Australian Journal of
Crop Science
AJCS

Aust J Crop Sci. 19(02):119-127 (2025) |
https://doi.org/10.21475/ajcs.25.19.02.p121
ISSN:1835-2707

Silicon mitigates cadmium toxicity and modulates positive anatomical changes in tree plants of African mahogany

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***Received:* 21/04/2024**

***Revised:* 16/09/2024**

***Accepted:* 11/10/2024**

Abstract: This study aimed to evaluate the effect of silicon mitigation (Si) on *Khaya ivorensis* exposed to cadmium (Cd) and anatomical changes promoted by Cd in roots and leaves tissues. O delineamento experimental foi inteiramente casualizado em esquema fatorial 4 x 4 com cinco repetições. Cd and Si treatments were 0, 25, 50, 75 mg L^{-1} and 0, 100, 150, and 300 mg L^{-1} respectively. At 25 mg L^{-1} mg, Cd has shown increases of 30%, 25%, and 33% in Epidermis thickness from adaxial (ETAd), spongy parenchyma thickness (SPT), and root cortex thickness (RCT). Also, 75 mg L^{-1} Cd decreased epidermis thickness from abaxial (ETAb) and root cortex thickness (RCT) by 70% and 81%. However, Si attenuated anatomical changes caused by Cd. It occurs especially in combination with treatments at 150 mg L^{-1} Si and 50 mg L^{-1} Cd. Under these conditions, ETAb increased by 33%. Both palisade parenchyma thickness (PPT) and spongy parenchyma thickness (SPT) were 48% and 55% thicker than tissues under 50 mg L^{-1} Cd. The *K. ivorensis* is tolerant to Cd up to 25 mg L^{-1} mg because it did not show considerable growth reduction. Si has shown modulation in tissue thickness. It has a positive impact on the vegetative growth of *K. ivorensis* . Therefore, this study indicates that *K. ivorensis* tolerates Cd toxicity up to 25 mg L^{-1} Cd and shows that Si induces anatomical modulations in leaves and roots.

Keywords: Si stress mitigation; Cd stress; mahogany; anatomical modifications; heavy metal.

Abbreviations: ETAd_Epidermis thickness from adaxial; ETAb_epidermis thickness from abaxial; PPT_palisade parenchyma thickness; SPT_spongy parenchyma thickness; PPT/SPT ratio_palisade parenchyma thickness/spongy parenchyma thickness ratio; RD_Root diameter; RCT_root cortex thickness; VCD_vascular cylinder diameter; VED_vessel element diameter.

Introduction

Cadmium (Cd) is a highly toxic heavy metal (HM) that affects plant growth in natural or artificial environments (Song et al., 2019). Cd is largely a pollutant, which is released into nature through anthropogenic action (e.g. power stations, heat systems, waste incinerators, urban traffic, cement factories, and phosphate fertilizers as by-products), but also weathering of rocks (Sanità di Toppi and Gabbrielli 1999; Andresen and Küpper 2013).

The Cd toxicity mechanisms in plants were studied and described in classic review articles (Das et al., 1997; Benavides et al., 2005). Plants affected by Cd have shown a reduction in their growth. It was caused by disorders such as physiological, nutritional, biochemical, and anatomical. For example, Cd hurts plants because it promotes reductions in gas exchange (Song et al., 2019). In addition, Cd reduces essential nutrient content (Gomes et al., 2013), induces oxidative stress (Pereira et al., 2018), affects nitrogen metabolism (Chaffei et al., 2004), reduces the size of endodermal cells and the promotes disintegration of root epidermis (Vitória et al., 2003; Liza et al., 2020).

Silicon (Si) is the second most abundant element in soils. It is not considered an essential element to plants (Epstein 1999; Marschner 2012), but it induces tolerance in plants, which was submitted to abiotic stresses such as HM toxicity (Ali et al., 2016; Pereira et al., 2018; Ali et al., 2019), water deficit (Saud et al., 2014; Safoora et al., 2018; Avila et al., 2020), salt stress (Liang 1999; Torabi et al., 2015; Raza et al., 2019). Furthermore, Si improves gas exchange, water use efficiency, and plant growth (Silva et al., 2015; Oliveira et al., 2019).

The mechanisms of promoting tolerance in plants to HM are diverse and modulated by Si. In vegetables, Si induces HM tolerance by reducing its absorption and promoting its immobilization in root apoplast. As a consequence, chelating HM, coprecipitating Si bound, reduces oxidative stress, improving gas exchange and increasing absorption of essential nutrients (Adrees et al., 2015).

The first organ to come into contact with HM is the root system which is present the in vegetal growth substrate. Also, the first to manifest morphological, anatomical and physiological results of HM toxicity (Pérez Chaca et al., 2014). On the other hand, leaves are organs that are exposed to the air environment, which becomes more sensitive and has plasticity characteristics to environmental changes compared to other organs (Vaculík et al., 2015). Thus, leaves are indicators organs that have shown how the surrounding environment of plants is, as well as morphological and structural characteristics that are ecological indicators of plant habitat (Farooq et al., 2016; Greger et al., 2016). In this context, HM toxicity is a stressful factor because promotes changes in the dimensions of leaf and root tissues, as well as on the surface of organs, such as stomata, epidermis and plant attachments (Li et al., 2007; Shi and Cai 2009; Cui et al., 2017; Pereira et al., 2017; Xu et al., 2017).

African mahogany or *Khaya ivorensis* A. Chev. (*K. ivorensis*) is a tree species of african origin belonging to the Meliaceae family (Ribeiro et al., 2017). The species has great economic potential for commercialization because of its noble wood, which can be used in industry, naval and civil constructions, panels and laminates industries, among others (Pinheiro et al., 2011). *K. ivorensis* is a heliophile species, tolerant shade during its juvenile stage, which was classified as a pioneer and secondary species emerging (Budowski 1965; Denslow 1987; Foli 2000). In the literature, there is no record of the mitigating action of Si on the effects of toxic levels of Cd on the growth and anatomical characteristics of *K. ivorensis* plants since the species is not an accumulator of Si and Cd.

