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Synergistic Effects of Exogenous Nutrient Ions on the Real-Time Cadmium Extraction by an Accumulator

Siqi Wang [1,*], Huiping Dai [2,*], Dandan Ji [3], Shuang Cui [1], Chengzhi Jiang [1], Lidia Skuza [4], Lianzhen Li [5], Shuhe Wei [3] and Lijun Zhang [6]

1 Key Laboratory of Wastewater Treatment Technology of Liaoning Province, Academy of Environmental and Chemical Engineering, Shenyang Ligong University, Shenyang 110159, China; ccshuang@163.com (S.C.); zebra_jiang1@163.com (C.J.)
2 College of Biological Science & Engineering, Shaanxi Province Key Laboratory of Bio-Resources, Qinling-Bashan Mountains Bioresources Comprehensive Development C.I.C, State Key Laboratory of Biological Resources and Ecological Environment Jointly Built by Qinba Province and Ministry, Shaanxi University of Technology, Hanzhong 723001, China
3 Key Laboratory of Pollution Ecology and Environment Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China; jidandan13@mails.ucas.ac.cn (D.J.); shuhewei@iae.ac.cn (S.W.)
4 Institute of Biology, Centre for Molecular Biology and Biotechnology, University of Szczecin, 71-415 Szczecin, Poland; lidia.skuza@usz.edu.pl
5 College of Environmental Sciences and Engineering, Qingdao University, Qingdao 266071, China; lianzhen.li@foxmail.com
6 Shenbei New District Rural Area Revitalization and Development Center, Shenyang 110121, China; sbxqzbz@126.com
* Correspondence: wang3331336637@126.com (S.W.); daihp72@snut.edu.cn (H.D.)

Abstract: *Bidens tripartita* L. is a cadmium (Cd) accumulator. However, the real-time influx or efflux of Cd^{2+} around its root apex has not yet been performed. The object of this experiment was to compare the roles of added ions in solution on dynamic Cd extraction by *B. tripartita* root tip. Quartz sand was used to grow the seedling of *B. tripartite*. The Cd concentrations of all samples were determined by using ICP-OES after digestion. The Cd^{2+} influx around the root apex was measured in vivo, i.e., using non-invasive micro-test technology (NMT). The results showed that the Cd^{2+} influx was found to be decreased by 35.9%, 43.7%, 20.6%, and 57.5% under 10 μM Cd combined with high content Ca^{2+}, Mg^{2+}, Fe^{3+}, or K^{+} (16 mM, 8 mM, 0.5 mM, 18 mM, respectively), compared to that under 10 μM Cd stress. But Cd treatments with low content ions with 0.05 mM Fe^{3+} or 0.5 mM S increased the Cd^{2+} influx in roots by 20.5% and 34.6%, respectively. It was also found that Cd treatment with high concentrations of Ca^{2+} or K^{+} increased the shoot biomass of *B. tripartita* seedlings. Chl a and b contents were significantly decreased in the Cd treatments with low concentrations of Fe^{3+} or S compared to those under Cd stress alone, and the dehydrogenase activity of the roots decreased in the treatment of Cd with 0.05 mM Fe^{3+} or 0.5 mM S. Our results indicate that the addition of 0.05 mM Fe^{3+} or 0.5 mM S promoted Cd^{2+} influx and Cd uptake by *B. tripartita.* Unlike traditional measurement, the Cd^{2+} movements of three-dimensional space around the *B. tripartita* root tip had been performed by NMT. It was suggested that the effects of S and Fe^{3+} on the remediation potential of *B. tripartita* need to be further researched in the future. The results of this study provided a real-time and micro-dynamic theoretical basis for phytoremediation mechanisms.

Keywords: Cd^{2+}; NMT; biomass; chlorophyll; dehydrogenase

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1. Introduction

Bidens tripartita L. (Asteraceae) was reported as a Cd accumulator by Wei et al. [1]. Using pot culture and concentration gradient experiments, *B. tripartita* was found that the mean values of the Cd enrichment factor and translocation factor were all higher than 1. Its biomass did not obviously decrease compared to that of the controls under soil Cd concentrations of 10 mg/kg. However, the Cd content of the shoot did not exceed 100 mg/kg, which does not fulfil the criteria for a Cd hyperaccumulator. Therefore, *B. tripartita* was identified as a Cd accumulator only. Zheljazkov et al. [2] also affirmed the Cd extraction characteristics of *B. tripartite*. It was proposed that Ca or Fe deficiency treatments promoted the absorption of Cd by edible amaranth (*Amaranthus mangostanus* L.). The transmembrane transports of Cd^{2+} and Ca^{2+} in amaranth roots were realised by the same channels. The low-Ca varieties showed lower Cd absorption ability than the high-Ca varieties [3]. Gao et al. [4] determined the effects of Cd on essential Fe in *Malus xiaojinensis* seedlings and discovered that chlorophyll and Fe concentrations in leaves were significantly reduced under Cd treatment, but Fe concentrations were markedly increased in the roots. In tomato plants, López-Millán et al. [5] reported that 10 μM Cd stresses induced a moderate Fe deficiency and 100 μM Cd treatments caused alterations in nutrient homeostasis. Thus, exploring the roles of such exogenous nutrient ions on *B. tripartite*-accumulating Cd is more interesting.

Usually, the comparison of synergistic or antagonistic effects between elements in plants is determined after sampling by chemical methods. Non-invasive, i.e., in vivo and real-time determination, is difficult. NMT (non-invasive micro-test technology) is a recently developed in vivo micro-measurement technology [6]. Using NMT, Zhang et al. [7] explored the effects of ammonium on Cd^{2+} influx around the root tips of *S. nigrum* and discovered that NH^{4+} decreased Cd^{2+} influx and increased Cd retention in root cell walls, alleviating toxicity. Wu et al. [8] found the impact of added Zn on Cd extraction by *pakchoi* root. Wang et al. [9] compared 20 soybean varieties using NMT under 5 μM Cd treatment and found that the differences around the root tip are 20 times between the highest and lowest Cd^{2+} influx. Li et al. [10] observed the greatest inrush of Cd^{2+} in the meristematic zone of *Phragmites australis*. Sun et al. [11] investigated the hyperaccumulating characteristics of *Sedum alfredii* and discovered that the hyperaccumulation mechanisms involved rapid uptake at a specific site from the root apex, fast translocation from the root to the shoot, and efficient Cd-transmembrane transport. Tao et al. [12] compared longitudinal variations in root tips between the hyperaccumulator and non-hyperaccumulator ecotypes of *S. alfredii* and found that the hyperaccumulating ecotype exhibited a higher Cd influx than the non-hyperaccumulating ecotype.

