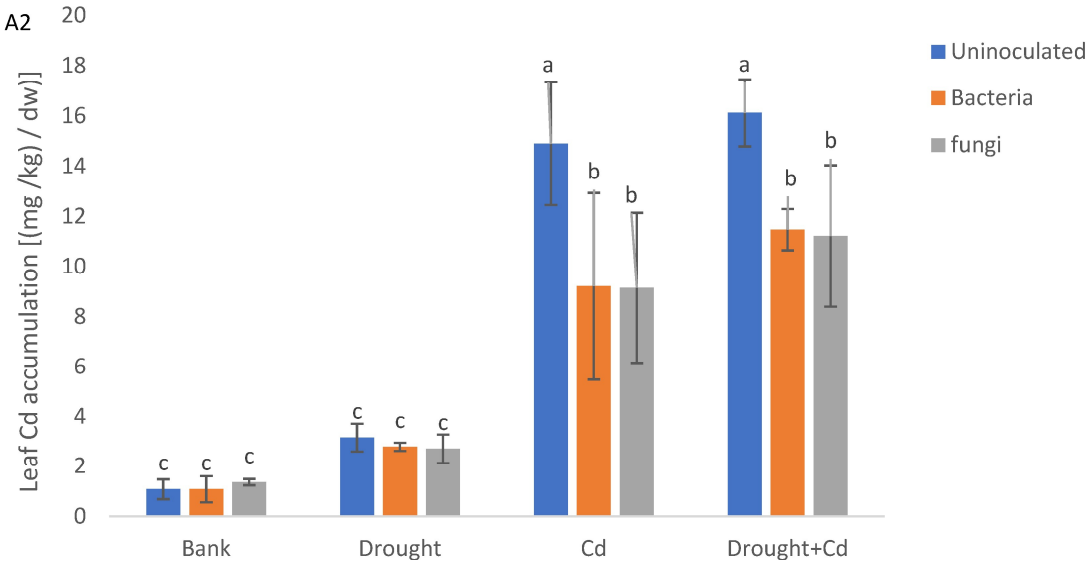
The results of the analysis of variance (Table 4) demonstrated that the effects of the stress groups and microorganism treatments, and their interaction on the leaf and root Cd accumulation were all found to be statistically significant. According to (Figure 9(A2)), under both Cd and drought + Cd stress, fungal and bacterial inoculation were able to significantly reduce Cd accumulation in the leaf (compared with the uninoculated) (38.19–38.59% reduction in Cd stress group, and 28.85–30.39% reduction in drought + Cd group, respectively).

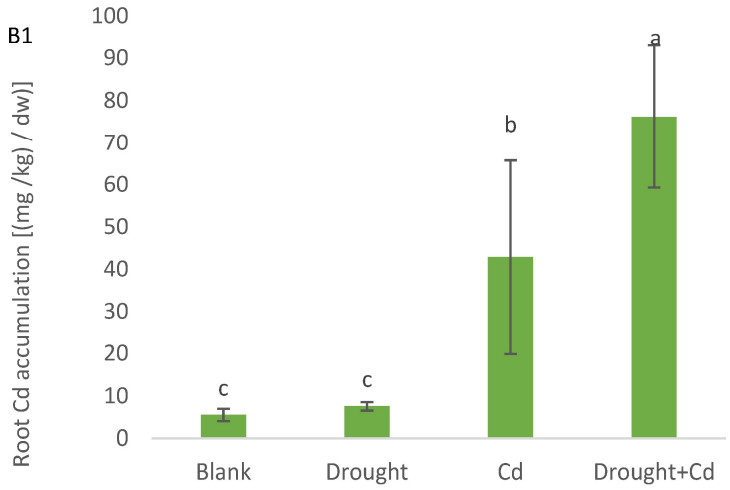
Results of Cd accumulation in the root showed that under Cd stress, fungal and bacterial inoculation were able to significantly enhance Cd accumulation in the root (compared with the uninoculated), by 207.06% and 159.88%, respectively. The highest Cd accumulation in roots under drought + Cd stress was observed at fungal inoculation (83.43 mg/kg DW) (Figure 9(B2)). Accumulation of Cd in the soil was also significantly affected by both the stress groups and microorganisms separately (Table 4). Drought stress caused a significant increase in Cd accumulation in the soil (with an 8.43% increase compared with Cd stress alone). Moreover, fungal and bacterial inoculation significantly decreased the Cd accumulation in the soil (by 12.69 and 9.21%, respectively) (Figure 9(C1)).

3.10. Principle Component Analysis (PCA) of the Studied Traits of Z. mays under Various Treatments

In the biplot of the PCA, the first two PCs explained 65.7% of the variations in the studied traits among the treatments (Figure 10). The first PC explained 44.3% of the variation and comprised Cd accumulation, and root and leaf enzymatic activity. In contrast, the second PC accounted for 21.4% of the variations in the traits and treatments. The cosine of the angles between the vectors in the biplot and PC analysis displays the strength of the correlation between the traits. The acute angles (<90°) represent positive correlations, whereas wide obtuse angles (<90°) designate negative correlations. The correlation was found to be stronger for angles close to 0° and 180°, and the length of the vectors connecting the traits to the origin can indicate the extent of the variation (Figure 10).