The hypothesis guiding this study was that increasing levels of Si improve the growth and anatomical parameters of *K. ivorensis* under toxic levels of Cd and that this species tolerates low levels of Cd. Research aimed to evaluate the mitigation effects of Si on *K. ivorensis* exposed to cadmium, as well as anatomical changes promoted by Cd in root and leaf tissues.

Results

Cadmium and silicon content

According to polynomial adjustment ($Z_{Cd\ leaf}$ and $Z_{Cd\ root}$), Cd content in leaves (Figure 1B), and roots (Figure 1A) was high in Cd toxic treatments. Leaves and roots showed different Cd levels in response to Cd treatments. At 25 mg L^{-1} Cd treatment, Cd content followed decreased order of root (19.78 mg kg^{-1} DM) > leaf (11.47 mg kg^{-1} DM). However, as Cd treatment increased, there was an increase in Cd content in leaves. In the treatment of 75 mg L^{-1}, there was a high accumulation of Cd in leaves, which reached a maximum point of 57 mg kg^{-1} Cd DM. Cd content was found at 45% higher than the root submitted to the same treatment and 87% higher than the control.

The Si supply reduced Cd levels in different evaluated organs of *K. ivorensis* . Canonical analysis showed 38 mg L^{-1} Cd and 141 mg L^{-1} Si interaction as the lowest Cd content obtained in leaves (Figure 2C and 2B). In these interactions, Cd levels were 60% less than levels found at 50 mg L^{-1} Cd. The minimum Cd content point was in the root system (Figure 2C), with the value of 4.8 mg kg^{-1} Cd DM which occurred in the presence of 150 mg L^{-1} Si and the absence of Cd. However, there was a reduction of 26% of Cd root content in the interaction between 50 mg L^{-1} Cd and 150 mg L^{-1} Si. The concentrations of 25 mg L^{-1} Cd and 50 mg L^{-1} Si did not affect Si content in all evaluated organs. The minimum levels of Si contents in leaves (0.012 mg kg^{-1} Si DM) and roots (0.18 mg kg^{-1} Si DM) were observed only at 75 mg L^{-1} Cd (Figure 2C, 2B and, 2A).

As Si concentration increased, also there was an increase in Si content in evaluated organs of *K. ivorensis* . The polynomial equation ($Z_{Si\ leave}$ and $Z_{Si\ root}$) showed maximum points of Si contents at 150 mg L^{-1} Si concentration, which reached a mean of 4.9 and 7.7 mg kg^{-1} Si DM in leaves and roots respectively. Si accumulation in *K. ivorensis* was observed in root > leave (Figures 2A and 2B). According to the polynomial equation in plants treated with Cd, high Si content in leaves and roots was obtained between the interaction of Si and Cd at 27 mg L^{-1} Cd and 132 mg L^{-1}Si; 30 mg L^{-1} Cd and 151 mg L^{-1} Si. These also resulted in the highest levels of Si in leaves and roots compared to plants at 50 mg L^{-1} Cd concentration.

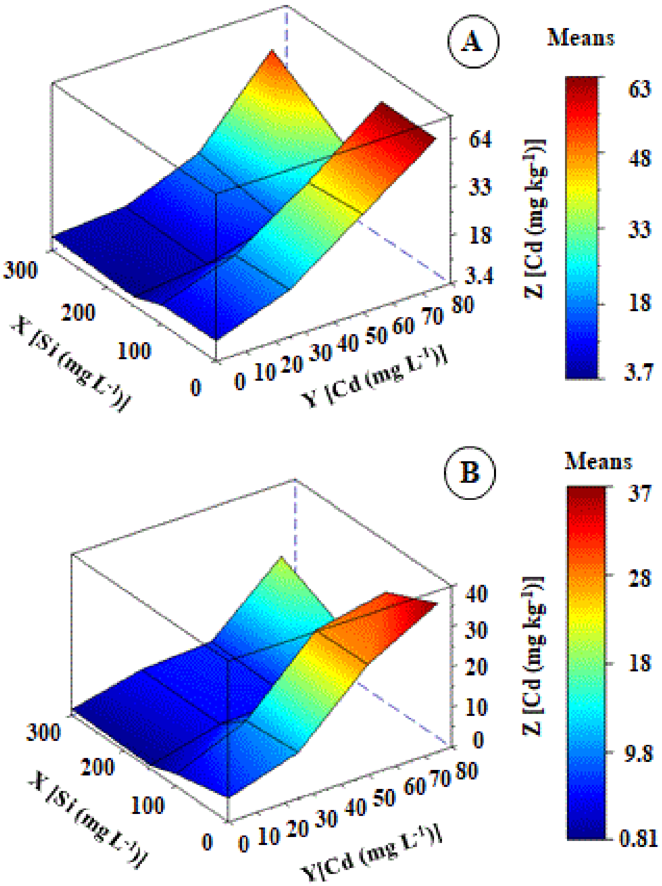

Fig 1. Cd content (mg kg^{-1} DM) in roots (A) and leaves (B) of *Khaya ivorensis* plants under silicon and cadmium treatments. $Z_{Cd\ Leaf} = 3.96858^{**} + 8.51915^{*}X - 0.62525^{ns}Y + 1.33539^{**}X^2 - 0.20243^{ns}YX + 0.84597^{ns}Y^2$ $R^2 = 0.81$; $Z_{Cd\ Root} = 20.09769^{*} - 11.15876^{*}X - 1.12187^{ns}Y - 2.08924^{*}X^2 - 1.75749^{*}YX - 0,4.17401^{ns}Y^2$ $R^2 = 0.89$; * significant diferences at 5%; ** significant diferences at 1%; ns: no significant.

Growth parameters

Height (H), leaf area (LA), and root length (RL) get through similar variations in response to the increased value of Cd treatments. However, combined treatments between Cd and Si have shown that Si promoted improvements in the growth of *K. ivorensis* (Figure 3). The growth parameters H and LA were reduced by 49 and 60% in 75 mg L^{-1} Cd treatment compared to control, respectively (Figure 3A and 3B).

