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Calcium-Associated Anions Play a Dual Role in Modulating Cadmium Uptake and Translocation in Wheat

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Abstract: Cadmium accumulation in wheat as a daily food, even in low concentrations, is a serious threat to human health. Previous studies have reported conflicting results on the impact of calcium treatments on cadmium uptake and translocation in plants due to the complex soil conditions. Our hydroponic study offers clearer insights into how specific calcium treatment parameters influence cadmium uptake and translocation in wheat. The hydroponic medium was contaminated by cadmium ($CdCl_2$) and the following treatments were applied: $CaCO_3$, $CaSO_4$, $CaCl_2$, $CaCO_3$ + $CaSO_4$, $CaCO_3$ + $CaCl_2$, and $CaSO_4$ + $CaCl_2$. After harvesting, the wheat was analyzed for Cd^{2+} uptake characteristics including translocation factor, bioconcentration factor, and uptake. Furthermore, physiological growth parameters and plant nutrients were also determined. Applying $CaCO_3$ significantly decreased wheat Cd^{2+} concentration by about three times in $CaCO_3$ and two times in $CaCO_3$ + $CaSO_4$ and $CaCO_3$ + $CaCl_2$ treatments than in Cd-control. This study clearly elucidates that pH and CO_3^{2-} were crucial in reducing Cd^{2+} concentration in wheat. SO_4^{2-}, Cl^-, and Ca^{2+} showed no effect on Cd^{2+} concentration. Ca^{2+} only reduced the translocation factor (TF) of Cd^{2+} in plants. $CaCO_3$ also declined cadmium interference in the Mg^{2+}, Mn^{2+}, and Cu^{2+} uptake. Therefore, this study provides novel insight into the pure effects of calcium treatments on controlling cadmium contamination in plants, independent of soil effect.

Keywords: cadmium; uptake; translocation; calcium treatments; wheat

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1. Introduction

Increasing concern about the introduction of heavy metals into the human food chain, especially in agricultural activities, has been noticed in recent years. Among all heavy metals, cadmium (Cd^{2+}) has received considerable attention due to its high solubility and mobility in soils and its toxicity for plants and humans [1]. In many epidemiological studies, the correlation between the environmental exposure of humans to cadmium and diseases such as stroke, ischemia, renal and hepatic dysfunction, anemia, osteoporosis, and diabetes has been discussed. Cadmium poisoning has been reported worldwide, causing many deaths annually [2]. Cadmium exposure mainly results from eating cadmium-contaminated food. It has been estimated that more than 80% of dietary cadmium intake comes from cereals (especially rice and wheat), vegetables (especially leafy greens), and root vegetables (especially potatoes and carrots) [3]. Cadmium is easily uptaken by plant roots and accumulates in plants. A growing number of epidemiological evidence suggests that between current cadmium exposure levels and the threshold for adverse health effects, there is no margin of safety; therefore, reducing cadmium consumption by humans is an essential need. Understanding the mechanisms of cadmium retention in the root and its translocation from root to shoot and grain enables the development of low Cd-accumulating crops [4].

Cd in soil exists primarily as Cd(II), $Cd(OH)_2$, $CdCO_3$ solids, and aqueous Cd sulfates, with interactions with P, As, Cr, and other anions influenced by soil pH and chemical

factors. Cd(II) in soil can be immobilized via adsorption, precipitation, reduction, ion exchange, surface complex formation, hydrogen bonding, interactions, and pore filling [5].

