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The Effects of Coexisting Elements (Zn and Ni) on Cd Accumulation and Rhizosphere Bacterial Community in the Soil-Tomato System

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Abstract: The increasing cadmium (Cd) levels in agricultural soils have become a worldwide concern for food crop security. Cd accumulation in the soil-plant system is closely related to other coexisting factors. In this study, the effects of different levels of Zn or Ni on Cd accumulation in tomato plants and on the rhizosphere soil bacterial community structure were analyzed by coupling pot experiments with high-throughput sequencing. The results demonstrated that tomato plants (*Lycopersicon esculentum*) in Zn-Cd and Ni-Cd co-contaminated soils exhibited lower relative growth rates. Co-contamination at low levels tended to reduce the bioaccumulation of heavy metals in the roots of plants, whereas increased contaminant concentrations produced the opposite effect. In the presence of 200 mg/kg Zn or 20 mg/kg Ni, the biomass of plant roots increased by 4.95–23.16% and the Cd content of the plant roots decreased by 17.36–68.93% due to the antagonistic effects between Cd and Zn/Ni. In addition, the richness and diversity of the bacterial community were significantly altered under HMs co-contamination, and the number of special bacteria was positively correlated with the level of heavy metals in the rhizosphere soil. The relative abundance of *Proteobacteria* increased and that of *Actinobacteria* decreased in soils with low levels of heavy metals. This may improve the tolerance of plant roots to heavy metals and reduce the accumulation of Cd in plant roots. These findings highlight the important role of coexisting elements in the inhibition of Cd accumulation in tomatoes and offer important information for the production of safe crops.

Keywords: tomato; cadmium; co-contamination; microbial community

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1. Introduction

The accumulation of heavy metals (HMs) in soil has become a significant environmental problem with rapid urbanization and industrialization [1]. The latest nationwide survey on the status of soil contamination in China revealed that 16.1% of all samples analyzed exceeded the environmental quality standards [2,3], with HMs being the main pollutants (82.4%). The pollution status of HMs in soils from China between 1977 and 2020 was investigated by Shi et al. [4], with the results indicating that cadmium (Cd) was the most widespread pollutant in agricultural soils. The mean concentration of Cd was 5.73 times higher than the limit for agricultural soils when comparing the metal concentration relative to the standard limit [5]. Importantly, Cd is one of the most toxic and dangerous HMs for living organisms, even at low concentrations [6]. Moreover, increasing Cd pollution seriously affects the yield of crops [7]. Cd can also be transferred from contaminated soil to crops and plants and accumulate in organisms through the food chain, posing severe threats to human health [8]. Tomatoes (*Lycopersicon esculentum*, L. *esculentum*), a vital vegetable crop worldwide, provide numerous health benefits to humans [9].

Therefore, a better understanding of the fate of Cd in the soil-tomato system and the influencing factors is critical for protecting food security.

Many studies have examined the mechanisms underlying Cd toxicity and the uptake, translocation, and accumulation of Cd in crops [10–12]. The toxicity of Cd inhibits plant growth, disturbs photosynthesis and transpiration, and reduces the synthesis of carbohydrates and nucleic acids [13–15]. The uptake and accumulation of Cd in crops are influenced by many factors, including plant type, Cd concentration, soil pH, and organic matter [12]. Moreover, coexisting elements in the soil may significantly influence the uptake and transport of Cd in the crop through antagonistic, additive, and synergistic effects between HMs [16,17]. While zinc (Zn) and nickel (Ni) are essential micronutrients that are required for many structural and biochemical functions in plants, they are toxic at high concentrations [18,19]. Ni in the soil can reduce the absorption of Cd in the roots of maize and rice [20,21]. Moreover, the presence of Zn can significantly reduce Cd accumulation and toxicity in many crops (such as wheat, rice, maize, and leaf vegetables) because Cd and Zn have similar absorption systems and ion competition exists [22–24]. However, several studies have demonstrated that the application of Zn or Ni does not inhibit Cd accumulation in some crops [22,25]. Although the influences of coexisting mineral factors on Cd accumulation in different crops have been widely investigated, the existing research on the interactions between HMs in the same crop is conflicting. In addition, there is a scarcity of studies on the interactive effects of Ni combined with Cd treatment on tomato plants.

As an important medium for the exchange of plant and soil resources, the rhizosphere microbiota plays an important role in crop growth [26]. Generally, HM pollution has negative effects on the rhizosphere microbial community [27]. As a toxic element in soil, Cd can affect the microbial distribution, and the diversity of the bacterial community decreases with increasing levels of Cd [28]. High HMs levels can also result in the enrichment of some microbial species that are tolerant to very high concentrations of HMs [29]. For crops, changes in the soil microbial community composition and structure can affect the absorption of Cd and micronutrients (such as Fe, Zn, Mg, and Ca) [30]. Recently, several studies have explored the impact of changes in the structure of rhizosphere microbial communities on the accumulation of heavy metals in plants through inoculation with specific bacterial strains or the application of substances to the soil [24,31,32]. Nonetheless, our understanding of how the whole indigenous soil microbiome affects Cd accumulation in crop plants remains limited, especially when considering the complex interactions between Cd and other HMs in soil-plant systems.

In this study, a pot experiment was conducted, and tomatoes were exposed to different levels of contamination with Cd, Zn, Ni, and various combinations of these HMs. The plant growth and HMs concentrations of the tomato plant tissues were determined at the end of the treatment. Pyrosequencing of the 16S rRNA genes was conducted to detect differences in the bacterial communities of the rhizosphere soil under the different treatments. The aims of this study were to (I) explore the growth characteristics of tomatoes under Cd, Zn, and Ni stress; (II) determine the fates of Cd, Zn, and Ni in soil-tomato systems under single/combined contamination; and (III) investigate the interaction effects between the rhizosphere microbial community structure and the HMs uptake and accumulation processes in tomato plants. It was hypothesized that the coexistence of Cd and other HMs in tomato-soil systems would significantly affect the migration and accumulation of Cd in tomato plants through complex interactions between the plant and the rhizosphere microbial community. These findings can inform the development of strategies for the safe cultivation of tomato crops in areas contaminated with Cd, Zn, and Ni.

2. Materials and Methods

2.1. Material Preparation

Cherry tomatoes (*Lycopersicon esculentum var. cerasiforme*) are a high Cd-accumulating fruit and typically contain two to three times the recommended Cd concentration for fruits

and vegetables [13,33]. In this study, a commonly cultivated type of cherry tomato (Meiwei, a high Cd accumulation cultivar) was selected based on the findings of Xu et al. [34]. The cherry tomato seedlings were obtained from a registered retailer in Songjiang, Shanghai, China. The seedlings were propagated in a half-strength modified Hoagland's nutrient solution in order to maintain suitable seedling development and were grown in a growth chamber (SGZ1000A, China) under cool white fluorescent lights (450 μmol photons·m^{-2} s^{-1}) at 25 ± 2 °C with a 12-h light/dark photoperiod. The modified Hoagland nutrient solution was composed of $Ca(NO_3)_2{\cdot}4H_2O_2$ (0.473 g/L), KNO_3 (0.253 g/L), NH_4NO_3(0.40 g/L), KH_2PO_4 (0.068 g/L), $MgSO_4$ (0.12 g/L), and microelements. The cultured tomato seedlings with a similar growth status (plant height: 8 ± 2 cm) were randomly assigned to the treatment groups.

The pot experiment was conducted at the Donghua University Songjiang Campus (31°02′52.92″ N, 121°13′37.44″ E). The soil samples were collected from an agricultural field located near Songjiang, Shanghai, at a depth of 0–20 cm. The method of topsoil sampling was as described in the Technical Specification for Soil Environmental Monitoring [35]. The soil samples were transported to the laboratory, air-dried at room temperature, and passed through a 2-mm nylon sieve before use in the experiments. Following the method described by Jiang et al. [36], the pH value of the soil samples was measured at a soil:water ratio of 1:2.5 using a multi-parameter tester (HQ40d, Hach Water Quality Analytical Instruments (Shanghai) Co., Ltd., Shanghai, China). The soil texture was determined by a combination of sieving and sedimentation (ISO 11277). The soil organic matter was determined using wet digestion by the potassium dichromate method. The content of total nitrogen was measured according to the micro-Kjeldahl method, and the total phosphorus content was colorimetrically determined by wet digestion with HF–$HClO_4$. The contents of available nitrogen and phosphorus were measured by the micro-diffusion technique and the Olsen method, respectively. For the total Cd, Zn, and Ni concentrations in the soil, 0.2 g of soil samples were digested with a mixture of nitric acid (HNO_3), hydrofluoric acid (HF), and perchloric acid ($HClO_4$) in Teflon crucibles on a hot plate (GDANA-HT10, China). The physicochemical properties of the initial soil samples were as follows: heavy loam; pH, 7.63; 15.90 g·kg^{-1} organic matter; 16.96 mg·kg^{-1} available phosphorus (P); 125.67 mg·kg^{-1} available nitrogen (N); 307.26 mg·kg^{-1} total P; 660.05 mg·kg^{-1} total N; total Cd, Zn, and Ni concentrations of 0.10, 2.96, and 0.13 mg·kg^{-1}, respectively.

