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The Effects of Calcium and Sulfur Fertilizers Accompanied by Different Side Elements on the Growth and Cd Uptake of *Spinacia oleracea* Grown in Cd-Contaminated Alkaline Soil

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Abstract: The detoxification of crops grown in Cadmium (Cd)-contaminated acid soil has been widely studied, but for contaminated alkaline soil, there is still inadequate research or information. In order to investigate the effects of calcium and sulfur fertilizers, accompanied by different side elements, on the growth and Cd uptake of *Spinacia oleracea* grown in Cd-contaminated alkaline soil, the plants were subjected to five treatments, including calcium silicate (Ca-Si), calcium biphosphate (Ca-P), magnesium sulfate (S-Mg), ferric sulfate (S-Fe), and zinc sulfate (S-Zn), and a control group. The results showed that the S-Fe achieved the highest shoot fresh mass and dry mass and the highest shoot Cd concentration and accumulation, which were 30%, 68%, 4.6%, and 73% higher than the control group, respectively. The Ca-Si, Ca-P, S-Mg, and S-Zn reduced the root Cd concentration by 18%, 42%, 7%, and 49%, respectively, and reduced the shoot Cd concentration by 25%, 36%, 15%, and 27%, respectively, as compared to the control. S-Fe increases plant N uptake and photosynthesis, which is beneficial to biomass accumulation. Ca-P improves soil and plant P nutrition status, as well as plant K and Ca status, and helps alleviate plant Cd stress. Overall, calcium fertilizers accompanied by phosphorus have the potential to reduce plant Cd contamination risk, while sulfur fertilizers accompanied by iron show potential for enhancing Cd extraction.

Keywords: heavy metal; iron; leafy greens; macronutrients; vegetable production; zinc

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1. Introduction

As a heavy metal that extensively exists in the environment [1,2], Cd is characterized by easy movement, difficult degradation, and strong toxicity [3,4] in terms of environmental toxicity. Cd can enter farmland soils [5,6] through human activities such as mining, metal smelting, atmospheric sedimentation, sewage irrigation, and the use of Cd-containing fertilizers and pesticides. As a result, Cd may pose serious threats to crop production, food safety, and human health [7–9].

The safe use of Cd-contaminated farmland soils is an urgent demand [10]. Decreasing the crop's Cd uptake is the key to the safe use of mildly and moderately Cd-contaminated soils [11]. Specifically, controlling the plant's Cd uptake through physiology control technology is the most economic and effective pathway to relieve the crop's Cd stress and decrease the crop's Cd uptake [12–14].

The phytotoxicity of Cd lies in its impediments to leaf photosynthesis [15] and disturbances to the nutrient balance of plants, thus restricting their growth and development of plants [16,17]. Therefore, optimizing mineral nutrient supply to decrease the Cd toxicity of plants and avoid Cd penetrating into the food chain could be an effective management

approach [8]. In addition, decreasing the Cd uptake of crops by optimizing the mineral fertilizer input is a green technology for controlling Cd uptake by crop, thus conforming to the clean production philosophy and the long-term goal of carbon neutralization [18,19].

The effects of mineral nutrients on the Cd concentration of plants depend on fertilizer types [20] and nutrient forms [20,21]. In the control of plant Cd uptake, the fertilizer type and supply form should be optimized according to the specific crop. Zou et al. (2018) [22] studied the effects of different phosphorus fertilizers on Cd accumulation in three-colored amaranth, and the results demonstrated that the reduction in plant Cd concentration by the use of different phosphorus fertilizers showed different efficacy, which followed the order of: calcium magnesium phosphate fertilizer > ground phosphorite > superphosphate > potassium dihydrogen phosphate. Similarly, Wang et al. (2020) [23] investigated the effects of different nitrogen fertilizers on Cd accumulation in the three-colored amaranth grown in yellow-brown soils, and the fertilizers showed different efficacy on increasing the plant Cd concentration, and the increase amplitude is as follows: urea > ammonium nitrate > ammonium sulfate > sodium nitrate. Hence, the effects of mineral fertilizers on Cd uptake by crops are related to both fertilizer types and accompanying ions, and optimizing the accompanying elements of the mineral fertilizers to manipulate soil to plant Cd transportation has both theoretical importance and practical guidance.