Studies have revealed that calcium (Ca^{2+}) treatments can decline Cd^{2+} availability in soils and their uptake by plants. Adding lime into the soil increases pH and Cd^{2+} can precipitate as $CdCO_3$ and Cd $(OH)_2$, thereby decreasing Cd^{2+} availability for plant uptake [6], though most of the micronutrients tend to be less available when soil pH is above 7.5. The production of $CdSO_4$ complexes is less available than Cd^{2+} [7]. In contrast, some studies showed an increase in Cd^{2+} concentration in plants through applying gypsum [8]. It is reported that sulfate application increased $CdSO_4$ proportion in the growth medium and subsequently in plants because the diffusion of $CdSO_4$ is faster than Cd^{2+} in plants [9]. In addition, another study found that gypsum only reduced Cd^{2+} uptake in plants in the soil sample with the highest cation exchange capacity (CEC) and the highest Ca^{2+} concentration in solution, because of possible competition between Ca^{2+} and Cd^{2+} for root uptake [10]; the study investigated six soil types with variable CECs and soil solutions with Ca^{2+}. It has been stated that with the increasing concentration of supplemented $CaCl_2$, Cd^{2+} uptake in plants and its concentration in the aboveground parts of plants increased significantly [11]. The availability of Cd^{2+} can increase in the presence of Cl^- through the formation of soluble inorganic chloride complexes such as $CdCl^+$ and $CdCl_2$ [12]. Another study showed that although applying $CaCl_2$ in the rhizosphere increased Cd^{2+} uptake into roots, the transport of Cd^{2+} from roots through the stem to leaves was suppressed by Ca^{2+} treatment [13]. The uptake of Cd^{2+} ions occurs via the same transmembrane carriers used for the uptake of other divalent cations including Ca^{2+}, Fe^{2+}, and Mg^{2+}. In addition, the membrane potential of root epidermal cells creates a strong driving force for the cation's uptake. Therefore, the presence of excess Ca^{2+} in the rhizosphere may have resulted in the depolarization of membrane potential, and therefore Cd^{2+} uptake was increased [13]. The decreasing concentration of Cd^{2+} in the root apoplast may be attributed to the competition of Ca^{2+} with Cd^{2+} for binding sites in the cell walls. Ca^{2+} affects Cd^{2+} transport by reducing its mobility within the plant and enhancing its sequestration into cellular compartments, such as vacuoles, which limits Cd's translocation from roots to shoots and its overall toxicity [14]. Therefore, in the competition between Ca^{2+} and Cd^{2+} for root uptake, applying Ca^{2+} can decrease Cd^{2+} concentration and bioaccumulation in plants [10]. Calcium (Ca^{2+}) reduces the mobility of cadmium within the plant and enhances its sequestration into cellular compartments, such as vacuoles, thereby limiting the translocation of cadmium from roots to shoots and reducing its overall toxicity [6,10,13].

The results about the effect of Ca^{2+} treatments on Cd^{2+} availability and its translocation in plants are inconsistent. The factors that can affect the Cd^{2+} uptake by plants under Ca^2 treatments are Ca^{2+}, along with anions, and their effect on pH. These three factors and their interactions with the soil conditions determine the effect of calcium treatments on Cd^{2+} uptake and translocation in plants. By using different calcium treatments individually and in combination, we could better identify the more effective and dominant parameters (Ca^{2+}, anions, and pH) in cadmium uptake and its translocation in plants. Since the soil is a complex environment with adsorption and desorption processes, we decided to perform this experiment hydroponically. In this way, the effect of soil clay particles on the surface adsorption of Ca^{2+} and Cd^{2+}, the possibility of washing anions to the lower layers of the soil, the effect of the buffering capacity of soils on soil pH, and the presence of organic substances were eliminated. Therefore, we could clarify the net effect of calcium treatments.

Additionally, some studies showed that the presence of cadmium leads to mineral nutrient deficiencies. The concentration of Mg^{2+}, Ca^{2+}, and K^+ was decreased in cucumber, maize, tomato, and lettuce due to high levels of Cd^{2+} in the soil [14]. Toxic heavy metals can compete with the transport systems operating for nutrient uptake, and this occurs by using the same transmembrane carriers for the uptake of Ca^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+}, and Zn^{2+} ions [15,16]. The effect of Ca^{2+} treatments applied in Cd^{2+}-contaminated situations on nutrient uptake by the plant has not been investigated yet, which is substantially important for selecting the better Ca^{2+} treatment.

In this research, the following hypotheses are studied: (1) $CaCl_2$ treatment will increase $CdCl_2$ in solution which increases Cd^{2+} uptake by wheat, and $CaSO_4$ as well as $CaCO_3$ treatments will precipitate cadmium as $CdSO_4$ and $CdCO_3$ in solution and decrease Cd^{2+} concentration in plant. (2) Cd^{2+} uptake and translocation in wheat can be suppressed by Ca^{2+} treatments. (3) Metal nutrient (Mg^{2+}, Mn^{2+}, Fe^{2+}, $Cu^{2+,}$ and Zn^{2+}) uptake will be decreased with increasing pH in $CaCO_3$ treatments.

The results of other studies about the effect of Ca^{2+} treatments on Cd^{2+} uptake and translocation are not the same due to complex soil conditions. This research funding provides a novel insight about the pure effects of Ca^{2+} treatments on Cd^{2+} uptake and translocation in wheat, independent of their interaction with soil parameters (pH, buffering capacity, soil texture, etc.). By attaining a thorough understanding of the direct effects of Ca^{2+} treatments, it becomes possible to predict how these treatments will function in controlling cadmium contamination across diverse soils with varying characteristics.