2.2. Experimental Design

Three experimental treatment groups were examined in this study: 1. the effect of Cd treatment on tomato seedlings; 2. the effect of Cd with Zn co-treatment on tomato seedlings; 3. the effect of Cd with Ni co-treatment on tomato seedlings. In this experiment, $CdCl_2{\cdot}2.5H_2O$ solution, $ZnSO_4{\cdot}7H_2O$ solution, and $Ni(NO_4)_2{\cdot}6H_2O$ solution were used as the sources of Cd, Zn, and Ni, respectively. The selection of Cd, Zn, and Ni contamination levels was mainly based on extreme pollution situations that have been reported earlier [37–39]. As shown in Table 1, the three experimental treatment groups comprised sub-treatments, as follows: (1) the Cd treatment group included four levels of Cd contamination (0, 1, 20, 50 mg kg^{-1} soil); (2) the Cd with Zn co-treatment group included four levels of Cd contamination (0, 1, 20, 50 mg kg^{-1}) which were respectively supplemented with 1, 20, 50 mg Zn kg^{-1} soil; (3) the Cd with Ni co-treatment group included four levels of Cd contamination (0, 1, 20, 50 mg kg^{-1}) which were respectively supplemented with 20, 80, 160 mg Ni kg^{-1} soil. The treated soils were transferred into plastic pots (16 cm × 14 cm), and all soil samples were cultured at room temperature for 30 days in the dark. In total, the study comprised 28 experimental treatment combinations, which were replicated three times in a completely randomized block design.

Table 1. Concentrations (mg·kg^{-1} DW soil) of heavy metals added to the soil.

Treatment Groups	Cd Concentration	Zn Concentration	Ni Concentration	Nomenclature
Cd single treatment	0	0	0	Cd_0(CK)
	1	0	0	Cd_1
	20	0	0	Cd_2
	50	0	0	Cd_3
Cd combined with Zn treatments	0	200	0	Zn_1Cd_0
	1	200	0	Zn_1Cd_1
	20	200	0	Zn_1Cd_2
	50	200	–	Zn_1Cd_3
	0	500	0	Zn_2Cd_0
	1	500	0	Zn_2Cd_1
	20	500	–	Zn_2Cd_2
	50	500	–	Zn_2Cd_3
	0	1000	0	Zn_3Cd_0
	1	1000	0	Zn_3Cd_1
	20	1000	0	Zn_3Cd_2
	50	1000	0	Zn_3Cd_3
Cd combined with Ni treatments	0	0	20	Ni_1Cd_0
	1	0	20	Ni_1Cd_1
	20	0	20	Ni_1Cd_2
	50	0	20	Ni_1Cd_3
	0	0	80	Ni_2Cd_0
	1	0	80	Ni_2Cd_1
	20	0	80	Ni_2Cd_2
	50	0	80	Ni_2Cd_3
	0	0	160	Ni_3Cd_0
	1	0	160	Ni_3Cd_1
	20	0	160	Ni_3Cd_2
	50	0	160	Ni_3Cd_3

2.3. Plant Culture

Each pot contained 0.85 kg of treated soil and one pre-germinated tomato seedling. Plant culture was conducted in a phytotron (SGZ-1000 A, Hangzhou Shuolian Instrument Co., Ltd., Hangzhou, China) at 25–30 °C under a 12-h light/dark photoperiod with a relative humidity of 80%. During the 65 days of culture, the soil in each treatment was maintained at a water-holding capacity of 65–70% by adding deionized water. The plant growth conditions remained the same, and the plots were rotated weekly to eliminate spatial variability in the growth chamber.

2.4. Determination of Plant Growth

All tomato plants were harvested for analysis at the end of the seedling period and before the fruiting period [38]. At the end of the 65-day treatment, the fresh weight and length of the plants were measured to evaluate the plant growth response. The relative growth rate (RGR, g·d^{-1}) of the different treatments was calculated as follows: $RGR = (lnW_2 \_ lnW_1)/t$, where W_1 and W_2 are the initial fresh weight and final fresh weight (g), respectively, after 65 days of treatment, and t represents the treatment time (days).

2.5. Determination of HM Contents

After 65 days of treatment, the plants were removed from the pots and rinsed in deionized water. After harvesting, they were divided into roots, stems, and leaves for analysis of the HMs concentrations. Before analysis, the harvested plants were washed with distilled water and blotted with tissue paper. The roots, stems, and leaves were dried at 75 °C for 48 h. After drying, the ground plant samples were passed through 149 μm nylon

sieves to obtain a uniform particle size. The ground samples of the roots and shoots were analyzed for HMs accumulation by the wet digestion method, as described by Miller [40]. A 1 g plant sample, 10 mL of HNO_3, and 5 mL of $HClO_4$ were added to a glass flask, and digestion was performed to obtain the HMs solution. After complete digestion, the resulting solution was transferred to a volumetric flask and diluted to 25 mL. The total Cd, Zn, and Ni concentrations of all samples were determined by an atomic absorption spectrophotometer (TAS-986, USA).

2.6. Microbial Analysis of the Soil Samples

When the plants were harvested, rhizosphere soil samples from the CK, Cd_1, Cd_3, minimum and maximum Zn-Cd co-contamination treatments, and Ni-Cd co-contamination treatments were also collected for soil bacterial community analysis. The genomic DNA in the rhizosphere soil was extracted using an Invitrogen kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification of the V4-V5 regions of the bacterial 16S rRNA gene was performed with the primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAG TTT-3′). PCR reactions were performed, and the PCR products were purified, as described in a previous study [41]. Furthermore, all PCR products were sequenced on an Illumina Miseq system (Mayo Biotechnology Co., Ltd., Shanghai, China).

2.7. Data Analysis

Three replicates were performed for each experiment, and the experimental data are expressed as the mean ± SE. The Cd, Zn, and Ni concentrations in the tomato plants were expressed on a plant dry weight basis (mg kg−1 DW). All figures were plotted using Prism. Heat maps were generated by Heml to reflect the differences in the abundance distributions of species between the different groups. All statistical analyses were performed in SPSS. One-way analysis of variance (ANOVA) with Tukey's test ($p < 0.05$) was used to evaluate significant differences in the plant growth rate and HMs contents of the plant, with the Cd, Zn, and Ni contamination levels as single factors.

3. Results and Discussion

3.1. Effects of Cd, Zn, and Ni on Plant Growth

After 65 days of treatment, the results revealed that plant growth was significantly affected by the concentrations of Cd, Zn, and Ni. As shown in Figure 1, under the single Cd contamination treatment, the RGR of *L. esculentum* was negatively correlated with the soil Cd level. Studies have confirmed the negative impact of Cd on the growth of tomato plants [42,43]. As shown in Table S1, the weight of the tomato plant in the 1 mg kg^{-1} Cd treatment significantly increased by 8.45% compared to the control (13.73 g·$plant^{-1}$), but in the 20–100 mg kg^{-1} Cd treatments, the plant weight decreased by 6.26–61.83% compared to the control (13.73 g·$plant^{-1}$). This may be because metal ions may activate the enzymes involved in cytokinin metabolism, which accelerates plant growth [44]. However, Cd has toxic effects on plant cells when the Cd concentration exceeds a certain level [45]. Similar observations were reported by Valencia-Hernandez et al. [46].

For the Zn-Cd and Ni-Cd co-contamination treatments, the RGR values of the plants were lower than those of the Cd alone treatment. Zn, as an enzyme co-factor, participates in the physiological processes of plants and has an important effect on plant growth [47]. The results indicated that the application of Cd supplemented with Ni or Zn further caused a significant reduction in the fresh weight of the plant (reduction of 23.90–82.18%) as compared to the Cd alone treatment (Table S1). It has been reported that Zn might be the dominant factor underlying reduced plant growth at higher levels of Zn-Cd treatment than at lower levels [48]. Thus, the reduced biomass of the plants treated with Zn or Zn-Cd might be due to the toxic effect of excess Zn and the impact of the excess Zn on the homeostasis of metal ions in tomato plants [23]. Compared with other HMs, Ni has the highest toxicity, mainly due to the low Ni requirement of crops [14]. Further, plant

cells might have greater respiration rates under Cd and Ni stress, which significantly affect cell elongation and meristematic activity [49]. The lower biomass of plants exposed to Ni and Zn is also related to the inhibitory effects of Ni and Zn on the activities of enzymes involved in the photosynthetic carbon reduction cycle [50,51]. In summary, the relative growth rate and biomass of tomato plants were reduced with exposure to Zn-Cd and Ni-Cd co-contaminated soil, and these effects are likely attributed to alterations in numerous physiological processes as a result of HM exposure.

