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Lead-Resistant *Morganella morganii* Rhizobacteria Reduced Lead Toxicity in *Arabidopsis thaliana* by Improving Growth, Physiology, and Antioxidant Activities

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Abstract: Biological remediation serves as a powerful technique for addressing heavy metals toxicity in metals-contaminated soils. The present study aimed to evaluate the efficacy of lead (Pb)-resistant rhizobacterial strains on growth, photosynthetic traits, and antioxidant activities of the *Arabidopsis* plant under lead toxicity in pot conditions. Two pre-isolated and pre-characterized Pb-resistant *Morganella morganii* (ABT3) and *Morganella morganii* (ABT9) strains were used for inoculating *Arabidopsis* plants grown under varying Pb concentrations (1.5 mM and 2.5 mM) using $PbNO_3$ as the lead source. The treatments were set up in a completely randomized design with four replications. Data on growth parameters, physiological characteristics, lipid peroxidation, and antioxidant activities were recorded at harvesting. It was observed that Pb contamination caused a significant reduction in *Arabidopsis* growth, chlorophyll content and quantum yield at both lead concentrations. The Pb concentration of 2.5 mM, showed a substantial decrease in all parameters, including shoot fresh weight (58.72%), shoot dry weight (59.31%), root fresh weight (67.31%), root dry weight (67.28%), chlorophyll content (48.69%), quantum yield (62.36%), catalase activity (65.30%), superoxide dismutase (60.88%), and peroxidase activity (60.54%) while increasing lipid peroxidation (113.8%). However, the inoculation with Pb-resistant *M. morganii* strains (ABT3 and ABT9) improved plant growth, photosynthesis and antioxidant activities, while reduced the malondialdehyde content of Arabidopsis compared to control plants without inoculation. The *M. morganii* strain ABT9 showed a maximum increase in the shoot fresh weight (67.18%), shoot dry weight (67.96%), root fresh weight (94.04%), root dry weight (93.92%), shoot length (148.88%), root length (123.33%), chlorophyll content (52.53%), quantum yield (58.57%), catalase activity (39.46%), superoxide dismutase (21.84%), and peroxidase activity (22.34%) while decreasing lipid peroxidation (35.28%). PCA analysis further showed that all nine treatments scattered differently across the PC1 and PC2, having 81.4% and 17.0% data variance, respectively, indicating the efficiency of Pb-resistant strains. The heatmap further validated that the introduction of Pb-resistant strains positively correlated with the growth parameters, quantum yield, chlorophyll content and antioxidant activities of Arabidopsis seedlings. Both Pb-resistant strains improved *Arabidopsis* plant growth and photosynthetic efficiency under lead stress conditions. Thus, both *Morganella morganii* ABT3 and *Morganella morganii* ABT9 strains can be considered as bio-fertilizer

for reducing lead toxicity thereby improving plant growth and physiology in metal-contaminated agricultural soils.

Keywords: lead; phytoremediation; antioxidant enzymatic activity; *Arabidopsis*; plant-growth promoting rhizobacteria

1. Introduction

Agriculture is one of the most important sectors of Pakistan's economy. It accounts for 18.9% of the country's GDP. Pakistan's economy has had agriculture-dependent growth since its beginning, which helps in promoting economic stability in the country [1]. Moreover, sustainable agriculture is also needed to solve food security issues to fulfil the demands of a growing global population. However, pathogenic attack, drought, low and high temperatures, nutrient deficiencies, soil salinity, and toxic metals are the primary causes of yield loss, reducing agricultural output by 50–80% depending on geographical region and crop type (Galanakis 2018) [2]. Due to increased urbanization and industrialization, heavy metal contamination of the air, water, and soil has caused major threats to the environment and agriculture. There is a need to devise strategies to minimize the metal toxicity that harms our ecosystem and decreases agricultural production.

The second most hazardous heavy metal prevalent in our environment is lead (Pb) [3]. Various human activities have increased Pb pollution in the air, water, and soil. Weathering of Pb-containing rocks, waste disposal, usage of Pb-gasoline in automobiles, shedding paint chips, and irrigation with sewage water are all potential causes of Pb toxicity in the soil [4]. The Pb concentrations in plants and soil in several parts of Pakistan are above the toxic threshold limit [5]. The amount of Pb in uncontaminated soil is less than 50 mg kg^{-1}, but contaminated soil has 500–800 mg kg^{-1} of Pb. The toxicity of Pb in plants inhibits key mechanisms such as nitrogen metabolism, ultimately reducing the growth and yield of plants [6]. Pb toxicity impairs chloroplast ultrastructure and disturbs the electron transport chain, Calvin cycle, and plastoquinone, causing a significant decrease in photosynthesis and leading to a substantial decrease in yield [7]. High Pb concentrations in plants induce oxidative stress that leads to the production of reactive oxygen species (ROS) and causes membrane damage in the form of lipid peroxidation [8]. Pb-induced lipid peroxidation disrupts biological membranes and impairs the function of cellular organelles including chloroplasts, mitochondria, and peroxisomes (Malecka et al., 2009) [9].

To deal with the detrimental effects of Pb toxicity, plants have evolved a controlled and sophisticated homeostatic network for ensuring an appropriate supply of essential defense metabolites while preventing the toxic buildup of Pb in the whole plant and at cellular levels [6]. Moreover, in response to Pb toxicity, plants produce different antioxidants, both enzymatic and non-enzymatic, including ascorbate peroxidase, proline, glutathione, guaiacol peroxidase (GPX), catalase (CAT), and peroxidase (POD) to reduce the detrimental effects of oxidative stress (Thakur et al., 2022) [10]. During oxidative phosphorylation, mitochondrial ROS generated in the membrane-linked electron transport chain stimulates antioxidant activity in plants. According to Qureshi et al. (2007) [11], Pb-induced oxidative stress in Indian senna significantly increased antioxidants such as CAT and glutathione reductase (GR) (Qureshi et al., 2007) [11]. Another study reported that Pb toxicity causes oxidative damage by increased concentration of malondialdehyde (MDA) in physic nuts; however, improved activity of several antioxidant enzymes was also observed in response to oxidative stress (Shu et al., 2012) [12].

Over the last few decades, significant progress has been made in decreasing heavy metal-induced toxicity by using different strategies, including chemical, physical, and biological methods. The traditional physicochemical approaches are costly and ineffective for low concentrations of heavy metals compared to biological remediation [13]. Biological remediation is a cost-effective and environmentally friendly approach that involves

the application of metal-tolerant bacterial strains and reduces heavy metal toxicity and improves plant growth [14,15]. Microorganisms (fungi, algae, bacteria) have been reported to be particularly effective in the remediation of lead-contaminated soils due to their ability to sequester, precipitate, or modify the lead oxidation state (Kang et al., 2016) [16]. Pb-resistant bacterial strains endure heavy metal stress through different processes such as intracellular bioaccumulation, biosorption, and heavy metal transformation by enzymatic or oxidative reduction, bio-precipitation, and metal volatilization [17,18]. Various heavy metal-resistant plant growth-promoting rhizobacteria (PGPR) strains can promote phytoextraction, while others can inhibit heavy metal mobility to different parts of the plant by producing metal-chelating compounds [19,20]. Various PGPR strains have been reported that produce growth regulators such as cytokinin, gibberellin, and auxin for improving plant growth while counteracting the toxic effects of heavy metals [21,22]. The heavy metal-resistant rhizobacterial strains also improve nutrient availability in water and soil by increasing the solubility of different minerals such as potassium, phosphorus, zinc, nitrogen, and iron [23]. They also improve the antioxidant machinery of plants and reduce heavy metal-induced oxidative stress [24,25]. Studies reported that potential PGPR strains adsorb lead into their cell surface and convert it into a less toxic form while reducing the uptake of lead in plants [26,27]. The Pb-tolerant PGPR strain improved antioxidant activity and photosynthetic performance of sunflower plants in Pb-contaminated soil [28,29]. Some potential Pb-resistant rhizobacterial strains include species of *Bradyrhizobium*, *Bacillus*, *Klebsiella*, *Enterobacter*, *Micrococcus*, *Ralstonia*, *Pseudomonas*, *Serratia*, *Variovorax*, *Stenotrophomonas*, etc. [28–30].

To date, numerous metal-resistant PGPR strains have been isolated and characterized; however, their practical application remains a challenge. More research is needed in this area to investigate the impact of already reported novel metal-resistant PGPR strains on plant growth and their survival potential in harsh conditions, with the ultimate goal of achieving sustainable agriculture. *Arabidopsis thaliana* has been used as the research model for dicotyledonous plants for decades. The application of Arabidopsis to plant heavy metal tolerance (Pb) study would be the beginning to shed light on the tolerance mechanisms underlying dicotyledonous plants. Therefore, the present study was conducted on Arabidopsis and aimed to investigate the impact of Pb-resistant rhizobacterial strains (ABT3 and ABT9) on the growth, chlorophyll content and antioxidant activities of *Arabidopsis* plants in Pb-contaminated soil.