According to bivariate analysis, the highest means of H, LA, and RL were observed in the absence of Cd and the intermediate concentration of Si. Treatment with 150 mg L^{-1} of Si induced increases in all growth variables, with more significant effects on RL (Figure 3). The concentration of 150 mg L^{-1} of Si promoted a maximum point of 20 cm $root^{-1}$. This average was 76% higher than that of plants treated with 50 mg L^{-1} of Cd.

The significant interaction between Cd and Si concentrations improved growth variables. Canonical analysis resulted in optimal growth levels occurring in 41 mg L^{-1} Cd and 145 mg L^{-1} Si interaction. In this interaction, there were increases of 33% in H and LA (Figure 3A and 3B). About RL, there was a 58% increase compared to 50 mg L^{-1} Cd (Figure 3C).

Leaf anatomical parameters

Concentration of 25 mg L^{-1} Cd increased the thickness of the abaxial (ETAb) and adaxial (ETAd) epidermis of *K. ivorensis* between 25% and 30% compared to control, respectively (Figure 4A and 4B). Treatment with 50 mg L^{-1} Cd causes the thickness the of vascular bundle sheath (Figur C). The concentration of 25 mg L^{-1} Cd increases spongy tissue thickness (SPT) (Figure 4D).

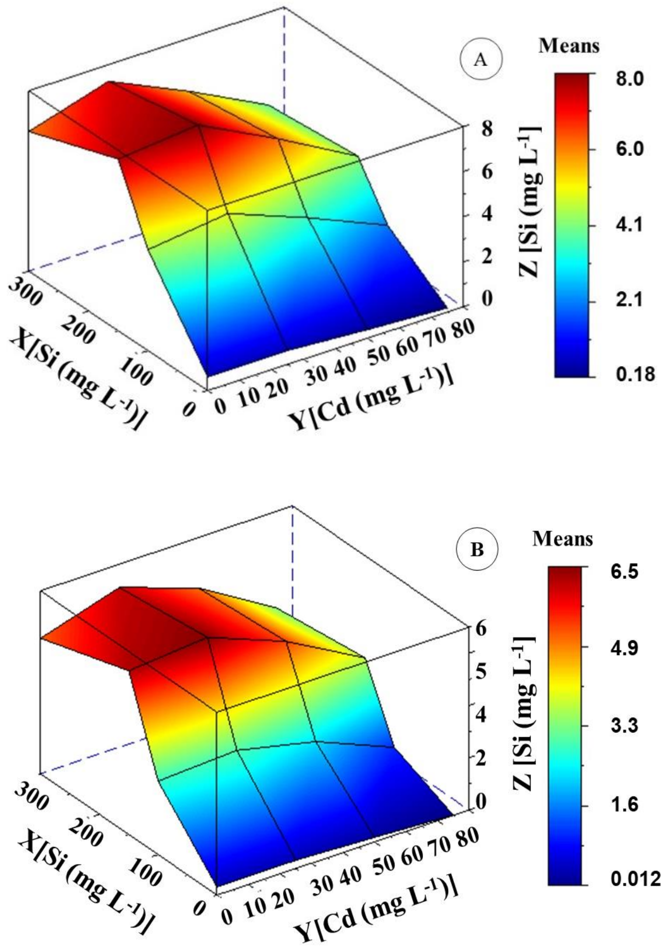

Fig 2. Si content (mg kg^{-1} DM) in roots (A) and leaves (B) of *Khaya ivorensis* plants under silicon and cadmium treatments. $Z_{Si\ leaf} = -7.60881^{**} + 6.75170^{**}X + 2.37499^{**}Y - 0.85133^{**}X^2 - 0.27441^{**}YX - 0.45728^{**}Y^2$ $R^2 = 0.95$; $Z_{Si\ root} = -7.55413^{**} + 7.58789^{**}X + 2.12173^{**}Y + 1.02241^{**}X^2 - 0.24372^{**}YX - 0.45438^{**}Y^2$ $R^2 = 0.96$; * significant diferences at 5%; ** significant diferences at 1%; ns: no significant.

Even though the magnitude of the increase was smaller than in the ETAb and ETAd, it was little significant in the thickness of spongy parenchyma (PPT). The lowest mean of the PPT/SPT ratio was observed at 25 mg L^{-1} Cd (Figure 4E). Above 25a mg L^{-1} Cd there was reduction in ETAp, ETAd, PPT and SPT of *K. ivorensis* lea,ves (Figure 4A, 4B, 4C and 4D). However, this effect was not observed in the PPT/SPT ratio, which increased with increasing Cd concentration (Figure 4E). The interaction between Cd and Si considerably increased STA,b, ETAd, and SPT (Figure 4A, 4B and 4D). These anatomical parameters have shown the best interaction between Cd and Si which was between 50 mg L^{-1} Cd and 150 mg L^{-1} Si.

Concerning cells from STAb, ETAd, and SPT tissues of plants treated with 75 mg L^{-1} Cd there was a loss of cell structure and reductions in intercellular spaces compared to the control plants (Figure 6E). Minimum thickness points of STAb, ETAd, and SPT were 4.5µm, 5.8µm and 17µm. These values were 73%, 70%, and 68% lower than control.

There was a compact arrangement of epidermal cells in the 150 mg L^{-1} Si and 50 mg L^{-1} Cd interaction. Furthermore, in this interaction, there was an increase in the epidermal thickness of leaf tissues (Figure 6D). Canonical analysis has shown optimal points between the interaction of Cd and Si, which occurred in combination with 45 mg L^{-1} Cd and 165 mg L^{-1} Si with an increase in the thickness of leaf tissues (Figure 6).

Root anatomical parameters

There were wide variations in root tissue thickness, as well as Cd concentrations increased in the culture medium (Figures 5 and 7). Even though *K. ivorensis* was treated with 25 mg L^{-1} Cd, they did not show anatomical changes compared to the control. Except for root cylinder thickness (RCT) (Figure 5B) and root vessel element diameter (VED) (Figure 5D). The concentration of 25 mg L^{-1} Cd increased by 33% and 28% RCT and VED. According to the fit of the regression equation, the maximum point of the RCT and VED were 120 and 55 µm (Figure 5B and D). According to surface analysis of response, RCT and VED at 75 mg L^{-1} Cd were reduced by 81% and 80%, respectively (Figure 5B and 5D). However, Si has been minimized to the toxic effect of Cd between the interaction of 40 mg L^{-1} Cd and 150 mg L^{-1} Si. These interactions, resulted in values of RD 250 µm and VED 58µm. These averages above of DR and VED represent increases of 53% and 56% compared to plants at 50 mg L^{-1} Cd, respectively.