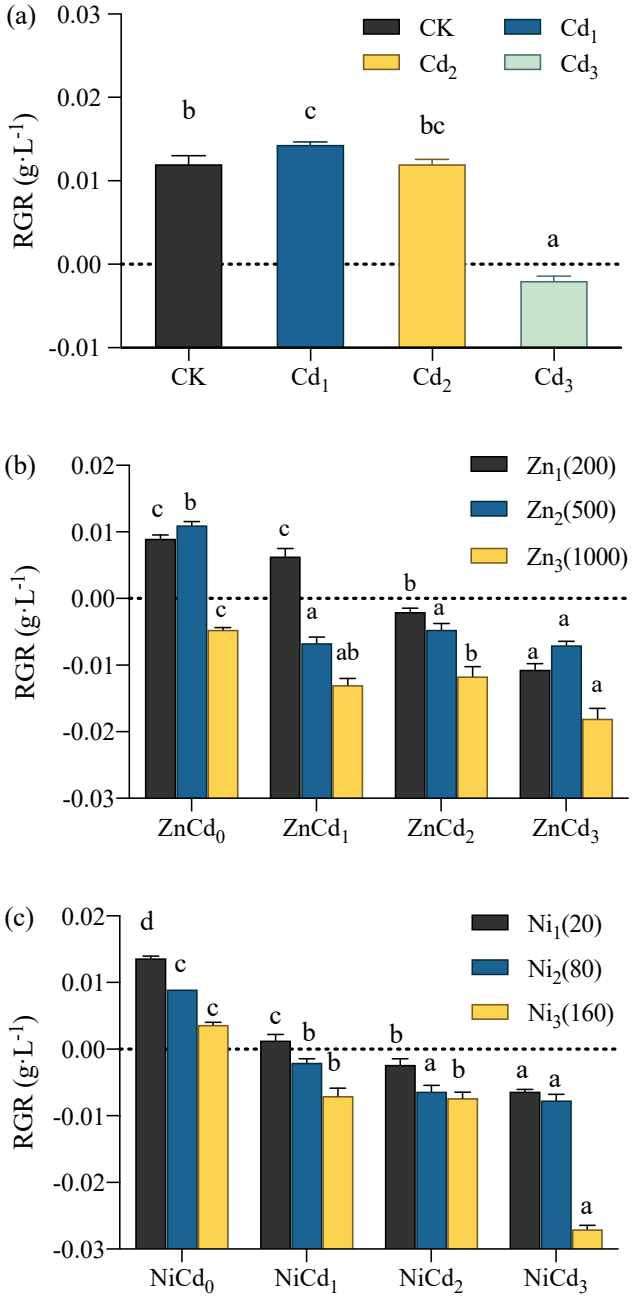

Figure 1. RGR of plants after 65 days of growth under single (**a**), Zn-Cd co-contamination conditions (**b**), and Ni-Cd co-contamination conditions (**c**). The error bars represent the mean ± S.E. (n = 3). The different letters within the different treatments indicate the significant difference at the $p < 0.05$.

In contrast, the application of certain concentrations of Zn (200 mg·kg^{-1}) or Ni (20 mg·kg^{-1}) with 1–20 mg·kg^{-1} Cd increased the root fresh weight by 77.13% and 50.95%, respectively, in comparison to the Cd treatment alone (Table S1). It has been claimed that

Cd has a harmful impact on tomato roots, greatly impairing cell elongation and resulting in cell death [52]. The increase in the root fresh weight might be due to the reduced accumulation of Cd in the plant roots as a result of competition between Cd and Zn/Ni [21,53,54]. However, the high Zn/Ni levels with Cd treatment led to a significant reduction in the fresh weight of the plants (Table S1). It has been reported that higher Zn levels combined with Cd can increase the Cd concentration in plants; meanwhile, higher Zn treatment (300 mg·kg^{-1}) significantly reduced the plant weight and caused oxidative stress in plants [48].

3.2. Accumulation and Transformation of Cd, Zn, and Ni in Tomato Plants

The concentrations of Cd, Zn, and Ni in the shoots and roots of *L. esculentum* are shown in Figure 2. Overall, the Cd, Zn, and Ni concentrations in the plant increased with increasing HMs contamination of the soil. The average HMs content of the different parts of the plant for all treatments was as follows: root > stem > leaf. The HMs concentration in the shoots of *L. esculentum* was 2–3 times lower than that in the roots. Previous studies indicated that more than 70% of Cd accumulates in the tomato roots under Cd stress [52,55]. This may serve as a defense mechanism against Cd stress to reduce the toxic effect of Cd on the aboveground parts of the tomato plant.

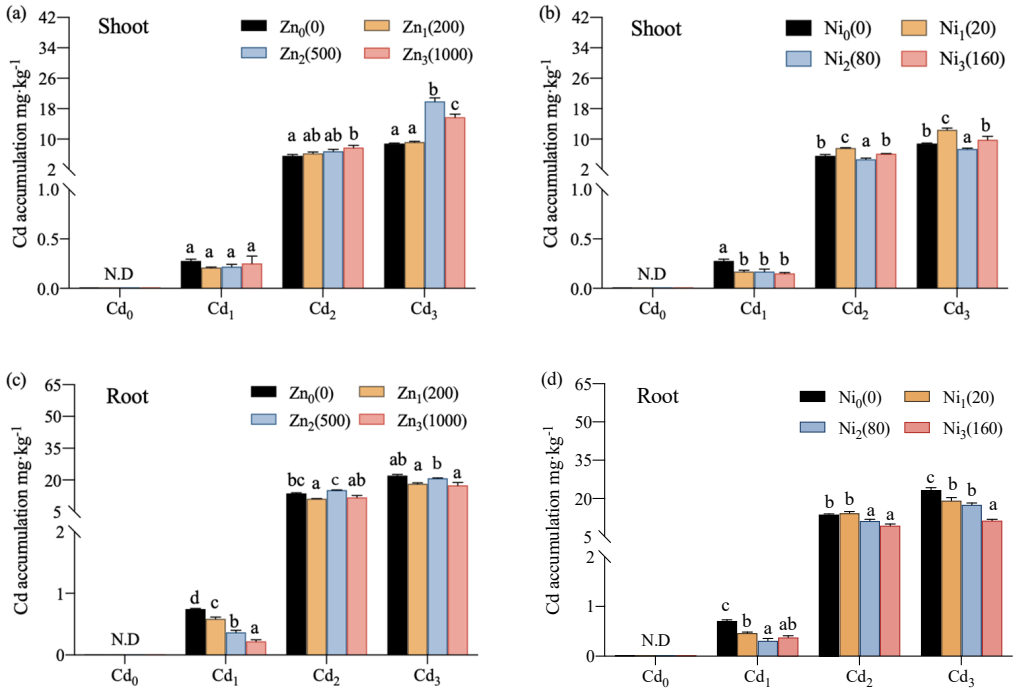

Figure 2. Concentrations of Cd in the shoots (**a**,**b**) and roots (**c**,**d**) of *L. esculentum* under single or co-contamination conditions. The error bars represent the mean ± S.E. (n = 3). The different letters within the different treatments indicate the significant difference at the $p < 0.05$.

The impact of co-pollution on the accumulation and migration of HMs in the soil-plant system is related to the degree of pollution. Compared with the single Cd treatment, the 200 mg kg^{-1} Zn with Cd treatment resulted in a decrease in Cd accumulation in the roots (17.36–22.12%). There is no special transport protein for Cd in plants; Cd enters the roots of the plant through essential element transport proteins [56]. Cd and Zn have similar chemical properties and absorption transporters (such as OsIRT1, OsHMA2, and OsZIP7), and this may be the reason for the antagonistic effect between Cd and Zn [57,58]. Cd might be extruded from the roots by Zn application due to the specific up-regulation of Cd-transporting ATPase; this could contribute to the lower Cd accumulation in the roots under the Cd + Zn treatment compared to the Cd only treatment. Additionally, Zn supplementation could activate metallothionein expression and increase the content

of phenolic compounds in the roots, which could chelate HMs or remove free radicals, attenuating oxidative damage under Cd stress [59,60]. However, higher concentrations of Zn can not only aggravate the toxic effects of Cd on plants but can also facilitate the translocation of Cd [61,62]. Therefore, high supplementation of Zn in the Cd treatment resulted in a slight increase in Cd accumulation in the roots and shoots as compared with the single Cd treatment.

The effects of Zn-Cd co-contamination on the Zn content of plants are shown in Figure 3. The combination of Cd+Zn produced a 5.97–37.74% reduction in Zn accumulation in the roots and a 36.80–123.07% increase in Zn accumulation in the shoots, as compared to the Zn only treatment. Moreover, the Zn contents of the roots and shoots significantly decreased with increasing Cd supply. Increased Cd accumulation in the plant is likely to negatively affect Zn accumulation because Cd and Zn compete for the same transporter and binding compounds. This is in accordance with the results observed in Cd-hyperaccumulators (*Cosmos bipinnatus* and *Catharanthus roseus*) [61,63] and other crops (wheat, rice, and maize) [22,23,64]. In addition, under excessive Cd and Zn stress, more Zn is transported to the shoots and more Cd is trapped in the roots due to the important role of Zn in enzyme reactions and gene expression to maintain shoot growth and photosynthesis.

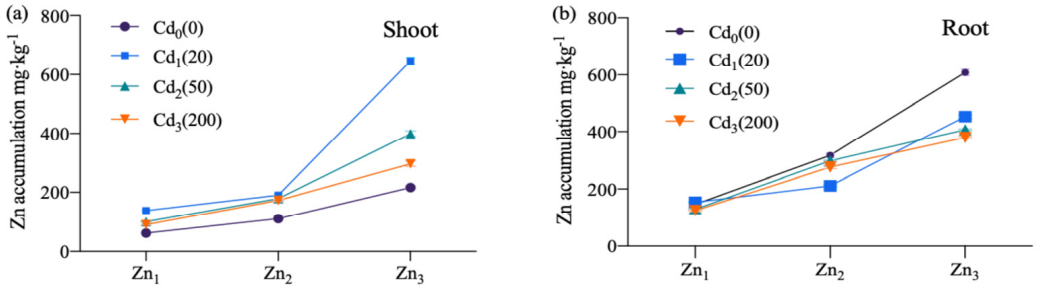

Figure 3. Concentrations of Zn in the shoots (**a**) and roots (**b**) of *L. esculentum* under single or co-contamination conditions. The error bars represent the mean ± S.E. (n = 3).