2. Materials and Methods

The pot experiment was conducted in the growth room of the Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan to evaluate the phytoremediation potential of pre-isolated and pre-characterized heavy metal-resistant rhizobacterial strains (*Morganella morganii* ABT3 (accession no. ON316873) and *Morganella morganii* ABT9 (accession no. ON316874)) on the physiology, growth, and antioxidant activities of *Arabidopsis* plants in Pb-contaminated soil. Both strains were isolated from the salt-affected area of Pakistan and were identified as *Morganella morganii*. All chemicals/reagents used in this study were purchased from Sigma Aldrich (Darmstadt, Germany).

2.1. Lead Tolerance of Rhizobacterial Isolates

The screening of Pb resistance was performed by using the spot inoculation technique. Briefly, the freshly grown pure rhizobacterial isolates (ABT3 and ABT9) were inoculated on LB media amended with different $PbNO_3$ concentrations ranging between 0.5 mM to 5 mM and were allowed to grow for 24 h at 28 ± 2 °C. The Pb minimum inhibitory concentration (MIC) was selected as the highest Pb concentration at which the ABT3 and ABT9 cultures showed their growth.

2.2. Inoculum Preparation

For inoculum preparation, both ABT3 and ABT9 strains were grown in 100 mL LB media in a conical flask (250 mL), followed by incubation at 28 ± 2 °C in a shaking incubator at 100 rpm for 2 days. The cells were harvested by centrifugation for 5 min, and the pellet was resuspended in sterile distilled water. The optical density of both strains was observed at 535 nm using a spectrophotometer and maintained at 10^8 CFU mL^{-1} [31]. The obtained suspension was then used for the inoculation of Arabidopsis seeds.

2.3. Arabidopsis Seed Sterilization and Inoculation

Arabidopsis thaliana (Col-0) seeds were obtained from the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. Seeds were sterilized using 50% (*v*/*v*) bleach solution for 1 min and washed 3–4 times with sterile distilled water. The seeds were again dipped in 70% ethanol for 1 min and washed 5–6 times with sterile distilled water. Washed seeds were dipped in the inoculum of each strain for 30 min. The second inoculation was given after 3 weeks of germination by mixing it in Hoagland solution [32].

2.4. Pot Experiment

The pot experiment was performed in a completely randomized design (CRD) with four replicates of all nine treatments. The treatments consisted of water control, two different concentrations of lead (1.5 mM and 2.5 mM), bacterial inoculations (*Morganella morganii* ABT3 and *Morganella morganii* ABT9) and combined treatment of each bacterial inoculation with different lead concentrations. Inoculated seeds were sown in sterilized sand and were placed in the growth room under controlled conditions: photoperiod: 16/8 light/dark, temperature: 18–22 °C, and 75% humidity. Plants were watered with Hoagland nutritive media on alternate days after germination [32]. Five-week-old plants were treated with $PbNO_3$ starting from 0.5 mM with a gradual increase up to the desired concentration of 2.5 mM. After 2 days of the last treatment, plants were harvested for estimation of growth parameters, chlorophyll, and quantum yield analysis, while for biochemical experimentation, plants were preserved in liquid nitrogen and stored at −80 °C.

2.5. Estimation of Growth Parameters

The fresh weight of the shoot and root of the *Arabidopsis* plant was measured using a weighing balance, while for dry weight, shoot and shoot were oven-dried for 10 days at 65 °C. Length of root and shoot was measured with a scale.

2.6. Chlorophyll Content and Quantum Yield

Chlorophyll content was measured with a chlorophyll meter (SPAD-502). Three readings were taken from each replicate and their average was considered a standard value. Quantum yield (Fv/Fm) was estimated using the Flour-Pen FP100 (PSI, CZ). For this, a 5 cm area of *Arabidopsis* plant leaves was exposed to a strong light (3000 μmol m^{-2} s^{-1}) pulse, and the readings were taken as reported previously [33].

2.7. Lipid Peroxidation

Lipid peroxidation was determined by measuring (malonaldehyde) MDA content. Two hundred milligrams of leaves were homogenized in a 5 mL 0.1% trichloroacetic acid (TCA) solution followed by centrifugation at 10,000 rpm for 5 min. The supernatant (1 mL) was mixed with 1% TBA (thiobarbituric acid) and 5% TCA (3 mL) solution. The final mixture was heated in a hot water bath at 95 °C for 30 min. After heating, the mixture was centrifuged at 5000 rpm for 5 min. The supernatant was collected in a separate tube, and the absorbance of the sample mixture was measured at 532 and 600 nm with the help of a UV-VIS spectrophotometer. The actual concentration was estimated by comparing it with the extinction co-efficient value (155 μM^{-1} cm^{-1}) [34].

2.8. Antioxidant Enzymes Estimation

Antioxidant enzyme activity was estimated by homogenizing 0.2 g of leaves with 50 mM potassium phosphate buffer in a pre-chilled pestle mortar following centrifugation for 20 min at 12,000 rpm. The collected supernatant was used for the antioxidant analysis.

2.8.1. Catalase (CAT)

CAT activity was measured by observing hydrogen peroxide (H_2O_2) decomposition at 240 nm for 2 min using a spectrometer. The reaction mixture contained 200 µL of enzyme extract, 300 µL of 0.1 M H_2O_2, 1.5 mL of 50 mM potassium phosphate buffer (7.8 pH) and 1 mL dH2O. CAT activity was expressed as mmol/g fresh weight/min [35].

2.8.2. Peroxidase (POD)

POD activity was analyzed following the protocol of Chance and Maehly [36] with minor modifications. The reaction mixture contained: 50 µL of enzyme extract, 50 mM potassium phosphate buffer (pH 7.8) and 0.2 mL of 20mM guaiacol. The reaction was initiated by adding 0.25 mL of 40 mM H_2O_2. The absorbance value was measured with a spectrometer at 470 nm every 20 sec up to 2 min. POD activity was expressed as µ/min.

2.8.3. Superoxide Dismutase (SOD)

The activity of SOD was determined through the nitro blue tetrazolium (NBT) photoreduction (Dhindsa and Matowe 1981) [37]. The reaction mixture consisted of: 33 mM of NBT (50 µL), 10 mM of L-methionine (100 µL), 0.003 mM of riboflavin (50 µL), 50 mM potassium phosphate buffer (250 µL) and enzyme extract (100 µL). This mixture was kept under a 15 W lamp for 30 min. Then, the light was turned off to cease the reaction. For control, illuminated and non-illuminated reactions without enzyme extract were used. At 560 nm, the absorbance was measured. The SOD concentration necessary to cause a 50% inhibition of NBT was defined as one unit of SOD activity, and the enzyme activity was expressed as the unit mg/protein.

2.9. Statistical Analysis

One-way ANOVA (analysis of variance) was performed for analyzing data, and the mean of each treatment was compared by standard Fisher's LSD test (5% probability) using the Statstix 8.1 software. The significant level was as follows: $p \leq 0.5$ (*); $p \leq 0.005$ (**); $p \leq 0.001$ (***). For heatmap and principal component analysis (PCA), Origin-Pro 2021 software was used.

3. Results

The phytoremediation potential of Pb-resistant *M. morganii* strains (ABT3 and ABT9) was evaluated on the growth, photosynthetic machinery, and antioxidant activity of the Arabidopsis plant under Pb stress. Results showed that shoot fresh and dry weight was significantly reduced due to Pb contamination compared to control uninoculated plants without contamination (Figure 1). It was observed that Pb stress at 2.5 mM concentration showed a more severe decrease in shoot fresh and dry weight. At 2.5 mM Pb contamination, the shoot fresh and dry weight were reduced up to 58.72% and 59.31%, respectively, compared to control uninoculated Arabidopsis plants. However, the application of Pb-resistant *M. morganii* strains (ABT3 and ABT9) promoted the fresh and dry weight of shoots at both levels of Pb contamination. The *M. morganii* strain ABT9 showed a maximum increase in shoot fresh and dry weight (67.18 and 67.96%, respectively) at 2.5 mM Pb stress compared to *Arabidopsis* seedlings grown at the same stress level without inoculation.

Similarly, Pb contamination significantly reduced the root fresh and dry weight compared to control uninoculated *Arabidopsis* plants (Figure 2). The decrease in the root fresh and dry weight increased with increasing Pb concentrations. Maximum reduction in root fresh and dry weight of *Arabidopsis* plants was observed at 2.5 mM Pb stress compared to uninoculated controls. Results showed that 67.31 and 67.28% decreases,

respectively, were observed in root fresh and dry weight at 2.5 mM Pb contamination compared to control plants without inoculation. However, the inoculation of Pb-resistant *M. morganii* strains (ABT3 and ABT9) improved the root fresh and dry weight at both Pb levels. Pb-resistant rhizobacteria (ABT9) increased the root fresh and dry weight by 94.04 and 93.92%, respectively, compared to Arabidopsis plants grown at 2.5 mM lead contamination without inoculation.