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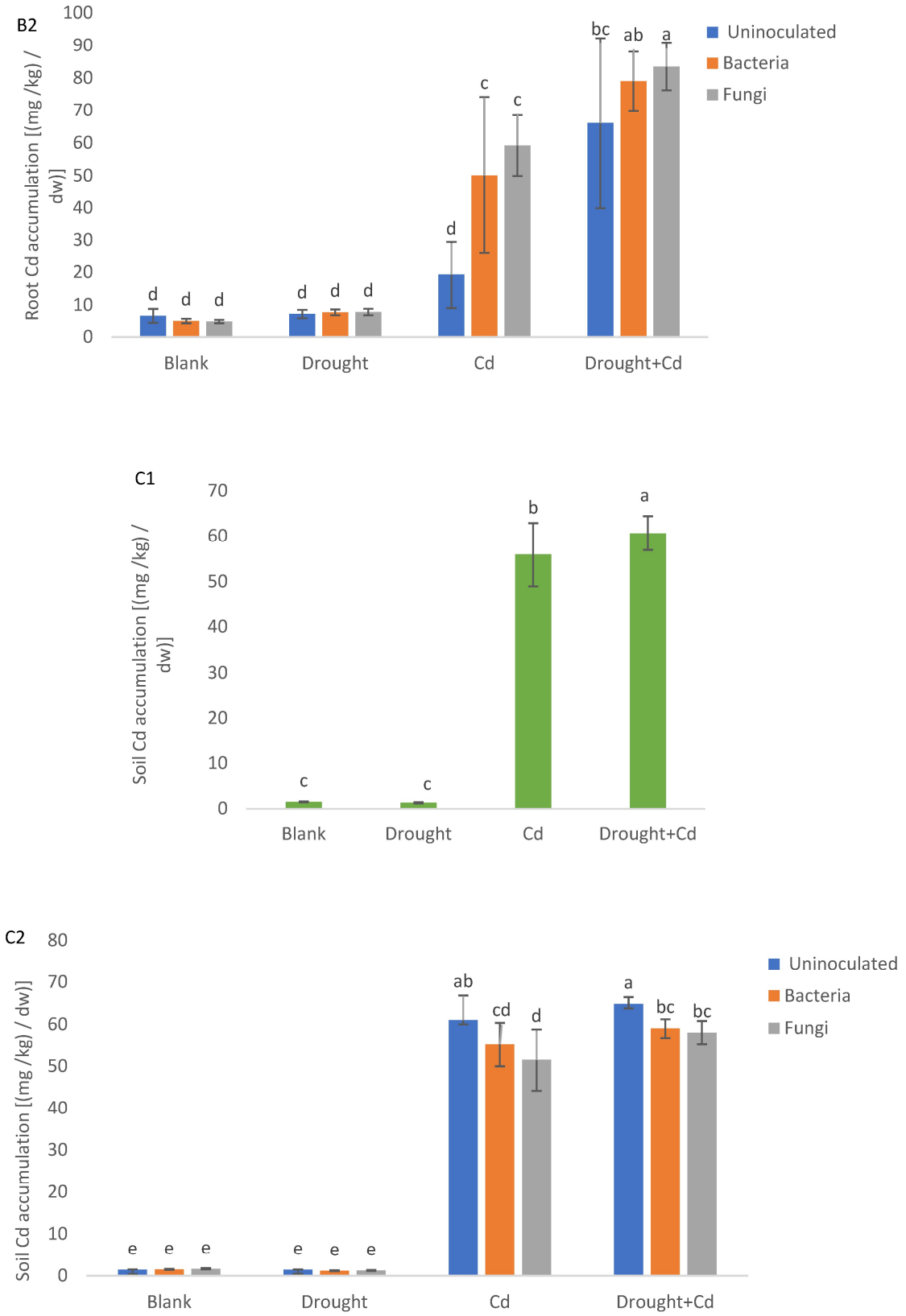

Figure 9. Variation in the maize cadmium (Cd) accumulation in the leaf, root, and soil under the studied treatments. (**A1**,**B1**,**C1**) represent the effects of the individual treatments, and (**A2**,**B2**,**C2**) represent the effects of the interaction between two factors. Mean values (±standard deviation) with the same letters are not significantly different ($p < 0.05$), according to the LSD test.

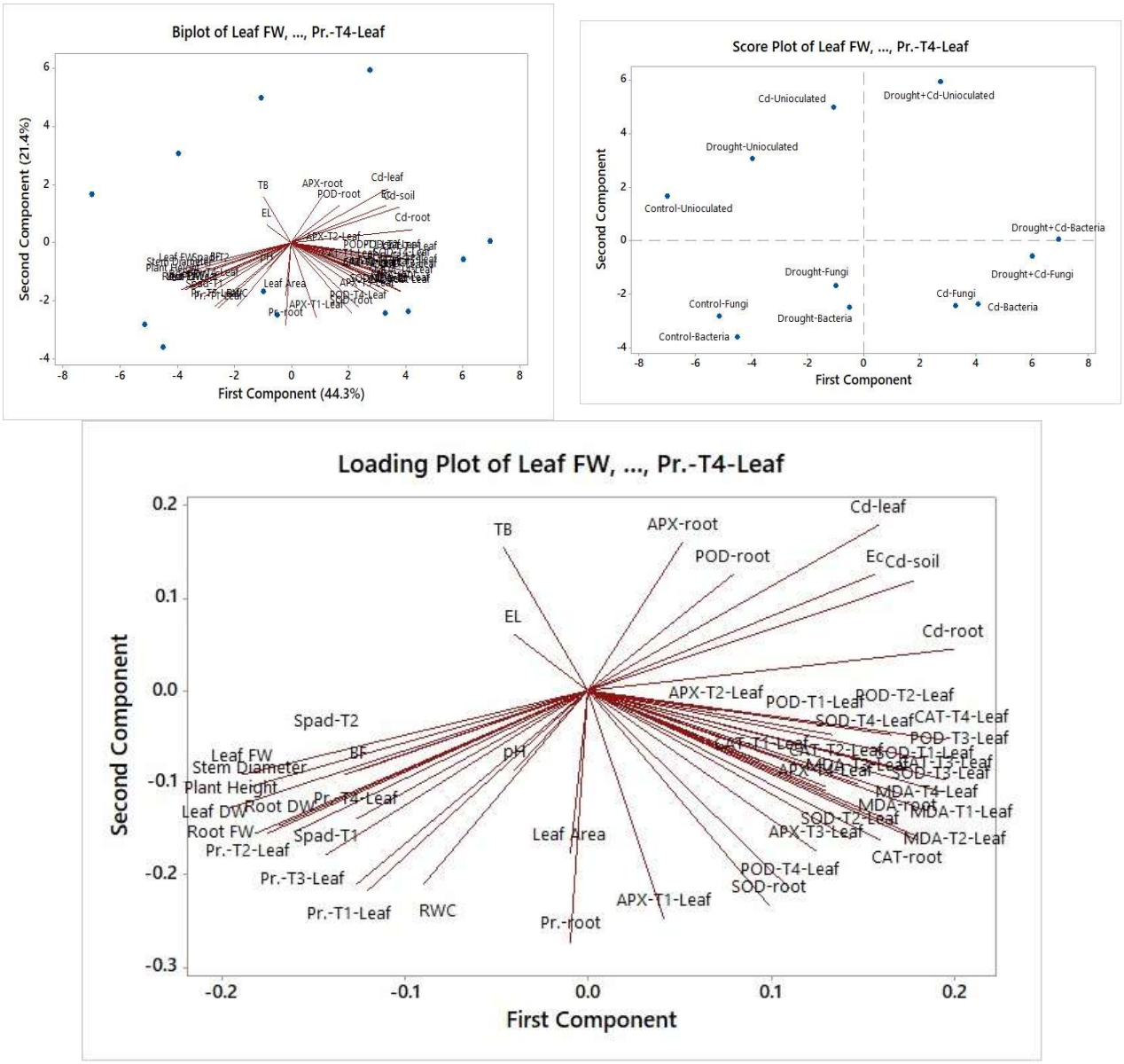

Figure 10. Projection of the assessed treatments and measured parameters on the first and second principal components in the studied plants.