Unlike the Zn-Cd treatment, the Cd and Ni co-contamination treatment successively decreased the Cd concentration in the roots of *L. esculentum* by 15.74–56.08% compared with the individual Cd treatment (Figure 2b). For the Ni-Cd treatments, increasing the Ni contamination level from 0 to 20 mg/kg increased the total Cd accumulation in plant shoots from 0.28 to 12.42 mg/kg dry weight of plant, while further increases in the contamination level to 160 mg/kg did not cause further increases in Cd accumulation. Under the same concentrations of Cd and Ni in the culture medium, the Cd absorption capacity of the plant is more than twice that of Ni due to the different binding affinities of metal transporters in plants [65]. This may be the reason for the high accumulation of Cd in the plant shoots under the combined contamination of low-concentration (20 mg kg^{-1}) Ni and 20 mg kg^{-1} Cd.

In the current study, the Ni content of the roots of *L. esculentum* was dependent on the Ni level. Compared to the Ni-only treatment, the Ni-Cd treatment at lower levels decreased the Ni concentration of the tomato roots by 20.39–31.14% and the Ni concentration of the tomato shoots by 28.96–39.68% (Figure 4). These results suggest that the interaction pattern between Cd and Ni in tomato plants is antagonistic. As shown in Figure 2, Ni contamination at higher levels inhibited Cd enrichment in the plant roots and reduced Cd transport to the shoots. Previous research has revealed that the concentration of Cd in the roots of rice decreases with increasing Ni addition, and Cd is mainly distributed in the root cell walls and soluble fractions [21]. The polysaccharides and proteins contained in the plant cell wall can provide polar substances such as hydroxyl or carboxyl groups that bind to HM ions, thereby inhibiting the further transport of HM ions. This is consistent with the results of Zhang et al. [21], but contrary to the results of Khaliq et al. [66], where the addition of Cd enhanced the uptake of Ni by the roots.

In general, Cd-Zn and Cd-Ni co-contamination at low levels tended to reduce the bioaccumulation of HMs in the roots of tomato plants. However, according to the maxi-

mum allowable value specified in the Chinese food standard [67], the Cd contents in the aboveground parts of the plant greatly exceeded the threshold for cereal food security (0.05 mg/kg for Cd), at least for the present growth stage. This indicates that these levels of contamination will pose serious threats to human health if humans consume foods derived from crops grown in these Cd-contaminated soils. Therefore, proactive measures are still needed to prevent human exposure to crops grown on Cd-contaminated agricultural soils in the future.

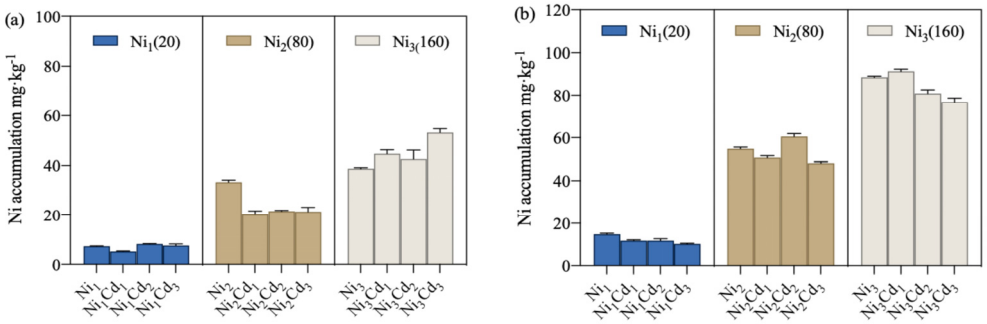

Figure 4. Concentrations of Ni in the shoots (**a**) and roots (**b**) of *L. esculentum* under single or co-contamination conditions. The error bars represent the mean ± S.E. (n = 3).

3.3. Effects of Cd, Zn, and Ni on the Community Diversity of the Soil Microorganisms

The microbial richness and diversity of the rhizosphere soil under the different HM treatments are shown in Table 2. The Chao, Ace, and Shannon indices in the soils contaminated with Cd alone were much smaller than those of the uncontaminated soil, indicating that community diversity in these contaminated soils decreased. As some microorganisms are highly sensitive to HM toxicity, they will become extinct after exposure to high concentrations of HM [68]. However, compared to the single Cd treatment (Cd_1 and Cd_3), the Zn-Cd and Ni-Cd co-treatments increased the diversity and richness of the soil bacterial community. The findings revealed that certain concentrations of Zn or Ni tended to enhance the microbial diversity of the rhizosphere soil in the co-contamination treatments.

Table 2. Microbial diversity indices in the soil under different heavy metal treatments.

System	Shannon	Simpson	Ace	Chao	Coverage
CK	6.991645	0.004494	4196.907	4198.966	0.982454
Cd_1	6.072421	0.006859	2857.150	2846.419	0.984998
Cd_3	5.823361	0.020838	3063.735	3014.942	0.984634
Zn_1Cd_1	6.070820	0.007931	2932.663	2938.172	0.985992
Zn_3Cd_3	6.757506	0.004557	4106.077	4030.048	0.985002
Ni_1Cd_1	5.706493	0.011493	2876.45	2862.277	0.986712
Ni_3Cd_3	6.066191	0.012557	3875.397	3810.785	0.979581

3.4. Effects of Cd, Zn, and Ni on the Community Structure of the Soil Microorganisms

3.4.1. Microbial Community at the Phylum Level

Figure 5 below shows the bacterial community composition at the phylum level. In all soil samples, *Proteobacteria* (27.9–52.0%) was the most dominant phylum, followed by *Actinobacteria* (6.6–21.1%), *Bacteroidetes* (4.2–26.9%), *Acidobacteria* (4.6–18.6%), and *Chloroflexi* (3.6–12.5%). Among these, *Proteobacteria* are ubiquitous in the rhizosphere environments of different plant species; they have good tolerance to HM contamination [69–71]. Wang et al. also found that *Proteobacteria*, *Bacteroidetes*, *Acidobacteria,* and *Actinobacteria* are the most dominant phyla in Cd-contaminated wheat rhizosphere soil [72].

Microorganisms will accumulate richness by interacting with HMs; this leads to the evolution of some HM-tolerant groups that are more adapted to the current environment. As shown in Figure 5a, the relative abundance of *Proteobacteria* decreased with increasing

Cd concentration. *Proteobacteria* play an important role in soil C and N cycling, and their relative abundance is positively correlated with several C and N cycling enzyme activities [73]. Thus, the current results indicate that high Cd levels may influence soil nutrient cycling, thus inhibiting the root growth of *L. esculentum*. Compared with the soil contaminated with Cd alone, the relative abundance of *Proteobacteria* in the Zn-Cd and Ni-Cd co-contaminated soils increased with increasing Cd levels. High concentrations of HMs and toxic substances serve as a source of energy and nutrition for *Proteobacteria* [74]. Thus, an increase in the relative abundance of *Proteobacteria* in contaminated soil might be helpful in maintaining a stable nutritional level for the growth of *L. esculentum* and may improve the ability of *L. esculentum* to resist Cd toxicity [75].

