



Cadmium, silicon and nutrient accumulation by maize plants grown on a contaminated soil amended with a diatomaceous Earth fertilizer

Josévaldo Ribeiro Silva¹  Venâncio de Lima Veloso¹  Fernando Bruno Vieira da Silva¹ 
Clístenes Williams Araújo do Nascimento^{1*} 

¹Departamento de Agronomia, Universidade Federal Rural de Pernambuco (UFRPE), 52171-900, Recife, PE, Brasil. E-mail: cwascimento@yahoo.com.

*Corresponding author.

ABSTRACT: *Given that cadmium (Cd) poses high persistence in the environment and toxicity to humans, strategies to either decrease or avoid Cd entry in the trophic chain are fundamental to secure food safety. Here we assessed the effects of applying rates of Si as a diatomaceous Earth-based fertilizer on the amelioration of Cd toxicity towards maize plants grown on soil with or without pH correction. Besides determining Si and Cd concentrations, we also evaluated plant accumulation of nutrients (N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn) as a function of Si doses applied to the soil. Results showed that both the Si application and the liming had a positive effect on biomass and nutrient uptake, but the Si effect on plants' performance took place irrespectively to the soil pH. Silicon ameliorated Cd phytotoxicity in both limed and unlimed soils by decreasing Cd concentration in shoots and improving biomass yield and plant nutrition. Silicon alleviation of Cd-inhibitory effects on plants was more effective in the unlimed soil owing to the higher Cd availability in acidic soils. Also, taking into account the reduced transfer of Cd to shoots driven by Si, lower Cd accumulation in maize grains is likely, with implications to food safety. So, further studies on field conditions are warranted.*

Key words: heavy metals, plant nutrition, Zea mays, Cd, phytotoxicity.

Acúmulo de cádmio, silício e nutrientes por plantas de milho cultivadas em solo contaminado e adubado com fertilizante terra diatomáceas

RESUMO: *Considerando que o cádmio (Cd) apresenta alta persistência no ambiente e toxicidade aos seres humanos, estratégias para diminuir ou evitar a entrada de Cd na cadeia trófica são fundamentais para garantir a segurança alimentar. Neste contexto, avaliamos os efeitos de silício (Si) aplicado como fertilizante a base de terra diatomácea na amenização da toxicidade do Cd em milho cultivado em solo contaminado, com e sem correção de pH. Além de determinar as concentrações de Si e Cd, o acúmulo de nutrientes nas plantas (N, P, K, Ca, Mg, Fe, Mn, Cu e Zn) em função das doses de Si aplicadas ao solo foi avaliado. Os resultados mostraram que tanto a calagem quanto o Si tiveram efeitos positivos na biomassa e na absorção de nutrientes, mas o efeito do Si no desenvolvimento das plantas foi independente da reação do solo. O Si reduziu a fitotoxicidade por Cd em ambos os solos, corrigidos e não corrigidos, diminuindo a concentração de Cd na parte aérea. A amenização da toxicidade de Cd foi mais efetiva em solos sem correção, devido à maior disponibilidade de Cd em solos ácidos. Os benefícios da aplicação de Si sobre a produção de biomassa, composição mineral e fitotoxicidade do Cd foram observados em plantas cultivadas em solos com e sem correção. Considerando a transferência reduzida de Cd para a parte aérea promovida pelo Si, é provável que ocorra menor acúmulo de Cd nos grãos de milho, com implicações para a segurança alimentar.*

Palavras-chave: metais pesados, nutrição de planta, Zea mays, Cd, fitotoxicidade.

INTRODUCTION

Cadmium (Cd) is one of the most toxic metals for humans, plants, and animals (ATSDR, 2017). Anthropogenic activities such as mining, waste disposal, and the use of fertilizers and agrochemicals have increased Cd availability in soils. The entry of Cd into the food chain is facilitated by the metal high mobility in both soils

and plants, which, in turn, reduces the uptake of nutrients and water, inhibits photosynthesis and alters the expression of genes and proteins. Since food consumption is the main route of human exposure to contaminants, high Cd mobility is a threat to food safety (LAMB et al., 2016). Therefore, strategies that aim to decrease the mobility and consequent transfer of Cd from soil to plants are essential to reduce the risk of Cd food chain contamination.