2. Materials and Methods

2.1. Plant Growing Conditions

Wheat (Scirocco variety) was hydroponically cultivated under standard greenhouse conditions (18 °C day/14 °C night cycle, and a 14 h photoperiod). Wheat seedlings (15 days old) were transferred into containers with an aerated nutrient solution of the following composition: 2.0 mM $Ca(NO_3)_2$, 0.2 mM KCl, 0.2 mM KH_2PO_4, 0.5 mM $MgSO_4$, 2 mM $CaCl_2$, 1 mM K_2SO_4, 0.2 mM Fe-EDTA, 5 µM H_3BO_3, 0.3 µM $CuSO_4$, 0.5 µM $ZnSO_4$, 2 µM $MnSO_4$, and 0.01 µM $(NH_4)_6Mo_7O_{24}$, and nutrient solutions were replaced every week. After 2 weeks, the following treatments were established: Cd^{2+} ($CdCl_2$: 10 µM) and Ca^{2+} treatments (control: 0, $CaCO_3$ (4 mM)), $CaSO_4$ (4 mM), $CaCl_2$ (4 mM), $CaCO_3$ and $CaSO_4$ (4+4 mM), $CaCO_3$ and $CaCl_2$ (4 + 4 mM) and $CaSO_4$ and $CaCl_2$ (4 + 4 mM) in four replications. After three weeks of exposure, plants were harvested and analyzed.

2.2. Elemental Analyses

The fresh weight of the aerial parts and roots was considered as an indicator of fresh shoot and root biomass. Plant tissues were ground and digested (HNO_3: $HClO_4$; 4:1) and analyzed for total Cd^{2+} concentrations and nutrients of Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+}, determined through inductively coupled plasma (ICP-OES and ICP-MS) (Agilent Technologies 7700 Series, Boebelingen, Germany). Nutrients were determined to understand the effect of treatments under Cd^{2+} contamination on nutrient uptake. Translocation factor (TF), bioconcentration factor (BCF), and uptake were determined as follows:

$$\text{Translocation factor (Roots-Shoots) (TF): Concentration in shoot/Concentration in roots} \quad (1)$$

$$\text{Bioconcentration factor (BCF): Plant tissue concentration (mg kg}^{-1}\text{)/Concentration in water (mg L}^{-1}\text{)} \quad (2)$$

$$\text{Uptake: [Concentration in shoot} \times \text{Shoots dry weight] + [Concentration in root} \times \text{Roots dry weight]} \quad (3)$$

Visual MINTEQ 4.1 is a software chemical equilibrium model for the calculation of metal speciation, solubility equilibria, sorption, etc., in liquids. In this study, soluble and precipitated forms of cadmium in the nutrient solution were calculated by inputting elements of the plant growth medium through the chemical speciation model, Visual 95 MINTEQ.

2.3. Statistical Analysis

The statistical analysis was conducted by using SPSS 17.0 statistical software. ANOVA was used for calculating the statistical significance of differences. Means values were compared using the Duncan test (at a test level of $\alpha = 0.05$). Relations between pH, Ca^{2+}, and anions with Cd^{2+} in plant and nutrient uptake were calculated using Pearson's correlation.

3. Results

3.1. Effect of Ca^{2+} Treatments on Cd^{2+} Uptake and Translocation in Wheat

Applying Ca^{2+} treatments has a significant effect on the pH of the nutrition solution, fresh root and shoot biomass, the Cd^{2+} concentration in the shoot and root, and the Cd^{2+} bioconcentration factor (BCF). However, the Cd^{2+} translocation factor (TF) does not significantly change with the application of Ca^{2+} treatments.

3.1.1. Effect of Ca^{2+} Treatments on pH of Nutrition Solution

By adding $CaCO_3$ treatments, pH increased significantly from 6.6 in the control solution to 7.8 as the maximum pH (Table 1). Additionally, $CaCO_3$ increased pH in its combination treatments (($CaCO_3$ + $CaSO_4$) and ($CaCO_3$ + $CaCl_2$)) rather than control. However, both $CaSO_4$ and $CaCl_2$ treatments and their combination treatment decreased the nutrient solution pH significantly, rather than the control. The lowest pH (5.8) was observed in the treatment $CaCl_2$ (Table 1).

Table 1. Nutrient solution pH under different Ca^{2+} treatments.

Treatment	pH
Control	6.62 ± 0.02 c
$CaCO_3$	7.82 ± 0.03 a
$CaSO_4$	6.44 ± 0.02 d
$CaCl_2$	5.78 ± 0.04 f
$CaCO_3$ + $CaSO_4$	6.72 ± 0.02 b
$CaCO_3$ + $CaCl_2$	6.79 ± 0.02 b
$CaSO_4$ + $CaCl_2$	6.30 ± 0.04 d

Note: Values (±SE) are means of four independent pot replicates. Letters indicate significant differences between treatments ($p \leq 0.05$).