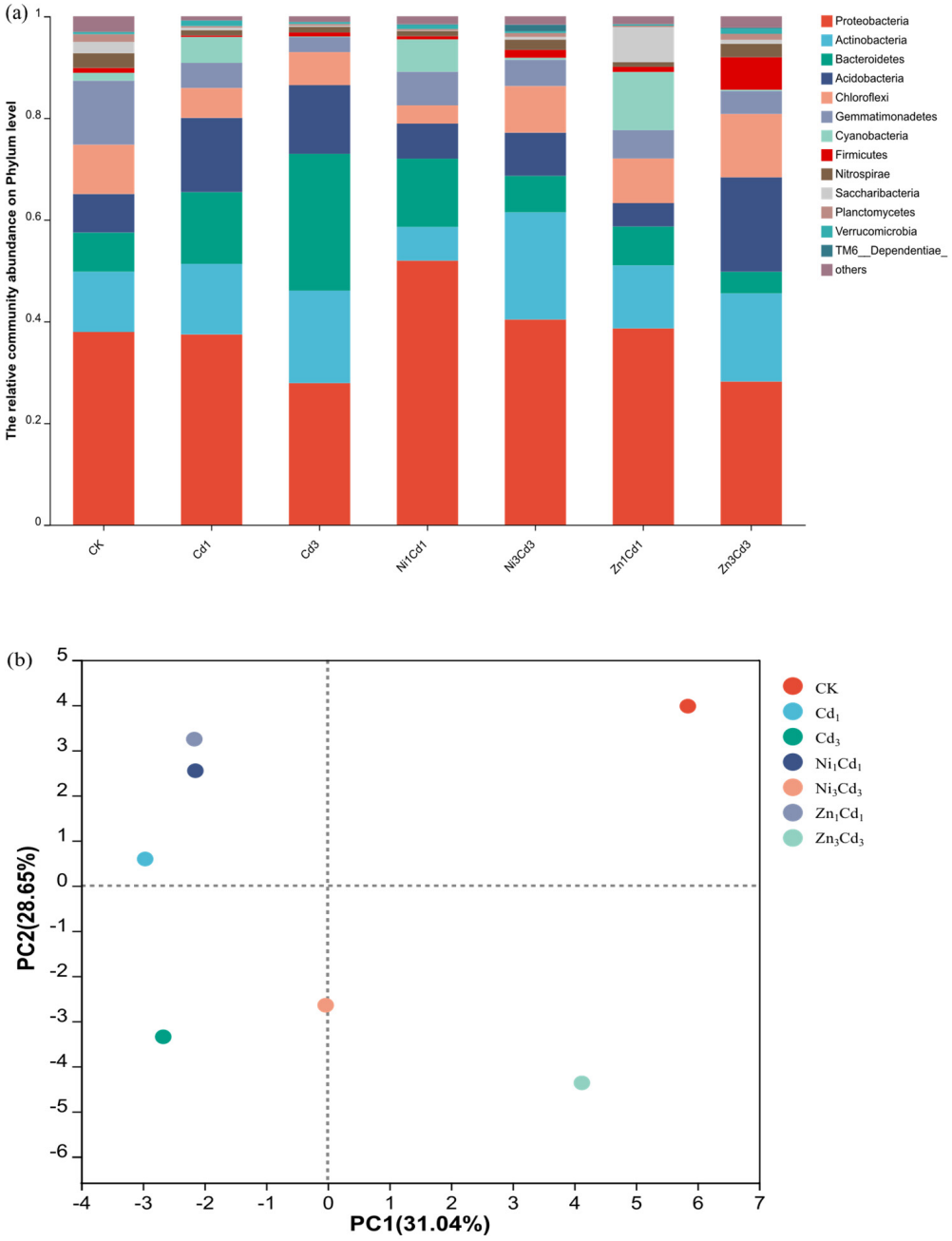

Figure 5. (**a**) The compositions (**b**) and principal components analysis (PCA) of the bacterial communities at the phylum level of different soils contaminated with heavy metals. Note: Only phyla with a relative abundance higher than 1.0% are shown in (**a**).

In addition, the phyla *Actinobacteria*, *Bacteroidetes,* and *Acidobacteria* were more enriched in the Cd-treated rhizosphere soil in comparison to the uncontaminated soil. *Acidobacteria* and *Bacteroidetes* are reported to resist HM toxicity due to their complexation and adsorption capacities [76]. However, the relative abundances of these microorganisms (*Actinobacteria*, *Bacteroidetes*, and *Acidobacteria*) were significantly lower in the Zn-Cd and Ni-Cd co-polluted soils than in the soils polluted with the same concentrations of Cd. Moreover, *Actinobacteria* were positively associated with the available Cd in the contaminated soils [77], indicating that the significant reduction in Cd accumulation in the roots in the presence of Zn or Ni might be related to a decrease in the available Cd concentration in the rhizosphere soil.

In principal component analysis (PCA), a shorter distance between the microbial samples represents higher structural similarity. The current PCA revealed that the bacterial communities in the different soils were clustered according to their HMs concentrations. As shown in Figure 5b, the samples with severe HMs concentrations were closely packed on the negative axis of PC2, while the samples with lower HMs concentrations were clustered together on the positive axis of PC2. Meanwhile, the bacterial communities of the Cd_1, Ni_1Cd_1, and Zn_1Cd_1 treatments exhibited similar structures. Together, these findings demonstrate that the microbial community compositions of the rhizosphere soil were significantly influenced by the different concentrations of Cd contamination, with greater effects in the case of a high concentration of Cd combined with other HM pollution.

3.4.2. Microbial Community at the Genus Level

The heat map in Figure 6 highlights the top 30 genera that accounted for the most sequences. The right side of the figure shows the meaning of the color gradient. The higher the abundance, the more red the color, and the lower the abundance, the more blue the color [77,78]. The rhizosphere of the Cd-contaminated soil exhibited higher abundances of *unclassified_Acidobacteria* and *unclassified_Cytophagaceae* as compared to the uncontaminated soil. *Unclassified_Acidobacteria* and unclassified_*Cytophagaceae* are affiliated with the phyla *Acidobacteria* and *Bacteroidetes*, respectively. *Acidobacteria* and *Bacteroidetes* are more tolerant of HMs contamination [79], and when the Cd concentration is high, *Bacteroides* can promote the transformation of nutrients. On the contrary, the depletion of *Acidobacteria* and *Bacteroidetes* in the rhizosphere soil of the Zn-Cd and Ni-Cd co-pollution treatments might be an indirect effect due to increases in the relative abundances of other microbial taxa [80]. The highest abundance of *Sphingomonas,* which belongs to *Alphaproteobacteria* at the class level and *Proteobacteria* at the phylum level, was observed in the uncontaminated rhizosphere soil (8.43%). *Sphingomonas* can promote the growth of host plants by stimulating the root secretion of plant auxins and cytokinins [81,82] and immobilizing HMs [83]. The current results indicate that single and combined HMs pollution of soils may have a negative influence on the enrichment of the *Sphingomonas* genera, thus inhibiting the root growth of the plant.

Additionally, the relative abundances of *Pseudomonas* (belonging to the *Proteobacteria* phylum) and *Leptolyngbya* (belonging to the *Cyanobacteria* phylum) in the Ni_1Cd_1, Ni_3Cd_3, Zn_1Cd_1, and Zn_3Cd_3 treatments were higher than those in the Cd_1 and Cd_3 treatments. The abundance of the bacterial genus *Pseudomonas* was inhibited in soil treated with Cd [84], whereas it was enriched in the Zn-Cd and Ni-Cd co-polluted rhizosphere soil, and this could affect plant growth and HMs absorption. A previous study found that *Pseudomonas,* a well-known plant growth-promoting bacteria (PGPR), reduced metal-induced stress in plants [70,82], and thus, the enrichment of some special species of *Pseudomonas* (such as *Pseudomonas* sp. *TCd-1*) might form a natural barrier to decrease Cd uptake by plants [85]. Thus, it can be assumed that the changes in the relative abundances of *Proteobacteria* and *Actinobacteria* at the phylum level and *Pseudomonas* at the genus level in HMs-co-polluted soil may enhance the Cd tolerance of plants and reduce the Cd accumulation in plants.

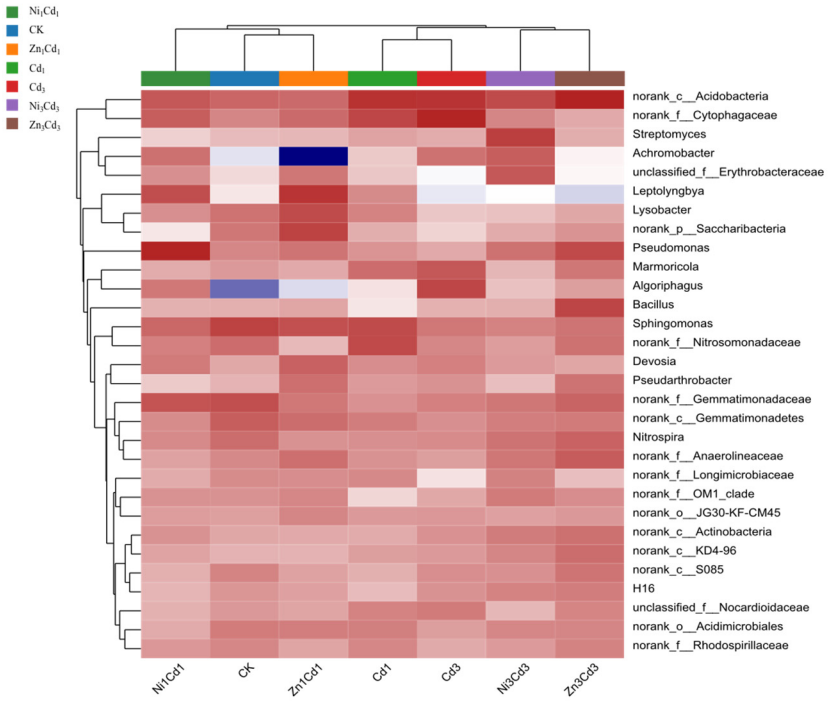

Figure 6. A genus-level heat map of different soils treated with HMs.

4. Conclusions

The absorption and transport behaviors of Cd in a soil-tomato system under different pollution conditions were studied in a pot experiment. The results demonstrated that exposure to multiple contamination (Zn-Cd and Ni-Cd) stresses significantly alters the microbial diversity of the rhizosphere soils, which indirectly affects the absorption and accumulation processes of Cd in the roots of tomato plants. Furthermore, several bacterial groups are stimulated by Cd-Zn or Cd-Ni co-stress, likely contributing to the tolerance and bioaccumulation behavior of Cd in plants. These findings improve our empirical and theoretical understanding of the interaction effects between Cd, Zn, and Ni in soil-crop systems. Although the accumulation of pollutants in the growth stage of the tomato plant was preliminarily discussed in this study, the effects in other stages of the plant cycle require further investigation. In future studies, the uptake and accumulation of Cd in the tomato fructification stage should be investigated in order to evaluate the risk of Cd exposure in the food chain.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11051523/s1, Table S1: The growth attributes of *L. esculentum* plants after treatment with single element (Cd, Zn, or Ni) or co-contaminated conditions for 65d. Data are shown as mean ± SD, n = 3.