Silicon (Si) has beneficial effects on growth, development, and yield of various crops (NASCIMENTO et al., 2019). The element is absorbed by the roots in the form of monosilicic acid (H_4SiO_4) and transported to aboveground parts, where Si deposition occurs as amorphous silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) in the leaf epidermis. Silica in plant tissues has been related to reduction of plant tipping, increased tolerance to fungal and insect attacks, and water deficit (WANG, Y. et al., 2019).

Silicon also plays a role in alleviating heavy metal stress in plants. For example, silicon addition to nutrient solution reduced by 20% the translocation of Cd from roots to maize shoots, with effects on damage reduction to the photosynthetic apparatus (SILVA et al., 2017). Rice plants (*Oryza sativa*) cultivated under Cd stress and Si-treated showed an increase in chlorophyll content, photosynthetic rate, and growth (REHMAN et al., 2019). Silicon application to soil had a positive effect on the biomass production of pigeon pea plants grown under Cd and Zn stress; besides, silicon reduced uptake and translocation of these metals to the shoots (GARG & SINGH, 2018). Cadmium is also able to change the plant mineral composition; therefore, silicon ability to improve uptake, transport, and nutrient distribution in plants may play a role in Cd stress tolerance (SILVA et al., 2017).

The soil pH is also an important factor in determining Cd availability to plants. Maize plants grown in limed soil had higher biomass yield and lower Cd accumulation compared to plants on the unlimed soil (CUNHA & NASCIMENTO, 2009). SHAREEF et al. (2016) reported that increasing soil pH significantly diminished the accumulation of Cd in roots, shoots, and grains of maize. KIRKHAM (2006) data revealed that both the increased soil pH and the Si amendment were efficient in reducing the Cd availability in soil.

Si-mediated immobilization of Cd, which can occur both in the soil and in the plant itself, can potentially reduce the risks of Cd accumulation by crops and, therefore, be valuable in the contaminated soils management and reduction of risks associated with food chain contamination. In this scenario, studying the effects of Si on Cd phytotoxicity attenuation should involve assessing the effects of Si on both the plant and soil. Our objective was to evaluate the effect of Si doses using a diatomaceous Earth source on the biomass production and uptake of Cd, Si, and nutrients by maize plants (*Zea mays* L.) grown on soil contaminated with cadmium. Diatomaceous earth is reported to optimize soil fertility and plant nutrition through improved water, physical

and chemical soil properties (NASCIMENTO et al., 2019). Since pH is probably the most crucial isolated soil factor determining Cd availability to plants, we studied Si effects in the same soil with and without pH correction.

MATERIALS AND METHODS

The soil chemical characteristics of the Ultisol used in the experiment were analyzed and displayed the following results: $\text{pH}_{\text{H}_2\text{O}}$ 4.6; 3.16 mg dm^{-3} P; $6.41 \text{ cmolc dm}^{-3} \text{ H}^+ + \text{Al}^{3+}$; $0.25 \text{ cmolc dm}^{-3} \text{ Al}^{3+}$; $0.01 \text{ cmolc dm}^{-3} \text{ Na}^+$; $0.01 \text{ cmolc dm}^{-3} \text{ K}^+$; $0.80 \text{ cmolc dm}^{-3} \text{ Ca}^{+2}$; $0.60 \text{ cmolc dm}^{-3} \text{ Mg}^{+2}$; 13 g kg^{-1} organic carbon; 340 g kg^{-1} clay; 32 g kg^{-1} silt; and 628 g kg^{-1} sand. Cadmium concentration in soil (0.7 mg kg^{-1}) was determined after acid extraction (USEPA, 2002). The Si available concentration of 2.3 mg kg^{-1} was obtained after extraction with acetic acid (KORNDÖRFER et al., 1999).