Some specific mineral nutrients, such as calcium [24] and sulfur [25], have the potential to reduce the plant's Cd uptake. Calcium fertilizer plays a prominent role in adverse signal transduction, maintains cell stabilization and ion balance, relieves Cd stress in plants, and improves crop growth [24,26]. Research on Arabidopsis indicates that plasma membrane-associated calcium signaling helps modulate Cd tolerance, which is one of the key strategies to cope with Cd stress [27]. Another report showed that adding Calcium alleviated the physiological toxicity of Cd in S. matsudana and reduced its Cd uptake [28]. Sulfur fertilizer is conducive to eliminating adverse stress-free radicals, decreasing the Cd toxicity of plants, and improving crop growth [29,30]. Li et al. (2023) [31] found that the application of S significantly reduced Cd concentration in brown rice at maturity stage by reducing soil Cd availability, inhibiting Cd transfer from other tissues to seeds of brown rice, and diluting Cd in brown rice through increased plant biomass. Zia-ur-Rehman et al. (2023) [32] evaluated the efficiency of S (0.1 and 0.2%) for remediation of Cd-contaminated soils (50 and 100 $mg \cdot kg^{-1}$) and depicted that S supply increased shoot biomass and Cd concentrations in the shoot and root of spider plants. However, Ca and S involvements to alleviate Cd stress in plants remain evasive. In terms of the studied parameter, chlorophyll II fluorescence has always been an important evaluation parameter that cannot be ignored in plant responses to Cd stress. Cai et al. (2010) [33] indicated that Cd stress causes a decrease in maximal photochemical efficiency of PSII (Fv/Fm), inhibits leaf photosynthesis in rice, and that the alleviation of Cd toxicity by GSH relates to improvement of leaf photosynthesis. The role of fluorescence remains to be further expanded. The mineral nutrient status of plants was very sensitive to the aggravation or relief of soil Cd stress. Altaf et al. (2023) indicated that Cd toxicity greatly reduced the growth of pepper by impeding its nutrient uptake [15], and Hakeen et al. (2022) reported that exogenous application of Ca reduces the Cd uptake of Fagopyrum esculentum by restoring its mineral content and minimizing its Cd toxicity [24]. The role of mineral nutrient supply in detoxifying plant Cd stress and reducing plant Cd uptake requires more in-depth research. Cd contamination is dispersed over a wide area in farmland, which has both acidic and alkaline soil. Although Cd toxicity is less harmful to plants in alkaline soils than it is in acidic soils, incidents of crop Cd hazards in alkaline soils have been repeatedly reported. Up until now, the research on the prevention and control of Cd contamination in crops in alkaline soils has not yet received enough attention compared with that in acidic soils.

The studies working on the acid soil mainly aim to increase soil pH by adding exogenous alkaline materials so as to deactivate the soil's available Cd, but this method is not applicable for alkaline soil. Thus, we turned to exploring the potential use of exogenous nutrient supply to mitigate Cd toxicity and then reduce crop Cd uptake. To investigate

the effects of calcium or sulfur fertilizers on the leafy vegetable's Cd uptake as well as compare the differences between various company elements, a greenhouse experiment was conducted. *Spinacia oleracea* (spinach), a Cd-enriched vegetable [34], was chosen in the present study. The effects of the different calcium and sulfur fertilizers with various side elements on the Cd uptake, growth, and nutrient uptake of the plants growing in the Cd-contaminated alkaline soil were compared. Their impacts on the soil's physicochemical properties and Cd availability were also evaluated. This study aims to provide technological guidance to optimize the use of fertilizers in controlling the Cd uptake by leafy vegetables. We hypothesized that some specific side elements could amplify the detoxification effect of calcium and sulfur on the spinach grown in the Cd-contaminated alkaline soil, further reduce plant Cd uptake, and increase plant biomass accumulation.

2. Materials and Methods

2.1. Experiment Set-Up

The pot experiment was carried out from 15 January 2022 to 16 March 2022 in the gutting-connected greenhouse of the Beijing Academy of Agriculture and Forestry Sciences, located in Haidian district, Beijing, China. The average greenhouse inside temperature was 25~30 °C in daytime and 10–15 °C in nighttime during the trial, and the average duration of daylight was 11 h.

The top soil layer (0–20 cm) from a Cd contaminated farmland (the previous crop was maize) was collected for the present pot experiment. The soil was cinnamon soil, and its basic physicochemical properties are as follows: pH—7.74, organic matter—11.1 $g \cdot kg^{-1}$, electric conductivity—0.0147 $s \cdot m^{-1}$, total nitrogen—1 $g \cdot kg^{-1}$, total phosphorus—1.5 $g \cdot kg^{-1}$, total potassium—16 $g \cdot kg^{-1}$, available phosphorus—23 $mg \cdot kg^{-1}$, available potassium—155 $mg \cdot kg^{-1}$. The soil had an initial total Cd (TCd) concentration of 1.87 $mg \cdot kg^{-1}$ and an initial available Cd concentration (ACd) of 0.60 $mg \cdot kg^{-1}$. According to the National Soil Quality Standards for Vegetable Field issued in 2006 (HJ333-2006), the threshold of total soil Cd content for safe vegetable production in soil with pH > 7.5 was 0.4 $mg \cdot kg^{-1}$ [35], and the soil TCd content in this experiment was 367% higher than the threshold, which was defined as highly contaminated soil.

The experiment was a completely randomized block design with five replicates for each treatment, and five different fertilizers were tested. The main effective elements in the fertilizers were Ca and S, and each of them had different company elements, which were Si and P for the Ca and Mg, Fe, and Zn for the S. Therefore, there were five treatments: calcium silicate ($CaSiO_3$, Ca-Si), calcium biphosphate ($Ca_2(H_2PO4)_3$, Ca-P), magnesium sulfate ($MgSO_4$, S-Mg), ferric sulfate ($Fe_2(SO_4)_3$, S-Fe), and zinc sulfate ($ZnSO_4$, S-Zn), with one control group (control) without fertilizer amendment. One pot of plant was considered one biological replicate, so there were a total of 30 pots of plants in the experiment. And the fertilizers were applied three times at 0 days, 10 days, and 20 days after sowing, respectively, with the same dosage at each time, resulting in a total addition of 1.8 g pot^{-1} as 0.6 g kg^{-1}.

For each pot (diameter: 16 cm, height: 13 cm), 3 kg of experiment soil was filled in, thoroughly watered, and kept still until the soil water content was stable at 100% of field capacity. Then, the 15 seeds of *S. oleracea* (Hongfu Hybridization Generation I, Beijing Juping Xingli Agricultural Science and Technology Co. Ltd., Beijing, China) were sown into the pot, and the seedlings were thinned to five plants per pot right before the second time of fertilization. Pots were weighed every day, and when the pot soil water content was lower than 75% of field moisture capacity, they were watered back to 100% field capacity with deionized water. Moreover, the positions of the pots were changed every day to assure uniform illumination for each treatment group.