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References

1. Zhao, K.; Zhang, L.; Dong, J.; Wu, J.; Ye, Z.; Zhao, W.; Ding, L.; Fu, W. Risk Assessment, Spatial Patterns and Source Apportionment of Soil Heavy Metals in a Typical Chinese Hickory Plantation Region of Southeastern China. *Geoderma* **2020**, *360*, 114011. [CrossRef]
2. Ministry of Environmental Protection of the People's Republic of China (MEP); Ministry of Land and Resources of China (MLR). *National Soil Pollution Investigation Bulletin*; MEP: Beijing, China, 2014. (In Chinese)
3. National Environmental Protection Agency of the People's Republic of China (NEPA); China State Administration for Market Regulation (CSAMR). *GB15618-1995*; Environmental Quality Standards for Soils. Standards Press of China: Beijing, China, 1995. (In Chinese)
4. Shi, J.; Zhao, D.; Ren, F.; Huang, L. Spatiotemporal Variation of Soil Heavy Metals in China: The Pollution Status and Risk Assessment. *Sci. Total Environ.* **2023**, *871*, 161768. [CrossRef]
5. Ministry of Environmental Protection of the People's Republic of China (MEP); China State Administration for Market Regulation (CSAMR). *GB 15618-2018*; Soil Environmental Quality-Risk Control Standard for Soil Contamination of Agricultural Land. Standards Press of China: Beijing, China, 2018. (In Chinese)
6. Shahid, M.; Dumat, C.; Khalid, S.; Schreck, E.; Xiong, T.; Niazi, N.K. Foliar Heavy Metal Uptake, Toxicity and Detoxification in Plants: A Comparison of Foliar and Root Metal Uptake. *J. Hazard. Mater.* **2017**, *325*, 36–58. [CrossRef]
7. Hussain, I.; Ashraf, M.A.; Rasheed, R.; Iqbal, M.; Ibrahim, M.; Zahid, T.; Thind, S.; Saeed, F. Cadmium-Induced Perturbations in Growth, Oxidative Defense System, Catalase Gene Expression and Fruit Quality in Tomato. *Int. J. Agric. Biol.* **2017**, *19*, 61–68. [CrossRef]
8. Clemens, S.; Aarts, M.G.M.; Thomine, S.; Verbruggen, N. Plant Science: The Key to Preventing Slow Cadmium Poisoning. *Trends Plant Sci.* **2013**, *18*, 92–99. [CrossRef]
9. Sofy, A.R.; Sofy, M.R.; Hmed, A.A.; Dawoud, R.A.; Alnaggar, A.E.-A.M.; Soliman, A.M.; El-Dougdoug, N.K. Ameliorating the Adverse Effects of Tomato Mosaic Tobamovirus Infecting Tomato Plants in Egypt by Boosting Immunity in Tomato Plants Using Zinc Oxide Nanoparticles. *Molecules* **2021**, *26*, 1337. [CrossRef]
10. Rizwan, M.; Ali, S.; Adrees, M.; Ibrahim, M.; Tsang, D.C.W.; Zia-ur-Rehman, M.; Zahir, Z.A.; Rinklebe, J.; Tack, F.M.G.; Ok, Y.S. A Critical Review on Effects, Tolerance Mechanisms and Management of Cadmium in Vegetables. *Chemosphere* **2017**, *182*, 90–105. [CrossRef] [PubMed]
11. Rizwan, M.; Ali, S.; Adrees, M.; Rizvi, H.; Zia-ur-Rehman, M.; Hannan, F.; Qayyum, M.F.; Hafeez, F.; Ok, Y.S. Cadmium Stress in Rice: Toxic Effects, Tolerance Mechanisms, and Management: A Critical Review. *Environ. Sci. Pollut. Res.* **2016**, *23*, 17859–17879. [CrossRef]
12. Li, Z.; Liang, Y.; Hu, H.; Shaheen, S.M.; Zhong, H.; Tack, F.M.G.; Wu, M.; Li, Y.-F.; Gao, Y.; Rinklebe, J.; et al. Speciation, Transportation, and Pathways of Cadmium in Soil-Rice Systems: A Review on the Environmental Implications and Remediation Approaches for Food Safety. *Environ. Int.* **2021**, *156*, 106749. [CrossRef] [PubMed]
13. López-Millán, A.-F.; Sagardoy, R.; Solanas, M.; Abadía, A.; Abadía, J. Cadmium Toxicity in Tomato (*Lycopersicon esculentum*) Plants Grown in Hydroponics. *Environ. Exp. Bot.* **2009**, *65*, 376–385. [CrossRef]
14. Küpper, H.; Andresen, E. Mechanisms of Metal Toxicity in Plants. *Metallomics* **2016**, *8*, 269–285. [CrossRef] [PubMed]
15. Qiao, K.; Tian, Y.; Hu, Z.; Chai, T. Wheat Cell Number Regulator CNR10 Enhances the Tolerance, Translocation, and Accumulation of Heavy Metals in Plants. *Environ. Sci. Technol.* **2019**, *53*, 860–867. [CrossRef]
16. Hu, B.; Shao, S.; Fu, T.; Fu, Z.; Zhou, Y.; Li, Y.; Qi, L.; Chen, S.; Shi, Z. Composite Assessment of Human Health Risk from Potentially Toxic Elements through Multiple Exposure Routes: A Case Study in Farmland in an Important Industrial City in East China. *J. Geochem. Explor.* **2020**, *210*, 106443. [CrossRef]
17. Sharma, A.; Nagpal, A.K. Contamination of Vegetables with Heavy Metals across the Globe: Hampering Food Security Goal. *J. Food Sci. Technol.* **2020**, *57*, 391–403. [CrossRef]
18. Kumar, A.; Jigyasu, D.K.; Kumar, A.; Subrahmanyam, G.; Mondal, R.; Shabnam, A.A.; Cabral-Pinto, M.M.S.; Malyan, S.K.; Chaturvedi, A.K.; Gupta, D.K.; et al. Nickel in Terrestrial Biota: Comprehensive Review on Contamination, Toxicity, Tolerance and Its Remediation Approaches. *Chemosphere* **2021**, *275*, 129996. [CrossRef]
19. Hassan, Z.; Aarts, M.G.M. Opportunities and Feasibilities for Biotechnological Improvement of Zn, Cd or Ni Tolerance and Accumulation in Plants. *Environ. Exp. Bot.* **2011**, *72*, 53–63. [CrossRef]
20. Huang, D.; Gui, H. Distribution Features and Internal Relations of Heavy Metals in Soil–Maize System of Mining Area, Anhui Province, Eastern China. *Hum. Ecol. Risk Assess. Int. J.* **2019**, *25*, 863–881. [CrossRef]
21. Zhang, Z.; Lu, Y.; Li, H.; Gao, Y.; Yang, Z. The Role of Nickel in Cadmium Accumulation in Rice. *Sci. Total Environ.* **2023**, *859*, 160421. [CrossRef] [PubMed]
22. Li, X.; Zhou, Q.; Sun, X.; Ren, W. Effects of Cadmium on Uptake and Translocation of Nutrient Elements in Different Welsh Onion (*Allium fistulosum* L.) Cultivars. *Food Chem.* **2016**, *194*, 101–110. [CrossRef]