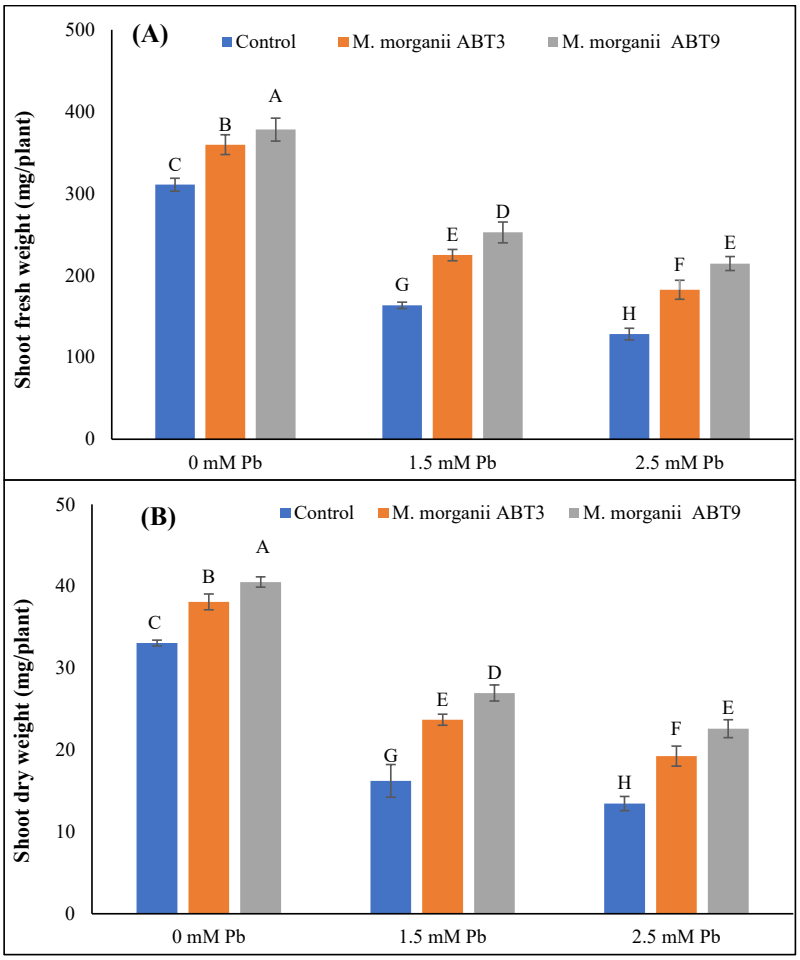

Figure 1. Effect of Pb-resistant *Morganella morganii* (ABT3 and ABT9) strain inoculation on shoot biomass of *Arabidopsis* seedlings grown in lead-contaminated soil. (**A**) Shoot fresh weight; (**B**) Shoot dry weight. Values are the mean of four replicates ± standard deviation. Number of biological replicates = 4. Letters A, B, C, and D, etc. represent a significant difference between means of different treatments.

Shoot and root length of *Arabidopsis* significantly decreased due to Pb contamination compared to control plants without inoculation, while improvement in both shoot and root length was observed under all Pb stress by the inoculation of Pb-resistant *M. morganii* strains (ABT3 and ABT9) in Pb-contaminated soil (Figure 3). Results showed that Pb-resistant rhizobacteria (ABT9) promoted shoot and root length by 148.88 and 123.33%, respectively, compared to Arabidopsis plants grown in 2.5 mM lead-contaminated soil without inoculation.

Further, the efficacy of Pb-resistant strains *M. morganii* strains (ABT3 and ABT9) on photosynthetic efficiency of *Arabidopsis* plants was observed in terms of quantum yield (Fv/Fm) and chlorophyll content. Results showed that Pb stress significantly reduced chlorophyll content and Fv/Fm, while inoculation with Pb-resistant *M. morganii* strains (ABT3 and ABT9) promoted these parameters significantly (Figure 4). The highest Pb concentration (2.5 mM) caused a more severe decrease in chlorophyll content (by 48.69%)

and Fv/Fm (by 62.36%) compared to that in control plants without inoculation. Pb-resistant rhizobacteria (ABT9) yielded a maximum increase in chlorophyll content and Fv/Fm by 52.53% and 58.57%, respectively, compared to Arabidopsis plants grown under 2.5 mM lead contamination without inoculation.

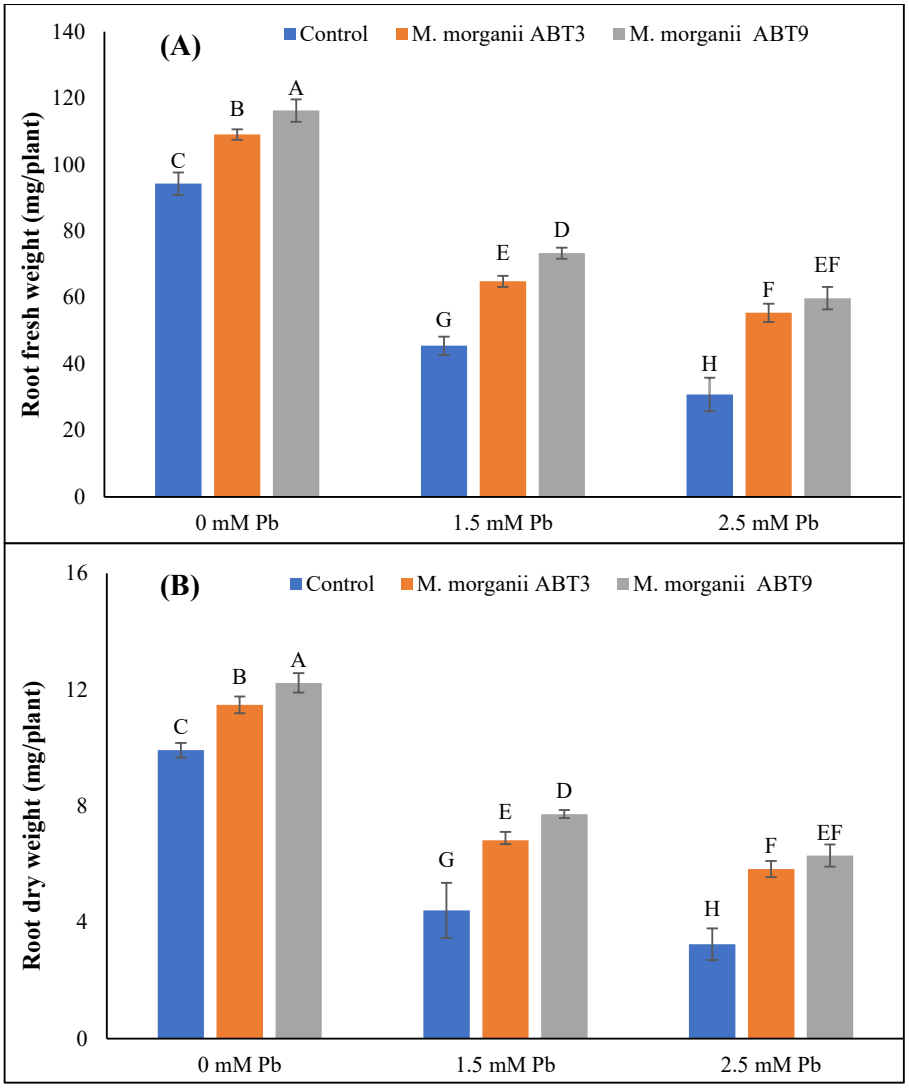

Figure 2. Effect of Pb-resistant *Morganella morganii* (ABT3 and ABT9) strain inoculation on root biomass of Arabidopsis seedlings grown in lead-contaminated soil. (**A**) Root Fresh weight; (**B**) root dry weight. Values are the mean of four replicates ± standard deviation. Number of biological replicates = 4. Letters A, B, C, and D, etc. represent a significant difference between means of different treatments.

An increase in lipid peroxidation and antioxidant enzyme activities (SOD, POD and CAT) was observed with increasing Pb concentration compared to non-stressed un-inoculated *Arabidopsis* plants (Figure 5). Maximum increases in lipid peroxidation, SOD, POD and CAT, by 113.8, 60.88, 60.54, and 65.30%, respectively, were observed at the 2.5 mM Pb concentration compared to control plants without inoculation. Inoculation with Pb-resistant *M. morganii* strains (ABT3 and ABT9) significantly decreased lipid peroxidation while causing considerable increases in SOD, POD and CAT enzyme activities in PGPR-inoculated Pb-stressed *Arabidopsis* plants. At 1.5 mM Pb stress, both strains reduced lipid peroxidation by 41.42 and 34.01%, respectively, while at 2.5 mM Pb stress, decreases of 38.24 and 35.28%, respectively, were observed. At both Pb concentrations, the maximum improvements in SOD (26.36 and 21.84%, respectively), POD (29.25 and 22.34%,

respectively) and CAT (28.80 and 39.46%, respectively) activities were observed in ABT9 strain-inoculated Arabidopsis plants compared to plants grown under 1.5 mM and 2.5 mM lead contamination without inoculation.