2.2. Leaf Chlorophyll Fluorescence and Actual Photochemical Efficiency

Chlorophyll fluorescence parameters of plants were measured after 30 days of sowing with a portable pulse modulation chlorophyll florescence instrument (FluorPen FP100, PSI, Brno, Czech Republic). After the dark treatment of plant leaves for 30 min, the initial

florescence (Fo), maximum florescence (Fm), variable fluorescence (Fv, from the formula of Fm-Fo), the potential efficiency of PSII (Fv/Fo), the maximum photochemical efficiency of PSII (Fv/Fm), and the actual photochemical efficiency of PSII (Qy) [36,37] were measured.

2.3. Plant Biomass and Nutrient Uptake

The plants were harvested after 60 days of sowing and divided into aboveground and underground parts at the boundary of soil level, which were defined as shoot and root, respectively. The plant parts were rinsed with distilled water and dried with air-laid paper, then weighed for fresh mass. After that, the plants were preheated at 105 °C for 10 min, and dried at 75 °C to a constant weight. The dry mass of aboveground and underground parts was weighed, ground, crushed, and prepared for testing.

For the determination of plant total nitrogen, phosphorus, and potassium (TN, TP, and TK, respectively), the sulfuric acid–hydrogen peroxide digestion method was first adopted [38]. Total nitrogen (TN) in digestion solution was measured by the Kjeldahl method [39], total phosphorus (TP) was analyzed with the ammonium-molybdophosphoric blue color method [40], and total potassium (TK) was determined by the flame photometer method [41]. The plant total calcium and total magnesium (TCa and TMg, respectively) concentrations were measured by the nitric acid-perchloric acid digestion method [42], followed by inductively coupled plasma optical emission spectrometry [43].

2.4. Soil Physicochemical Properties and Nutrient Content

The soil physico-chemical parameters were studied as follows: soil pH was measured in an aqueous suspension (1:2.5 = soil:water ratio) with a PHS-3C pH meter (Leici, INASE Scientific Instrument Co., LTD, Shanghai, China) (Rodriguez-Berbel et al. 2022). Soil electrical conductivity (EC) was determined on a soil-water suspension (1:2.5 = soil:water ratio) with a TZS-ECW-GA digital conductivity meter (Zhejiang Top Cloud-agri Technology Co., Ltd., Hangzhou, China) [44]. Soil total organic matter (OM) was analyzed with the potassium dichromate volume-external heating method [45]. Soil total nitrogen content (TN) was digested in a sulfuric acid-hydrogen peroxide solution and tested by the Kjeldahl method with an automatic Kjeldahl nitrogen analyzer (KDY-9820, KETUO, Beijing, China) [46]. Soil available phosphorus (AP) concentration was extracted using sodium bicarbonate and measured through the vanadium molybdate yellow colorimetric method with a spectrophotometer (Model 722, Modern Science Ltd., Shanghai, China) [47]. Soil-available potassium (AK) was extracted by ammonium acetate and tested following the flame photometer method (6400A, Precision Scientific Instrument Co., Ltd., Shanghai, China) [48].

2.5. Plant Cd Uptake and Soil Cd Content

Plant total Cd concentration (TCd) was measured by digesting leaf and root samples with a mixture of nitric and perchloric acid (3:1, *v*/*v*) [49]. Soil-available Cd (ACd) was obtained by filtering the soil with DTPA after immersion for 2 h at a soil-liquid ratio of 1:2.5 (w:w) [50]. Soil TCd was determined by digesting soil with a mixture of aqua regia and perchloric acid [51]. The Cd contents in the leach liquor and digestion solution were filtrated and tested by an inductive coupling plasma spectrogenerator (ICP-OES 5110, Agilent, Palo Alto, CA, USA) [52].

The Cd uptake capacity of plant shoot and root was reflected by the bioconcentration factor (BF_{shoot} and BF_{root}, respectively). The transfer capacity of absorbed Cd from roots to the aboveground part was reflected by the transfer coefficient (TF). Calculation methods were as follows:

$$BF_{Shoot} = \text{Shoot TCd}/\text{Soil TCd} \quad (1)$$

$$BF_{root} = \text{Root TCd}/\text{Soil TCd} \quad (2)$$

$$TF = \text{Shoot TCd}/\text{Root TCd} \quad (3)$$

2.6. Statistical Analysis

The analysis of variance (ANOVA) was performed with JMP Pro 16 (SAS Institute Inc., Cary, NC, USA) to detect the treatment effect on plant growth, nutrient uptake, and soil physicochemical properties. The Pearson correlation and principal component analysis were performed with Origin Pro 2021 (OriginLab Corporation, Northampton, MA, USA) to detect the relationship between the different traits measured in the experiment. The box plots were plotted with Origin Pro 2021 (the same as above). All the data were presented as mean ± standard error.

3. Results

3.1. Leaf Fluorescence and Plant Growth

The spinach leaf fluorescence influenced by different fertilizers is shown in Table 1. Among the three traits assessed, only Qy was influenced by the treatment; the plants that received sulfur fertilizers had a significantly higher actual quantum yield of PSII than the control group. The spinach biomass influenced by different fertilizers is shown in Figure 1. The S-Fe group achieved the highest shoot fresh mass and the highest shoot dry mass, but no significance was detected among the treatments. The S-Fe group achieved a 30% higher shoot fresh mass and a 68% higher shoot dry mass than the control group.