Discussion

Increased Cd content in leaves and roots was observed in *K. ivorensis* (Figure 1). However, above 25 mg L^{-1} Cd high levels of Cd were observed in shoots and roots coinciding with a reduction in H, LA, and RL. Thus, indicates that even in this concentration *K. ivorensis* tolerates the toxicity of Cd. Cd content treatment in roots of *K. ivorensis* was 42% higher than in leaves. These results suggest the potential of *K. ivorensis* to accumulate Cd in the leaf (Cd content of 11.47 mg kg^{-1} DM) and root (Cd content of 19.78 mg kg^{-1} DM). These Cd concentrations in leaves and roots of *K. ivorensis* plants exceed the values of Cd concentrations observed in other plant species such as in Populus alba (Rafati et al., 2011) and Prosopis juliflora (Varun et al., 2011) which are phytoremediation potential plants. Cd is a non-essential element that negatively affects plant growth (Das et al., 1997; Benavides et al., 2005). Fan et al., (2011) in a study with plants of *Swietenia macrophylla* showed that this species accumulated 154 mg kg^{-1} Cd DM in the stem, with a reduction of 25% and 31% in the dry mass of the stem and the whole plant, respectively. Decreases in plant growth functionally connected to the negative impact of Cd on gas exchange parameters (López-Climent et al., 2011), chlorophyll fluorescence (Piršelová et al., 2016), chlorophyll content (Li et al., 201,2) and nutritional status of Cd treated plants (Jibril et al., 2017). However, exogenous Si supply minimizes the effects of Cd on growth because it reduces Cd uptake and transport to the aerial part of plants. This mechanism of action of Si improves gas exchange (Farooq et al., 2013), chlorophyll fluorescence (Howladar et al., 2018), chlorophyll contents (Ali et al., 20,19) and nutritional status (Alzahrani et al., 2018), which allows growth gains even under conditions of mild to moderate Cd toxicity in plants, as evidenced in these study.

Until 25 mg L^{-1} mg Cd *K. ivorensis* presented thickness of evaluated tissues (ETad, ETAb, PPT, and SPT). The epidermis cell wall accumulates a considerable amount of negative charges from functional groups such as -OH, -COOH and -SH (Krzesłowska et al., 2011). The thickness of the epidermis can expand its function as a metal ion filter (Araújo and Silva 2013). However, high Cd treatments reduced the thickness of tissues evaluated (ETad, ETAb, P,PT and SPT), size of cells and induced loss of cell shape (Figure 6), in specific of treatment at 75 mg L^{-1} Cd. Toxic Cd affected the expansion of leaf tissues of tomatoes (Djebali et al., 2010). In bean, Cd reduced relative water contents and inhibited cell expansion, which indicates that HM negatively affects cell extensibility (Poschenrieder et al., 1989).

Anatomical modifications were induced by Cd, which implies decreases in leaf area and, consequently, a reduction in the photosynthetic capacity of plants (Chugh and Sawhney 1999; Di Cagno et al., 1999). Cotton plants have shown a reduction in the thickness of up and low epidermal and spongy parenchyma in response to 200 µM of Cd (Ozyigit et al., 2013). Similarly, *Alternanthera tenella* treated with 150 µM has shown a reduction