We applied Si under two soil pH conditions: original (pH 4.6) and corrected to pH 6.5. The soil was corrected using calcium carbonate (CaCO_3) and magnesium oxide (MgO) with a Ca:Mg molar ratio of 3:1, based on a previous acidity neutralization curve. Then, Cd was applied to the soil at the concentration of 10 mg kg^{-1} as cadmium chloride ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$); this concentration was chosen based on previous studies that identified a soil Cd toxic critical level for maize growing (CUNHA et al., 2008). After ten days of Cd contact with soil, Si was applied as a granular fertilizer (2-5 mm) derived from diatomaceous earth (AgrisilicaTM, Agripower Australia Limited), with a total concentrations of 26% Si, 2% Ca, 1% Mg, and 1% Fe (NASCIMENTO et al., 2019). Treatments consisted of doses equivalent to 0, 100, 150, 200, 300 mg kg^{-1} of Si (equivalent to 200, 300, 400, and 600 kg ha^{-1} of Si), mixed up in the total soil volume, homogenized, and incubated for ten days at 80% of the maximum water retention capacity before maize sowing.

The experiment was disposed in a randomized block design arranged in a 2 x 5 factorial scheme (2 soil pHs and 5 Si doses), with three replicates. Soil fertilization was done simultaneously with the application of Si, at the rates of 50, 400, 150 and 40 mg dm^{-3} of N, P, K, and S, respectively, applied as $(\text{NH}_4)_2\text{SO}_4$ and KH_2PO_4 . Micronutrients were also added as follow (mg dm^{-3}): 3.7 of Mn ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$); 4 of Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$); 1.3 of Cu (CuSO_4), 1.6 of Fe ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$); and 0.15 of Mo ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$).

Maize seeds (*Zea mays* L.) were sown

directly in the pot and, ten days after germination, only one plant was maintained until the end of the 30 days cultivation period. The soil in the pots was maintained at 80% of soil water-holding capacity by daily watering with distilled water. At the end of cultivation, shoots and roots were collected, washed in tap and distilled water, and oven-dried at 70 °C for 72 h; biomass was then measured and tissues were ground to plant chemical analyses.

The concentrations of Cd, Ca, Mg, Cu, Mn and Zn in the digestion extract (HClO_4 : HNO_3 , 1: 3 v/v) were determined by optical emission spectrometry (ICP-OES) (Perkin-Elmer/ICP-OES 7000), while P was obtained by photocolimetry and K by flame photometry. The N concentration was determined in a sulfuric extract (SILVA et al., 2009). For Si determination, the plant samples were digested using hydrogen peroxide and sodium hydroxide in an autoclave. The Si in the extracts was measured by photocolimetry at a wavelength of 410 nm (NI 2000UV, Nova Instruments, Brazil), using ammonium molybdate as a complexing agent (KORNDÖRFER et al., 2004).

The main and interaction effects were analyzed by applying ANOVA (F test). Correlation and regression analyses ($p \leq 0.05$) were used to investigate the relationship of Si rates applied to the soil with biomass yield and plant concentrations of Si, Cd, and nutrients. All statistical analyses were

accomplished with the software Statistica 10.1.

RESULTS AND DISCUSSION

Biomass production

The correlations between biomass yield and Si concentration in shoots were highly significant (pH 4.6, $r = 0.98^{**}$ and pH 6.5, $r = 0.94^{\circ}$). The large Si uptake by gramineous species, such as maize, sugarcane, rice, and wheat, is one of the most known effects of Si on plants. Also, some studies showed that adding Si to Cd-contaminated soil enhanced biomass of maize plants (CUNHA & NASCIMENTO, 2009; VACULÍK et al., 2012). There was an interaction between the Si doses and the pH values on shoots and roots biomass production ($p \leq 0.01$); although, Si doses increased plant biomass regardless of the soil pH. Plants cultivated in the soil with pH 6.5 had higher Si concentration, but the increment in Si uptake was proportionally higher for plants cultivated in the lower pH (Figure 1A). Soil pH correction and hence higher roots growth is a cause for increased Si and nutrient uptake as either shoot and roots biomass were doubled by liming (Figure 1A and 1B). However, it is clear that the higher the Si concentration in soil, the higher the biomass yield; therefore, the increased plant biomass was likely a combination of Si supply and Si alleviation of Cd stress.