Table 1. The spinach leaf chlorophyll fluorescence traits at the final harvest. The average potential efficiency of PSII (Fv/Fo), the maximum photochemical efficiency of PSII (Fv/Fm), and the actual photosynthetic quantum yield of PSII (Qy) in the plant leaves were shown. The numbers sharing different letters were in different Tukey's HSD homogenous subsets.

	Fv/Fo	Fv/Fm	Qy
Control	1.82 ± 0.09 a	0.65 ± 0.01 a	0.38 ± 0.03 b
Ca-Si	1.86 ± 0.17 a	0.65 ± 0.02 a	0.45 ± 0.01 ab
Ca-P	1.84 ± 0.19 a	0.64 ± 0.03 a	0.45 ± 0.01 ab
S-Mg	2.13 ± 0.10 a	0.68 ± 0.01 a	0.47 ± 0.02 a
S-Fe	2.33 ± 0.04 a	0.70 ± 0.00 a	0.48 ± 0.01 a
S-Zn	2.11 ± 0.14 a	0.68 ± 0.01 a	0.47 ± 0.00 a
ANOVA			*

Note: '*' for $p < 0.05$.

The spinach nutrient uptake of shoots influenced by different fertilizers is shown in Table 2. The concentrations of P, K, Ca, and Mg in the spinach shoot, but not N, were significantly influenced by the treatments. The Ca-P increased the shoot P by 569% compared to the control and increased the shoot K by 16% compared to the control. However, the S-Fe significantly reduced the shoot K by 36.3% compared to the control. The Ca-P also increased the shoot Ca by 27.2% and 60.3%, respectively, compared to S-Mg and S-Fe. However, the Ca-P reduced the shoot Mg by 87.2% compared to the control.

3.2. Plant Cd and Soil Cd Content

The spinach shoot Cd concentration and accumulation based on the dry mass were shown in Figure 2a,b. The S-Fe addition resulted in both the highest shoot Cd concentration and accumulation, while the Ca-P addition resulted in the lowest in both traits. S-Fe resulted in 63.0% higher shoot Cd concentration and 214% higher shoot Cd accumulation compared to Ca-P. Compared to control, Ca-Si, Ca-P, S-Mg, and S-Zn decreased root Cd concentration by 18%, 42%, 7%, and 49%, respectively, and reduced shoot Cd concentration by 25%, 36%, 15%, and 27%, respectively. The soil TCd and ACd concentrations are shown in Figure 2c,d. The S-Mg significantly reduced the soil ACd compared to the control, but the S-Zn resulted in a significantly higher soil ACd than the S-Mg and S-Fe did.

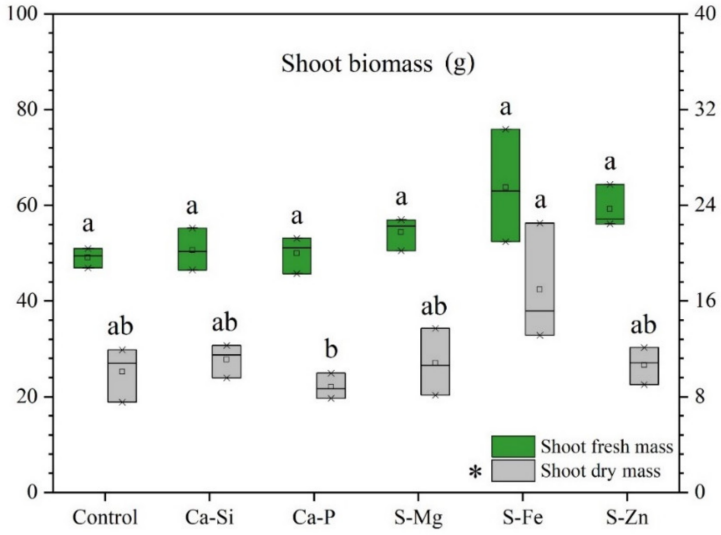

Figure 1. The shoot biomass at the final harvest. The average spinach shoot fresh mass and dry mass were shown, with the X-axis showing the treatments and the Y-axis showing the corresponding mass units. The numbers sharing different letters were in different Tukey's HSD homogenous subsets. * in the figure means significant differences existed (lower case a and b) in the parameter of shoot dry mass among different Ca and S treatments.

Table 2. The nutrient concentration in the spinach shoots at the final harvest. The average concentrations of N, P, K, Ca, and Mg in the plant shoot were shown. The numbers sharing different letters were in different Tukey's HSD homogenous subsets.

	N	P	K	Ca	Mg
	g kg^{-1}	**mg kg^{-1}**	**g kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**
Control	13.03 ± 1.21 a	23.90 ± 1.85 c	2.95 ± 0.23 ab	119.69 ± 10.19 abc	96.65 ± 12.44 a
Ca-Si	14.17 ± 0.49 a	56.57 ± 16.55 bc	2.79 ± 0.11 ab	123.54 ± 9.16 abc	49.48 ± 19.80 ab
Ca-P	12.27 ± 0.45 a	160.00 ± 8.91 a	3.41 ± 0.04 a	150.83 ± 7.67 a	12.34 ± 0.39 b
S-Mg	13.05 ± 0.90 a	64.20 ± 4.80 bc	2.54 ± 0.13 bc	118.59 ± 4.05 bc	87.41 ± 1.94 a
S-Fe	15.60 ± 0.41 a	34.10 ± 11.30 bc	1.88 ± 0.13 c	94.08 ± 0.39 c	87.99 ± 18.98 a
S-Zn	14.56 ± 0.22 a	70.93 ± 6.19 b	2.43 ± 0.18 bc	138.70 ± 2.75 ab	37.27 ± 5.20 ab
ANOVA		***	***	**	**

Note: '**' for $p < 0.01$, '***' for $p < 0.001$.