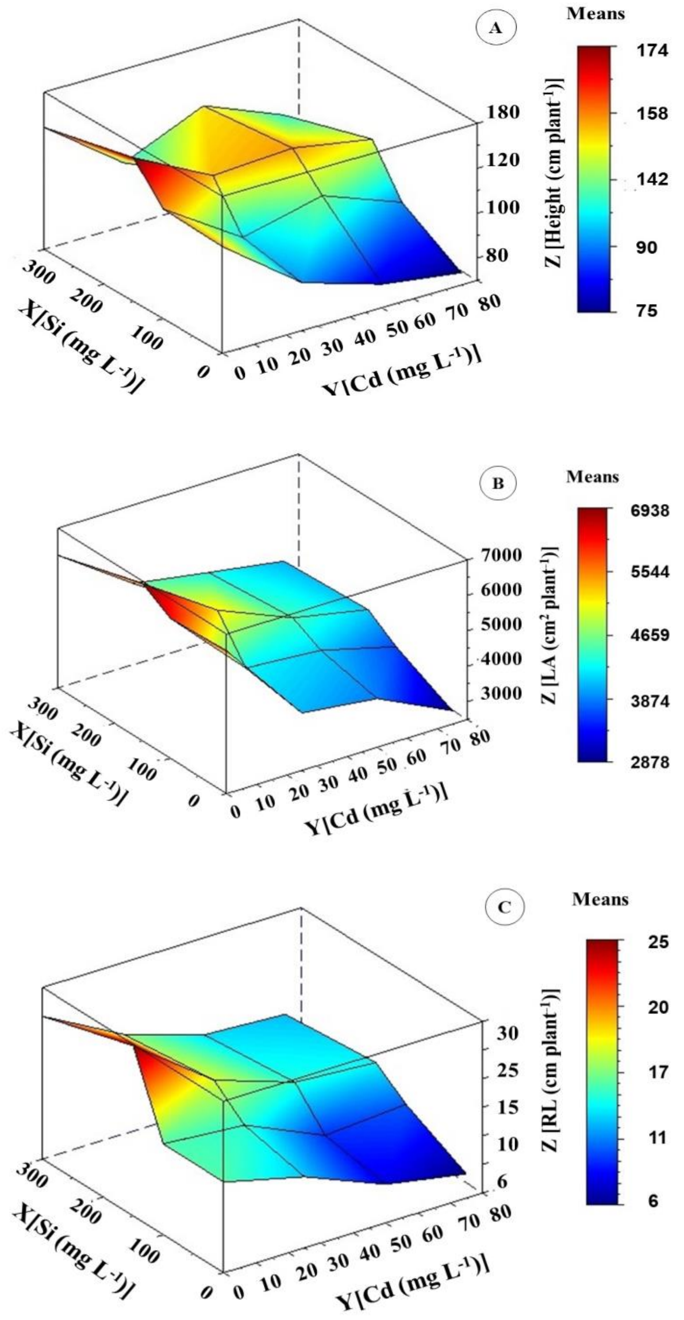

Fig 3. Height (A), leaf area (B), and root length (C) of *Khaya ivorensis* plants under silicon and cadmium treatments.
$Z_H = 110.27469^{**} - 0.83716Y^{**} + 0.21975X^{**} + 0.00498Y^{2**} + 0.00095XY^{**} - 0.00062X^{2**}$ $R^2 = 0.77$; $Z_{AF} = 6189.17485^{**} - 78.16847Y^{**} + 6.58098X^{**} + 0.47423Y^{2**} + 0.05197XY^{**} - 0.02076X^{2**}$ $R^2 = 0.91$; $Z_{CR} = 15.65691^{**} - 0.26992Y^{**} + 0.07626X^{**} + 0.00169\,Y^{2**} - 0.00010XY^{ns} - 0.00015X^{2**}$ $R^2 = 0.82$; * significant diferences at 5%; ** significant diferences at 1%; ns: no significant.

in thickness of up and low epidermis, and palisade and spongy parenchyma (Rodrigues et al., 2017).

The 25 mg L^{-1} Cd treatment increased the thickness of VCD and VED. However, high Cd treatments reduced the thickness of these evaluated root tissues. In addition, there was great accumulation and transport of Cd from the root system to shoot in high Cd treatments. The great thickness of the epidermis and endodermis acts as a barrier to Cd transport to shoot. Due to the presence of negative charges on the cell wall of these tissues (Melo Marques et al., 2011; Krzesłowska et al., 2011). The epidermis and endoderm also represent an important apoplastic barrier for the radial transport of ions and water in the vascular system (Enstone et al.,, 2002; Enstone and Peterson, 2005). Root anatomy results found in these studies suggest that *K. ivorensis* is sensitive to concentrations greater than 25 mg L^{-1} Cd. Chickpea plants treated with Cd (250 - 1000 μM) have shown considerable reduction in root diameter and root tissue thickness (Liza et al., 2020), similar to results found in the present study. In rice crops, Cd was deformed and disarranged cells in the cortex (Fan et al., 2016). On the other hand, Brachiaria decumbens grown in soil contaminated with Cd (mixture of soil without Cd + soil containing 10.5 mg kg^{-1} Cd in proportions of 7.5 and 15%) have shown higher thickness of endoderm, exoderm, and cell wall of cortical cells (Gomes et al., 2011).

Treatments combined between Si with Cd improved shoot and root growth. This improvement was connected to positive changes in leaf anatomy caused by Si. Supply of Si reduced the negative impact of Cd on growth and anatomical variables, specifically in the treatment of 150 mg L^{-1} Si. Several studies report the role of Si in the mitigation of harmful effects of HMs on the growth of rice (Fan et al., 2016; Bari et al., 2020), corn (Cunha et al., 2009; Vakulík et al., 2012), cotton (Anwaar et al., 2015; Ali et al., 2016), wheat (Ali et al., 2019) and alfalfa (Wu et al., 2015). In wheat and rice, Si increases the suberization of ectoderm and endoderm. It also decreases Cd transport to shoot, which contributes to improving plant growth (Fleck et al., 2011; Wu et al., 2019). In addition, Si induces ion chelation through exudates released from the root or by decreases in the amount of free ions in vegetable organs. These two strategies reduce the transport of toxic ions to aerial parts of plants (Adrees et al., 2015). According to surface analysis, a concentration of 150 mg L^{-1} Si improved growth and anatomical parameters evaluated in leaves and roots. These results coincided with a great accumulation of Si and a reduction in the accumulation of Cd in *K. ivorensis* (shoot and root).

The study has shown that *K. ivorensis* tolerates Cd toxicity up to 25 mg L^{-1} Cd because plant growth was little affected compared to control. Also, this research has shown that a concentration of 150 mg L^{-1} Si improved the thickness of leaf tissues important to vegetative growth, specifically STAb, ETAb and SPT. These tissues are important to diffuse CO_2 from the environment to the carboxylation site in chloroplasts (Ennajeh et al., 2010). The high STAb, ETAb favors less water loss because the epidermis is the layer that contributes to the efficiency of water use, which reduces its loss during the transpiration process (Javelle et al., 2011). Higher VED and VCD induced by Si in *K. ivorensis* indicate that the great thickness of these tissues can facilitate the transport of water and nutrients through symplast (Meyer et al., 2011).

Materials and methods

Plant material, growth conditions, and experimental design

The experiment was carried out in a greenhouse at the Federal Rural University of Amazon, Belém, Pará - Brazil (01° 28'03" S and 48° 29'18" W). During the experimental period, averages of temperature and relative humidity of air were recorded in the inside greenhouse at 30 ± 4 ° C and 90 ± 3%, respectively. Young plants of african mahogany, *Khaya ivorensis* A. Chev (*K. ivorensis*), 120 days old and average height of 60 cm were transferred to pots of 5 L, which contains a nutrient solution with 25% ionic strength (Sarruge, 1975). The changed nutrient solution was made in intervals of seven days. The pH of the solution was maintained at 5.8 ± 0.2. The plants remained under these conditions for 60 days. After that, the ionic strength of the nutrient solution was changed to 100% where Cd and Si treatments were applied for 60 days. The experimental design used was randomized blocks organized in a 4 x 4 factorial scheme (cadmium and silicon concentrations). Assessments were carried out when the plants reached 120 days old.