3.1.2. Effect of Calcium Treatments on Root and Shoot Fresh Biomass

The total root fresh weight in the control solution polluted with Cd^{2+} was 1.4 (g pot^{-1}), which is enhanced significantly to 8.0 (g pot^{-1}) in $CaCO_3$ treatment and 7.5 (g pot^{-1}) in $CaCO_3$ + $CaSO_4$. The total root fresh biomass was also improved in $CaCO_3$ + $CaCl_2$ treatment than in the control, but it was not significant. The difference in root fresh biomass between other treatments was not significant compared with the control (Figure 1).

The shoot fresh biomass in different Ca^{2+} treatments had the same trend as the root fresh biomass. Only the addition of $CaCO_3$ and its combined treatments $CaCO_3$ + $CaSO_4$ and $CaCO_3$ + $CaCl_2$ could significantly increase the shoot fresh biomass to 16.5, 14.3, and 11.2 (g pot^{-1}), respectively, while the biomass was 3.8 g pot^{-1} in control (Figure 1a). These results can also be seen in the photos taken of the plant in Figure 1b,c.

3.1.3. Effect of Ca^{2+} Treatments on Cd^{2+} Concentration in Wheat and Bioconcentration Factor

The Cd^{2+} concentration in the wheat root was 1092 (μg/g dry weight) in the control treatment. Both $CaCO_3$ and $CaCO_3$ + $CaSO_4$ treatments could reduce the Cd^{2+} concentration in the root by about half (Figure 2).

The concentration of Cd^{2+} in the shoot in the control was 104 (μg/g dry weight). $CaCO_3$ treatment decreased wheat shoot Cd^{2+} concentration by about three times (37 (μg/g dry weight)). The combination treatments (($CaCO_3$ + $CaSO_4$) and ($CaCO_3$ + $CaCl_2$)) could also decline shoot Cd^{2+} concentration by about two times (Figure 2).

Figure 3 shows the Cd^{2+} bioconcentration factor (BCF) in whole parts of wheat under different Ca^{2+} treatments. The two treatments of $CaCO_3$ and $CaCO_3$ + $CaSO_4$ significantly declined the Cd^{2+} bioconcentration factor from 1064 in the control treatment to around half of it, at 472 and 616, respectively.