Table 3 showed the average root TCd, BF_{shoot}, and BF_{root}, as well as TF. The Ca-P and S-Zn significantly reduced the root TCd by 42.1% and 49.3% compared to control, respectively, and they also resulted in the lowest and second lowest BF_{shoot}, but Tukey's HSD multiple comparison did not separate them into another homogenous subset. In addition, Ca-P and S-Zn significantly reduced the BF_{root} by 45.1% and 51.6%, respectively, compared to the control. However, none of the treatments reduced the TF when compared to the control, and S-Zn significantly increased the TF compared to Ca-Si and S-Mg.

3.3. Soil Physicochemical Properties and Nutrient Condition

Table 4 shows the average soil pH, EC, and concentrations of OM, TN, AP, and AK. All three sulfur fertilizers significantly reduced the soil pH compared to control, while the two kinds of calcium fertilizers decreased the soil EC compared to control. The Ca-P increased soil AP by 99%, and other soil condition traits were not impacted by the treatments.

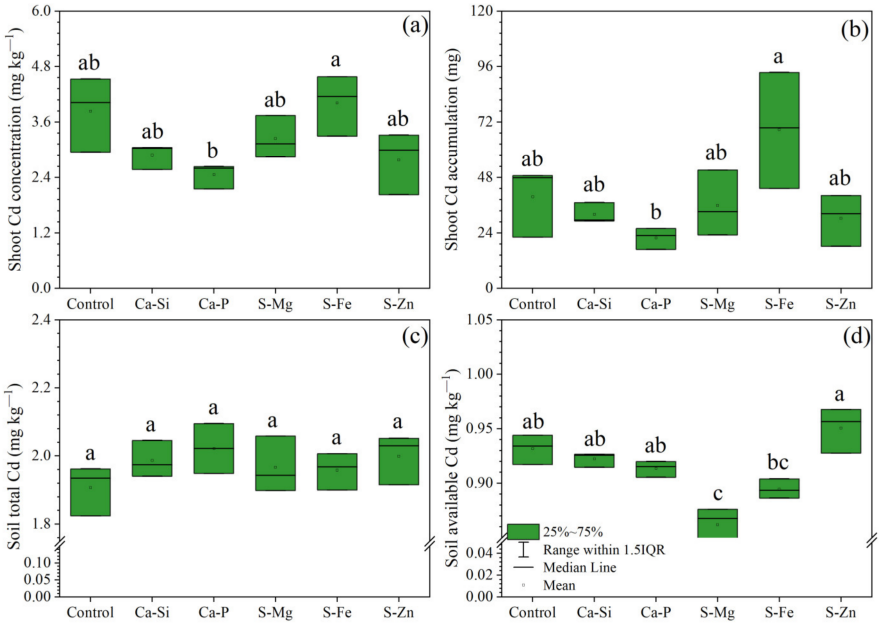

Figure 2. The spinach shoot Cd and soil Cd levels at the final harvest. The dry mass basis average total Cd concentration in the plant shoot (**a**), the dry mass basis average total Cd accumulation in the plant shoot per plant (**b**), the average soil total Cd concentration (**c**), and the average soil available Cd concentration (**d**) were shown. The numbers sharing different letters were in different Tukey's HSD homogenous subsets.

Table 3. The Cd concentration, Cd bioconcentration factors, and Cd translocation factors at the final harvest. The average root total Cd concentration (Root TCd), the shoot Cd bioconcentration factor (BF_{shoot}), the root Cd bioconcentration factor (BF_{root}), and the root-to-shoot Cd translocation factor (TF) were shown. The numbers sharing different letters were in different Tukey's HSD homogenous subsets.

	Root TCd	**BF_{shoot}**	**BF_{root}**	**TF**
	mg kg^{-1}			
Control	2.92 ± 0.14 a	2.01 ± 0.25 a	1.53 ± 0.10 a	1.31 ± 0.11 ab
Ca-Si	2.40 ± 0.11 ab	1.45 ± 0.08 a	1.21 ± 0.05 ab	1.20 ± 0.01 b
Ca-P	1.69 ± 0.21 b	1.22 ± 0.10 a	0.84 ± 0.12 b	1.49 ± 0.18 ab
S-Mg	2.71 ± 0.12 a	1.65 ± 0.16 a	1.38 ± 0.09 a	1.19 ± 0.05 b
S-Fe	2.85 ± 0.29 a	2.05 ± 0.19 a	1.46 ± 0.15 a	1.46 ± 0.26 ab
S-Zn	1.48 ± 0.27 b	1.40 ± 0.21 a	0.74 ± 0.14 b	1.92 ± 0.12 a
ANOVA	***	*	**	*

Note: '*' for $p < 0.05$, '**' for $p < 0.01$, '***' for $p < 0.001$.