Regarding roots biomass, we observed a distinct behavior for limed and non-limed soils. A linear effect of Si doses on roots biomass of plants

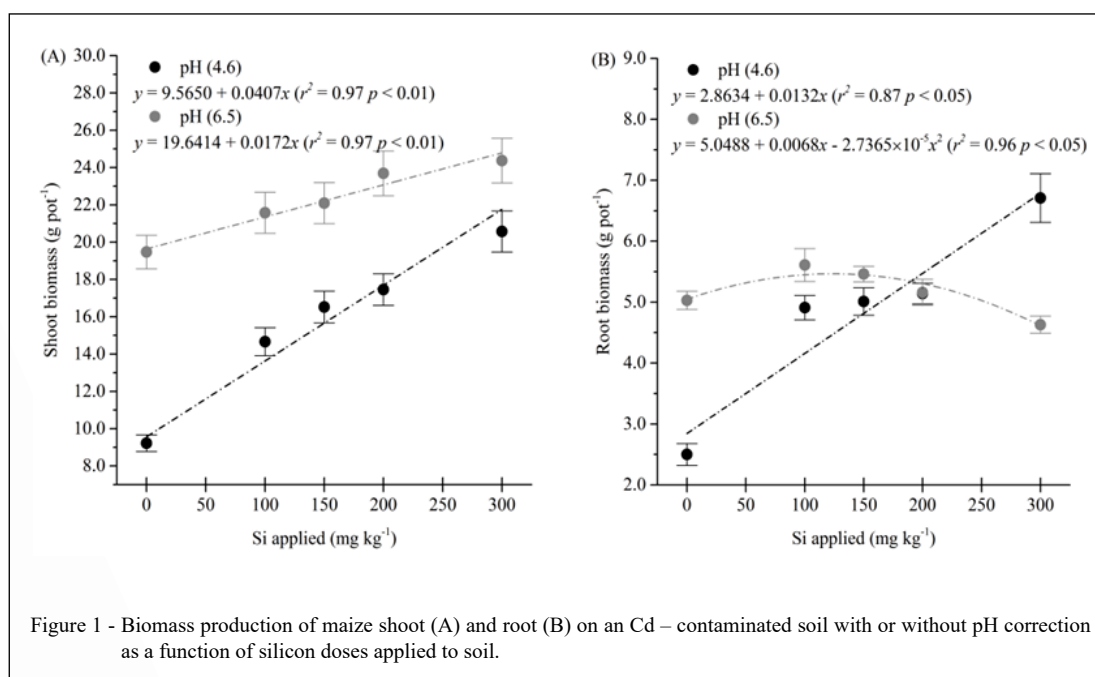


Figure 1 - Biomass production of maize shoot (A) and root (B) on an Cd – contaminated soil with or without pH correction as a function of silicon doses applied to soil.

grow on the acidic soil (Figure 1B) suggested that Si alleviation of Cd-inhibitory effects on maize plants is less effective in soils with higher pH, in which carbonates can partially precipitate cadmium. It is well known that soil acidity is one of the main factors affecting Cd bioavailability, with higher Cd mobility in acidic soils (YU et al., 2016). Soil liming can reduce the availability of Cd by up to 76% (WANG et al., 2014), which along with Si effects on Cd phytotoxicity is responsible for the lower metal damage and higher roots biomass reported in the limed soil (Figure 1B).

Accumulation of silicon and cadmium by plants

Silicon concentration in shoot and roots showed an interaction ($p \leq 0.01$) with Si doses and

pH values. Accumulation of Si had a linear response, except for roots in the corrected soil (Figure 2A and 2B), which is in line with root biomass response in this soil condition (Figure 1B). Both Si concentrations were higher in plants growing on the acidic soil. Decreased Si accumulation with an increase of pH is a likely result of Si lower solubility in the soil as dissociation of silicic acid (H_4SiO_4) is strongly pH-dependent (YU et al., 2016). Therefore, the lower Si removal from the corrected soil can be related to the monosilicic acid dissociation into $H_3SiO_4^-$, which is specifically-adsorbed onto iron and aluminum oxides, reducing the Si availability to plants (KEEPING et al., 2017).