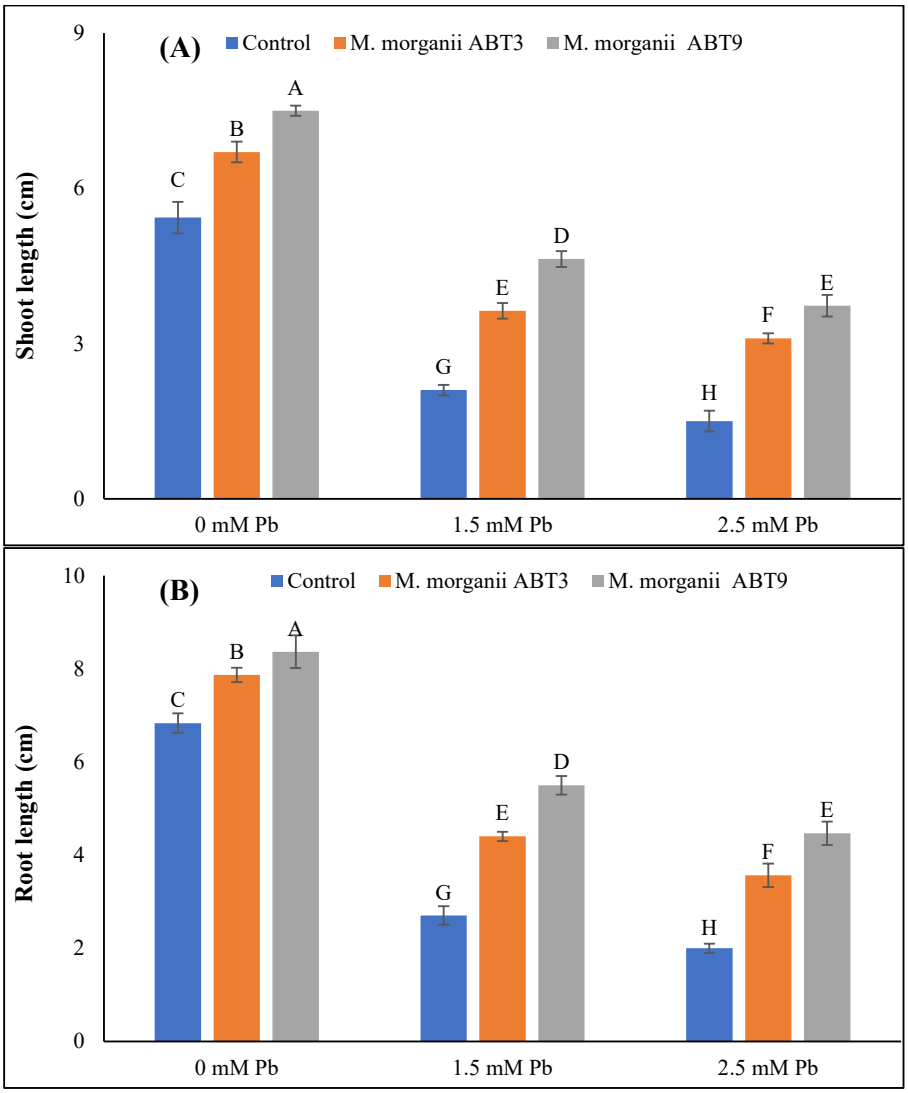

Figure 3. Effect of Pb-resistant *Morganella morganii* (ABT3 and ABT9) strain inoculation on shoot and root length of *Arabidopsis* seedlings grown in lead-contaminated soil. (**A**) Shoot length; (**B**) root length. Values are the mean of four replicates ± standard deviation. Number of biological replicates = 4. Letters A, B, C, and D, etc. represent a significant difference between means of different treatments.

The PCA analysis showed the phytoremediation potential of treatments with Pb-resistant rhizobacterial strains ABT3 and ABT9 on Arabidopsis plants under Pb contamination. All nine treatments scattered differently across the PC1 and PC2, indicating the efficiency of Pb-resistant strains. PC1 showed 75.5% data variance, while PC2 showed 22.9% data variance (Table 1).

PC1 consisted of inoculated treatments with or without Pb stress, and a positive correlation was observed in all growth parameters, photosynthetic parameters, and antioxidant activities of the Arabidopsis plants except lipid peroxidation (Figure 6).

In the heatmap, it was observed that the treatments with Pb-resistant strains (ABT3 and ABT9) were positively correlated with the growth parameters, quantum yield, chlorophyll content and antioxidant activities of *Arabidopsis* seedlings (Figure 7), and these treatments were more isolated compared to all other treatments, thus indicating remarkable differences in inoculated and uninoculated *Arabidopsis* plants.

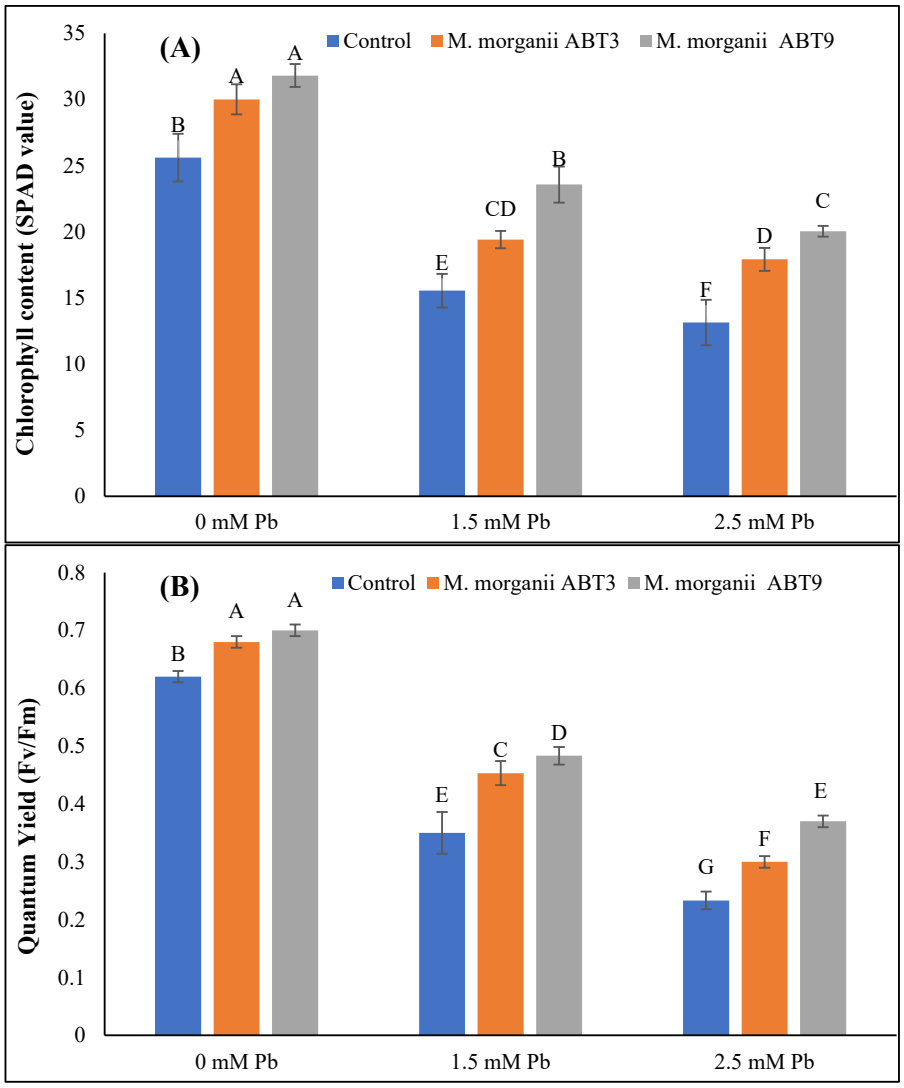

Figure 4. Effect of Pb-resistant *Morganella morganii* (ABT3 and ABT9) strain inoculation on photosynthetic parameters of *Arabidopsis* seedlings grown in lead-contaminated soil. (**A**) Chlorophyll content; (**B**) quantum yield. Values are the mean of four replicates ± standard deviation. Number of biological replicates = 4. Letters A, B, C, and D, etc. represent a significant difference between means of different treatments.

Table 1. Percentage variance for different treatments on Arabidopsis plants obtained by PCA analysis.