3.4. The Relationship between Plant Growth, Plant Cd Uptake, Soil Physicochemical Properties, and Nutrient Conditions

Figure 3 shows the Pearson correlation matrix built between the plant growth traits, plant Cd uptake traits, and soil physicochemical properties traits. The Y-axis listed the leaf fluorescence traits, plant biomass, and plant Cd uptake traits from top to bottom. The X-axis listed the plant nutrients uptake and soil physicochemical properties from left to right. The plant shoot N concentration had a positive correlation with leaf fluorescence traits, plant biomass traits, and shoot Cd accumulation, and it was significantly positively correlated with shoot fresh mass (shoot FM). The shoot P was negatively correlated to most of the leaf fluorescence traits, plant biomass, and plant Cd uptake traits, and it had a significant negative correlation with the shoot total Cd concentration (shoot TCd), BF_{shoot}, shoot Cd accumulation, root TCd, and BF_{root}. Similarly, shoot K was significantly negatively correlated to the Fv/Fo, Fv/Fm, shoot FM, shoot DM, and shoot Cd accumulation, while

shoot Ca was significantly negatively correlated to the shoot DM, shoot TCd, BF_{shoot}, shoot Cd accumulation, root TCd, and BF_{root}. On the contrary, the shoot Mg was positively correlated to most of the traits assessed and had a significant positive correlation with shoot TCd, BF_{shoot}, shoot Cd accumulation, root TCd, and BF_{root}.

Table 4. The soil physicochemical properties and nutrients condition at the final harvest. The average soil pH, electricity conductivity (EC), organic matter concentration, total nitrogen concentration (TN), available phosphorus concentration (AP), and available potassium concentration (AK) were shown. The numbers sharing different letters were in different Tukey's HSD homogenous subsets.

	pH	EC	OM	TN	AP	AK
			g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}
Control	8.24 ± 0.06 a	247.00 ± 4.04 a	18.23 ± 0.42	1.12 ± 0.01	8.97 ± 0.12 b	160.85 ± 2.72
Ca-Si	7.95 ± 0.08 ab	186.00 ± 7.81 b	18.88 ± 0.56	1.15 ± 0.01	9.92 ± 0.40 b	168.22 ± 7.25
Ca-P	7.90 ± 0.06 ab	188.83 ± 5.89 b	19.82 ± 1.15	1.15 ± 0.10	17.85 ± 0.86 a	181.77 ± 9.29
S-Mg	7.64 ± 0.06 b	239.67 ± 5.49 a	18.66 ± 0.76	1.17 ± 0.03	8.98 ± 0.05 b	165.62 ± 1.89
S-Fe	7.77 ± 0.06 b	254.00 ± 3.00 a	19.02 ± 0.60	1.16 ± 0.01	9.22 ± 0.41 b	162.68 ± 2.05
S-Zn	7.71 ± 0.11 b	238.33 ± 3.71 a	18.88 ± 0.80	1.14 ± 0.01	9.23 ± 0.43 b	172.83 ± 9.81
ANOVA	**	***			***	

Note: '**' for $p < 0.01$, '***' for $p < 0.001$.

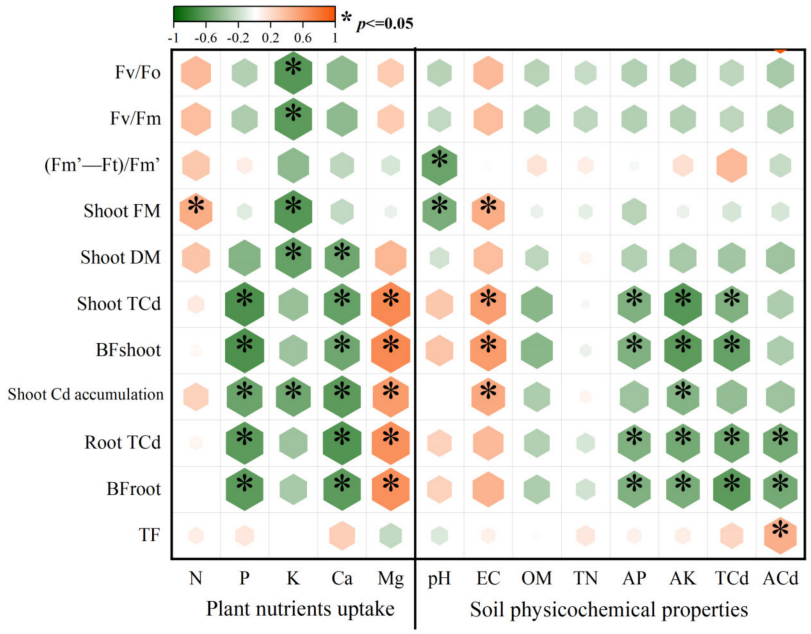

Figure 3. The Pearson correlation between spinach growth traits, spinach Cd uptake, and soil conditions. The Y-axis is lining the Fv/Fo, Fv/Fm, (Fm' − Ft)/Fm', shoot fresh mass (shoot FM), shoot dry mass (shoot DM), shoot total Cd concentration (shoot TCd), Cd bioconcentration factor of shoot (BFshoot), total Cd accumulation in plant shoot (shoot Cd accumulation), root total Cd concentration (root TCd), Cd bioconcentration factor of root (BFroot), and root to shoot Cd translocation factor (TF). The X-axis lines the concentration of total nitrogen (N), total phosphorus (P), total potassium (K), total calcium (Ca), and total magnesium (Mg) in the plant shoot, and the soil pH, electric conductivity (EC), organic matter (OM), total nitrogen (TN), available phosphorus (AP), available potassium (AK), total Cd (TCd), and available Cd (ACd).

The soil pH was significantly negatively correlated to (Fm' − Ft)/Fm' and shoot FM, but the soil EC was significantly positively correlated to shoot FM, shoot TCd, BF_{shoot}, and shoot Cd accumulation. The soil AP, AK, and TCd were significantly negatively correlated

to shoot TCd, BF_{shoot}, root TCd, and BF_{root}, while the soil ACd was significantly negatively correlated to root TCd and BF_{root} but positively correlated to TF.