Recently, some studies have focused on the Cd extraction mechanisms of *B. tripartita* [1,2,13,14]. However, the dynamic process and influence factor of Cd extraction by *B. tripartita* roots have not yet been performed. Thus, the purpose of this experiment was to compare the roles of some exogenous nutrient ions on real-time Cd^{2+} influx around *B. tripartita* roots using NMT. We hypothesised that the addition of ions would have some inhibition or promotion effects on Cd uptake by *B. tripartita* roots. Though NMT could obtain the most real physiological signals of *B. tripartita* roots accurately, there is no corresponding mechanism combined with Cd^{2+} influx to form a comprehensive system that reflects the dynamic changes in Cd uptake. So, the chlorophyll content of the leaf was determined due to its synthesis, and its stability was closely related to the excess or deficiency of nutrient ions.

2. Materials and Methods

2.1. Experiment Conduction

The sand culture experiments were conducted in a control room because the Cd adsorption by the quartz sand was very small, and the total Cd is assumed to be the soluble Cd. Particularly, plant grow well and remain healthy when they are taken out under such conditions. Seeds of *B. tripartita* were harvested from a farmland in Shenyang City at maturity. Before planting, the seeds of *B. tripartite* were sterilised (using sodium hypochlorite) and settled evenly in seedling trays. The trays were then transferred to

a growth chamber (Snijders Scientific B.V., Tilburg, The Netherlands) for 5 days at a temperature of 27 °C and 80% relative humidity. After germination, six uniform *B. tripartita* seedlings were transplanted to each pot (φ24 × 20 cm^2) with sand and mixed with 1/5 strength Hoagland solution + 10 μM Cd (as $CdCl_2$ 2.5 H_2O) compared with controls without Cd. The solution's pH was adjusted to 5.5 using morpholinoethanesulphonic acid. The 10 μM Cd treatments combined with two concentration gradients were selected for each nutrient ion (e.g., Ca^{2+} was supplied as Ca $(NO_3)_2$, Mg^{2+} as $MgSO_4$, Fe^{3+} as Fe-EDDHA, S as $MgSO_4$, and K^+ as KNO_3) by adjusting the corresponding ionic concentrations in 1/5 strength solutions. The 12 treatments with a 1/5 strength Hoagland solution were listed as follows: (1) without Cd; (2) 10 μM Cd^{2+}; (3) 10 μM Cd^{2+} + 1 mM Ca^{2+}; (4) 10 μM Cd^{2+} + 16 mM Ca^{2+}; (5) 10 μM Cd^{2+} + 0.5 mM Mg^{2+}; (6) 10 μM Cd^{2+} + 8 mM Mg^{2+}; (7) 10 μM Cd^{2+} + 0.05 mM Fe^{3+}; (8) 10 μM Cd^{2+} + 0.5 mM Fe^{3+}; (9) 10 μM Cd^{2+} + 0.5 mM S; (10) 10 μM Cd^{2+} + 8 mM S; (11) 10 μM Cd^{2+} + 2 mM K^+; (12) 10 μM Cd^{2+} + 18 mM K^+. The Cd treatment groups with low concentration ions were set by simulating the concentrations comparably to those present in the plants stressed by the deficiency of exogenous nutrient ions [15–18]. But the designs of the Cd treatment group with high concentrations of ions were according to the results of the pre-experiments. After the above treatments were completed, 50 mL of sterile nutrient solutions corresponding to each treatment were poured into pots every two days. The seedlings were grown in the control room (25 ± 2 °C, 16 h/8 h light/dark cycle, and 65% humidity). An ordinary incandescent lamp (15 W) was used for white light illumination, but exposure to red and blue light was implemented using an LED lamp (ISL-150 × 150-RB, TOSHIBA Lighting and Technology, Tokyo, Japan) at the wavelengths of 660 (red) and 470 (blue) nm. There were three replications for different treatments. Every pot was randomly placed in the room. All plant samples with a root length of 10–15 cm were collected after 30 days.

2.2. Measurements of Cd^{2+} Fluxes by NMT

The calibration of the cadmium ion selective micro-electrode was implemented as follows: the cadmium ion selective micro-electrode and Ag/AgCl reference electrode were immersed in a measuring solution. The state of the micro-electrode was observed by camera and video capture software to ensure there were no ion exchanger leaks from the tip. Meanwhile, the electric potential values were recorded by the NMT system. The initial calibration solution was 50, 100, and 500 μM $Cd(NO_3)_2$. The calibration process was not completed until the potential values were kept constant in different calibration solutions. The slope of the micro-electrode could be calculated by the Nernst equation according to the activity of the cadmium ion in the correction solution and the measured potential value. The above methods are used to explore the characteristics of micro-electrodes under ideal conditions. When micro-electrode technology was applied to monitor plant samples, it was necessary to use a buffer solution to maintain biological activity, so the measuring solution consisted of 0.1 mmol/L $Cd(NO_3)_2$, 0.1 mmol/L KCl, 0.1 mmol/L $CaCl_2$, 1.0 mmol/L NaCl, and 0.1 mmol/L $MgSO_4$. The pH of the measuring solution was maintained at 6.0 ± 0.2 by adding 0.15 mmol/L 2-(N-Morpholino) ethanesulfonic acid (MES).

The measuring solution was added to the culture dish, and the primary (longest) root of *B. tripartita* was fixed with filter paper and resin blocks to expose the root tip in the dish. The cadmium-ion selective micro-electrode was immersed in the measuring solution after calibration, and the micro-electrode was close to the root apex by adjusting the operator. As shown in Figure 1, the scanning locations from the root apex were from 0 to 600 μm every 100 μm; the electric potential values were recorded by the data collection system after parameters were set, and the potential values were converted into Cd^{2+} fluxes by using Mage Flux [9,19].