Together with the soil pH effects previously discussed, the relationship between Si

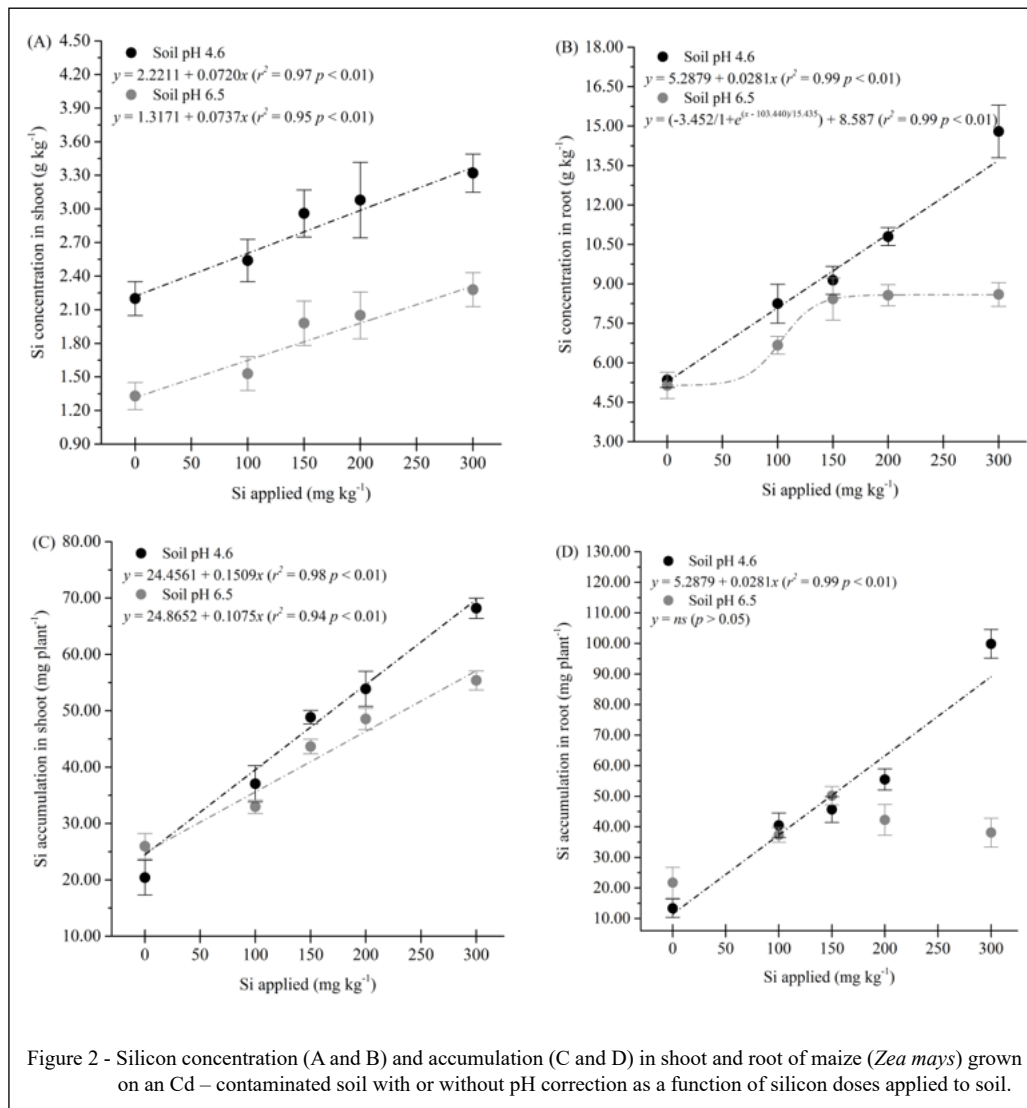


Figure 2 - Silicon concentration (A and B) and accumulation (C and D) in shoot and root of maize (*Zea mays*) grown on a Cd - contaminated soil with or without pH correction as a function of silicon doses applied to soil.

solubility and soil pH seems to be a significant factor accounting for Cd concentration in our plants. At both pH conditions, shoot Cd concentrations decreased linearly with increasing Si doses (Figure 3A). The reduced Cd concentrations in shoots rely not only on the decreased bioavailability of the metal in Si-treated soils but also on the Cd retention in roots and avoidance of root-to-shoot translocation (CUNHA & NASCIMENTO et al., 2009). For instance, VACULÍK et al. (2012) reported the development of an endodermal barrier in maize roots treated with Si that was correlated with Cd concentration in plants. Also, CUNHA & NASCIMENTO (2009) suggested that the silica deposition visualized in the endodermis and pericycle of roots played a role in the maize tolerance to Cd stress.