4. Discussion

For many years, cadmium has been well-known due to its high toxicity, and has been regarded as an exceptionally significant contaminant. Although Cd is a non-essential element for plant physiology, it is readily absorbed by roots and translocated to the shoots [44]. Moreover, biomass studies have indicated that Cd and drought stress can adversely affect the plant growth and pigment content by interfering with cell elongation, water flow, mineral uptake, and chromium biosynthesis [45]. In addition, the plants grown under individual stress environments displayed less growth in the combined stress environments of Cd and drought. Therefore, it is possible for excessive amounts of heavy metals to damage plants directly or indirectly, disturb metabolic processes, and inhibit their growth [46]. The abiotic, stress-tolerant PGPB improves plant growth and survival under both individual and combined stress by establishing beneficial interactions with the host plants [15]. The experiments revealed that both fungal and bacterial inoculation were efficient in protecting *Z. mays* from growth inhibition caused by the individual (Cd or drought) and combined stresses (Cd + drought), as strongly supported by the plant biomass and morphological

data. By preventing cell elongation, water flow, mineral uptake, chl biosynthesis, etc., drought stress and Cd can thereby impair the plant growth and pigment content. Furthermore, compared to plants growing under individual stress situations, the combined stress circumstances (Cd + drought) worsened the impacts and lowered plant development [47]. Recently, *Trifolium arvense*'s growth and pigment content showed the greatest decline under combined (drought + multiple) stress conditions, according to researchers [48]. In the present study, regardless of the stress condition, the biomass and chl content of *Z. mays* were enhanced by the inoculation of microorganisms. These could be attributed to the potential of PGPB on the production of siderophores and IAA-stressed conditions. It is well known that siderophores synthesized by PGPB boost chl levels, and hence enhance plant health when exposed to abiotic stress. Researchers have investigated the effects of siderophores on iron and chlorophyll levels in plants, and discovered that siderophores produced by *Bacillus subtilis* promote *Arabidopsis* growth [49]. Moreover, *Bacillus subtilis* siderophores chelate Fe^{3+}, which leads to a rise in iron levels, chl content, and photosynthetic efficiency in *Arabidopsis* [50]. Similar to this, a greater plant growth was also noted in ACCD-producing PGPB, which was inoculated into *Helianthus annuus* [51] and maize [45] that had been exposed to heavy metal stress and drought stress. By reducing plant ethylene levels through accelerating the conversion of ACC to ammonia and α-ketobutyrate, ACCD thereby plays a crucial part in the alleviation of metal and drought stress [52]. Moreover, by generating IAA, PGPB can enhance root elongation and cell proliferation, resulting in a larger root surface for nutrient and water uptake [53]. Through changing the plants' RWC, enzymatic and non-enzymatic antioxidants, etc., [53] PGPB can thereby enhance the plant stress tolerance, biomass output, and protein content, particularly under abiotic stress conditions [54]. In the current study, Cd + drought caused a drastic decrease in the protein content of *Z. mays*, which was possibly due to an altered protein metabolism and homeostasis caused by the excess toxicity of abiotic stress [55]. The inoculation of PGPB, however, considerably boosted plant protein content under Cd + drought stress over the uninoculated plants, thereby demonstrating that PGPB was able to preserve the physiological status of plants to deal with the stressful circumstances [9,53]. Another study found that *Enterobacter ludwigii,* when inoculated with metal-exposed *Triticum aestivum,* resulted in a higher protein content [56]. The increased protein content observed under stress caused by microorganisms indicates that the bacteria helped plants to diminish the stress effects of Cd and drought [57]. As demonstrated previously in *T. aestivum*, the increase in the plant protein under heavy metal stress following PGPB inoculation may have been associated with an enhanced plant RWC. This may be explained by the reduction in plant development caused by the loss in both turgor and hydraulic conductance brought on by the toxicity of concurrent metal and drought stress [56]. However, PGPB inoculation raised the RWC under varied stress situations, indicating that PGPB may be therefore able to affect stomatal function and retain water potential to stop additional water loss [53]. Previously, an increased RWC in *P. azotoformans*-inoculated plants subjected to metal + drought stress was also observed, and indicated that this was due to an improved hydraulic conductance and decreased negative effects of abiotic stresses [58]. By promoting root growth through a variety of PGP metabolites, such as siderophore generation, ACCD activity, phosphate solubilization, and IAA synthesis, the inoculation of PGPB can thereby increase the plant's RWC [9]. ROS-mediated lipid peroxidation produces MDA, which is employed as a biomarker of membrane damage brought on by diverse abiotic stressors [59]. The lower MDA observed in inoculated plants may be due to the ability of PGPB to protect the plants from oxidative damage by regulating the levels of secondary metabolites (proline and phenolics) and antioxidant activities. This finding is corroborated by the results of *Curtobacterium herbarum*-inoculated *Lactuca sativa*, which exhibited reduced MDA levels under aluminum (Al) and drought stress conditions due to Al bioaccumulation and the activity of enzymatical antioxidants [60]. Moreover, under heavy metal and drought stress circumstances, PGPB-inoculated plants have been shown to accumulate more proline and phenolics as an internal detoxifying mechanism [9]. As a redox buffer, low molecular