The principal component analysis of plant growth traits, plant Cd uptake, and soil conditions is shown in Figure 4. The components 1 and 2, respectively, explained 51.6% and 15.8% of the total variance of the data points representing the traits assessed. The data points clustered into two groups as two 95% confidence ellipses, grouping by the fertilizer's main effective contents, which were calcium and sulfur. The (Fm'-Ft)/Fm', shoot FM, soil EC, shoot Cd accumulation, and shoot DM were positively impacted by both component 1 and component 2. The shoot TCd, shoot Mg, BF_{root}, and root TCd were positively impacted by component 1 but negatively impacted by component 2. The shoot P, shoot K, soil AP, and soil pH were negatively impacted by both component 1 and component 2. While the TF, soil ACd, and shoot Ca were negatively impacted by component 1, they were positively impacted by component 2.

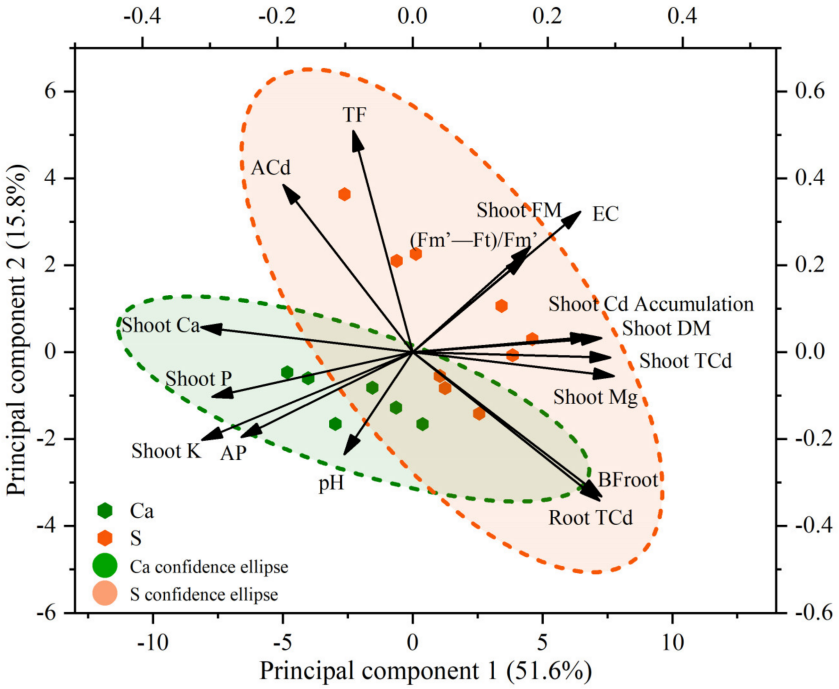

Figure 4. The principal component analysis of the spinach growth traits, spinach Cd uptake traits, and soil conditions. The growth traits included the (Fm' − Ft)/Fm', shoot fresh mass (shoot FM), shoot dry mass (shoot DM), and total phosphorus (shoot P), total potassium (shoot K), total calcium (shoot Ca), and total magnesium (shoot Mg) in the plant shoot. The spinach Cd uptake traits included shoot total Cd concentration (shoot TCd), total Cd accumulation in plant shoot (shoot Cd accumulation), root total Cd concentration (root TCd), Cd bioconcentration factor of root (BFroot), and root-to-shoot Cd translocation factor (TF). And the soil conditions traits included the pH, electric conductivity (EC), available phosphorus (AP), and available Cd (ACd).

4. Discussion

In the current experiment, we found that the Cd detoxification, Cd uptake, biomass accumulation, and photosynthesis capacity of spinach grown in alkaline soil could not be promoted at the same time. The fertilizers that suppressed Cd uptake would also suppress plant growth, while the fertilizers that promoted biomass accumulation also increased Cd concentration and total uptake. In the meantime, all the treatments decreased the soil pH, but their influence on the soil ACd did not consistent with their impact on the soil pH.

The phytotoxicity of Cd is usually reflected in growth suppression [53,54]; therefore, promoting biomass accumulation is a phenomenon of detoxification. In this case, the sulfur fertilizer performed better than the calcium fertilizer. Many studies focused on plant Cd

uptake control by adding sulfur have been reported. Adhikari et al. (2018) [8] investigated the influences of sulfate supplementation into culture media on the Cd uptake of corn, and the results demonstrated that adding sufficient sulfate markedly restored the plant shoot biomass and significantly reduced Cd uptake as well as tissue Cd accumulation, and the growth improvement was associated with sulfur-mediated relief of Cd stress. Similarly, Shen et al. (2021) [55] investigated the impacts of sulfur addition to soil on the Cd uptake of water spinach, and the results demonstrated that adding sulfur significantly increased the plant biomass and considerably reduced its shoot Cd concentration (31%). The promotion in the expression of PME and LAC genes, accompanied by an increase in PME activity and lignin content (cell wall components), can well explain the increase in biomass and the reduction of Cd transport to plant shoots [55].