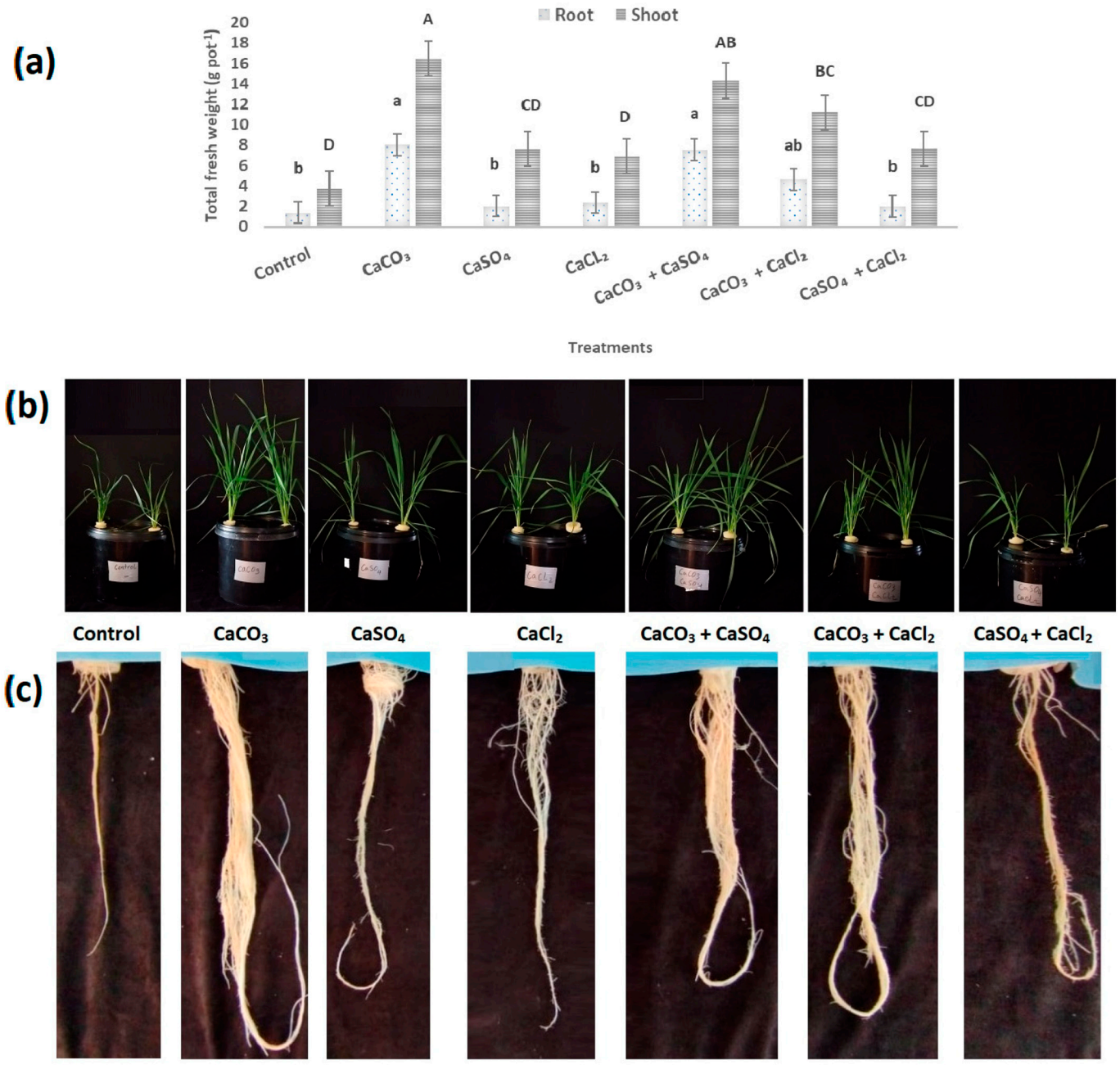

Figure 1. Ca^{2+} treatment's effect on fresh root and shoot biomass after growing for three weeks on a nutrient solution supplemented with 10 µmol Cd^{2+} and under control (Cd^{2+}) and Ca^{2+} treatments. Figure (**a**) shows weight of fresh root and shoot biomass (g pot^{-1}). The bars are means ± standard error of four replicates. Letters indicate significant differences between treatments ($p \leq 0.05$). Figure (**b**) and Figure (**c**) shoot and root of wheat, respectively.

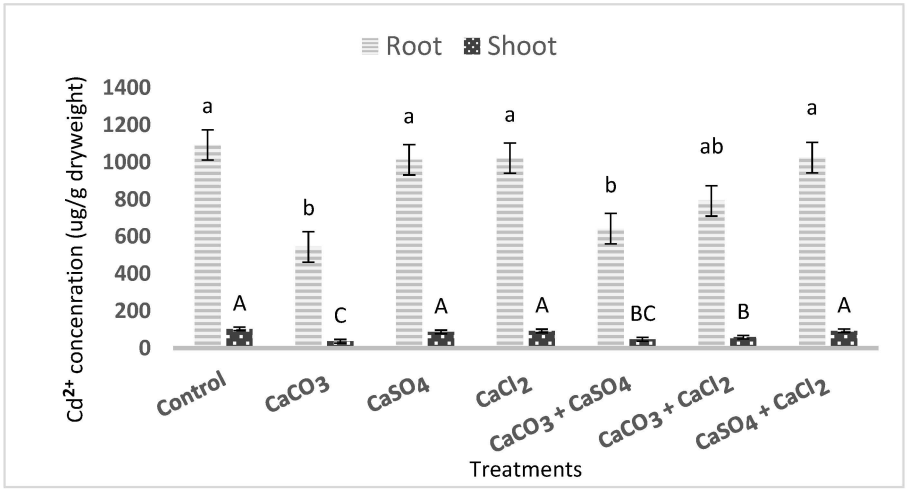

Figure 2. Effect of Ca^{2+} treatments on Cd^{2+} concentration in root and shoot. The bars are means ± standard error of four replicates. Letters indicate significant differences between treatments ($p \leq 0.05$).

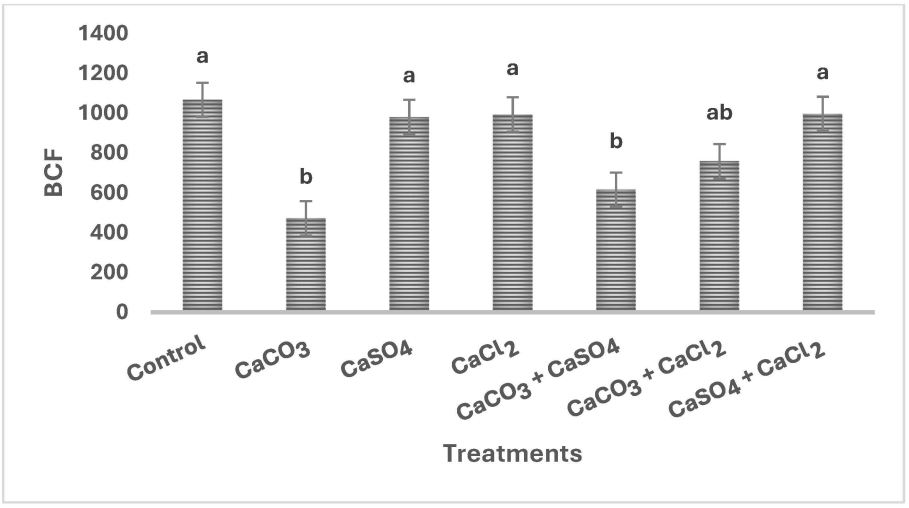

Figure 3. Effect of Ca^{2+} treatments on Cd^{2+} bioconcentration factor (BCF) in whole wheat. The bars are means ± standard error of four replicates. Letters indicate significant differences between treatments ($p \leq 0.05$).