23. Cai, Y.; Xu, W.; Wang, M.; Chen, W.; Li, X.; Li, Y.; Cai, Y. Mechanisms and Uncertainties of Zn Supply on Regulating Rice Cd Uptake. *Environ. Pollut.* **2019**, *253*, 959–965. [CrossRef]
24. Yu, Y.; Gu, C.; Bai, Y.; Zuo, W. Impact of Organic Amendments on the Bioavailability of Heavy Metals in Mudflat Soil and Their Uptake by Maize. *Environ. Sci. Pollut. Res.* **2022**, *29*, 63799–63814. [CrossRef]
25. Green, C.E.; Chaney, R.L.; Bouwkamp, J. Increased Zinc Supply Does Not Inhibit Cadmium Accumulation by Rice (*Oryza sativa* L.). *J. Plant Nutr.* **2017**, *40*, 869–877. [CrossRef]
26. Zhang, F.; Xu, N.; Zhang, Z.; Zhang, Q.; Yang, Y.; Yu, Z.; Sun, L.; Lu, T.; Qian, H. Shaping Effects of Rice, Wheat, Maize, and Soybean Seedlings on Their Rhizosphere Microbial Community. *Environ. Sci. Pollut. Res.* **2022**, *30*, 35972–35984. [CrossRef]
27. Abarenkov, K.; Henrik Nilsson, R.; Larsson, K.; Alexander, I.J.; Eberhardt, U.; Erland, S.; Høiland, K.; Kjøller, R.; Larsson, E.; Pennanen, T.; et al. The UNITE Database for Molecular Identification of Fungi—Recent Updates and Future Perspectives. *New Phytol.* **2010**, *186*, 281–285. [CrossRef] [PubMed]
28. Muhammad, A.; Xu, J.; Li, Z.; Wang, H.; Yao, H. Effects of Lead and Cadmium Nitrate on Biomass and Substrate Utilization Pattern of Soil Microbial Communities. *Chemosphere* **2005**, *60*, 508–514. [CrossRef]
29. Guo, H.; Nasir, M.; Lv, J.; Dai, Y.; Gao, J. Understanding the Variation of Microbial Community in Heavy Metals Contaminated Soil Using High Throughput Sequencing. *Ecotoxicol. Environ. Saf.* **2017**, *144*, 300–306. [CrossRef]
30. Huang, L.; Wang, X.; Chi, Y.; Huang, L.; Li, W.C.; Ye, Z. Rhizosphere Bacterial Community Composition Affects Cadmium and Arsenic Accumulation in Rice (*Oryza sativa* L.). *Ecotoxicol. Environ. Saf.* **2021**, *222*, 112474. [CrossRef]
31. Hong, Y.; Li, D.; Xie, C.; Zheng, X.; Yin, J.; Li, Z.; Zhang, K.; Jiao, Y.; Wang, B.; Hu, Y.; et al. Combined Apatite, Biochar, and Organic Fertilizer Application for Heavy Metal Co-Contaminated Soil Remediation Reduces Heavy Metal Transport and Alters Soil Microbial Community Structure. *Sci. Total Environ.* **2022**, *851*, 158033. [CrossRef]
32. Xiao, A.W.; Li, Z.; Li, W.C.; Ye, Z.H. The Effect of Plant Growth-Promoting Rhizobacteria (PGPR) on Arsenic Accumulation and the Growth of Rice Plants (*Oryza sativa* L.). *Chemosphere* **2020**, *242*, 125136. [CrossRef]
33. Hussain, M.M.; Saeed, A.; Khan, A.A.; Javid, S.; Fatima, B. Differential Responses of One Hundred Tomato Genotypes Grown under Cadmium Stress. *Genet. Mol. Res.* **2015**, *14*, 13162–13171. [CrossRef]
34. Xu, Z.-M.; Tan, X.-Q.; Mei, X.-Q.; Li, Q.-S.; Zhou, C.; Wang, L.-L.; Ye, H.-J.; Yang, P. Low-Cd Tomato Cultivars (*Solanum lycopersicum* L.) Screened in Non-Saline Soils Also Accumulated Low Cd, Zn, and Cu in Heavy Metal-Polluted Saline Soils. *Environ. Sci. Pollut. Res.* **2018**, *25*, 27439–27450. [CrossRef] [PubMed]
35. National Environmental Protection Agency of the People's Republic of China (NEPA). *HJ/T 166-2004*; The Technical Specification for soil Environmental monitoring. China Environment Publishing Group: Beijing, China, 2004. (In Chinese)
36. Jiang, Y.; Liang, Y.; Li, C.; Wang, F.; Sui, Y.; Suvannang, N.; Zhou, J.; Sun, B. Crop Rotations Alter Bacterial and Fungal Diversity in Paddy Soils across East Asia. *Soil Biol. Biochem.* **2016**, *95*, 250–261. [CrossRef]
37. Gharaibeh, M.A.; Marschner, B.; Heinze, S. Metal Uptake of Tomato and Alfalfa Plants as Affected by Water Source, Salinity, and Cd and Zn Levels under Greenhouse Conditions. *Environ. Sci. Pollut. Res.* **2015**, *22*, 18894–18905. [CrossRef]
38. Zhu, L.; Li, T.; Wang, C.; Zhang, X.; Xu, L.; Xu, R.; Zhao, Z. The Effects of Dark Septate Endophyte (DSE) Inoculation on Tomato Seedlings under Zn and Cd Stress. *Environ. Sci. Pollut. Res.* **2018**, *25*, 35232–35241. [CrossRef] [PubMed]
39. Amjad, M.; Ameen, N.; Murtaza, B.; Imran, M.; Shahid, M.; Abbas, G.; Naeem, M.A.; Jacobsen, S. Comparative Physiological and Biochemical Evaluation of Salt and Nickel Tolerance Mechanisms in Two Contrasting Tomato Genotypes. *Physiol. Plant.* **2020**, *168*, 27–37. [CrossRef]
40. Miller, R.O. Nitric-perchloric wet acid digestion in an open vessel. In *Handbook of Reference Methods for Plant Analysis*; Kalra, Y.P., Ed.; CRC Press: Washington, DC, USA, 1998; pp. 57–62.
41. Liu, X.; Zhang, X.; Li, R.; Wang, G.; Jin, Y.; Xu, W.; Wang, H.; Qu, J. Organic Amendment Improves Rhizosphere Environment and Shapes Soil Bacterial Community in Black and Red Soil under Lead Stress. *J. Hazard. Mater.* **2021**, *416*, 125805. [CrossRef] [PubMed]
42. Hou, J.; Liu, X.; Cui, B.; Bai, J.; Wang, X. Concentration-Dependent Alterations in Gene Expression Induced by Cadmium in Solanum Lycopersicum. *Environ. Sci. Pollut. Res.* **2017**, *24*, 10528–10536. [CrossRef]
43. Su, L.; Xie, Y.; He, Z.; Zhang, J.; Tang, Y.; Zhou, X. Network Response of Two Cherry Tomato (*Lycopersicon esculentum*) Cultivars to Cadmium Stress as Revealed by Transcriptome Analysis. *Ecotoxicol. Environ. Saf.* **2021**, *222*, 112473. [CrossRef] [PubMed]
44. Shentu, J.; He, Z.; Yang, X.-E.; Li, T. Accumulation Properties of Cadmium in a Selected Vegetable-Rotation System of Southeastern China. *J. Agric. Food Chem.* **2008**, *56*, 6382–6388. [CrossRef] [PubMed]
45. Clarkson, D.T.; Hanson, J.B. The Mineral Nutrition of Higher Plants. *Annu. Rev. Plant Physiol.* **1980**, *31*, 239–298. [CrossRef]
46. Valencia-Hernandez, J.-A.; Solano-Alvarez, N.; Rico-Rodriguez, M.-A.; Rodriguez-Ontiveros, A.; Torres-Pacheco, I.; Rico-Garcia, E.; Guevara-Gonzalez, R.-G. Eustressic Dose of Cadmium in Soil Induces Defense Mechanisms and Protection Against Clavibacter Michiganensis in Tomato (*Solanum lycopersicum* L.). *J. Plant Growth Regul.* **2023**, *42*, 407–414. [CrossRef]
47. Beyer, W.N.; Green, C.E.; Beyer, M.; Chaney, R.L. Phytotoxicity of Zinc and Manganese to Seedlings Grown in Soil Contaminated by Zinc Smelting. *Environ. Pollut.* **2013**, *179*, 167–176. [CrossRef] [PubMed]
48. Rizwan, M.; Ali, S.; Rehman, M.Z.U.; Maqbool, A. A Critical Review on the Effects of Zinc at Toxic Levels of Cadmium in Plants. *Environ. Sci. Pollut. Res.* **2019**, *26*, 6279–6289. [CrossRef]
49. Ghori, N.-H.; Ghori, T.; Hayat, M.Q.; Imadi, S.R.; Gul, A.; Altay, V.; Ozturk, M. Heavy Metal Stress and Responses in Plants. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 1807–1828. [CrossRef]