PC1 (75.5%)	PC2 (22.9%)	Loading Plot
	For treatments	
0.94946	−1.80178	0 mM Pb
−0.88433	−1.18867	1.5 mM Pb
−1.36361	−0.69363	2.5 mM Pb
1.25362	0.23881	*M. morganii* ABT3
−0.23369	0.36301	*M. morganii* ABT3 + 1.5 mM Pb
−0.73261	0.95396	*M. morganii* ABT3 + 2.5 mM Pb
1.4332	0.49424	*M. morganii* ABT9
0.06381	0.51591	*M. morganii* ABT9 + 1.5 mM Pb
−0.48585	1.11814	*M. morganii* ABT9 + 2.5 mM Pb

Table 1. *Cont.*

PC1 (75.5%)	PC2 (22.9%)	Loading Plot
	For parameters	
0.33069	0.04776	Shoot fresh weight
0.33025	0.05596	Shoot dry weight
0.33058	0.05043	Root fresh weight
0.33016	0.0608	Root dry weight
0.32596	0.10605	Shoot length
0.32949	0.0653	Root length
0.32642	0.09333	Chlorophyll
0.32929	−0.03247	Quantum yield
−0.3002	−0.1797	MDA
−0.12436	0.54935	CAT
−0.11951	0.56043	SOD
−0.11677	0.56117	POD

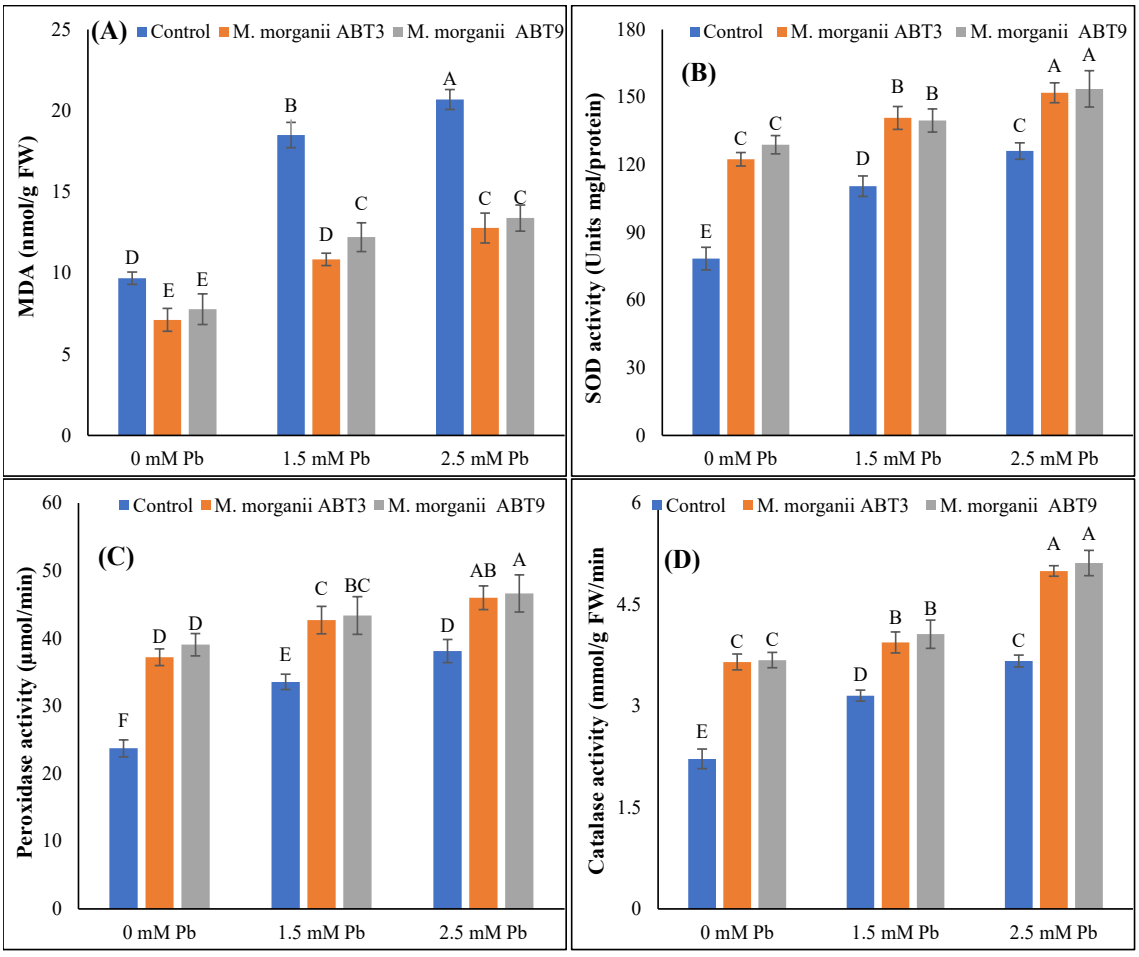

Figure 5. Effect of Pb-resistant *Morganella morganii* (ABT3 and ABT9) strain inoculation on lipid peroxidation and antioxidant activities of *Arabidopsis* seedlings grown in lead-contaminated soil. (**A**) MDA; (**B**) superoxide dismutase; (**C**) peroxidase activity; and (**D**) catalase activity. Values are the mean of four replicates ± standard deviation. Number of biological replicates = 4. Letters A, B, C, and D, etc. represent a significant difference between means of different treatments.

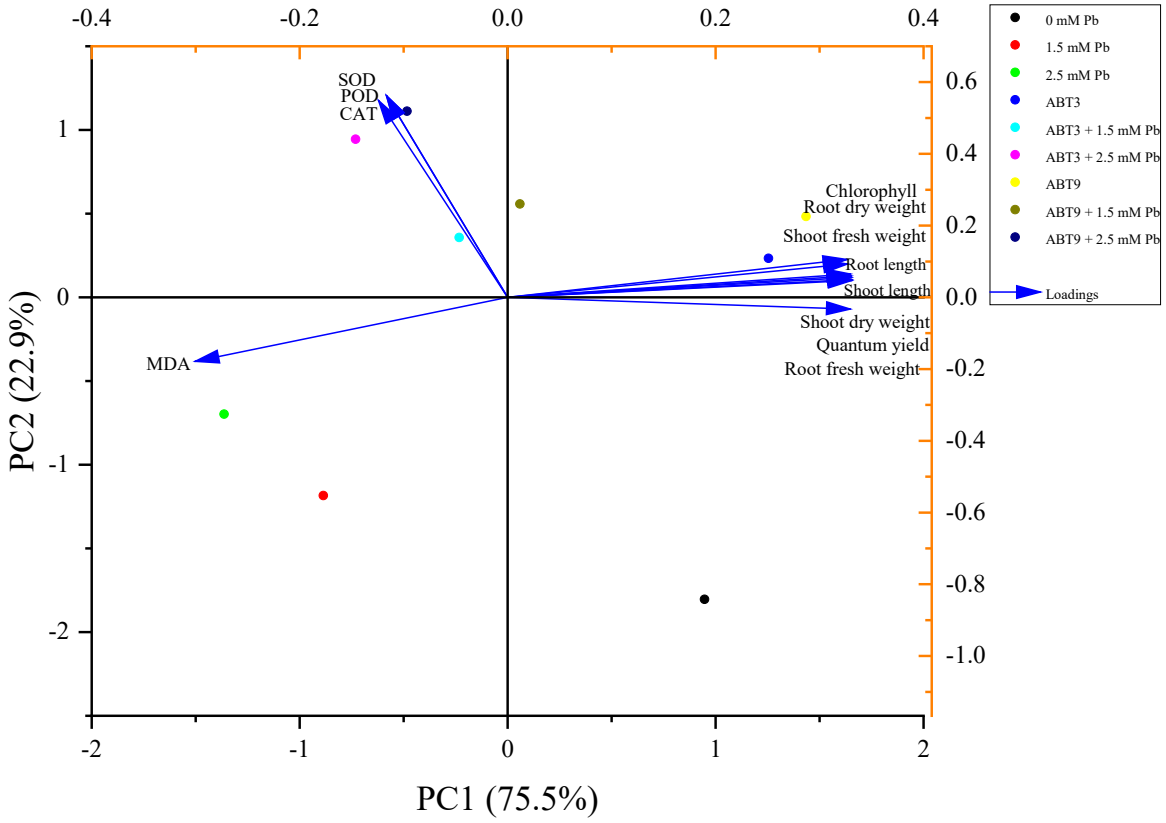

Figure 6. Principal component analysis of the effect of Pb-resistant (ABT3 and ABT9) strain inoculation on growth parameters, photosynthetic parameters and antioxidant enzyme activities of *Arabidopsis* plants grown under lead stress.