weight antioxidants, such as phenols can contribute electrons and hydrogen atoms to scavenge ROS-mediated free radicals and have an impact on both plant establishment and growth. Additionally, via regulating membrane stability, osmotic adjustment, and ROS scavenging processes, enhanced proline accumulation and SOD activity limit the negative consequences of stress [61]. Microorganism inoculation not only improves plant growth, but also ameliorates the deleterious effect of Cd and drought stress by regulating antioxidant activities and secondary metabolite accumulation in plants [45]. The improvements in the biochemical rate of CO_2 fixation that resulted from the increase in photosynthetic pigment content was deemed to be crucial for boosting the net photosynthesis and the growth rate of plants under abiotic stress [62]. Since the PGPB was found to significantly affect the metal bioavailability and its uptake by plants through a variety of mechanisms, including metal reduction and oxidation, metal methylation, formation of insoluble complexes, chelation through siderophores, bioaccumulation, and intracellular sequestration, the role of PGPB in improving heavy metal tolerance under drought stress conditions is noteworthy [9]. Previously conducted studies have pointed out that inoculation with metal-resistant plant growth-promoting rhizobacteria (PGPR) can reduce heavy metal toxicity and enhance plant growth in contaminated soil [46]. There are multiple heavy metal-resistant bacteria which are capable of stimulating plant growth in soils contaminated with Cd. Moreira et al. reported isolated Cd-resistant Gram-negative bacterium, including *Chryseobacterium humi*, *Ralstonia eutropha*, *Pseudomonas reactants*, and *Rhizobium radiobacter* from soil contaminated with EDTA. [63] When maize plants were injected with inoculum grown in mine soils, they grew taller and produced more biomass as a result; the effects increased with the size of the inoculum [64]. In the present study, a pot experiment demonstrated that *B. paralicheniformis* and *T. asperellum* were able to effectively protect corn from growth inhibition caused by either a drought, Cd, or a combined Cd along with drought stress, as supported by the plant, and led to improved growth factor. Studies have illustrated that Gram-negative cells contain heavy metal-bound functional groups. There are three main components in the cell walls of bacteria: ticoronic acid, peptide glycan, and teichoic acid [65]. All three have functional groups that are linked by deprotonation to metal cations. In contrast to Gram-positive cells, Gram-negative cells have fewer peptide glycans and a more complex extracellular membrane. Lypo-proteins, phospholipids, and various proteins are also found in the extracellular membrane of Gram-negative bacteria [65]. Various Cd-resistance mechanisms are present in different Cd-resistant bacteria groups. The mechanisms by which bacteria resist metals or complexes include degradation, conversion to fewer toxic compounds, or direct distribution inside the cell [15]. Metallothionein (MT) and phytochelatins are low molecular weight proteins that bind most of the heavy metals inside the cells [66]. Plant nutrient absorption and translocation are enhanced by the extensive extraradical hyphal network produced by fungi [65,67]. Aside from modifying the architecture and topography of the root system, fungi may also increase or decrease the nutrient absorption efficiency depending on the particular hosts and fungi [68]. Plants can be strengthened to handle environmental stresses, such as heavy metals, by fungi, in addition to promoting growth. Enhanced nutrient availability results in these positive effects on plant growth, and this is partly explained by the complex interactions occurring between the fungi and plants. [67] Mycorrhizal fungi have still not been proven to be effective in the tolerance of heavy metal-contaminated soils, contrary to numerous studies [69]. The type of plant, fungus, and metal utilized can all influence the amount of metal taken up by plants treated with fungi. A high concentration of heavy metals in the soil has been reported to inhibit mycorrhiza-plant associations, thereby reducing, delaying, or even eliminating fungi colonization. Nevertheless, fungi propagules never completely disappear from highly contaminated soils. According to present findings, *T. asperellum* fungi effectively protects *Z. mays* from drought, Cd, and a combination of both, resulting in an improvement in the growth factor [70].

A significant increase in H_2O_2 levels was documented in plants when drought stress and Cd were applied, with concomitant increases in both the lipid peroxidation and

membrane permeability. High levels of lipid peroxidation can occur as a result of the elevated levels of ROS, thereby damaging plant ultrastructure during water-stress conditions. ROS-mediated lipid peroxidation causes MDA to accumulate in membranes, serving as a biomarker of membrane damage [71]. In the present study, the MDA accumulation observed was high in maize plants that were subjected to water deficit conditions. It has been believed that oxidative enzymes are produced under abiotic stress, such as drought, as a result of a high oxidative stress. The ascorbic acid content in both water-stressed and non-stressed maize was found to be significantly increased by bio-seed primed bacterial treatments [72].

Both fresh and dry biomass of maize grown under water-limited conditions are significantly affected by ascorbic acid levels, as illustrated by the data. In crops becoming more tolerant to water stress, such as canola [73], wheat [74], and tomato [75], ascorbic acid levels increased. Plants may be protected from oxidative damage by bacteria and fungi due to their ability to reduce the toxicity of Cd, regulate secondary metabolites, such as phenolics and proline, and activate the antioxidant enzymes. According to Khan et al., exogenous PGPR strains reduce H_2O_2 and MDA levels significantly. Under aluminum (Al) and drought stress conditions, *Curtobacterium herbarum*-inoculated *Lactuca sativa* demonstrated reduced MDA levels due to Al bioaccumulation and enzymatic antioxidant activity [60]. During drought stress, antioxidant enzymes scavenge ROS, which are indicators of plant stress resistance [60]. Under heavy metal and drought stress conditions, increases in the proline and phenolic accumulation in plants as an internal detoxification mechanism occurs [76]. Therefore, plant proteins may be altered by drought exposure as a result of protein degradation or an enhanced antioxidative activity. In plants, small-molecule antioxidants, such as phenols, can act as redox buffers, which donate electrons and hydrogen atoms to scavenge free radicals generated by ROS [77]. APX, CAT, POD, and SOD are antioxidant enzymes that protect plants from oxidative stress [78]. *P. mirabilis* decreased metal toxicity and oxidative stress in PGPB-inoculated maize plants exposed to Cr stress, resulting in a decreased proline accumulation and SOD activity [79]. Many crops have successfully managed drought and other abiotic stresses with *Bacillus* genera [80]. Co-inoculation was shown to alleviate drought stress under non-sterile soil conditions in previous studies [80,81]. As evidenced by the results of the current study, both bacteria and fungi can improve phytostabilization in Cd-contaminated soils despite the presence of drought stress.