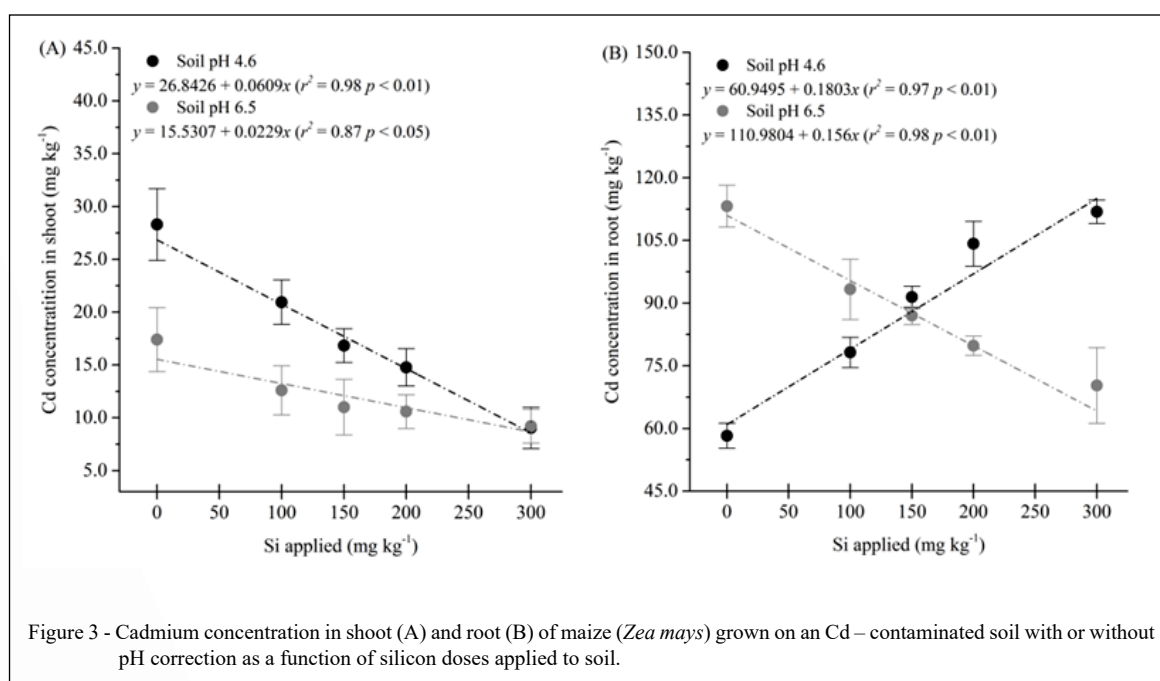
The increase of both Cd and Si concentrations in roots of plants grown on the acidic soil (Figure 3B) suggested that diminished Cd translocation to shoots relied on Cd-Si complexation in roots. This is confirmed by the ratio between Cd accumulated in shoots/Cd accumulated in roots, which decreased with the doses of Si applied to the soil (Figure 4). Conversely, for the limed soil, Cd-Si precipitation in soil was probably the main factor decreasing Cd concentration in roots; although, Si still played a role in reducing Cd transfer to shoots. It is worth pointing out that roots were more abundant