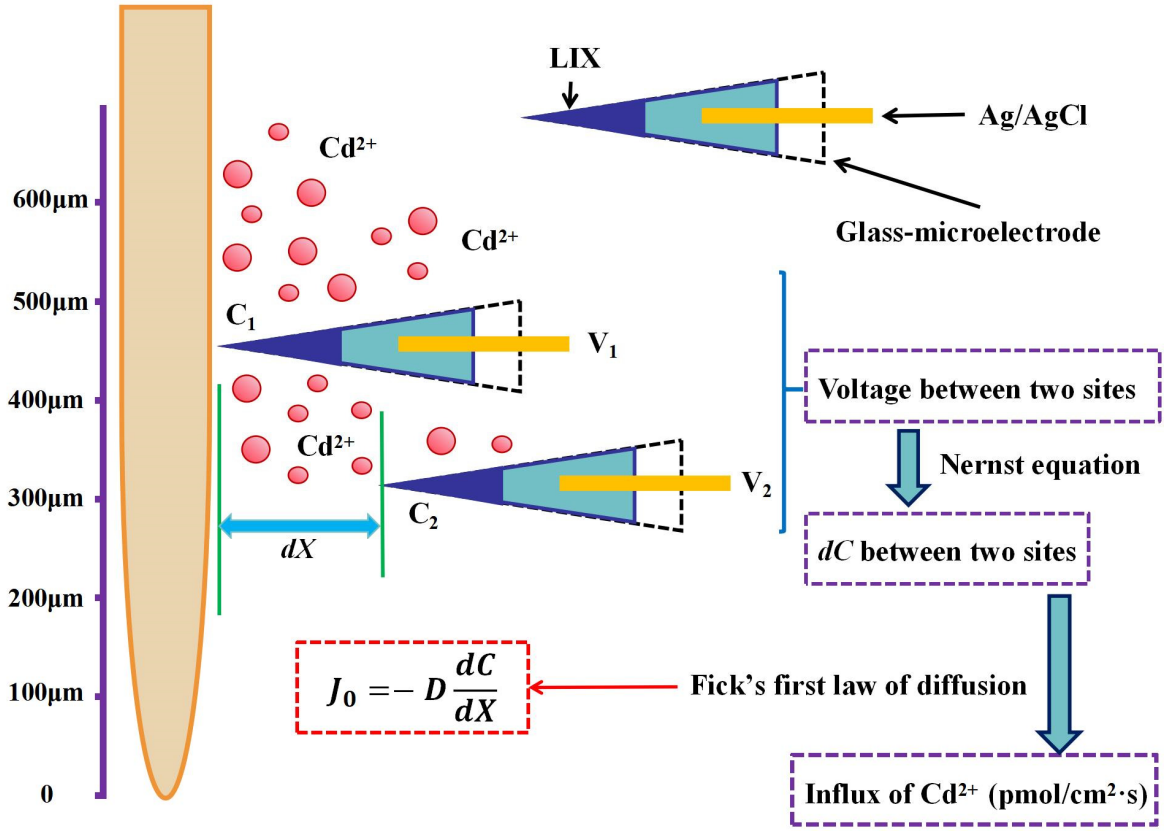

Figure 1. The principle of NMT and setting of probe on *Bidens tripartita* L. root surface based on Han et al. [6] with some modifications.

2.3. Measurements of Chlorophyll, Dehydrogenase Activity, Biomass and Cd Concentration

Chlorophyll is a very significant indicator for photosynthesis. Some fresh leaves of *B. tripartita* were used to make homogenates using 95% ethanol. Chl a and Chl b were separated after they were centrifuged at 5180× *g* for 20 min at 4 °C. The contents of Chl a and Chl b were determined using a UV-visible near-infrared spectrophotometer (UV-3600i Plus, SHIMADZU, Kyoto, Japan) at 663 and 645 nm, respectively [20].

Dehydrogenase activity is an important index to reflect root vigour, which is determined as the amount of triphenyl tetrazole chloride (TTC) reduced per unit time [21].

Biomass determination for the harvested samples of *B. tripartita* seedlings was conducted by the normal method using a balance with an accuracy of 0.001 g. Before measurement, the roots and shoots were separated and oven-dried until counterweight.

The Cd concentration in all samples was determined using inductively coupled plasma optical emission spectrometry (ICP-OES: iCAP 7000, Thermo Fisher Scientific Inc., Waltham, MA, USA) [13,22]. Before such determination, plant samples were digested with a mixture of concentrated HNO_3 and $HClO_4$ (87:13, *v*/*v*) [23]. For QA/QC, the standard reference material poplar leaf GBW07604 (GSV-3) (Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, Beijing, China) was used to verify the measured values of Cd [24]. The limits of detection (LOD) and quantification (LOQ) were 0.0017 and 0.0035 mg/kg, respectively. The measured value of Cd in certified materials was 0.29 ± 0.01 mg/kg, and the values of relative standard deviation (RSD) and Cd recovery rate were 3% and 91 ± 2%, respectively [1].

2.4. Data Analysis and Difference Comparison

Data processing, including average calculation and standard deviation (SD) analysis, was conducted by Excel 2020 SPSS (29.0) and DPS (V19.0) software were used to compare the significant difference at $p < 0.05$ [9,25].

3. Results

3.1. Cd^{2+} Flow Rate of *B. tripartita* Root Tip in Different Treatments

The Cd^{2+} influx (pmol/cm^2·s) round root apexes of 10 μM Cd-treated *B. tripartita* seeding were higher compared to those of controls, and a clear spatial and temporal organisation of the Cd^{2+} influx profile was observed. At 300 μm of the root apex, the Cd^{2+} influxes of *B. tripartite* were the highest for all treatments (Table 1). Compared to 10 μM Cd^{2+}, the flow rates of Cd^{2+} (at 300 μm) were decreased by 35.9% and 43.7% under 10 μM Cd^{2+} + 16 mM Ca^{2+} and 10 μM Cd^{2+} + 8 mM Mg^{2+}, respectively. Likewise, 10 μM Cd^{2+} + 0.5 mM Fe^{3+} or 18 mM K^+ also decreased the Cd^{2+} influxes by 20.6% and 57.5%, respectively. There were no significant effects at low concentrations of Ca^{2+} (1 mM), Mg^{2+} (0.5 mM), or K^+ (2 mM). However, Cd^{2+} influx increased by 34.6% under 10 μM Cd^{2+} + 0.5 mM S, even at higher concentration S (8 mM) without significant influence. Notably, 10 μM Cd^{2+} + 0.05 mM Fe^{3+} or 0.5 mM S significantly increased the Cd uptake rates by *B. tripartita* roots ($p < 0.05$).

Table 1. *B. tripartita* root tip Cd^{2+} influx in different treatments (pmol/cm^2·s).