5. Conclusions

Plant growth and physiological behavior were negatively affected by Cd exposure, drought, and especially under combined stress conditions. In the present work, it has been demonstrated that the inoculation of *B. paralicheniformis* bacteria or *T. asperellum* fungi may alleviate the negative effects of stress on plant health. These protective effects were manifested by increasing the RWC, decreasing MDA, and activating antioxidant enzyme activities. Additionally, both fungi and PGPB reduced Cd accumulation in the shoot by reducing Cd translocation from the root to the shoot, although they improved Cd content in the root. The effective role played by fungi PGPB on plant growth, stress tolerance, morphophysiological and biochemical traits, and Cd accumulation provides new knowledge, which may pave the way to utilize such microorganisms for effective and permanent phytostabilization in the Cd-contaminated arid and semiarid regions. To investigate the molecular pathways involved in the microbial-mediated reduction in Cd and drought stress in plants, more experiments, such as the investigation into the transcriptional changes occurring in inoculated *Z. mays* under Cd and drought stress, are now under way. The present data supports that bio-inoculants and bioremediation tools could be used in water-deficit and heavy metal environments synergistically with crop plants to produce sustainable food in water-deficit environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071675/s1, Figure S1: Electrical conductivity (Ec) variation in soil at the end of the experiment; Figure S2: pH variation in soil at the end of the experiment.

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References

1. Wild, A. *Soils, Land and Food: Managing the Land during the Twenty-First Century*; Cambridge University Press: Cambridge, UK, 2003.
2. Ojuederie, O.B.; Olanrewaju, O.S.; Babalola, O.O. Plant growth promoting rhizobacterial mitigation of drought stress in crop plants: Implications for sustainable agriculture. *Agronomy* **2019**, *9*, 712. [CrossRef]
3. Yousefi, Z.; Babanejad, E.; Mohammadpour, R.; Esbokolaee, H.N. Evaluation of Cd phytoremediation by Portulaca oleracea irrigated by contaminated water. *Environ. Health Eng. Manag. J.* **2023**, *15*, 286. [CrossRef]
4. Pacheco, D.D.R.; Santana, B.C.G.; Pirovani, C.P.; de Almeida, A.-A.F. Zinc/Iron-Regulated Transporter-Like Protein (ZIP) Gene Family in Theobroma cacao L: Characteristics, evolution, function and 3D structure analysis. *Front. Plant Sci.* **2023**, *14*, 511. [CrossRef] [PubMed]
5. Shahid, M.; Pourrut, B.; Dumat, C.; Nadeem, M.; Aslam, M.; Pinelli, E. Heavy-metal-induced reactive oxygen species: Phytotoxicity and physicochemical changes in plants. In *Reviews of Environmental Contamination and Toxicology*; Springer: Berlin/Heidelberg, Germany, 2014; Volume 232, pp. 1–44.
6. Kohli, S.K.; Handa, N.; Gautam, V.; Bali, S.; Sharma, A.; Khanna, K.; Arora, S.; Thukral, A.K.; Ohri, P.; Karpets, Y.V. ROS signaling in plants under heavy metal stress. In *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress*; Springer: Singapore, 2017; pp. 185–214.
7. Sachdev, S.; Ansari, S.A.; Ansari, M.I. ROS Generation in Plant Cells Orchestrated by Stress. In *Reactive Oxygen Species in Plants: The Right Balance*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 23–43.
8. Islam, M.; Sandhi, A. Heavy Metal and Drought Stress in Plants: The Role of Microbes—A Review. *Gesunde Pflanz.* **2022**. [CrossRef]
9. Vocciante, M.; Grifoni, M.; Fusini, D.; Petruzzelli, G.; Franchi, E. The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant's environmental stresses. *Appl. Sci.* **2022**, *12*, 1231. [CrossRef]
10. Wang, Z.; Li, G.; Sun, H.; Ma, L.; Guo, Y.; Zhao, Z.; Gao, H.; Mei, L. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. *Open Biol.* **2018**, *7*, bio035279. [CrossRef]
11. Zahedi, S.M.; Hosseini, M.S.; Fahadi Hoveizeh, N.; Kadkhodaei, S.; Vaculík, M. Physiological and Biochemical Responses of Commercial Strawberry Cultivars under Optimal and Drought Stress Conditions. *Plants* **2023**, *12*, 496. [CrossRef]
12. Chugh, V.; Kaur, N.; Gupta, A.K. Evaluation of oxidative stress tolerance in maize (*Zea mays* L.) seedlings in response to drought. *Indian J. Biochem. Biophys.* **2011**, *48*, 47–53.
13. More, S.J.; Bardhan, K.; Ravi, V.; Pasala, R.; Chaturvedi, A.K.; Lal, M.K.; Siddique, K.H.M. Morphophysiological responses and tolerance mechanisms in cassava (*Manihot esculenta Crantz*) under drought stress. *J. Soil Sci. Plant Nutr.* **2023**, *23*, 71–91. [CrossRef]
14. Shah, A.; Nazari, M.; Antar, M.; Msimbira, L.A.; Naamala, J.; Lyu, D.; Rabileh, M.; Zajonc, J.; Smith, D.L. PGPR in agriculture: A sustainable approach to increasing climate change resilience. *Front. Sustain. Food* **2021**, *5*, 667546. [CrossRef]
15. Pal, A.; Bhattacharjee, S.; Saha, J.; Sarkar, M.; Mandal, P. Bacterial survival strategies and responses under heavy metal stress: A comprehensive overview. *Crit. Rev. Microbiol.* **2022**, *48*, 327–355. [CrossRef]
16. Munir, N.; Hanif, M.; Abideen, Z.; Sohail, M.; El-Keblawy, A.; Radicetti, E.; Mancinelli, R.; Haider, G. Mechanisms and strategies of plant microbiome interactions to mitigate abiotic stresses. *Agronomy* **2022**, *12*, 2069. [CrossRef]
17. Ma, Y.; Rajkumar, M.; Zhang, C.; Freitas, H. Inoculation of Brassica oxyrrhina with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. *J. Hazard. Mater.* **2016**, *320*, 36–44. [CrossRef]
18. Barnawal, D.; Bharti, N.; Pandey, S.S.; Pandey, A.; Chanotiya, C.S.; Kalra, A. Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. *Physiol. Plant.* **2017**, *161*, 502–514. [CrossRef]