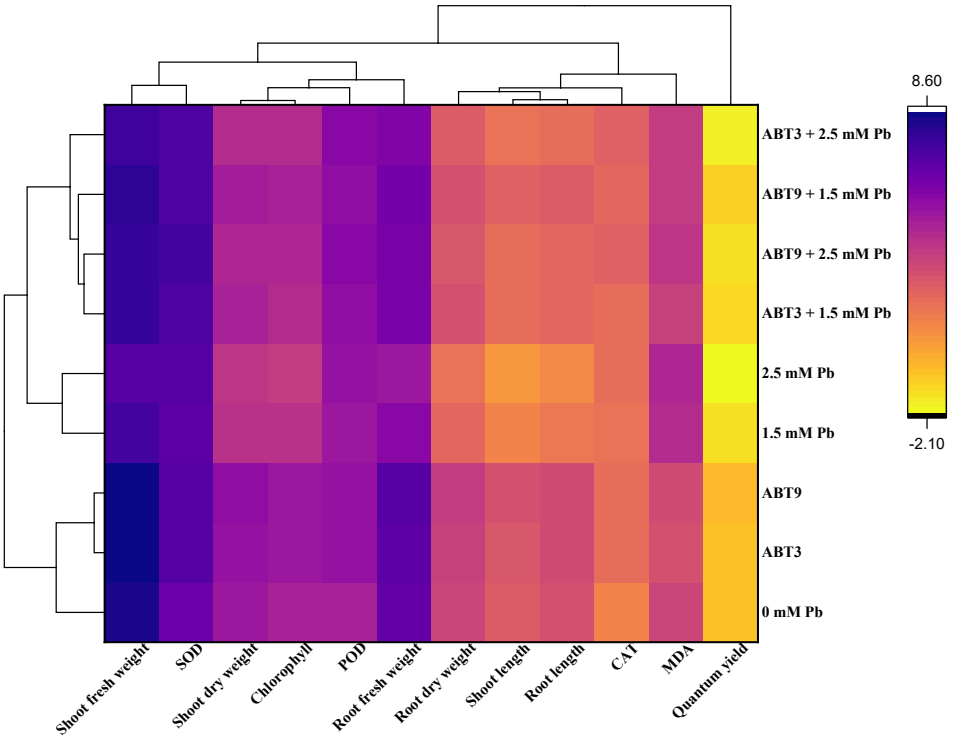

Figure 7. Heatmap for the mitigating effect of Pb-resistant (ABT3 and ABT9) strain inoculation on growth parameters, photosynthetic parameters and antioxidant enzyme activities of *Arabidopsis* plants grown under lead stress.

4. Discussion

Heavy metal-tolerant rhizobacterial strains are believed to protect plants against metal stress by modifying metabolism and decreasing the absorption or uptake of toxic heavy metals [19,20]. This study was conducted to evaluate the practical effectiveness of Pb-resistant rhizobacterial strains (ABT3 and ABT9) on *Arabidopsis* growth, photosynthetic efficiency, and antioxidant activity in lead-affected soil. Our findings demonstrated that Arabidopsis plants grown in lead-contaminated soil showed reduced growth compared to plants grown in uncontaminated soil. Our results are supported by previous studies conducted on different plants such as sunflower and sesame [28,38,39]. Lead stress may cause a reduction in the growth and development of plants because it reduces nutrient uptake, interferes with the respiration process, reduces plant photosynthetic activity, and disrupts the permeability of cell membrane [6]. Lead also induces structural damage, decreases physiological and biochemical activity, and impairs enzyme activities involved in the Calvin cycle and nitrogen and sugar metabolism [40]. Higher Pb concentrations may result in ROS production, triggering oxidative stress, cell membrane damage, and DNA strand breakage, thus resulting in decreased plant development [41,42].

However, inoculation with Pb-resistant rhizobacterial strains in Pb-contaminated soil reversed the toxic impact of lead and enhanced *Arabidopsis* growth compared to plants grown in uncontaminated soil. Several studies have shown that PGPR improves plant growth, yield, and physiology in the presence of metal toxicity [28,29,39]. Plant growth-promoting rhizobacteria have been shown to enhance nutrient uptake and availability through the recycling of organic waste [22]. Moreover, the production of 1-aminocyclopropane-1-carboxylate deaminase by PGPR also supports plant development by decreasing ethylene-mediated stress [43]. According to Kumar et al., *Rahnella aquatilis* and *Enterobacter aerogenes* reduced metal toxicity and enhanced the growth of *Brassica juncea* in a pot experiment [44], which is in agreement with our results. Inoculation with *Pseudomonas* strains reduced Pb-induced toxicity and improved the growth, yield and physiology of sunflower plants, as observed in the present study [28]. Our results are supported by previous studies of metal-stressed rice plants in which inoculation with *Bacillus cereus* and *Bacillus subtilis* remarkably increased shoot and root growth [45,46], which could be attributed to the phytohormone production and phosphate solubilization potential of inoculated rhizobacterial strains that help rice plants to acclimatize against the stressful environments [47]. Thus, it can be concluded that improvements in plant growth by Pb-resistant rhizobacterial strains in Pb-contamination soils could be due to phosphate solubilization, phytohormone production, siderophore production, and induced systemic resistance in metal-stressed plants [13,14,39].

Lead contamination also reduced physiological parameters including chlorophyll content and quantum yield. The decrease in these characteristics might be due to the Pb-induced inhibition of iron molecules into the chlorophyll phyto-porphyrin ring, resulting in decreased chlorophyll production in the Arabidopsis plant [7]. Lead also decreases the synthesis of chlorophyll by degrading its structure, inhibiting chlorophyllase activity and stopping plants from absorbing iron and magnesium [48], which can be one of the reasons for the reduced chlorophyll content and quantum yield observed in the present study. However, the toxic effect of Pb stress on the photosynthetic machinery of Arabidopsis plants were mitigated by the introduction of Pb-resistant rhizobacterial strains. Improvements in chlorophyll content and quantum yield of Arabidopsis plants by inoculation with Pb-resistant rhizobacterial strains could be due to PGPR-mediated increased iron uptake, which might have enhanced chlorophyll production, increased plants' growth and ultimately improved plant photosynthetic activities and quantum yield [49]. The results of the present study are in accordance with those of Ghori et al., who reported that inoculation with *Azotobacter chrococcum* increased the chlorophyll content of Pb-stressed maize plants [50,51], suggesting the protective role of microbial inoculation under metal toxicity.

Plants have protective mechanisms for metal detoxification and metal uptake to endure metal toxicity or keep metal concentrations within the physiological range [51]. Generally, heavy metal-induced oxidative stress causes several modifications in plant cells, such

as ROS production, decreased uptake of essential elements, and increased lipid peroxidation. Our results showed that Pb-induced oxidative stress affected the activities of antioxidant enzymes in *Arabidopsis* plants, while the increase in MDA content showed oxidative stress-induced membrane damage in Pb-stressed Arabidopsis plants. Significant increases in MDA content due to ROS-induced oxidative burst have been documented in earlier studies [52]. In maize, Cr-induced oxidative stress increased ROS production and electrolyte leakage, demonstrating the damage to membrane integrity [53]. Consistent with our findings, previous studies reported that metal toxicity resulted in higher lipid peroxidation and ROS accumulation [54,55]. Interestingly, the MDA content in Arabidopsis plants grown in Pb-contaminated soil inoculated with Pb-resistant *M. morganii* strains (ABT3 and ABT9) was significantly lower than in Pb-stressed plants, indicating the mitigating effect of Pb-resistant rhizobacterial strains. This decrease in MDA concentration as a result of inoculation led to a decrease in oxidative damage and, eventually, promoted the growth of *Arabidopsis* plants. Previous studies reported that inoculation of the soil with potential PGPR strains reduced ROS production and MDA accumulation in Cajanus cajan under Cd and Zn stress. The most plausible rationale for this mitigating effect is that the Pb-resistant strains either cause immobilization or exclusion of lead, eliminating the indirect inhibitory impact of Pb-induced oxidative stress on growth and antioxidant activities.

In this study, Pb toxicity upregulated superoxide dismutase, peroxidase and catalase enzyme activities compared to their levels in non-stressed plants, which is in accordance with the previous study conducted by Saleem et al., who reported that the antioxidant enzyme activities for scavenging Pb-generated ROS were significantly improved in sunflower plants, thus protecting the plants from oxidative stress [28]. It is interesting that after PGPR inoculation, the activity of antioxidant enzymes was amplified in Pb-stressed *Arabidopsis* plants. During metal stress, plant–microbe interaction enables plants to mitigate the detrimental effects of heavy metal toxicity by up-regulating their antioxidative ability [56]. Various enzymatic as well as non-enzymatic antioxidants control oxidative damage. SOD, an important component of the antioxidative defense system, is responsible for converting superoxide radicals into H_2O_2 rapidly and plays a vital role in protecting cells from the damage caused by oxygen species (Verma and Dubey 2003) [57]. Plants grown in soil supplemented with lead and inoculated with *M. morganii* were able to produce more SOD than plants grown in the untreated control soil. An increase in SOD enzyme activity is probably related to the de novo synthesis of enzyme proteins due to increased production of superoxide radicals (Fatima and Ahmad 2005) [58].

The second component involved in the ROS antioxidative process is CAT enzyme, which eliminates H_2O_2 by converting it into H_2O (Noctor and Foyer 1998) [59]. The increased CAT activity can be attributed to the increased production of hydrogen peroxide during stress conditions. In the present study, Pb stress significantly increased CAT activity, which is in agreement with previous studies [28,60]. Moreover, CAT activity significantly increased in *M. morganii*-inoculated plants subjected to Pb stress, which, in conjunction with the increased SOD activity, suggests improved oxidative stress tolerance in *Arabidopsis* plants. A similar increase in POD activity was also seen in plants grown in Pb-contaminated soil inoculated with bacteria and plants grown in Pb-contaminated soil alone. After *M. morganii* inoculation, it was observed that all antioxidative enzymes increased significantly, which facilitated plant growth under heavy metal stress conditions. The increased activities of antioxidants protect Arabidopsis plants from oxidative stress caused by high Pb concentrations by reducing the detrimental effects of ROS-induced lipid peroxidation [61]. Thus, it can be stated that Pb-resistant rhizobacterial strains stimulated the defense mechanism of plants [61].