Treatments	0	100 μm	200 μm	300 μm	400 μm	500 μm	600 μm
Without Cd	−19.59 ± 0.86 fH	−30.70 ± 1.54 eJ	−44.76 ± 1.62 cI	−61.66 ± 3.25 aF	−49.75 ± 1.95 bG	−36.39 ± 1.38 dF	−21.00 ± 1.15 fH
Cd alone	−46.58 ± 1.46 eD	−65.37 ± 3.54 cD	−75.76 ± 4.11 bD	−84.34 ± 3.32 aDC	−77.63 ± 2.43 bC	−63.85 ± 2.49 cB	−55.39 ± 2.94 dB
Cd with 1 mM Ca^{2+}	−38.42 ± 2.14 fE	−54.03 ± 2.49 dE	−67.22 ± 2.25 bF	−81.71 ± 2.34 aD	−66.90 ± 4.49 bE	−58.85 ± 3.62 cC	−48.82 ± 2.43 eD
Cd with 16 mM Ca^{2+}	−9.09 ± 1.94 eI	−28.29 ± 3.15 cK	−45.58 ± 2.77 bH	−54.04 ± 4.05 aG	−46.44 ± 3.52 bH	−29.26 ± 4.35 cG	−24.25 ± 2.17 dG
Cd with 0.5 mM Mg^{2+}	−54.83 ± 2.54 eC	−71.40 ± 2.43 cC	−79.63 ± 3.21 bC	−86.58 ± 3.13 aC	−80.74 ± 3.43 bB	−72.44 ± 3.78 cA	−66.95 ± 3.02 dA
Cd with 8 mM Mg^{2+}	−22.72 ± 1.22 gG	−33.08 ± 2.03 eI	−39.95 ± 1.90 cI	−47.48 ± 1.63 aH	−43.82 ± 2.38 bH	−36.67 ± 2.28 dF	−26.71 ± 1.72 fF
Cd with 0.05 mM Fe^{3+}	−61.14 ± 1.37 eA	−75.23 ± 1.97 cB	−87.56 ± 3.08 bB	−101.61 ± 3.67 aB	−87.33 ± 3.44 bA	−72.98 ± 2.68 cA	−69.12 ± 2.33 dA
Cd with 0.5 mM Fe^{3+}	−21.53 ± 0.62 fG	−36.11 ± 1.53 eH	−58.47 ± 3.27 bG	−66.97 ± 3.21 aE	−56.20 ± 2.09 bF	−49.87 ± 2.44 cE	−43.21 ± 4.03 dE
Cd with 0.5 mM S	−57.93 ± 3.15 fB	−85.73 ± 3.88 cA	−101.50 ± 5.06 bA	−113.54 ± 5.33 aA	−89.45 ± 4.44 cA	−74.07 ± 3.81 dA	−69.29 ± 3.57 eA
Cd with 8 mM S	−25.07 ± 1.40 gF	−47.75 ± 2.94 fF	−69.86 ± 1.86 bEF	−82.56 ± 4.57 aD	−65.64 ± 1.84 cE	−57.07 ± 2.08 dC	−52.07 ± 1.10 eC
Cd with 2 mM K^+	−25.41 ± 1.37 fF	−40.35 ± 1.93 eG	−70.94 ± 3.26 bE	−82.30 ± 3.54 aD	−71.45 ± 3.39 bD	−52.73 ± 2.31 cD	−47.78 ± 2.33 dD
Cd with 18 mM K^+	−10.59 ± 0.53 fI	−16.08 ± 0.69 eL	−31.71 ± 1.55 bJ	−35.85 ± 1.74 aI	−24.16 ± 1.26 cI	−24.47 ± 1.21 cH	−22.87 ± 0.96 dHG

Note: Negative sign means the influx direction from the measuring solution to the root. Data at different lines marked with the different lowercase letters or at different columns with capital letters were significantly different at $p < 0.05$.

3.2. Biomass and Cd Concentrations of *B. tripartita* in Different Treatments

In Table 2, Cd + 16 mM Ca^{2+} induced a promotion (14.5%) of shoot biomass, and Cd + 8 mM Mg^{2+} significantly increased shoot biomass of *B. tripartita* seedlings ($p < 0.05$). However, significant variations were not obtained between Cd + 1 mM Ca^{2+} or 0.5 mM Mg^{2+} ($p < 0.05$). The shoot biomass decreased by 27.3% under Cd + 0.05 mM Fe^{3+}. There were no significant effects of Cd + 8 mM S or 2 mM K^+ on shoot biomass relative to Cd treatment alone ($p < 0.05$). A significant reduction (21.2%) in *B. tripartita* shoot biomass was observed after Cd + 0.5 mM S ($p < 0.05$). Cd + 16 mM Ca^{2+} or 8 mM Mg^{2+} significantly reduced the Cd concentrations of *B. tripartita* ($p < 0.05$). The Cd contents of *B. tripartita* shoot were reduced significantly by 38.3% and 29.6% in Cd + 0.5 mM Fe^{3+} or 18 mM K^+, respectively, whereas Cd contents increased by 20.2% and 18.3% when the plants were Cd + 0.05 mM Fe^{3+} and 0.5 mM S, respectively ($p < 0.05$). However, the Cd content of *B. tripartita* under Cd + 8 mM S or 2 mM K^+ did not significantly change relative to Cd treatment alone ($p < 0.05$).

Shoot biomass of *B. tripartita* seedlings was higher under Cd + 8 mM Mg^{2+} or 18 mM K^+ than Cd alone. Cd + 0.05 mM Fe^{3+} also reduced the biomass. However, Cd + 0.5 mM Mg^{2+} or 8 mM S showed no significant impacts on biomass compared to the control, and Cd + 1 mM Ca^{2+} or 2 mM K^+ had no effects on the biomass of *B. tripartita* seedlings. The Cd content of *B. tripartita* plants was reduced under Cd + high concentrations of nutrient ions compared to Cd alone. However, Cd + low-concentration nutrient ions were all without any impacts on the Cd contents compared to 10 μM Cd^{2+}. Notably, *B. tripartita* can be used to extract Cd from soil contaminated with sulphur and Fe.

Table 2. The biomass, Cd concentration, Chl concentration of leaves, and dehydrogenase activity of roots from *Bidens tripartita* L. under Cd treatments with different exogenous nutrient ions.