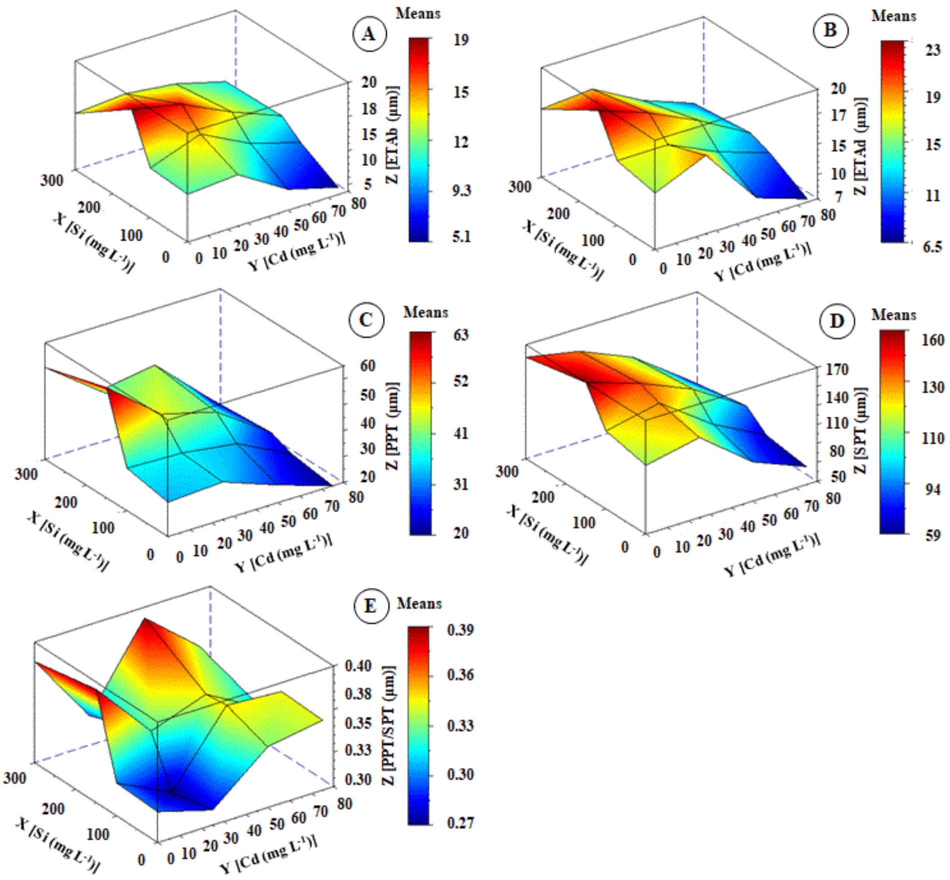

Fig 4. Change in epidermis thickness from abaxial - ETAb (A), epidermis thickness from adaxial - ETAd (B), palisade parenchyma thickness - PPT (C), spongy parenchyma thickness - SPT (D) and palisade parenchyma thickness and spongy parenchyma thickness ratio - PPT/SPT (E) of *Khaya ivorensis* plants under silicon and cadmium treatments. $Z_{ETAb} = 11.16610^{**} + 0.04756^{**}X - 0.00891^{ns}Y - 0.00014^{**}X^2 + 0.00016^{**}YX - 0.00113^{**}Y^2$ $R^2 = 0.83$; $Z_{ETAd} = 16.44439^{**} - 0.02913^{*}X + 0.04162^{**}Y - 0.00145^{*}X^2 + 0.00011^{*}YX - 0.00012^{**}Y^2$ $R^2 = 0.81$ $Z_{PPT} = 34.11487^{**} + 0.13758^{**}X - 0.05983^{*}Y - 0.00022^{**}X^2 - 0.00069^{**}YX - 0.00193^{**}Y^2$ $R^2 = 0.92$; $Z_{SPT} = 115.78797^{**} + 0.28610^{**}X + 0.08478^{*}Y - 0.00059^{**}X^2 - 0.00066^{**}YX - 0.01216^{**}Y^2$ $R^2 = 0.90$; $Z_{PPT/SPT} = 0.29183^{**} + 0.00316^{**}X - 0.00024^{*}Y - 0.0000014^{*}X^2 - 0.0000367^{**}YX - 0.0001189^{*}Y^2$ $R^2 = 0.88$; * significant diferences at 5%; ** significant diferences at 1%; ns: no significant.