In our experiment, the S-Fe achieved both the highest shoot fresh mass and the highest shoot dry mass, showing a preferable effect on growth promotion. The shoot N of plants subjected to S-Fe was the highest among all treatments. On this basis, the addition of S-Fe could facilitate the spinach's growth significantly, which was related to the increase in N uptake and utilization in the leaf chlorophyll, remediating the leaf's photosynthetic capacity. Additionally, spinach is an iron-rich crop, highly demanding in iron compared to other leafy greens [56]. The extra Fe^{3+} supplemented in the rhizosphere of plants can increase optical energy uptake, transfer, and electron transfer of the PSII of the plant's functional leaves and strengthen CO_2 carboxylation fixation in the photosynthetic dark reaction, thus increasing the net photosynthesis rate and biomass of plants [57]. However, at the same time, the shoot Cd of S-Fe plants was not reduced, so there was no biomass dilution effect happening like other researchers found [58], indicating a different mechanism of Cd detoxification and that the high Cd concentration inside the leaf would not hamper the photosynthesis process under the high Fe^{3+} availability. This inference was proved by the leaf fluorescence traits: the S-Fe plants had the highest Qy, which represented the actual photochemical efficiency of PSII [59]. The influence of Fe on plant Cd uptake not only closely relates to crop characteristics but also greatly depends on the Fe concentration tested. Research on rice showed that moderate Fe addition significantly decreased citric acid and increased the ionic soluble pectin content in roots, thereby providing more Cd-binding sites in the cell wall of roots and reducing Cd transport in the xylem. However, excess Fe enhanced Cd enrichment on the root of the plant by the formation of iron plaques, which indicates the coexistence of antagonistic and synergistic Fe-Cd interactions in some crops [60].

As shown in Figure 2, the changes in soil ACd followed a similar trend as the changes in plant shoot Cd accumulation, indicating that Cd availability was still the main factor impacting the total amount of plant Cd uptake. Among the three sulfur fertilizers, only S-Zn tended to reduce the Cd concentration in the plant shoot. In this experiment, a reduction of shoot Mg was observed after the S-Zn treatment, along with a reduction of the Cd concentration and accumulation in plant shoots. The S-Fe and S-Mg treatments achieved relatively higher Cd and Mg contents in the plant shoot than the other three fertilizers, indicating that Cd and Mg in the plant shoot had a similar change in trend. The positive correlation between shoot Mg and the traits of plant Cd uptake indicated the uptake of the two ions might have a synergistic effect. Whether a synergistic uptake between Mg and Cd in plants is present remains unknown, but Mleczek et al. (2011) [61] reported that increasing Mg content may reduce the Ca to Mg ratio, thereby increasing the Cd concentration of plants. Thus, controlling the soil Mg availability appropriately may be beneficial to decrease the Cd uptake of crops. Zn is viewed as an antagonistic element that restricts the Cd uptake of plants, and it might be due to the similar structures between Zn^{2+} and Cd^{2+}, leading to competition in root cell membrane uptake [9]. Hence, Zn^{2+} decreased the Cd uptake in the plant shoot significantly when used as an accompanying cation of sulfate. Umar et al. (2023) [62] proved that soil Zinc application significantly mitigated the toxic effects of Cd on the growth and physiological parameters of wheat, significantly increased Zn concentration, and decreased Cd concentration in grains of wheat, which is similar to the results in the present study.

The calcium fertilizers showed a better effect on reducing the spinach's Cd concentration than the sulfur fertilizers. In this experiment, a sharp reduction of shoot Mg was observed after the calcium fertilizer treatments, along with a reduction of the Cd concentration and accumulation in plant shoots. Ca^{2+} and Cd^{2+} have similar structures, resulting in competitive uptake on the root surface [63]. Hence, increasing the Ca^{2+} supply in the rhizosphere solution is likely to decrease the Cd uptake of the root by increasing the Ca uptake. Hakeem et al. (2022) [24] pointed out that exogenous calcium chloride treatment relieves the Cd toxicity of buckwheat and decreases the Cd uptake of buckwheat. The Cd uptake control effect follows the order: Ca-P > Ca-Si. By contrast, Ca-P and Ca-Si treatments decrease the Cd content in the aboveground part by decreasing the Cd uptake of the root. Ca-P treatment best controls Cd uptake in the plant shoot in this experiment. Rhizosphere P improvement is conducive to increasing photosynthesis and biomass accumulation in the aboveground part of plants, thus enabling the dilution of Cd concentration [64]. Besides, increasing plant P intake can help with the scavenging of reactive oxygen species (ROS) and chelation-mediated Cd detoxication [65]. The inner plant phosphorus regulates antioxidant enzyme activities, eliminates ROS, and alleviates lipid oxidation damage to the plant cytomembrane by increasing GSH [64]. On the other hand, P improvement forms PCs-Cd chelates by facilitating the synthesis of PCs, thus mediating the Cd detoxication of plants and decreasing the adverse effects of Cd stress on plants [66,67]. In the present study, Ca-P treatment also greatly improved plant K nutrition status, which might indirectly help alleviate the Cd stress and reduce Cd uptake through the "dilution effect" by increasing the plant biomass, as confirmed by studies on soybean, wheat, chickpea, and gladiolus [68,69].

5. Conclusions

Our research confirms that the company elements impact the detoxification effect of calcium and sulfur on the spinach due to the Cd stress. The calcium fertilizers had advantages in reducing shoot Cd uptake, especially when the company element was phosphorus, which resulted in the lowest shoot Cd concentration. The sulfur fertilizer had advantages in growth promotion, in which the photosynthesis capacity, macronutrient uptake, and biomass accumulation were all stimulated, and Fe was the best company element for sulfur, resulting in the highest biomass and photosystem quantum yield and Cd uptake. The decrease in pH within a certain range may not be associated with the available Cd in alkaline soil; rhizosphere metabolites mediated by changes in plant growth may be the reason. The potential value of Ca-P in reducing the Cd pollution risk of spinach and the auxiliary effects of S-Fe and S-Mg in enhancing Cd extraction by spinach deserve attention in further research and agricultural practice.

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