Treatments	Biomass (g/Plant)		Cd Concentration (mg/kg)		Concentration of Chl (mg/g FW)		Dehydrogenase Activity (mg/g·h)
	Root	Shoot	Root	Shoot	Chl a	Chl b	
Cd alone	0.25 ± 0.01 cd	1.65 ± 0.10 b	258 ± 15 cd	629 ± 26 cd	3.19 ± 0.21 b	2.12 ± 0.13 d	0.25 ± 0.01 ef
Cd with 1 mM Ca^{2+}	0.26 ± 0.02 c	1.68 ± 0.07 b	253 ± 15 d	641 ± 24 bc	3.11 ± 0.17 b	2.18 ± 0.11 cd	0.23 ± 0.01 ef
Cd with 16 mM Ca^{2+}	0.33 ± 0.02 b	1.89 ± 0.11 a	203 ± 11 f	510 ± 24 e	3.59 ± 0.09 a	2.58 ± 0.05 b	0.29 ± 0.01 d
Cd with 0.5 mM Mg^{2+}	0.24 ± 0.02 d	1.55 ± 0.05 b	277 ± 13 c	589 ± 24 d	3.16 ± 0.15 b	2.15 ± 0.14 d	0.25 ± 0.01 e
Cd with 8 mM Mg^{2+}	0.35 ± 0.01 a	1.95 ± 0.13 a	227 ± 11 e	543 ± 28 e	3.78 ± 0.10 a	2.47 ± 0.06 bc	0.33 ± 0.01 c
Cd with 0.05 mM Fe^{3+}	0.11 ± 0.02 f	1.20 ± 0.09 c	305 ± 13 b	756 ± 27 a	2.61 ± 0.13 c	1.69 ± 1.67 e	0.15 ± 0.01 g
Cd with 0.5 mM Fe^{3+}	0.36 ± 0.01 a	1.94 ± 0.11 a	117 ± 6 h	388 ± 13 g	3.70 ± 0.09 a	2.50 ± 0.06 b	0.35 ± 0.01 b
Cd with 0.5 mM S	0.13 ± 0.02 e	1.30 ± 0.07 c	328 ± 15 a	744 ± 30 a	2.76 ± 0.15 c	1.77 ± 0.11 e	0.16 ± 0.01 g
Cd with 8 mM S	0.24 ± 0.02 d	1.60 ± 0.10 b	256 ± 12 d	637 ± 34 c	3.11 ± 0.04 b	2.09 ± 0.05 d	0.24 ± 0.01 ef
Cd with 2 mM K^{+}	0.26 ± 0.02 c	1.63 ± 0.07 b	239 ± 10 de	616 ± 32 cd	3.03 ± 0.08 b	2.15 ± 0.05 d	0.23 ± 0.01 f
Cd with 18 mM K^{+}	0.34 ± 0.01 ab	1.88 ± 0.07 a	173 ± 10 g	443 ± 17 f	3.64 ± 0.05 a	3.01 ± 0.04 a	0.38 ± 0.01 a

Note: The differences between different means of each column marked with different letters were significant at $p < 0.05$.

3.3. Variations in Chlorophyll and Dehydrogenase Activity

As shown in Table 2, there were no significant differences among Cd + 1 mM Ca^{2+}, 0.5 mM Mg^{2+} and Cd alone ($p < 0.05$). The Chls concentrations in Cd + 16 mM Ca^{2+} or 8 mM Mg^{2+} were significantly higher than those under Cd alone ($p < 0.05$). Compared to Cd alone, the concentrations of Chls significantly increased by 16.0% and 17.9% under Cd + 0.5 mM Fe^{3+} ($p < 0.05$). But the concentrations of Chls decreased significantly by 18.0% and 20.6%, and 13.5% and 16.8%, respectively, under Cd + 0.05 mM Fe^{3+} or 0.5 mM S, respectively. Furthermore, the concentrations of Chls were increased by 14.2% and 41.9% in Cd + 18 mM K^{+}. However, there were no significant effects of 10 μM Cd^{2+} + 2 mM K^{+} on the contents of Chls ($p < 0.05$).

The dehydrogenase activities increased significantly in Cd + 16 mM Ca^{2+} or 8 mM Mg^{2+} compared to Cd alone ($p < 0.05$), whereas no significant differences happened among Cd + 1 mM Ca^{2+} and 0.5 mM Mg^{2+} and Cd alone (Table 2). The dehydrogenase activity was significantly increased by 41.9% under Cd + 0.5 mM Fe^{3+} ($p < 0.05$). However, Cd + 8 mM S had no significant effects on the dehydrogenase activity of *B. tripartita* roots relative to Cd alone ($p < 0.05$). A significant decrease (37.9%) in dehydrogenase activity was observed in the treatment of Cd + 0.05 mM Fe^{3+} compared to Cd alone ($p < 0.05$). Furthermore, dehydrogenase activity in Cd + 18 mM K^{+} was higher (55.4%) than that under Cd alone. There were no significant effects of Cd + 2 mM K^{+} on dehydrogenase activity compared to Cd alone ($p < 0.05$).

4. Discussion

4.1. Dynamic Uptake of Cd^{2+} Flux from Accumulator and Hyperaccumulator Roots under Cd Stress

Tao et al. [26] demonstrated that Cd transport via the apoplasmic pathway is mainly concentrated in the root tips and lateral roots of the hyperaccumulator *S. alfredii*. NMT scanning locations of our design were mainly concentrated around *B. tripartita* root tips from 0 to 800 μm every 100 μm, based on the aforementioned reference. Li et al. [10] determined the Cd^{2+} influx of *Sedum plumbizincicola* seedling roots under 50 μM Cd treatments and found the highest influxes at 300 μm from the root tips. The previous observations are basically the same as our results, and the highest Cd^{2+} influxes were at 300 μm too from the root apexes of *B. tripartita*; the Cd pretreatments promoted the Cd^{2+} influx as compared with controls. Lan et al. [27] found that Cd^{2+} influx in Cd-hyperaccumulator *Microsorum pteropus* leaves was significantly resisted with an exposure to 500 μM Cd. However, such status did not happen in stems both under 500 μM and 100 μM Cd treatments, and the Cd^{2+} root fluxes changed from influxes to effluxes when the exogenous Cd stress changed from 100 μM to 500 μM. By contrast, the net Cd^{2+} influx at 300 μm from the root tip of *B. tripartita* was −84.3 pmol/cm^2·s under 10 μM Cd treatments in this experiment.