5. Conclusions

This research concluded that Pb contamination reduced *Arabidopsis* growth by disturbing the photosynthetic machinery and membrane functioning. Inoculation with Pb-resistant

M. morganii strains (ABT3 and ABT9), on the other hand, proved effective in alleviating the Pb-induced reduction in *Arabidopsis* plant growth and photosynthetic parameters. Lead stress upregulated antioxidant activities, which were further improved with the application of Pb-resistant strains, leading to mitigation of oxidative stress. The present study strongly supports that Pb-resistant *M. morganii* strains (ABT3 and ABT9) improved heavy metal tolerance in *Arabidopsis* plants exposed to lead stress and can be applied as bio-fertilizers for improving plant growth and development in a metal-contaminated environment. However, the contribution of Pb-resistant *M. morganii* strains (ABT3 and ABT9) in enhancing stress tolerance through the activation of protective defense mechanisms needs to be evaluated further at the molecular level.

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References

1. Chandio, A.A.; Jiang, Y.; Rehman, A. Energy consumption and agricultural economic growth in Pakistan: Is there a nexus? *Int. J. Energy Sect. Manag.* **2020**, *13*, 597–609. [CrossRef]
2. Galanakis, C.M. *Sustainable Food Systems from Agriculture to Industry: Improving Production and Processing*; Academic Press: Cambridge, MA, USA, 2018.
3. Pratush, A.; Kumar, A.; Hu, Z. Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: A review. *Int. Microbiol.* **2018**, *21*, 97–106. [CrossRef] [PubMed]
4. Ali, H.; Khan, E.; Ilahi, I. Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *J. Chem.* **2019**, *2019*, 6730305. [CrossRef]
5. Waseem, A.; Arshad, J.; Iqbal, F.; Sajjad, A.; Mehmood, Z.; Murtaza, G. Pollution status of Pakistan: A retrospective review on heavy metal contamination of water, soil, and vegetables. *BioMed Res. Int.* **2014**, *2014*, 813206. [CrossRef] [PubMed]
6. Naz, H.; Naz, A.; Ayesha, A.S.; Khan, H. Effect of heavy metals (Ni, Cr, Cd, Pb, and Zn) on nitrogen content, chlorophyll, leghaemoglobin, and seed yield in chickpea plants in Aligarh city, UP, India. *Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 4387–4399.
7. Rai, R.; Agrawal, M.; Agrawal, S. Impact of heavy metals on physiological processes of plants: With special reference to photosynthetic system. In *Plant Responses to Xenobiotics*; Springer: Singapore, 2016; pp. 127–140.
8. Sidhu, G.P.S.; Singh, H.P.; Batish, D.R.; Kohli, R.K. Effect of lead on oxidative status, antioxidative response and metal accumulation in *Coronopus didymus*. *Plant Physiol. Biochem.* **2016**, *105*, 290–296. [CrossRef]
9. Malecka, A.; Piechalak, A.; Tomaszewska, B. Reactive oxygen species production and antioxidative defense system in pea root tissues treated with lead ions: The whole roots level. *Acta Physiol. Plant.* **2009**, *31*, 1053–1063. [CrossRef]
10. 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]
11. Qureshi, M.; Abdin, M.; Qadir, S.; Iqbal, M. Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. *Biol. Plant.* **2007**, *51*, 121–128. [CrossRef]
12. Shu, X.; Yin, L.; Zhang, Q.; Wang, W. Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. *Environ. Sci. Pollut. Res.* **2012**, *19*, 893–902. [CrossRef]
13. Li, C.; Zhou, K.; Qin, W.; Tian, C.; Qi, M.; Yan, X.; Han, W. A review on heavy metals contamination in soil: Effects, sources, and remediation techniques. *Soil Sediment Contam. Int. J.* **2019**, *28*, 380–394. [CrossRef]

14. Dixit, V.K.; Misra, S.; Mishra, S.K.; Joshi, N.; Chauhan, P.S. Rhizobacteria-mediated bioremediation: Insights and future perspectives. In *Soil Bioremediation: An Approach towards Sustainable Technology*; Wiley: Hoboken, NJ, USA, 2021; pp. 193–211.
15. Zubair, M.; Shakir, M.; Ali, Q.; Rani, N.; Fatima, N.; Farooq, S.; Shafiq, S.; Kanwal, N.; Ali, F.; Nasir, I.A. Rhizobacteria and phytoremediation of heavy metals. *Environ. Technol. Rev.* **2016**, *5*, 112–119. [CrossRef]
16. Kang, C.-H.; Kwon, Y.-J.; So, J.-S. Bioremediation of heavy metals by using bacterial mixtures. *Ecol. Eng.* **2016**, *89*, 64–69. [CrossRef]
17. Raklami, A.; Meddich, A.; Oufdou, K.; Baslam, M. Plants—Microorganisms-Based Bioremediation for Heavy Metal Cleanup: Recent Developments, Phytoremediation Techniques, Regulation Mechanisms, and Molecular Responses. *Int. J. Mol. Sci.* **2022**, *23*, 5031. [CrossRef] [PubMed]
18. Pal, A.K.; Sengupta, C. Isolation of cadmium and lead tolerant plant growth promoting rhizobacteria: *Lysinibacillus* varians and *Pseudomonas putida* from Indian Agricultural Soil. *Soil Sediment Contam. Int. J.* **2019**, *28*, 601–629. [CrossRef]
19. Etesami, H. Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: Mechanisms and future prospects. *Ecotoxicol. Environ. Saf.* **2018**, *147*, 175–191. [CrossRef]
20. Manoj, S.R.; Karthik, C.; Kadirvelu, K.; Arulselvi, P.I.; Shanmugasundaram, T.; Bruno, B.; Rajkumar, M. Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. *J. Environ. Manag.* **2020**, *254*, 109779. [CrossRef]
21. El-Tarabily, K.A. Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. *Plant Soil* **2008**, *308*, 161–174. [CrossRef]
22. Saleem, M.; Asghar, H.N.; Zahir, Z.A.; Shahid, M. Evaluation of lead tolerant plant growth promoting rhizobacteria for plant growth and phytoremediation in lead contamination. *Rev. Int. Contam. Ambient.* **2019**, *35*, 999–1009. [CrossRef]
23. Fahsi, N.; Mahdi, I.; Mesfioui, A.; Biskri, L.; Allaoui, A. Plant Growth-Promoting Rhizobacteria isolated from the Jujube (Ziziphus lotus) plant enhance wheat growth, Zn uptake, and heavy metal tolerance. *Agriculture* **2021**, *11*, 316. [CrossRef]
24. Ju, W.; Liu, L.; Fang, L.; Cui, Y.; Duan, C.; Wu, H. Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. *Ecotoxicol. Environ. Saf.* **2019**, *167*, 218–226. [CrossRef] [PubMed]
25. Zainab, N.; Khan, A.A.; Azeem, M.A.; Ali, B.; Wang, T.; Shi, F.; Alghanem, S.M.; Hussain Munis, M.F.; Hashem, M.; Alamri, S. PGPR-Mediated Plant Growth Attributes and Metal Extraction Ability of *Sesbania sesban* L. in Industrially Contaminated Soils. *Agronomy* **2021**, *11*, 1820. [CrossRef]
26. He, X.; Xu, M.; Wei, Q.; Tang, M.; Guan, L.; Lou, L.; Xu, X.; Hu, Z.; Chen, Y.; Shen, Z. Promotion of growth and phytoextraction of cadmium and lead in *Solanum nigrum* L. mediated by plant-growth-promoting rhizobacteria. *Ecotoxicol. Environ. Saf.* **2020**, *205*, 111333. [CrossRef] [PubMed]
27. Pietrini, I.; Grifoni, M.; Franchi, E.; Cardaci, A.; Pedron, F.; Barbafieri, M.; Petruzzelli, G.; Vocciante, M. Enhanced lead phytoextraction by endophytes from indigenous plants. *Soil Syst.* **2021**, *5*, 55. [CrossRef]
28. Saleem, M.; Asghar, H.N.; Zahir, Z.A.; Shahid, M. Impact of lead tolerant plant growth promoting rhizobacteria on growth, physiology, antioxidant activities, yield and lead content in sunflower in lead contaminated soil. *Chemosphere* **2018**, *195*, 606–614. [CrossRef]
29. Saran, A.; Imperato, V.; Fernandez, L.; Vannucchi, F.; Steffanie, N.; d'Haen, J.; Merini, L.; Vangronsveld, J.; Thijs, S. Bioaugmentation with PGP-trace element tolerant bacterial consortia affects Pb uptake by Helianthus annuus grown on trace element polluted military soils. *Int. J. Phytoremediation* **2021**, *23*, 202–211. [CrossRef]
30. Treesubsuntorn, C.; Dhurakit, P.; Khaksar, G.; Thiravetyan, P. Effect of microorganisms on reducing cadmium uptake and toxicity in rice (*Oryza sativa* L.). *Environ. Sci. Pollut. Res.* **2018**, *25*, 25690–25701. [CrossRef]
31. Naqqash, T.; Asma, I.; Sohail, H.; Shahid, M.; Afshan, M.; Javed, I.; Hanif, M.K.; Shaghef, E.; Malik, K.A. First report of diazotrophic *Brevundimonas* spp. as growth enhancer and root colonizer of potato. *Sci. Rep.* **2020**, *10*, 12893. [CrossRef]
32. Hoagland, D.R. The Water-Culture Method for Growing Plants without Soil. *Calif. Agric. Exp. Stn. Circ.* **1950**, *347*, 1–32.
33. Cessna, S.; Demmig-Adams, B.; Adams, W.W., III. Exploring photosynthesis and plant stress using inexpensive chlorophyll fluorometers. *J. Nat. Resour. Life Sci. Educ.* **2010**, *39*, 22–30. [CrossRef]
34. Heath, R.L.; Packer, L.J. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [CrossRef]
35. Aebi, H. (20) Catalase in vitro. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1984; Volume 105, pp. 121–126.
36. Chance, B.; Maehly, A. (136) Assay of catalases and peroxidases. *Methods Enzymol.* **1955**, *2*, 764–775.
37. Dhindsa, R.S.; Matowe, W. Drought tolerance in two mosses: Correlated with enzymatic defence against lipid peroxidation. *J. Exp. Bot.* **1981**, *32*, 79–91. [CrossRef]
38. Mehmood, S.; Saeed, D.A.; Rizwan, M.; Khan, M.N.; Aziz, O.; Bashir, S.; Ibrahim, M.; Ditta, A.; Akmal, M.; Mumtaz, M.A. Impact of different amendments on biochemical responses of sesame (*Sesamum indicum* L.) plants grown in lead-cadmium contaminated soil. *Plant Physiol. Biochem.* **2018**, *132*, 345–355. [CrossRef] [PubMed]
39. Teng, Z.; Shao, W.; Zhang, K.; Huo, Y.; Li, M. Characterization of phosphate solubilizing bacteria isolated from heavy metal contaminated soils and their potential for lead immobilization. *J. Environ. Manag.* **2019**, *231*, 189–197. [CrossRef]