19. Nguyen, P.-T.; Nguyen, T.-T.; Bui, D.-C.; Hong, P.-T.; Hoang, Q.-K.; Nguyen, H.-T. Exopolysaccharide production by lactic acid bacteria: The manipulation of environmental stresses for industrial applications. *AIMS Microbiol.* **2020**, *6*, 451. [CrossRef]
20. Looijesteijn, P.J.; Trapet, L.; de Vries, E.; Abee, T.; Hugenholtz, J. Physiological function of exopolysaccharides produced by *Lactococcus lactis*. *Int. J. Food Microbiol.* **2001**, *64*, 71–80. [CrossRef] [PubMed]
21. Castaldi, S.; Petrillo, C.; Donadio, G.; Piaz, F.D.; Cimmino, A.; Masi, M.; Evidente, A.; Isticato, R. Plant growth promotion function of *Bacillus* sp. strains isolated from salt-pan rhizosphere and their biocontrol potential against *Macrophomina phaseolina*. *Int. J. Mol. Sci.* **2021**, *22*, 3324. [CrossRef]
22. Liu, J.; Fimognari, L.; de Almeida, J.; Jensen, C.N.G.; Compant, S.; Oliveira, T.; Baelum, J.; Pastar, M.; Sessitsch, A.; Moelbak, L. Effect of *Bacillus paralicheniformis* on soybean (*Glycine max*) roots colonization, nutrient uptake and water use efficiency under drought stress. *J. Agron. Crop Sci.* **2023**. [CrossRef]
23. Hidangmayum, A.; Dwivedi, P. Plant responses to *Trichoderma* spp. and their tolerance to abiotic stresses: A review. *J. Pharmacogn. Phytochem.* **2018**, *7*, 758–766.
24. Doni, F.; Isahak, A.; Che Mohd Zain, C.R.; Wan Yusoff, W.M. Physiological and growth response of rice plants (*Oryza sativa* L.) to *Trichoderma* spp. inoculants. *AMB Express* **2014**, *4*, 45. [CrossRef]
25. Scudeletti, D.; Crusciol, C.A.C.; Bossolani, J.W.; Moretti, L.G.; Momesso, L.; Servaz Tubana, B.; De Castro, S.G.Q.; De Oliveira, E.F.; Hungria, M. *Trichoderma asperellum* inoculation as a tool for attenuating drought stress in sugarcane. *Front. Plant Sci.* **2021**, *12*, 645542. [CrossRef] [PubMed]
26. Gee, G.W.; Or, D. 2.4 Particle-size analysis. *Methods Soil Anal. Part 4 Phys. Methods* **2002**, *5*, 255–293.
27. Nelson, D.A.; Sommers, L.E. Total carbon, organic carbon, and organic matter. *Methods Soil Anal. Part 2 Chem. Microbiol. Prop.* **1983**, *9*, 539–579.
28. Rhoades, J. Salinity: Electrical conductivity and total dissolved solids. *Methods Soil Anal. Part 3 Chem. Methods* **1996**, *5*, 417–435.
29. Sumner, M.E.; Miller, W.P. Cation exchange capacity and exchange coefficients. *Methods Soil Anal. Part 3 Chem. Methods* **1996**, *5*, 1201–1229.
30. Watanabe, F.; Olsen, S. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Sci. Soc. Am. J.* **1965**, *29*, 677–678. [CrossRef]
31. Bremner, J.M. Nitrogen-total. *Methods Soil Anal. Part 3 Chem. Methods* **1996**, *5*, 1085–1121.
32. Knudsen, D.; Peterson, G.; Pratt, P.; Page, A. Methods of Soil Analysis Chemical and Microbiological Properties. *Am. Soc. Agron. Madison Wis. USA* **1982**, 225–246.
33. Zarei, M.; Saleh-Rastin, N.; Jouzani, G.S.; Savaghebi, G.; Buscot, F. Arbuscular mycorrhizal abundance in contaminated soils around a zinc and lead deposit. *Eur. J. Soil Biol.* **2008**, *44*, 381–391. [CrossRef]
34. Goodarzian Ghahfarokhi, M.; Mansurifar, S.; Taghizadeh-Mehrjardi, R.; Saeidi, M.; Jamshidi, A.M.; Ghasemi, E. Effects of drought stress and rewatering on antioxidant systems and relative water content in different growth stages of maize (*Zea mays* L.) hybrids. *Arch. Agron. Soil Sci.* **2015**, *61*, 493–506. [CrossRef]
35. Rizwan, M.; Ali, S.; Qayyum, M.F.; Ok, Y.S.; Zia-ur-Rehman, M.; Abbas, Z.; Hannan, F. Use of maize (*Zea mays* L.) for phytomanagement of Cd-contaminated soils: A critical review. *Environ. Geochem. Health* **2017**, *39*, 259–277. [CrossRef] [PubMed]
36. Xu, J.; Volk, T.A.; Quackenbush, L.J.; Stehman, S.V. Estimation of shrub willow leaf chlorophyll concentration across different growth stages using a hand-held chlorophyll meter to monitor plant health and production. *Biomass Bioenergy* **2021**, *150*, 106132. [CrossRef]
37. Lamacque, L.; Charrier, G.; Farnese, F.d.S.; Lemaire, B.; Améglio, T.; Herbette, S. Drought-induced mortality: Branch diameter variation reveals a point of no recovery in lavender species. *Plant Physiol.* **2020**, *183*, 1638–1649. [CrossRef] [PubMed]
38. Thalhammer, A.; Pagter, M.; Hincha, D.K.; Zuther, E. Measuring freezing tolerance of leaves and rosettes: Electrolyte leakage and chlorophyll fluorescence assays. In *Plant Cold Acclimation: Methods and Protocols*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 9–21.
39. Badr, A.; Brüggemann, W. Comparative analysis of drought stress response of maize genotypes using chlorophyll fluorescence measurements and leaf relative water content. *Photosynthetica* **2020**, *58*, 38–645. [CrossRef]
40. Khoubnasabjafari, M.; Jouyban, A. Challenges on determination of malondialdehyde in plant samples. *Arch. Crop Sci.* **2020**, *4*, 64–66.
41. Taban, A.; Saharkhiz, M.J.; Khorram, M. Formulation and assessment of nano-encapsulated bioherbicides based on biopolymers and essential oil. *Ind. Crop. Prod.* **2020**, *149*, 112348. [CrossRef]
42. Habibi, F.; Ramezanian, A.; Guillén, F.; Castillo, S.; Serrano, M.; Valero, D. Changes in bioactive compounds, antioxidant activity, and nutritional quality of blood orange cultivars at different storage temperatures. *Antioxidants* **2020**, *9*, 1016. [CrossRef]
43. Bose, S.; Jain, A.; Rai, V.; Ramanathan, A. Chemical fractionation and translocation of heavy metals in *Canna indica* L. *grown on industrial waste amended soil. J. Hazard. Mater.* **2008**, *160*, 187–193.
44. Haider, F.U.; Liqun, C.; Coulter, J.A.; Cheema, S.A.; Wu, J.; Zhang, R.; Wenjun, M.; Farooq, M. Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicol. Environ. Saf.* **2021**, *211*, 111887. [CrossRef]
45. Vishnupradeep, R.; Bruno, L.B.; Taj, Z.; Karthik, C.; Challabathula, D.; Kumar, A.; Freitas, H.; Rajkumar, M. Plant growth promoting bacteria improve growth and phytostabilization potential of *Zea mays* under chromium and drought stress by altering photosynthetic and antioxidant responses. *Environ. Technol. Innov.* **2022**, *25*, 102154. [CrossRef]