4.2. Effects of Exogenous Nutrient Ions on Cd^{2+} Fluxes

Lu et al. [28] verified that Cd^{2+} influx into *S. alfredii* roots sharply decreased when Ca^{2+} in the medium increased from 2 mM to 32 mM. This is consistent with our findings, which also showed that the Cd^{2+} influx of *B. tripartita* roots was reduced by 35.9% under Cd treatment with 16 mM Ca^{2+} compared with Cd stress alone. Greger et al. [29] suggested that Cd^{2+} in the apoplasm or vacuole of wheat cells was decreased by silicate down-regulation of cation channels. Our results indicate that high concentrations of Ca^{2+} have an antagonistic effect on the uptake of Cd^{2+}. It is possible that divalent cation concentrations compete with Cd^{2+} for uptake or that Ca^{2+} blocks Cd^{2+} influx in the cation channels. Jia et al. [30] found that pretreatment with 50 and 150 μmol/L NaHS decreased the Cd^{2+} influxes by 55.7% and 45.4% in *Isatis indigotica* root tips, respectively, and the application of NaHS effectively inhibited Cd^{2+} transmembrane transport. Zhang et al. [31] demonstrated that 10 μmol/L Cd and 25 μmol/L Fe-EDTA co-treatment could reduce the Cd influxes in *Vicia sativa* root tips, and the addition of Fe-EDTA significantly rescued Cd toxicity by inhibiting root uptake. Cd content decreased when plants were grown under Fe supply conditions. The findings from the current study are consistent with the discussed observations; the Cd^{2+} influxes of *B. tripartita* decreased under Cd treatment with 0.5 mmol/L Fe-EDDHA as compared to single Cd treatment. Yin et al. [32] elucidated the underlying physiological and biochemical mechanisms under optimal boron supply levels and found that, as a reliable Cd uptake inhibitor, boron decreased Cd^{2+} influx into cells and promoted the biosynthesis of cell wall components.

4.3. Biomass and Cd Accumulation Affected by Cd and Exogenous Nutrient Ions

Chen et al. [33] investigated the effects of Ca addition on root morphology, Cd translocation, and metabolic responses in *Catharanthus roseus* under Cd stress and found that the addition of 5 mmol/L $Ca(NO_3)_2$ promoted lateral root occurrence on root tips and alleviated Cd-induced toxicity by reducing the content of MDA and H_2O_2 in plants. The exogenous application of Ca decreased Cd uptake by the roots and increased Cd translocation to the shoots. However, the results of the current study indicated that the shoot biomass of *B. tripartita* decreased under Cd treatment when the Ca^{2+} concentration was tripled (16 mmol/L). High concentrations of Ca^{2+} inhibit plant growth and affect root development. Wu et al. [34] investigated K^+ regulation in *Arabidopsis thaliana* in response to Cd stress and discovered that the addition of 3 mmol/L K^+ alleviated leaf chlorosis under Cd stress. Cd treatment with 3 mmol/L K^+ not only increased the content of hemicellulose in the root cell wall but also down-regulated the expression of genes related to Cd membrane transport, which led to Cd fixation and retention in the root cell wall. In the present study, the shoot biomass of *B. tripartita* increased by 13.9% after Cd treatment with 18 mmol/L K^+. It is possible that hemicellulose biosynthesis was promoted by the Cd treatment with a high concentration of K^+. Vaculík et al. [35] investigated the influences of Cd on element distributions in *Salix caprea* roots using energy-dispersive X-ray spectroscopy (EDX) and found that, after exposure to 0.5 mg/L Cd for 14 weeks, Ca and Mg were accumulated in the peripheral bark and K and S were enriched in the phloem. Iron fertilisers can reduce Cd uptake and accumulation in wheat plants. Yao et al. [36] investigated the effects of $FeCl_3$ and $Fe_2(SO_4)_3$ on Cd uptake and accumulation in wheat under Cd stress and found that $FeCl_3$ and $Fe_2(SO_4)_3$ decreased Cd accumulation, and 50 μM $FeCl_3$ is the optimal treatment. Optimising soil environmental Fe effectively reduces Cd accumulation in shoots by immobilising Cd in the roots. Zhang et al. [7] discovered that fertilisation with 500 μM EDTA-Na_2Fe increased the contents of the ionic soluble pectin and decreased citric acid in rice roots, which provided more Cd-binding sites in the root cell walls and reduced Cd mobility in the xylem. They also demonstrated that low-Fe treatments up-regulated the expression of Cd-transport-related genes, and excessive-Fe treatments enhanced Cd enrichment in the iron plaque and root cell wall. Interference of Cd with Fe nutrition has a strong effect on the development and efficiency of the photosynthetic apparatus. Sárvári et al. [37] researched the influence of Fe-citrate supplied to the chlorophyll–protein com-

plexes of poplar leaves and verified that the increased Fe content of chloroplasts was due to excessive-Fe-induced changes in the Fe distribution in the leaf. The selection of a suitable Fe-to-Cd ratio and a proper light environment is crucial to avoiding the potentially harmful effects of excessive Fe. The combination of sulphur and chicory promotes Cd bioaccumulation for the phytoremediation of Cd-contaminated alkaline soil. Liu et al. [38] reported the mechanism of sulphur-assisted Cd phytoremediation by chicory and suggested that the Cd bioconcentration factors significantly increased by 201.0% for shoots and 227.7% for roots under 1.2 g/kg (2700 kg/ha) sulphur treatment, as compared with the control. Elemental sulphur can enhance the efficiency of Cd phytoremediation. Zia-ur-Rehman et al. [39] found that Cd accumulation in the roots of spider plants increased by 62% with a 0.2% sulphur treatment relative to controls under 100 mg/kg Cd stress, and the application of treatments such as 0.2% sulphur also increased the photosynthetic rate in plants. Similarly, the findings of the current study showed that Cd + 0.05 mM Fe^{3+} or 0.5 mM S increased Cd^{2+} influx around *B. tripartita* roots. First, the addition of excess Fe may protect against Cd stresses and based on the results of the chlorophyll concentration of leaves, photosynthetic symptoms were recovered in plants subjected to Cd stress. Second, considering the effects of Fe on the real-time uptake of Cd by *B. tripartita* roots, we chose Fe-EDDHA as the chelating iron fertiliser to avoid the inorganic ferric salt-led precipitation of $Fe(OH)_3$ on the root surface. Third, sulphur is involved in the synthesis of several important metabolites, such as glutathione, phytochelatins, and non-protein thiols, which are related to the tolerance and detoxification mechanisms of Cd in plants.

5. Conclusions

Using NMT, real-time Cd uptake by *B. tripartita* root under Cd treatments with different exogenous nutrient ions was measured in this experiment. Cd + 0.05 mM Fe^{3+} or 0.5 mM S increased Cd^{2+} influx at 300 μm from *B. tripartita* root apexes. Meanwhile, the dehydrogenase activity of roots increased significantly under Cd + 0.05 mM Fe^{3+} or 0.5 mM S compared to Cd alone, but the chlorophyll concentration of leaves decreased evidently, which laid the foundation for monitoring the Cd uptake by the roots of the accumulator using NMT. In addition, the processes involved in Cd uptake of *B. tripartita* root, Cd translocation from root to shoot, and Cd accumulation in the root/shoot could be monitored by NMT in future research, especially Cd^{2+} sequestration into the vacuole and Cd^{2+} transport in the xylem vessels together with inorganic ions. Based on our results and other comprehensive studies, the focus should be on the effects of S and Fe^{3+} on the remediation potential of *B. tripartita* in the future with the application of NMT.