50. Benzarti, S.; Mohri, S.; Ono, Y. Plant Response to Heavy Metal Toxicity: Comparative Study between the Hyperaccumulator *Thlaspi Caerulescens* (Ecotype Ganges) and Nonaccumulator Plants: Lettuce, Radish, and Alfalfa. *Environ. Toxicol.* **2008**, *23*, 607–616. [CrossRef]
51. Noor, I.; Sohail, H.; Sun, J.; Nawaz, M.A.; Li, G.; Hasanuzzaman, M.; Liu, J. Heavy Metal and Metalloid Toxicity in Horticultural Plants: Tolerance Mechanism and Remediation Strategies. *Chemosphere* **2022**, *303*, 135196. [CrossRef]
52. Jia, H.; Wang, X.; Wei, T.; Zhou, R.; Muhammad, H.; Hua, L.; Ren, X.; Guo, J.; Ding, Y. Accumulation and Fixation of Cd by Tomato Cell Wall Pectin under Cd Stress. *Environ. Exp. Bot.* **2019**, *167*, 103829. [CrossRef]
53. Qaswar, M.; Hussain, S.; Rengel, Z. Zinc Fertilisation Increases Grain Zinc and Reduces Grain Lead and Cadmium Concentrations More in Zinc-Biofortified than Standard Wheat Cultivar. *Sci. Total Environ.* **2017**, *605–606*, 454–460. [CrossRef] [PubMed]
54. Wu, S.; Shi, K.; Hu, C.; Guo, J.; Tan, Q.; Sun, X. Non-Invasive Microelectrode Cadmium Flux Measurements Reveal the Decrease of Cadmium Uptake by Zinc Supply in Pakchoi Root (*Brassica chinensis* L.). *Ecotoxicol. Environ. Saf.* **2019**, *168*, 363–368. [CrossRef]
55. Cho, U.-H.; Park, J.-O. Distribution and Phytotoxicity of Cadmium in Tomato Seedlings. *J. Plant Biol.* **1999**, *42*, 49–56. [CrossRef]
56. Qiu, R.-L.; Thangavel, P.; Hu, P.-J.; Senthilkumar, P.; Ying, R.-R.; Tang, Y.-T. Interaction of Cadmium and Zinc on Accumulation and Sub-Cellular Distribution in Leaves of Hyperaccumulator Potentilla Griffithii. *J. Hazard. Mater.* **2011**, *186*, 1425–1430. [CrossRef]
57. Takahashi, R.; Ishimaru, Y.; Shimo, H.; Ogo, Y.; Senoura, T.; Nishizawa, N.K.; Nakanishi, H. The OsHMA2 Transporter Is Involved in Root-to-Shoot Translocation of Zn and Cd in Rice: Characterization of OsHMA2. *Plant Cell Environ.* **2012**, *35*, 1948–1957. [CrossRef] [PubMed]
58. Talebi, M.; Tabatabaei, B.E.S.; Akbarzadeh, H. Hyperaccumulation of Cu, Zn, Ni, and Cd in Azolla Species Inducing Expression of Methallothionein and Phytochelatin Synthase Genes. *Chemosphere* **2019**, *230*, 488–497. [CrossRef]
59. Chen, S.; Wang, Q.; Lu, H.; Li, J.; Yang, D.; Liu, J.; Yan, C. Phenolic Metabolism and Related Heavy Metal Tolerance Mechanism in Kandelia Obovata under Cd and Zn Stress. *Ecotoxicol. Environ. Saf.* **2019**, *169*, 134–143. [CrossRef]
60. Manquián-Cerda, K.; Escudey, M.; Zúñiga, G.; Arancibia-Miranda, N.; Molina, M.; Cruces, E. Effect of Cadmium on Phenolic Compounds, Antioxidant Enzyme Activity and Oxidative Stress in Blueberry (*Vaccinium corymbosum* L.) Plantlets Grown in Vitro. *Ecotoxicol. Environ. Saf.* **2016**, *133*, 316–326. [CrossRef]
61. Chen, Q.; Lu, X.; Guo, X.; Pan, Y.; Yu, B.; Tang, Z.; Guo, Q. Differential Responses to Cd Stress Induced by Exogenous Application of Cu, Zn or Ca in the Medicinal Plant Catharanthus Roseus. *Ecotoxicol. Environ. Saf.* **2018**, *157*, 266–275. [CrossRef]
62. Mongkhonsin, B.; Nakbanpote, W.; Hokura, A.; Nuengchamnong, N.; Maneechai, S. Phenolic Compounds Responding to Zinc and/or Cadmium Treatments in *Gynura pseudochina* (L.) DC. Extracts and Biomass. *Plant Physiol. Biochem.* **2016**, *109*, 549–560. [CrossRef]
63. Du, J.; Zeng, J.; Ming, X.; He, Q.; Tao, Q.; Jiang, M.; Gao, S.; Li, X.; Lei, T.; Pan, Y.; et al. The Presence of Zinc Reduced Cadmium Uptake and Translocation in Cosmos Bipinnatus Seedlings under Cadmium/Zinc Combined Stress. *Plant Physiol. Biochem.* **2020**, *151*, 223–232. [CrossRef]
64. Liu, Y.-M.; Liu, D.-Y.; Zhang, W.; Chen, X.-X.; Zhao, Q.-Y.; Chen, X.-P.; Zou, C.-Q. Health Risk Assessment of Heavy Metals (Zn, Cu, Cd, Pb, As and Cr) in Wheat Grain Receiving Repeated Zn Fertilizers. *Environ. Pollut.* **2020**, *257*, 113581. [CrossRef]
65. Thomas, M. A Comparative Study of the Factors Affecting Uptake and Distribution of Cd with Ni in Barley. *Plant Physiol. Biochem.* **2021**, *162*, 730–736. [CrossRef]
66. Khaliq, M.A.; James, B.; Chen, Y.H.; Ahmed Saqib, H.S.; Li, H.H.; Jayasuriya, P.; Guo, W. Uptake, Translocation, and Accumulation of Cd and Its Interaction with Mineral Nutrients (Fe, Zn, Ni, Ca, Mg) in Upland Rice. *Chemosphere* **2019**, *215*, 916–924. [CrossRef] [PubMed]
67. National Health and Family Planning Commission of P.R. China (NHFPC); National Medical Products Administration (NMPA). *GB 27622017*; National Standard for Food Security-the Maximum Allowable Levels of Contaminants in Foods. Standards Press of China: Beijing, China, 2017. (In Chinese)
68. Margesin, R.; Płaza, G.A.; Kasenbacher, S. Characterization of Bacterial Communities at Heavy-Metal-Contaminated Sites. *Chemosphere* **2011**, *82*, 1583–1588. [CrossRef] [PubMed]
69. Kudjordjie, E.N.; Sapkota, R.; Steffensen, S.K.; Fomsgaard, I.S.; Nicolaisen, M. Maize Synthesized Benzoxazinoids Affect the Host Associated Microbiome. *Microbiome* **2019**, *7*, 59. [CrossRef] [PubMed]
70. Li, X.; Chen, D.; Li, B.; Yang, Y. Cd Accumulation Characteristics of Salvia Tiliifolia and Changes of Rhizospheric Soil Enzyme Activities and Bacterial Communities under a Cd Concentration Gradient. *Plant Soil* **2021**, *463*, 225–247. [CrossRef]
71. Li, S.; Li, G.; Huang, X.; Chen, Y.; Lv, C.; Bai, L.; Zhang, K.; He, H.; Dai, J. Cultivar-Specific Response of Rhizosphere Bacterial Community to Uptake of Cadmium and Mineral Elements in Rice (*Oryza sativa* L.). *Ecotoxicol. Environ. Saf.* **2023**, *249*, 114403. [CrossRef]
72. Wang, J.; Liu, T.; Sun, W.; Chen, Q. Bioavailable Metal(Loid)s and Physicochemical Features Co-Mediating Microbial Communities at Combined Metal(Loid) Pollution Sites. *Chemosphere* **2020**, *260*, 127619. [CrossRef]
73. Acosta-Martínez, V.; Bell, C.W.; Morris, B.E.L.; Zak, J.; Allen, V.G. Long-Term Soil Microbial Community and Enzyme Activity Responses to an Integrated Cropping-Livestock System in a Semi-Arid Region. *Agric. Ecosyst. Environ.* **2010**, *137*, 231–240. [CrossRef]
74. Wang, L.; Zou, R.; Li, Y.C.; Tong, Z.; You, M.; Huo, W.; Chi, K.; Fan, H. Effect of Wheat-Solanum Nigrum L. Intercropping on Cd Accumulation by Plants and Soil Bacterial Community under Cd Contaminated Soil. *Ecotoxicol. Environ. Saf.* **2020**, *206*, 111383. [CrossRef]

75. Yang, G.-L.; Zheng, M.-M.; Liao, H.-M.; Tan, A.-J.; Feng, D.; Lv, S.-M. Influence of Cadmium and Microplastics on Physiological Responses, Ultrastructure and Rhizosphere Microbial Community of Duckweed. *Ecotoxicol. Environ. Saf.* **2022**, *243*, 114011. [CrossRef]
76. Wang, X.; Fang, L.; Beiyuan, J.; Cui, Y.; Peng, Q.; Zhu, S.; Wang, M.; Zhang, X. Improvement of Alfalfa Resistance against Cd Stress through Rhizobia and Arbuscular Mycorrhiza Fungi Co-Inoculation in Cd-Contaminated Soil. *Environ. Pollut.* **2021**, *277*, 116758. [CrossRef]
77. Liu, H.; Wang, C.; Xie, Y.; Luo, Y.; Sheng, M.; Xu, F.; Xu, H. Ecological Responses of Soil Microbial Abundance and Diversity to Cadmium and Soil Properties in Farmland around an Enterprise-Intensive Region. *J. Hazard. Mater.* **2020**, *392*, 122478. [CrossRef] [PubMed]
78. Rawlings, D.E.; Johnson, D.B. The Microbiology of Biomining: Development and Optimization of Mineral-Oxidizing Microbial Consortia. *Microbiology* **2007**, *153*, 315–324. [CrossRef] [PubMed]
79. Chen, Y.; Chen, H.; Zhong, Q.; Yun, Y.-H.; Chen, W.; Chen, W. Determination of Microbial Diversity and Community Composition in Unfermented and Fermented Washing Rice Water by High-Throughput Sequencing. *Curr. Microbiol.* **2021**, *78*, 1730–1740. [CrossRef] [PubMed]
80. Lin, Y.; Ye, Y.; Hu, Y.; Shi, H. The Variation in Microbial Community Structure under Different Heavy Metal Contamination Levels in Paddy Soils. *Ecotoxicol. Environ. Saf.* **2019**, *180*, 557–564. [CrossRef]
81. Shi, S.; Nuccio, E.; Herman, D.J.; Rijkers, R.; Estera, K.; Li, J.; da Rocha, U.N.; He, Z.; Pett-Ridge, J.; Brodie, E.L.; et al. Successional Trajectories of Rhizosphere Bacterial Communities over Consecutive Seasons. *mBio* **2015**, *6*, e00746-15. [CrossRef] [PubMed]
82. Xia, Y.; Lu, D.; Qi, Y.; Chen, H.; Zhao, Y.; Bai, Y.; Zhu, L.; Geng, N.; Xu, C.; Hua, E. Removal of Nitrate from Agricultural Runoff in Biochar Electrode Based Biofilm Reactor: Performance and Enhancement Mechanisms. *Chemosphere* **2022**, *301*, 134744. [CrossRef]
83. Cheng, C.; Wang, R.; Sun, L.; He, L.; Sheng, X. Cadmium-Resistant and Arginine Decarboxylase-Producing Endophytic *Sphingomonas* Sp. C40 Decreases Cadmium Accumulation in Host Rice (*Oryza Sativa Cliangyou* 513). *Chemosphere* **2021**, *275*, 130109. [CrossRef]
84. Tangaromsuk, J.; Pokethitiyook, P.; Kruatrachue, M.; Upatham, E.S. Cadmium Biosorption by Sphingomonas Paucimobilis Biomass. *Bioresour. Technol.* **2002**, *85*, 103–105. [CrossRef]
85. Xiao, X.; Fan, M.; Wang, E.; Chen, W.; Wei, G. Interactions of Plant Growth-Promoting Rhizobacteria and Soil Factors in Two Leguminous Plants. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 8485–8497. [CrossRef]