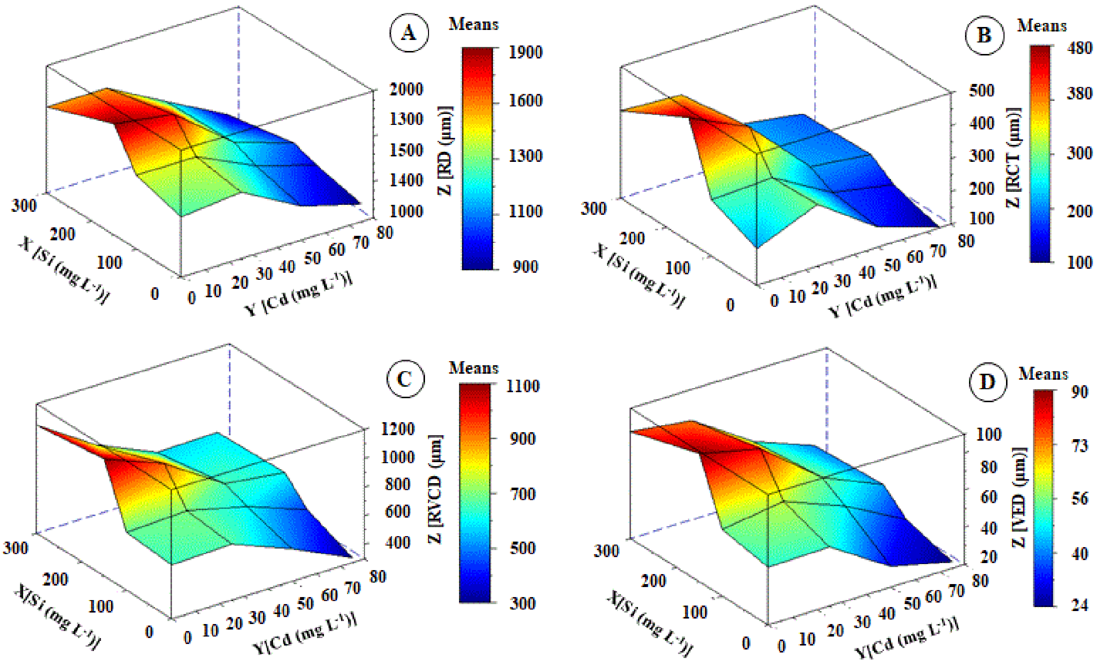

Fig 5. Change in root diameter - RD (A), root cortex thickening - RCT (B), vascular cylinder diameter - VCD (C) and vessel element diameter - VED (D) of *Khaya ivorensis* plants under silicon and cadmium treatments. $Z_{RD} = 1297.07468^{**} + 3.49697^{**}X - 0.76551^{*}Y - 0.00783^{*}X^2 - 0.01056^{**}YX - 0.08971^{**}Y^2$ $R^2 = 0.76$; $Z_{RCT} = 253.81416^{**} + 1.14101^{**}X - 1.16890^{*}Y - 0.00219^{*}X^2 - 0.00391^{*}YX - 0.01289^{*}Y^2$ $R^2 = 0.80$; $Z_{VCD} = 701.38552^{**} + 2.52964^{**}X - 4.42899^{**}Y - 0.00505^{**}X^2 - 0.00619^{*}YX + 0.724^{*}Y^2$ $R^2 = 0.87$; $Z_{VED} = 50.58553^{**} + 0.25031^{**}X - 0.07655^{*}Y - 0.00050^{**}X^2 - 0.00056^{**}YX - 0.00453^{*}Y^2$ $R^2 = 0.89$; * significant diferences at 5%; ** significant diferences at 1%; ns: no significant.

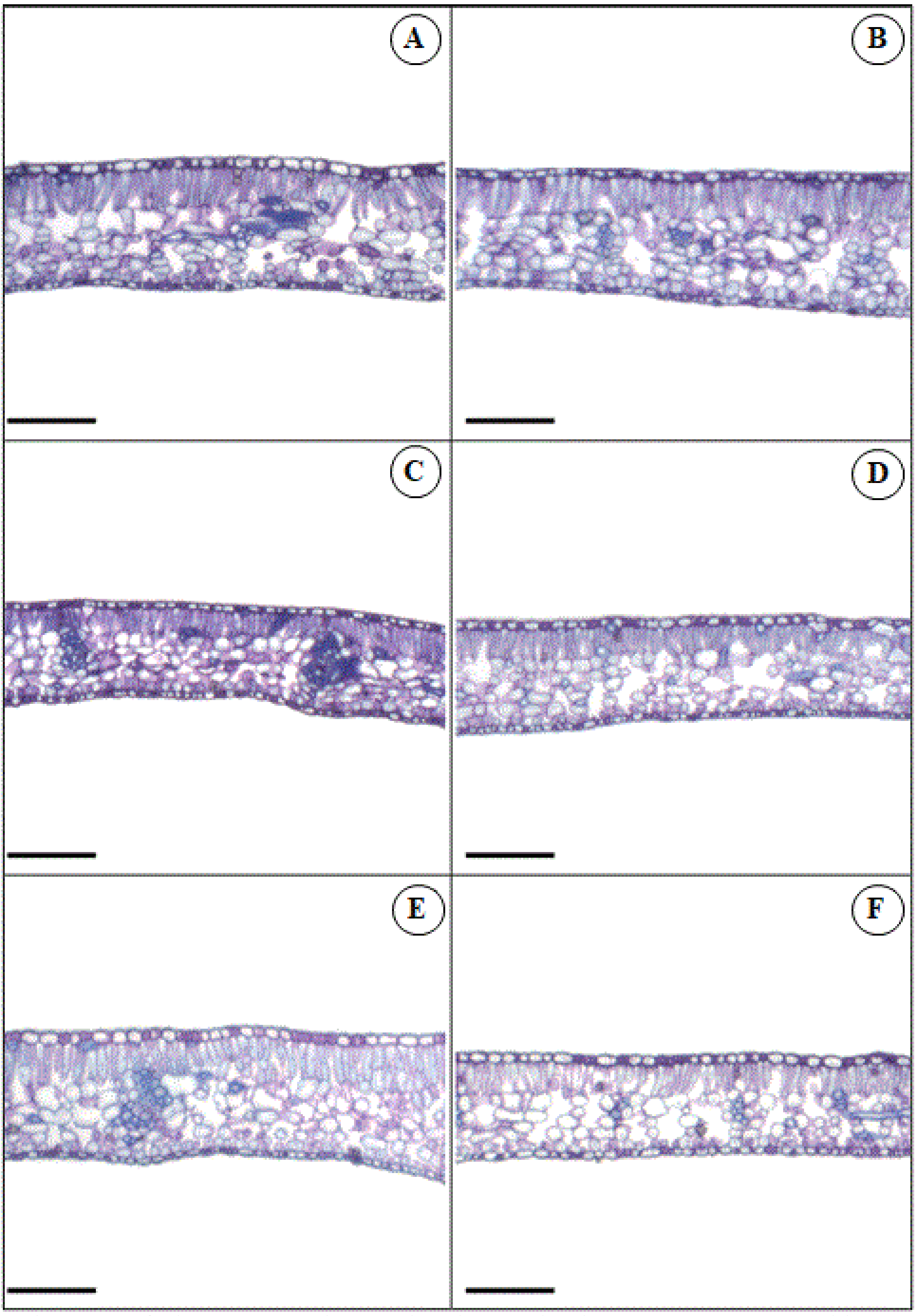

Fig 6. Leaf transversal sections of *Khaya ivorensis* plants under silicon and cadmium treatments. Capital letters represent Cd and Si treatments in mg L^{-1}. A (Cd 0 x Si 0); B (Cd 0 x Si 150); C (Cd 50 x Si 0); D (Cd 50 x Si 150); E (Cd 75 x Si 0); F (Cd 75 x Si 150). Bars: 200 µm.

Cadmium and silicon treatments

During 60 days, plants were exposed to cadmium and silicon treatment interaction. For Cd (cadmium chloride) concentrations were 0, 25, 50, and 75 mg L^{-1}. For Si were 0, 100, 150, and 300 mg L^{-1} (sodium metasilicate) with five repetitions.