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References

1. Wei, S.; Niu, R.; Srivastava, M.; Zhou, Q.; Wu, Z.; Sun, T.; Hu, Y.; Li, Y. *Bidens tripartite* L.: A Cd-accumulator confirmed by pot culture and site sampling experiment. *J. Hazard. Mater.* **2009**, *170*, 1269–1272. [CrossRef] [PubMed]
2. Zheljazkov, V.D.; Jeliazkova, E.A.; Kovacheva, N.; Dzhurmanski, A. Metal uptake by medicinal plant species grown in soils contaminated by a smelter. *Environ. Exp. Bot.* **2008**, *64*, 207–216. [CrossRef]
3. He, B.Y.; Yu, D.P.; Chen, Y.; Shi, J.L.; Xia, Y.; Li, Q.S.; Wang, L.L.; Ling, L.; Zeng, E.Y. Use of low-calcium cultivars to reduce cadmium uptake and accumulation in edible amaranth (*Amaranthus mangostanus* L.). *Chemosphere* **2017**, *171*, 588–594. [CrossRef] [PubMed]
4. Gao, C.; Wang, Y.; Xiao, D.S.; Qiu, C.P.; Han, D.G.; Zhang, X.Z.; Wu, T.; Han, Z.H. Comparison of cadmium-induced iron-deficiency responses and genuine iron-deficiency responses in *Malus xiaojinensis*. *Plant Sci.* **2011**, *181*, 269–274. [CrossRef] [PubMed]
5. 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]
6. Han, M.; Yang, H.; Yu, G.; Jiang, P.; You, S.; Zhang, L.; Lin, H.; Liu, J.; Shu, Y. Application of Non-invasive Micro-test Technology (NMT) in environmental fields: A comprehensive review. *Ecotoxicol. Environ. Saf.* **2022**, *240*, 113706. [CrossRef] [PubMed]
7. Zhang, Q.; Huang, D.; Xu, C.; Zhu, H.; Feng, R.W.; Zhu, Q. Fe fortification limits rice Cd accumulation by promoting root cell wall chelation and reducing the mobility of Cd in xylem. *Ecotoxicol. Environ. Saf.* **2022**, *240*, 113700. [CrossRef] [PubMed]
8. 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] [PubMed]
9. Wang, S.; Dai, H.; Skuza, L.; Chen, Y.; Wei, S. Difference in Cd^{2+} flux around the root tips of different soybean (*Glycine max* L.) cultivars and physiological response under mild cadmium stress. *Chemosphere* **2022**, *297*, 134120. [CrossRef] [PubMed]
10. Li, L.Z.; Tu, C.; Wu, L.H.; Peijnenburg, W.J.; Ebbs, S.; Luo, Y.M. Pathways of root uptake and membrane transport of Cd^{2+} in the zinc/cadmium hyperaccumulating plant *Sedum plumbizincicola*. *Environ. Toxicol. Chem.* **2017**, *36*, 1038–1046. [CrossRef] [PubMed]
11. Sun, J.; Wang, R.; Liu, Z.; Ding, Y.; Li, T. Non-invasive microelectrode cadmium flux measurements reveal the spatial characteristics and real-time kinetics of cadmium transport in hyperaccumulator and nonhyperaccumulator ecotypes of *Sedum alfredii*. *J. Plant Physiol.* **2013**, *170*, 355–359. [CrossRef] [PubMed]
12. Tao, Q.; Liu, Y.; Li, M.; Li, J.; Luo, J.; Lux, A.; Kováč, J.; Yuan, S.; Li, B.; Li, Q.; et al. Cd-induced difference in root characteristics along root apex contributes to variation in Cd uptake and accumulation between two contrasting ecotypes of *Sedum alfredii*. *Chemosphere* **2020**, *243*, 125290. [CrossRef] [PubMed]
13. Wei, S.H.; Zhou, Q.X.; Liu, R. Utilization of weed resource in the remediation of soils contaminated by heavy metals. *J. Nat. Res.* **2005**, *20*, 432–440.
14. Wei, H.; Huang, M.; Quan, G.; Zhang, J.; Liu, Z.; Ma, R. Turn bane into a boon: Application of invasive plant species to remedy soil cadmium contamination. *Chemosphere* **2018**, *210*, 1013–1020. [CrossRef] [PubMed]
15. Wang, S.; Dai, H.; Ji, D.; Cui, S.; Jiang, C.; Skuza, L.; Li, L.; Grzebelus, D.; Wei, S. Influencing factors of *Bidens pilosa* L. hyperaccumulating cadmium explored by the real-Time uptake of Cd^{2+} influx around root apexes under different exogenous nutrient ion levels. *Toxics* **2023**, *11*, 227. [CrossRef] [PubMed]
16. Wang, Q.; Ma, L.; Zhou, Q.; Chen, B.; Zhang, X.; Wu, Y.; Pan, F.; Huang, L.; Yang, X.; Feng, Y. Inoculation of plant growth promoting bacteria from hyperaccumulator facilitated non-host root development and provided promising agents for elevated phytoremediation efficiency. *Chemosphere* **2019**, *234*, 769–776. [CrossRef] [PubMed]
17. Jenkins, B.; Basu, S. Regulation of boron, iron, and cadmium transporters in maintaining proper balance between their deficiency and excess in plants. In *Metal and Nutrient Transporters in Abiotic Stress*; Academic Press: Cambridge, MA, USA, 2021; pp. 237–250.
18. Lewandowska, M.; Wawrzyńska, A.; Moniuszko, G.; Łukomska, J.; Zientara, K.; Piecho, M.; Hodurek, P.; Zhukov, I.; Liszewska, F.; Nikiforova, V.; et al. A contribution to identification of novel regulators of plant response to sulfur deficiency: Characteristics of a tobacco gene UP9C, its protein product and the effects of UP9C silencing. *Mol. Plant* **2010**, *3*, 347–360. [CrossRef] [PubMed]
19. Ma, W.; Xu, W.; Xu, H.; Chen, Y.; He, Z.; Ma, M. Nitric oxide modulates cadmium influx during cadmium-induced programmed cell death in tobacco BY-2 cells. *Planta* **2010**, *232*, 325–335. [CrossRef] [PubMed]
20. Krause, G.H.; Winter, K. Photoinhibition of photosynthesis in plants growing in natural tropical forest gaps: A chlorophyll fluorescence study. *Plant Biol.* **2015**, *109*, 456–462. [CrossRef]
21. Wang, Q.; Chen, L.; Xu, H.; Ren, K.; Xu, Z.; Tang, Y.; Xiao, J. The effects of warming on root exudation and associated soil N transformation depend on soil nutrient availability. *Rhizosphere* **2021**, *17*, 100263. [CrossRef]
22. Hamid, Y.; Tang, L.; Yaseen, M.; Hussain, B.; Zehra, A.; Aziz, M.Z.; He, Z.; Yang, X. Comparative efficacy of organic and inorganic amendments for cadmium and lead immobilization in contaminated soil under rice-wheat cropping system. *Chemosphere* **2019**, *214*, 259–268. [CrossRef]