40. Nawab, J.; Ghani, J.; Khan, S.; Khan, M.A.; Ali, A.; Rahman, Z.; Alam, M.; Hesham, A.E.-L.; Lei, M. Nutrient Uptake and Plant Growth Under the Influence of Toxic Elements. In *Sustainable Plant Nutrition under Contaminated Environments*; Springer: Cham, Switzerland, 2022; pp. 75–101.
41. Khan, F.; Hussain, S.; Tanveer, M.; Khan, S.; Hussain, H.A.; Iqbal, B.; Geng, M. Coordinated effects of lead toxicity and nutrient deprivation on growth, oxidative status, and elemental composition of primed and non-primed rice seedlings. *Environ. Sci. Pollut. Res.* **2018**, *25*, 21185–21194. [CrossRef]
42. Jeena, A.S.; Pandey, D. Metal induced genotoxicity and oxidative stress in plants, assessment methods, and role of various factors in genotoxicity regulation. In *Induced Genotoxicity and Oxidative Stress in Plants*; Springer: Singapore, 2021; pp. 133–149.
43. Nascimento, F.X.; Rossi, M.J.; Glick, B.R. Ethylene and 1-Aminocyclopropane-1-carboxylate (ACC) in plant–bacterial interactions. *Front. Plant Sci.* **2018**, *9*, 114. [CrossRef]
44. Kumar, K.V.; Srivastava, S.; Singh, N.; Behl, H. Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. *J. Hazard. Mater.* **2009**, *170*, 51–57. [CrossRef]
45. Etesami, H.; Srivastava, A.K. Bacterial induced alleviation of cadmium and arsenic toxicity stress in plants: Mechanisms and future prospects. In *Rhizosphere Engineering*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 445–469.
46. Jabeen, Z.; Irshad, F.; Habib, A.; Hussain, N.; Sajjad, M.; Mumtaz, S.; Rehman, S.; Haider, W.; Hassan, M.N. Alleviation of cadmium stress in rice by inoculation of Bacillus cereus. *PeerJ* **2022**, *10*, e13131. [CrossRef]
47. Zulfiqar, U.; Jiang, W.; Xiukang, W.; Hussain, S.; Ahmad, M.; Maqsood, M.F.; Ali, N.; Ishfaq, M.; Kaleem, M.; Haider, F.U. Cadmium Phytotoxicity, Tolerance, and Advanced Remediation Approaches in Agricultural Soils; A Comprehensive Review. *Front. Plant Sci.* **2022**, *13*, 773815. [CrossRef]
48. Kohli, S.K.; Handa, N.; Sharma, A.; Gautam, V.; Arora, S.; Bhardwaj, R.; Wijaya, L.; Alyemeni, M.N.; Ahmad, P. Interaction of 24-epibrassinolide and salicylic acid regulates pigment contents, antioxidative defense responses, and gene expression in *Brassica juncea* L. seedlings under Pb stress. *Environ. Sci. Pollut. Res.* **2018**, *25*, 15159–15173. [CrossRef] [PubMed]
49. Samaniego-Gámez, B.Y.; Garruña, R.; Tun-Suárez, J.M.; Kantun-Can, J.; Reyes-Ramírez, A.; Cervantes-Díaz, L. *Bacillus* spp. inoculation improves photosystem II efficiency and enhances photosynthesis in pepper plants. *Chil. J. Agric. Res.* **2016**, *76*, 409–416. [CrossRef]
50. Rizvi, A.; Khan, M.S. Heavy metal induced oxidative damage and root morphology alterations of maize (*Zea mays* L.) plants and stress mitigation by metal tolerant nitrogen fixing *Azotobacter chroococcum*. *Ecotoxicol. Environ. Saf.* **2018**, *157*, 9–20. [CrossRef] [PubMed]
51. Ghori, N.-H.; Ghori, T.; Hayat, M.; Imadi, S.; Gul, A.; Altay, V.; Ozturk, M. Heavy metal stress and responses in plants. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 1807–1828. [CrossRef]
52. De Dios Alché, J. A concise appraisal of lipid oxidation and lipoxidation in higher plants. *Redox Biol.* **2019**, *23*, 101136. [CrossRef] [PubMed]
53. Mahajan, P.; Sharma, P.; Singh, H.P.; Rathee, S.; Sharma, M.; Batish, D.R.; Kohli, R.K. Amelioration potential of β-pinene on Cr (VI)-induced toxicity on morphology, physiology and ultrastructure of maize. *Environ. Sci. Pollut. Res.* **2021**, *28*, 62431–62443. [CrossRef]
54. Jawad Hassan, M.; Ali Raza, M.; Ur Rehman, S.; Ansar, M.; Gitari, H.; Khan, I.; Wajid, M.; Ahmed, M.; Abbas Shah, G.; Peng, Y. Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two sorghum cultivars. *Plants* **2020**, *9*, 1575. [CrossRef]
55. Cheng, C.-H.; Ma, H.-L.; Deng, Y.-Q.; Feng, J.; Jie, Y.-K.; Guo, Z.-X. Oxidative stress, cell cycle arrest, DNA damage and apoptosis in the mud crab (*Scylla paramamosain*) induced by cadmium exposure. *Chemosphere* **2021**, *263*, 128277. [CrossRef]
56. Khanna, K.; Kohli, S.K.; Bali, S.; Kaur, P.; Saini, P.; Bakshi, P.; Ohri, P.; Mir, B.A.; Bhardwaj, R. Role of micro-organisms in modulating antioxidant defence in plants exposed to metal toxicity. In *Plants under Metal and Metalloid Stress*; Springer: Singapore, 2018; pp. 303–335.
57. Verma, S.; Dubey, R. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.* **2003**, *164*, 645–655. [CrossRef]
58. Fatima, R.A.; Ahmad, M. Certain antioxidant enzymes of Allium cepa as biomarkers for the detection of toxic heavy metals in wastewater. *Sci. Total Environ.* **2005**, *346*, 256–273. [CrossRef]
59. Noctor, G.; Foyer, C.H. Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Biol.* **1998**, *49*, 249–279. [CrossRef] [PubMed]
60. Shabaan, M.; Asghar, H.N.; Akhtar, M.J.; Ali, Q.; Ejaz, M. Role of plant growth promoting rhizobacteria in the alleviation of lead toxicity to *Pisum sativum* L. *Int. J. Phytoremediation* **2021**, *23*, 837–845. [CrossRef] [PubMed]
61. Janmohammadi, M.; Bihamta, M.; Ghasemzadeh, F. Influence of rhizobacteria inoculation and lead stress on the physiological and biochemical attributes of wheat genotypes. *Cercet. Agron. Mold.* **2013**, *46*, 49–67. [CrossRef]