Since different parameters of Ca^{2+} treatments have different effects on Cd^{2+} uptake and translocation in the plant, statistical correlations were used to clarify their effects (Table 2). The pH showed a very significant negative correlation with root and shoot Cd^{2+} concentration and BCF. However, pH did not correlate with the TF of Cd^{2+}. Ca^{2+} showed a significant negative correlation with the shoot Cd^{2+} concentration and TF. All four factors, Cd^{2+} concentration (root and shoot), BCF, and TF, showed a significant negative correlation with $CO_3{}^{2-}$. However, $SO_4{}^{2-}$ and Cl^- did not show a significant correlation with any of the parameters (Table 2).

To estimate different forms of Cd^{2+} in the nutrient solution, visual MINTEQ 4.1 software was used. The results showed that applying $CaCO_3$ and its combination treatments (($CaCO_3$ + $CaSO_4$) and ($CaCO_3$ + $CaCl_2$)) precipitated around 40% Cd^{2+} as $CdCO_3$. $CaSO_4$ and ($CaSO_4$ + $CaCl_2$) treatment only precipitated 14% and 9%, respectively, with Cd^{2+} as $CdSO_4$. In $CaCl_2$ and ($CaSO_4$ + $CaCl_2$) treatments, some parts of free Cd^{2+} changed to $CdCl^+$ at 30% and 26%, respectively (Table 3).

Table 2. Statistical correlations between pH, Ca^{2+}, CO_3^{2-}, SO_4^{2-}, and Cl^- with Cd^{2+} concentration (root and shoot), BCF, and TF.

	Cd^{2+} Concentration		BCF	TF
	Root	**Shoot**		
pH	−0.57 **	−0.65 **	−0.59 **	−0.25 ^{ns}
Ca^{2+}	−0.26 ^{ns}	−0.40 *	−0.28 ^{ns}	−0.41 *
CO_3^{2+}	−0.69 **	−0.87 **	−0.71 **	−0.47 *
SO_4^{2+}	0.07 ^{ns}	0.05 ^{ns}	0.07 ^{ns}	−0.08 ^{ns}
Cl^-	0.23 ^{ns}	0.24 ^{ns}	0.24 ^{ns}	−0.004 ^{ns}

ns: non-significant, * and **: significant at 0.05% and 0.01% probability levels, respectively.

Table 3. The percentage of dominant forms of cadmium in the nutrient solution was calculated using visual MINTEQ 4.1 software.

	Cd^{2+}	$CdCl^+$	CdEDTA	$CdSO_4$ (p)	$CdCO_3$ (p)	$Cd(CO_3)_2$
Control	62.14	14.98	16.18	5.94	0	0
$CaCO_3$	4.89	1.11	18.32	0.40	41.59	33.60
$CaSO_4$	56.19	11.98	16.19	14.35	0	0
$CaCl_2$	49.10	30.21	15.96	3.25	0	0
$CaCO_3$ + $CaSO_4$	7.13	1.48	18.47	1.68	42.18	28.89
$CaCO_3$ + $CaCl_2$	7.86	4.70	18.41	0.48	42.07	26.22
$CaSO_4$ + $CaCl_2$	46.68	26.44	15.98	9.23	0	0

p precipitated form of cadmium.

3.2. Effect of Ca^{2+} Treatments on Nutrient Uptake

The result of the analysis of variance of the effect of Ca^{2+} treatments on Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} uptake by wheat under Cd^{2+} contamination showed that applying Ca^{2+} significantly affected Mg^{2+}, Mn^{2+}, and Cu^{2+} uptake by wheat. However, the uptake of Fe^{2+} and Zn^{2+} did not change significantly with applying Ca^{2+} treatments.

Mg^{2+} uptake by wheat in the control treatment which was contaminated by Cd^{2+} was 2.5 (mg pot^{-1}). Applying $CaCO_3$ + $CaSO_4$ treatment significantly increased Mg^{2+} uptake by wheat around 3.7 times more than the control. Other treatments did not change Mg^{2+} uptake significantly compared to the control.