46. Wang, Y.; Narayanan, M.; Shi, X.; Chen, X.; Li, Z.; Natarajan, D.; Ma, Y. Plant growth-promoting bacteria in metal-contaminated soil: Current perspectives on remediation mechanisms. *Front. Microbiol.* **2022**, *13*, 966226. [CrossRef]
47. Saleem, M.H.; Parveen, A.; Khan, S.U.; Hussain, I.; Wang, X.; Alshaya, H.; El-Sheikh, M.A.; Ali, S. Silicon fertigation regimes attenuates cadmium toxicity and phytoremediation potential in two maize (*Zea mays* L.) cultivars by minimizing its uptake and oxidative stress. *Sustain. Sci.* **2022**, *14*, 1462. [CrossRef]
48. Ma, Y.; Rajkumar, M.; Moreno, A.; Zhang, C.; Freitas, H. Serpentine endophytic bacterium Pseudomonas azotoformans ASS1 accelerates phytoremediation of soil metals under drought stress. *Chemosphere* **2017**, *185*, 75–85. [CrossRef]
49. Rajkumar, M.; Ae, N.; Prasad, M.N.V.; Freitas, H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol.* **2010**, *28*, 142–149. [CrossRef] [PubMed]
50. Woo, O.-G.; Kim, H.; Kim, J.-S.; Keum, H.L.; Lee, K.-C.; Sul, W.J.; Lee, J.-H. Bacillus subtilis strain GOT9 confers enhanced tolerance to drought and salt stresses in Arabidopsis thaliana and Brassica campestris. *Plant Physiol. Biochem.* **2020**, *148*, 359–367. [CrossRef] [PubMed]
51. Gupta, P.; Kumar, V.; Usmani, Z.; Rani, R.; Chandra, A.; Gupta, V.K. A comparative evaluation towards the potential of *Klebsiella* sp. and *Enterobacter* sp. in plant growth promotion, oxidative stress tolerance and chromium uptake in *Helianthus annuus* (L.). *J. Hazard. Mater.* **2019**, *377*, 391–398. [CrossRef] [PubMed]
52. BÖLÜKbaŞI, E. Methylation Modelling and Epigenetic Analysis of Sunflower (*Helianthus annuus* L.) Seedlings Exposed to Cadmium Heavy Metal Stress. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım Doğa Derg.* **2022**, *25*, 467–475. [CrossRef]
53. Bouremani, N.; Cherif-Silini, H.; Silini, A.; Bouket, A.C.; Luptakova, L.; Alenezi, F.N.; Baranov, O.; Belbahri, L. Plant Growth-Promoting Rhizobacteria (PGPR): A Rampart against the Adverse Effects of Drought Stress. *Water* **2023**, *15*, 418. [CrossRef]
54. Mousavi, S.S.; Karami, A.; Maggi, F. Photosynthesis and chlorophyll fluorescence of Iranian licorice (*Glycyrrhiza glabra* L.) accessions under salinity stress. *Front. Plant Sci.* **2022**, *13*, 984944. [CrossRef] [PubMed]
55. Sharma, P.; Jha, A.B.; Dubey, R.S. Oxidative stress and antioxidative defense system in plants growing under abiotic stresses. In *Handbook of Plant and Crop Stress*, 4th ed.; CRC Press: Boca Raton, FL, USA, 2019; pp. 93–136.
56. Singh, R.P.; Mishra, S.; Jha, P.; Raghuvanshi, S.; Jha, P.N. Effect of inoculation of zinc-resistant bacterium *Enterobacter ludwigii* CDP-14 on growth, biochemical parameters and zinc uptake in wheat (*Triticum aestivum* L.) plant. *Ecol. Eng.* **2018**, *116*, 163–173. [CrossRef]
57. Abdelaal, K.; AlKahtani, M.; Attia, K.; Hafez, Y.; Király, L.; Künstler, A. The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. *Biology* **2021**, *10*, 520. [CrossRef]
58. Rashid, U.; Yasmin, H.; Hassan, M.N.; Naz, R.; Nosheen, A.; Sajjad, M.; Ilyas, N.; Keyani, R.; Jabeen, Z.; Mumtaz, S. Drought-tolerant Bacillus megaterium isolated from semi-arid conditions induces systemic tolerance of wheat under drought conditions. *Plant Cell Rep.* **2021**, *41*, 549–569. [CrossRef] [PubMed]
59. Ali, B.; Wang, X.; Saleem, M.H.; Hafeez, A.; Afridi, M.S.; Khan, S.; Ullah, I.; Amaral Júnior, A.T.d.; Alatawi, A.; Ali, S. PGPR-mediated salt tolerance in maize by modulating plant physiology, antioxidant defense, compatible solutes accumulation and bio-surfactant producing genes. *Plants* **2022**, *11*, 345. [CrossRef]
60. Silambarasan, S.; Logeswari, P.; Valentine, A.; Cornejo, P. Role of *Curtobacterium herbarum* strain CAH5 on aluminum bioaccumulation and enhancement of *Lactuca sativa* growth under aluminum and drought stresses. *Ecotoxicol. Environ. Saf.* **2019**, *183*, 109573. [CrossRef] [PubMed]
61. Zandi, P.; Schnug, E. Reactive oxygen species, antioxidant responses and implications from a microbial modulation perspective. *Biology* **2022**, *11*, 155. [CrossRef] [PubMed]
62. Tyagi, J.; Mishra, A.; Kumari, S.; Singh, S.; Agarwal, H.; Pudake, R.N.; Varma, A.; Joshi, N.C. Deploying a microbial consortium of *Serendipita indica*, *Rhizophagus intraradices*, and *Azotobacter chroococcum* to boost drought tolerance in maize. *Environ. Exp. Bot.* **2023**, *206*, 105142. [CrossRef]
63. Moreira, H.; Marques, A.P.; Franco, A.R.; Rangel, A.O.; Castro, P.M. Phytomanagement of Cd-contaminated soils using maize (*Zea mays* L.) assisted by plant growth-promoting rhizobacteria. *Environ. Sci. Pollut. Res.* **2014**, *21*, 9742–9753. [CrossRef]
64. Liu, Y.; Tie, B.; Li, Y.; Lei, M.; Wei, X.; Liu, X.; Du, H. Inoculation of soil with cadmium-resistant bacterium *Delftia* sp. B9 reduces cadmium accumulation in rice (*Oryza sativa* L.) grains. *Ecotoxicol. Environ. Saf.* **2018**, *163*, 223–229. [CrossRef]
65. Lima e Silva, A.A.d.; Carvalho, M.A.; de Souza, S.A.; Dias, P.M.T.; Silva Filho, R.G.d.; Saramago, C.S.; Bento, C.A.; Hofer, E. Heavy metal tolerance (Cr, Ag and Hg) in bacteria isolated from sewage. *Braz. J. Microbiol.* **2012**, *43*, 1620–1631. [CrossRef]
66. Thakur, M.; Praveen, S.; Divte, P.R.; Mitra, R.; Kumar, M.; Gupta, C.K.; Kalidindi, U.; Bansal, R.; Roy, S.; Anand, A. Metal tolerance in plants: Molecular and physicochemical interface determines the "not so heavy effect" of heavy metals. *Chemosphere* **2022**, *287*, 131957. [CrossRef]
67. Begum, N.; Qin, C.; Ahanger, M.A.; Raza, S.; Khan, M.I.; Ashraf, M.; Ahmed, N.; Zhang, L. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Front. Plant Sci.* **2019**, *10*, 1068. [CrossRef] [PubMed]
68. Kaur, H.; Singh, S.; Kumar, P. Reconditioning of plant metabolism by arbuscular mycorrhizal networks in cadmium contaminated soils: Recent perspectives. *Microbiol. Res.* **2022**, *268*, 127293. [CrossRef]
69. Yang, Y.; Huang, B.; Xu, J.; Li, Z.; Tang, Z.; Wu, X. Heavy metal domestication enhances beneficial effects of arbuscular mycorrhizal fungi on lead (Pb) phytoremediation efficiency of Bidens parviflora through improving plant growth and root Pb accumulation. *Environ. Sci. Pollut. Res.* **2022**, *29*, 32988–33001. [CrossRef] [PubMed]