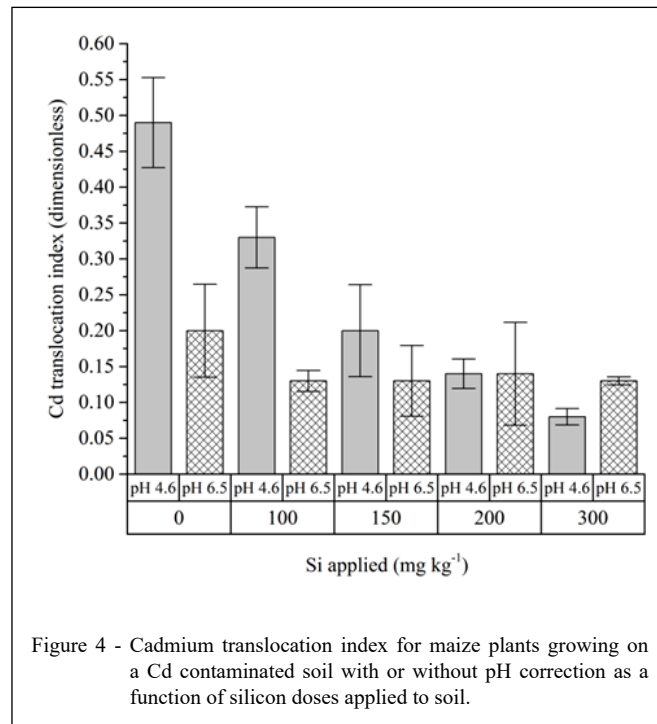
in the limed soil than in the acidic soil (Figure 1B), which explains the higher uptake of Cd and Si in these plants.

Accumulation of nutrients by plants

The regression analyses showed a significant and positive effect of the Si doses on the nutrients N, K, Ca, Mg, Fe, Mn, Cu, and Zn accumulated in maize shoots, irrespective of soil pH (Figure 5). Phosphorus was the only nutrient not significantly affected by Si application to soil (Figure 5B). Other studies have shown that high rates of Si applied to soil can diminish P uptake owing to antagonistic effects between H_2PO_4^- and H_3SiO_4^- (NASCIMENTO et al., 2019). Also, Si can increase P solubility in soil solution, which in turn can have an indirect effect on Cd precipitation in soil and roots as cadmium phosphate [$\text{Cd}_3(\text{PO}_4)_2$]. Indeed, P and Cd negative correlation in the shoot (-0.65^{**}) indicates that precipitation might have taken place.

As expected, owing to the higher roots biomass and hence greater nutrient uptake, macronutrient concentrations were, in general, higher in the limed soil (Figure 5), but Si addition improved nutrient accumulation regardless of soil pH condition. Silicon might enhance the efficient use of N by increasing its concentration in plants; increases in the accumulation of K, Ca, and Mg driven by Si have





also been reported for several crops (NASCIMENTO et al., 2019).