Mn^{2+} uptake was enhanced significantly through applying $CaCO_3$ from 101 (μg pot^{-1}) in the control treatment to 942, 820, and 471 (μg pot^{-1}), respectively, in the $CaCO_3$, ($CaCO_3$ + $CaSO_4$), and ($CaCO_3$ + $CaCl_2$) treatments. The maximum amount of Mn^{2+} uptake belonged to $CaCO_3$ and $CaCO_3$ + $CaSO_4$ treatments (Table 4).

Table 4. The uptake of Mg^{2+} (mg pot^{-1}), Mn^{2+} (μg pot^{-1}) and Cu^{2+} (μg pot^{-1}) by wheat in different Ca^{2+} treatments.

	Uptake		
	Mg^{2+}	Mn^{2+}	Cu^{2+}
Control	2.45 ± 0.7 b	101 ± 28 c	15 ± 5 c
$CaCO_3$	5.39 ± 0.6 ab	942 ± 32 a	70 ± 27 a
$CaSO_4$	1.79 ± 0.4 b	53 ± 8 c	37 ± 10 bc
$CaCl_2$	1.69 ± 0.3 b	51 ± 4 c	28 ± 11 bc
$CaCO_3$ + $CaSO_4$	9.01 ± 0.6 a	820 ± 32 a	82 ± 29 a
$CaCO_3$ + $CaCl_2$	3.13 ± 0.9 b	471 ± 20 b	54 ± 2 ab
$CaSO_4$ + $CaCl_2$	1.69 ± 0.3 b	65 ± 9 c	31 ± 3 bc

Note: Values (±SE) are means of four independent pot replicates. Letters indicate significant differences between treatments ($p \leq 0.05$).

The effect of Ca^{2+} treatments on Cu^{2+} uptake was the same as Mn^{2+} uptake. Applying Ca^{2+} treatments increased Cu^{2+} uptake significantly from 15 (μg pot^{-1}) in the control

treatment to 70 (μg pot^{-1}) and 82 (μg pot^{-1}) in $CaCO_3$, with ($CaCO_3$ + $CaSO_4$) as maximum amounts at 54 (μg pot^{-1}) in ($CaCO_3$ + $CaCl_2$) treatment (Table 4).

4. Discussion

4.1. Effect of Ca^{2+} Treatments on Cd^{2+} Uptake and Translocation in Wheat

Cd^{2+} can be uptaken by plants easily and accumulated in their tissues which is toxic for animals and humans, even in very low concentrations [1,2]. Using Ca^{2+} treatments can have both positive [6,7] and negative effects on the control of plant contamination with Cd^{2+} [8].

The control treatment showed the maximum amount of Cd^{2+} and bioconcentration factor (BCF). Previous studies have stated that the availability of Cd^{2+} increases in the presence of Cl^- through the formation of $CdCl^+$, and therefore it enhances Cd^{2+} uptake in plants and its concentration in plants [11,12]. In our study, $CaCl_2$ and its combination with $CaSO_4$ changed a small part of Cd^{2+} to soluble $CdCl^+$ and did not have a strong effect in reducing the amount of Cd^{2+} precipitated (Table 3). Also, the concentration of cadmium in wheat and BCF did not have a significant correlation with the Cl^- anion (Table 2). These results indicate that, contrary to previous reports which suggested the Cl^- anion as a factor enhancing cadmium uptake in plants, in our study the Cl^- anion had no significant effect on cadmium uptake and translocation in plants.

$CaSO_4$ treatment and its combination with $CaCl_2$ could only precipitate, respectively, 8% and 3% of Cd^{2+} as $CdSO_4$ more than control (Table 3). Therefore, the correlation between $SO_4{}^{2-}$ and Cd^{2+} concentration (root and shoot), BCF, and TF was not significant (Table 2). Other experiments reported that using $CaSO_4$, especially in the presence of extra Ca^{2+}, can decline Cd^{2+} concentration in plants by precipitating Cd^{2+} as $CdSO_4$ complexes in soil [7,10]. Also, they claimed $SO_4{}^{2-}$ has the potential to restrict Cd^{2+} translocation from root to shoot. Therefore, the results of the current study demonstrate the ineffectiveness of the $SO_4{}^2$ anion compared to other influential parameters on cadmium uptake and translocation in wheat.

In our experiment, only $CO_3{}^{2-}$ was able to precipitate nearly half of the cadmium in the solution phase as $CdCO_3$ and reduce the concentration of cadmium in the wheat and BCF. Carbonate also had a significant negative correlation with cadmium translocation from root to shoot (Tables 3 and 4). On the other hand, applying $CaCO_3$ to the nutrient solution led to enhanced pH (Table 1) and thereby decreased Cd^{2+} availability for plants [6], which caused a significant negative correlation with Cd^{2+} concentration in wheat and BCF (Table 2). Nevertheless, pH did not have an effect on Cd^{2+} translocation in wheat in our experiment. However, studies by Ali et al. (2020) on rice (*Oryza sativa* L.) in soil showed that Cd^{2+} translocation from root to shoot reached maximum levels at pH 6, due to the function of more genes (sNRAMP and OsHMA) responsible for the absorption and transfer of cadmium at this pH [17]. Therefore, among the calcium treatment anions, $CO_3{}^{2-}$ is identified as the most influential anion affecting cadmium uptake and its translocation in plants. This association is attributed to the $CO_3{}^{2-}$ ion's influence on pH and its role in precipitating a significant portion of Cd^{2+} from the solution phase.