23. Zeng, H.Y.; Chen, L.H.; Yang, Y.; Deng, X.; Zhou, X.H.; Zeng, Q.R. Basal and foliar treatment using an organic fertilizer amendment lowers cadmium availability in soil and cadmium uptake by rice on field micro-plot experiment planted in contaminated acidic paddy soil. Soil. Sediment. *Contam Int. J.* **2019**, *28*, 1–14. [CrossRef]
24. Masson, P.; Dauthieu, M.; Trolard, F.; Denaix, L. Application of direct solid analysis of plant samples by electrothermal vaporization-inductively coupled plasma atomic emission spectrometry: Determination of Cd and Si for environmental purposes. *Spectrochim. Acta B* **2007**, *62*, 224–230. [CrossRef]
25. Tang, Q.Y.; Feng, M.G. *DPS Data Processing System for Practical Statistics*; Science Press: Beijing, China, 2002. (In Chinese)
26. Tao, Q.; Jupa, R.; Luo, J.; Lux, A.; Kováč, J.; Wen, Y.; Zhou, Y.; Jan, J.; Liang, Y.; Li, T. The apoplasmic pathway via the root apex and lateral roots contributes to Cd hyperaccumulation in the hyperaccumulator *Sedum alfredii*. *J. Exp. Bot.* **2017**, *68*, 739–751. [PubMed]
27. Lan, X.Y.; He, Q.S.; Yang, B.; Yan, Y.Y.; Li, X.Y.; Xu, F.L. Influence of Cd exposure on H^+ and Cd^{2+} fluxes in the leaf, stem and root of a novel aquatic hyperaccumulator-*Microsorum pteropus*. *Chemosphere* **2020**, *249*, 126552. [CrossRef] [PubMed]
28. Lu, L.; Tian, S.; Zhang, M.; Zhang, J.; Yang, X.; Jiang, H. The role of Ca pathway in Cd uptake and translocation by the hyperaccumulator *Sedum alfredii*. *J. Hazard. Mater.* **2010**, *183*, 22–28. [CrossRef] [PubMed]
29. Greger, M.; Kabir, A.H.; Landberg, T.; Maity, P.J.; Lindberg, S. Silicate reduces cadmium uptake into cells of wheat. *Environ. Pollut.* **2016**, *211*, 90–97. [CrossRef]
30. Jia, H.; Wang, X.; Shi, C.; Guo, J.; Ma, P.; Ren, X.; Wei, T.; Liu, H.; Li, J. Hydrogen sulfide decreases Cd translocation from root to shoot through increasing Cd accumulation in cell wall and decreasing Cd^{2+} influx in *Isatis indigotica*. *Plant Physiol. Biochem.* **2020**, *155*, 605–612. [CrossRef]
31. Zhang, X.; Li, X.; Tang, L.; Peng, Y.; Qian, M.; Guo, Y.; Rui, H.; Zhang, F.; Hu, Z.; Chen, Y.; et al. The root iron transporter 1 governs cadmium uptake in *Vicia sativa* roots. *J. Hazard. Mater.* **2020**, *398*, 122873. [CrossRef] [PubMed]
32. Yin, A.; Huang, B.; Xie, J.; Huang, Y.; Shen, C.; Xin, J. Boron decreases cadmium influx into root cells of *Capsicum annuum* by altering cell wall components and plasmalemma permeability. *Environ. Sci. Pollut. R.* **2021**, *28*, 52587–52597. [CrossRef] [PubMed]
33. 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] [PubMed]
34. Wu, Q.; Zhu, X.; Zhao, X.; Shen, R. Potassium affects cadmium resistance in *Arabidopsis* through facilitating root cell wall Cd retention in a nitric oxide dependent manner. *Environ. Exp. Bot.* **2020**, *178*, 104175. [CrossRef]
35. Vaculík, M.; Konlechner, C.; Langer, I.; Adlassnig, W.; Puschenreiter, M.; Lux, A.; Hauser, M.T. Root anatomy and element distribution vary between two *Salix caprea* isolates with different Cd accumulation capacities. *Environ. Pollut.* **2012**, *163*, 117–126. [CrossRef]
36. Yao, Q.; Li, W.; Liu, Y.; Cheng, Y.; Xiao, X.; Long, D.; Zeng, J.; Wu, D.; Sha, L.; Fan, X.; et al. $FeCl_3$ and $Fe_2(SO_4)_3$ differentially reduce Cd uptake and accumulation in Polish wheat (*Triticum polonicum* L.) seedlings by exporting Cd from roots and limiting Cd binding in the root cell walls. *Environ. Pollut.* **2023**, *317*, 120762. [CrossRef] [PubMed]
37. Sárvári, É.; Solti, Á.; Basa, B.; Mészáros, I.; Lévai, L.; Fodor, F. Impact of moderate Fe excess under Cd stress on the photosynthetic performance of poplar (*Populus jacquemontiana* var. *glauca* cv. *Kopeczkii*). *Plant Physiol. Biochem.* **2011**, *49*, 499–505. [CrossRef] [PubMed]
38. Liu, H.; Luo, L.; Jiang, G.; Li, G.; Zhu, C.; Meng, W.; Zhang, J.; Jiao, Q.; Du, P.; Li, X.; et al. Sulfur enhances cadmium bioaccumulation in *Cichorium intybus* by altering soil properties, heavy metal availability and microbial community in contaminated alkaline soil. *Sci. Total Environ.* **2022**, *837*, 155879. [CrossRef] [PubMed]
39. Zia-ur-Rehman, M.; Mubsher, A.; Rizwan, M.; Usman, M.; Jafir, M.; Umair, M.; Alharby, H.; Bamagoos, A.; Alshamrani, R.; Ali, S. Effect of farmyard manure, elemental sulphur and EDTA on growth and phytoextraction of cadmium by spider plants (*Chlorophytum comosum* L.) under Cd stress. *Chemosphere* **2023**, *313*, 137385. [CrossRef]

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