70. Amanullah, F.; Khan, W.-u.-D. *Trichoderma asperellum* L. Coupled the Effects of Biochar to Enhance the Growth and Physiology of Contrasting Maize Cultivars under Copper and Nickel Stresses. *Plants* **2023**, *12*, 958. [PubMed]
71. Singh, A.; Mehta, S.; Yadav, S.; Nagar, G.; Ghosh, R.; Roy, A.; Chakraborty, A.; Singh, I.K. How to cope with the challenges of environmental stresses in the era of global climate change: An update on ROS stave off in plants. *Int. J. Mol. Sci.* **2022**, *23*, 1995. [CrossRef] [PubMed]
72. Mirzai, M.; Moeini, A.; Ghanati, F. Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. *J. Agr. Sci. Tech.* **2013**, *15*, 593–602.
73. Hasanuzzaman, M.; Raihan, M.R.H.; Alharby, H.F.; Al-Zahrani, H.S.; Alsamadany, H.; Alghamdi, K.M.; Ahmed, N.; Nahar, K. Foliar Application of Ascorbic Acid and Tocopherol in Conferring Salt Tolerance in Rapeseed by Enhancing K^+/Na^+ Homeostasis, Osmoregulation, Antioxidant Defense, and Glyoxalase System. *Agronomy* **2023**, *13*, 361. [CrossRef]
74. Zhou, Z.; Wei, C.; Liu, H.; Jiao, Q.; Li, G.; Zhang, J.; Zhang, B.; Jin, W.; Lin, D.; Chen, G. Exogenous ascorbic acid application alleviates cadmium toxicity in seedlings of two wheat (*Triticum aestivum* L.) varieties by reducing cadmium uptake and enhancing antioxidative capacity. *Environ. Sci. Pollut. Res.* **2022**, *29*, 21739–21750. [CrossRef]
75. Conti, V.; Romi, M.; Guarnieri, M.; Cantini, C.; Cai, G. Italian tomato cultivars under drought stress show different content of bioactives in pulp and peel of fruits. *Foods* **2022**, *11*, 270. [CrossRef]
76. Spormann, S.; Nadais, P.; Sousa, F.; Pinto, M.; Martins, M.; Sousa, B.; Fidalgo, F.; Soares, C. Accumulation of Proline in Plants under Contaminated Soils. *Antioxidants* **2023**, *12*, 666. [CrossRef]
77. Llauradó Maury, G.; Méndez Rodríguez, D.; Hendrix, S.; Escalona Arranz, J.C.; Fung Boix, Y.; Pacheco, A.O.; García Díaz, J.; Morris-Quevedo, H.J.; Ferrer Dubois, A.; Aleman, E.I. Antioxidants in plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. *Antioxidants* **2020**, *9*, 1048. [CrossRef] [PubMed]
78. Begum, N.; Ahanger, M.A.; Zhang, L. AMF inoculation and phosphorus supplementation alleviates drought induced growth and photosynthetic decline in Nicotiana tabacum by up-regulating antioxidant metabolism and osmolyte accumulation. *Environ. Exp. Bot.* **2020**, *176*, 104088. [CrossRef]
79. Islam, F.; Yasmeen, T.; Arif, M.S.; Riaz, M.; Shahzad, S.M.; Imran, Q.; Ali, I. Combined ability of chromium (Cr) tolerant plant growth promoting bacteria (PGPB) and salicylic acid (SA) in attenuation of chromium stress in maize plants. *Plant Physiol. Biochem.* **2016**, *108*, 456–467. [CrossRef]
80. Song, L.; Niu, X.; Zhou, B.; Xiao, Y.; Zou, H. Application of biochar-immobilized *Bacillus* sp. KSB7 to enhance the phytoremediation of PAHs and heavy metals in a coking plant. *Chemosphere* **2022**, *307*, 136084.
81. Azeem, M.; Haider, M.Z.; Javed, S.; Saleem, M.H.; Alatawi, A. Drought stress amelioration in maize (*Zea mays* L.) by inoculation of *Bacillus* spp. strains under sterile soil conditions. *Agriculture* **2022**, *12*, 50. [CrossRef]