Therefore, the results of this research show that the important influencing factors of calcium treatments in controlling Cd^{2+} uptake via wheat are carbonate and pH. It is for this reason that only $CaCO_3$ treatment and its combination with $CaSO_4$ or $CaCl_2$ can effectively suppress Cd^{2+} and bioconcentration factor in wheat (Figures 2 and 3).

Previous studies under soil conditions reported the positive effect of extra Ca^{2+} in decreasing Cd^{2+} concentration and bioaccumulation in plants, because of competition between Ca^{2+} and Cd^{2+} for root uptake [10]. Our study in hydroponic culture shows that adding Ca^{2+} and increasing its concentration in combined treatments did not affect Cd^{2+} concentration in the root (Table 2). However, a negative significant correlation between Ca^{2+} in nutrient solution and Cd^{2+} concentration in shoot and TF proves that the translocation of Cd^{2+} from root to shoot can be controlled significantly by applying and increasing Ca^{2+} concentration. According to Liu et al. (2023), Ca^{2+}, with its role in cell wall composition,

transporter gene expression, and transpiration, has a substantial role in Cd^{2+} resistance. The special proposed mechanisms can be the desorption of Cd^{2+} on the iron plaque of plant roots, maintaining the structural stability of cell walls, and inhibiting Cd^{2+} translocation by regulating transpiration [18]. Hayakawa et al. (2011) also reported that the presence of extra Ca^{2+} in the rhizosphere can restrict Cd^{2+} translocation because of Cd^{2+} detoxification in the vacuoles of root cells [13].

4.2. Effect of Ca^{2+} Treatments on Nutrient Uptake

Despite the positive effect of $CaCO_3$ on reducing Cd^{2+} availability and its uptake and transfer in the plant, it can lead to the reduction in essential metal nutrients uptake by the plant with an increase in pH. On the other hand, studies confirmed that the presence of Cd^{2+} contamination can disturb the equilibrium of metal nutrients and decrease their uptake [19,20]. The reason can be competition with the same transporters, a disturbance in water uptake, or an effect on key enzymes in the transport process [21].

No study shows the effect of Ca^{2+} treatments on nutrient uptake in a Cd^{2+}-contaminated environment. In this study, it was observed that the use of $CaCO_3$ alone and its combination with $CaSO_4$ and $CaCl_2$ in cadmium-contaminated environments has a positive effect on increasing the uptake of Mg^{2+}, $Mn^{2+,}$ and Cu^{2+} than the control treatment (Table 4), even in $CaCO_3$ treatment when the pH was more than 7.5 (Table 1). Therefore, the uptake disorders of nutrients in the environment contaminated with cadmium can be adjusted by applying $CaCO_3$. The reason for this was the effect of $CaCO_3$ on the precipitation of 40% of the cadmium from the soluble phase and therefore the decreasing cadmium disorder on the uptake of Mg^{2+}, Mn^{2+}, and Cu^{2+} (Table 2).

5. Conclusions

This study investigated the effectivity of Ca^{2+} treatment on Cd^{2+} uptake and translocation in wheat. The hydroponic experiment enabled us to streamline the system's complexity and identify the main influencing factors of Ca^{2+} treatments on controlling Cd^{2+} contamination in wheat. Without the influence of soil parameters (pH, buffering capacity, soil texture, etc.), the examination of the hypothesis revealed that $CaSO_4$ and $CaCl_2$ did not show a significant effect on Cd^{2+} uptake and translocation in wheat. The most effective factors of a Ca^{2+} treatment on controlling cadmium uptake via the plant are related to the effect of $CO_3{}^{2-}$ and pH. There was no significant correlation between anions of $SO_4{}^{2-}$ and Cl^- with Cd^{2+} uptake and translocation in wheat. Calcium ion has no role in reducing cadmium uptake by plants. However, cadmium translocation from roots to shoots was suppressed by Ca^{2+}. Therefore, the application of $CaCO_3$ treatment effectively leads to the reduction in cadmium concentration and its translocation from roots to aerial parts of the plant. Also, carbonate, by removing some part of cadmium from the solution phase, can reduce the negative effect of cadmium on other nutrient uptake disorders.

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