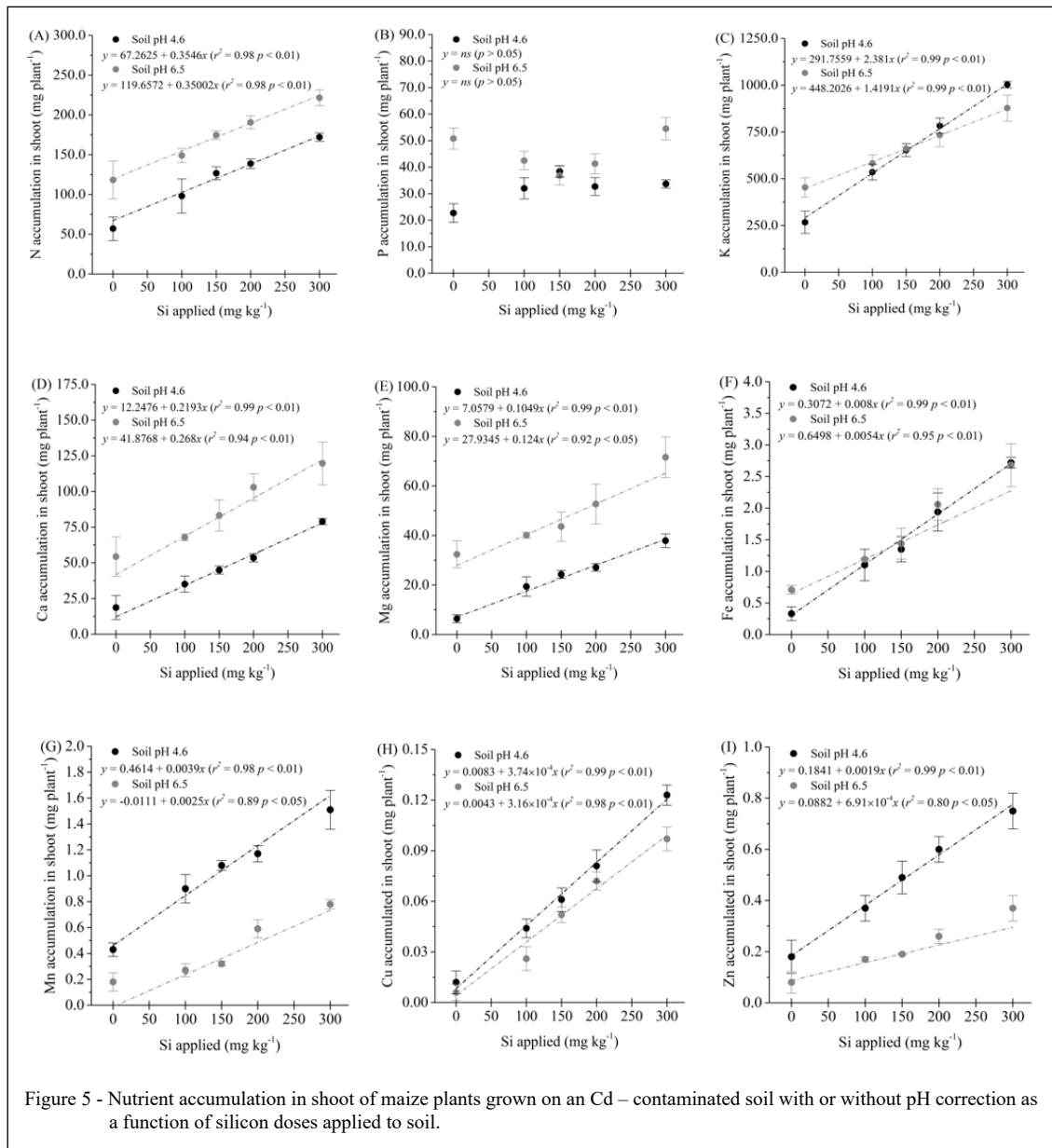
The plant nutrition improvement reported here can be due not only to Si effects on nutrient efficiency use, but also on the role that Si plays on Cd stress. The root plasma membrane is the primary barrier to Cd uptake, as well as the first target for metal toxicity. Cadmium can replace Ca^{+2} and affect membrane integrity. Besides, Cd can lead to modification of plasma membrane H^{+} -ATPase activity, which could diminish the gradient of protons across the membrane and hence decrease nutrient uptake. Therefore, the role of Si in maintaining membrane integrity is essential to prevent Cd uptake and to sustain ionic homeostasis of root cells (JANICKA-RUSSAK et al., 2012).

In contrast to macronutrients, an opposite trend with higher accumulation of Fe, Mn, Zn, and Cu in plants cultivated on the acidic soil was observed (Figure 5F to 5I). Cationic micronutrients decrease in availability as pH rises; for example, Zn, Cu, and Mn available concentrations in soil decrease 100 fold with every one unit increase in pH (PALMER et al., 2009). Our data showed that Si addition to both limed and non-limed soils caused an increase in micronutrient accumulation (Figure 5). Antagonism for plant uptake between Cd^{+2} and cationic micronutrients is well

known. Metal uptake relies mainly on a ubiquitous family of divalent metals transporters that mediates Fe, Zn, Mn, and Ni uptake from soil (GONZÁLEZ-GUERRERO et al., 2016). Therefore, the increased micronutrient accumulation for plants growing on Si-treated soils is likely a result of decreased competition between Cd and micronutrients owing to Si effects on Cd solubility, as previously discussed.

CONCLUSION

Our results showed that Si applied as a diatomaceous Earth fertilizer improves biomass and nutrient accumulation (N, K, Ca, Mg, Fe, Mn, Cu, and Zn) of maize plants grown on a Cd-contaminated soil. The Si application to soil and the liming had a positive effect on biomass and nutrient uptake, but the Si effect on plants' performance took place irrespectively to the soil pH. Silicon ameliorated Cd phytotoxicity in both limed and non-limed soils by decreasing Cd concentration in shoots; Si alleviation of Cd-inhibitory effects on plants was more effective in the non-limed soil owing to the higher Cd availability in acidic soils. Considering the reduced transfer of Cd to shoots driven by Si, lower Cd accumulation in maize grains is likely, with implications to food safety.



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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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