Anatomical analyses

Samples were collected from the middle region of the leaf branch. Leaves were fully expanded from the third node and 5 cm of root apex. After that, all material botanicals collected were fixed in FAA 70 for 24 hours dehydrated in ethanol, and soaked in Historesin Leica TM (Leica, Nussloch, Germany). Cross sections with a thickness of 5 µm were obtained through rotative microtome (model Leica RM 2245, Leica Biosystems). Sections were stained with toluidine blue (O'Brien *et al.,* 1964). Lamines were observed and photomicrographed under an optical microscope (Motic BA 310, Motic Group Co. LTD.) coupled to a digital camera (Motic 2500, Motic Group Co., LTD.). Previously calibrated with micrometer lamine, images were analyzed with Moticplus 2.0. Anatomical parameters evaluated were: epidermis thickness from adaxial (ETAd), epidermis thickness from abaxial (ETAb), palisade parenchyma thickness (PPT), spongy parenchyma thickness (SPT) and PPT/SPT ratio. Root diameter (RD), root cortex thickness (RCT), vascular cylinder diameter (VCD) and, vessel element diameter (VED) were measured in root samples

Cadmium and silicon content

The Cd content was determined according to Miyazawa *et al.,* (2009). Samples of 0.5g dry matter (leaf, stem or root) were digested in a digester tube with 8 mL of nitric acid and perchloric acid solution (3:1). The cadmium content was determined by atomic absorption spectrometry. The Si content was determined according to Kraska and Breitenbeck (2010) through wet digestion. In 0.1 g of dry matter (leaf, stem or root), 2 mL of 30% hydrogen peroxide and 0.1 M of sodium hydroxide were added.

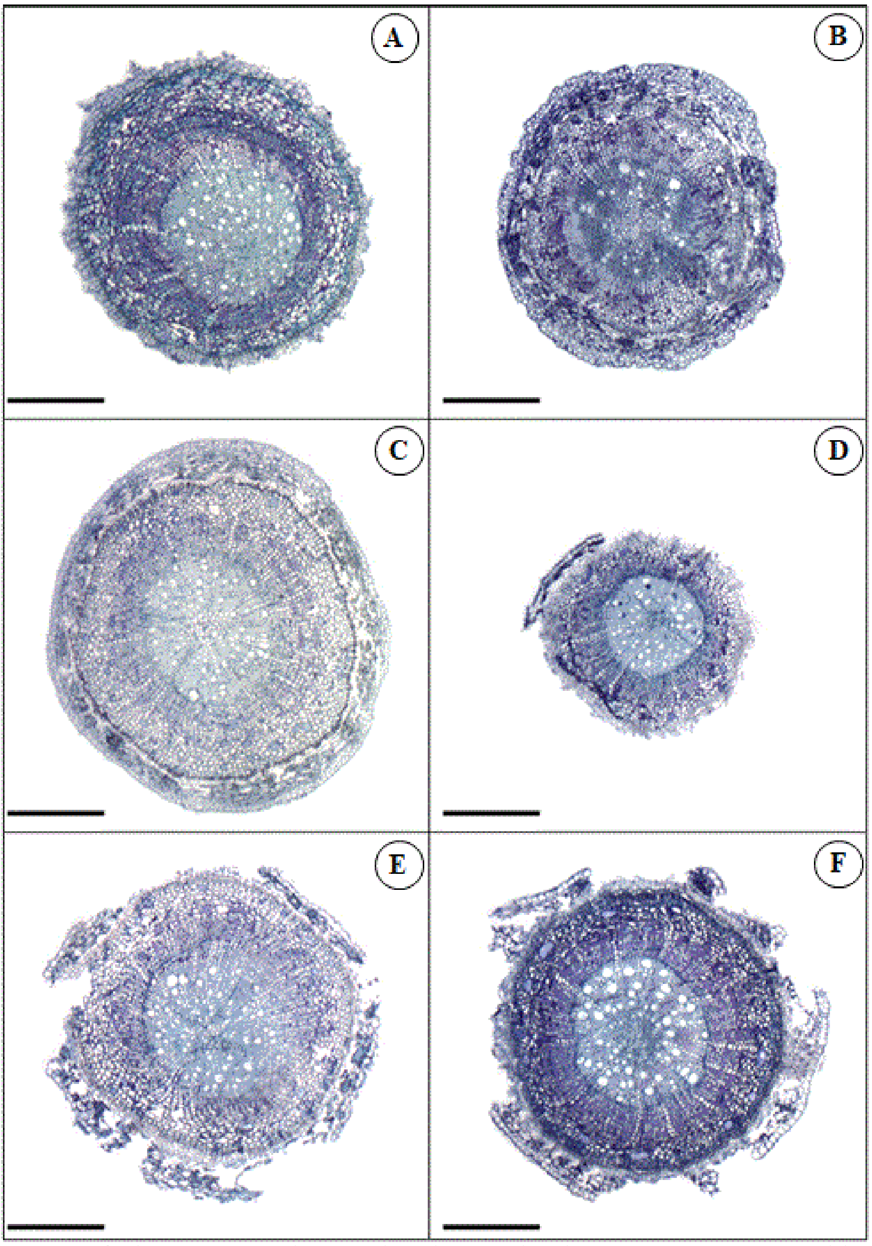

Fig 7. Root transversal sections of *Khaya ivorensis* plants under silicon and cadmium treatments. Capital letters represent Cd and Si treatments in mg L^{-1}. A (Cd 0 x Si 0); B (Cd 0 x Si 150); C (Cd 50 x Si 0); D (Cd 50 x Si 150); E (Cd 75 x Si 0); F (Cd 75 x Si 150). Bars: 200 µm.

The reaction was incubated the in oven at 95 °C for four hours. Ammonium fluoride (NH_4F) was added at 5 mM in samples to facilitate the formation of monosilicic acid. Absorbances were determined using a spectrophotometer at 630nm (Hallmark *et al.*, 1982).

Growth parameters

Plant height (H) and root length (RL) were measured with a millimeter ruler. The leaf area was determined after scanning and processing the images obtained using the ImageJ Software.

Statistical analysis

The statistical package "Statical Analysis System" (SAS Institute, 1999) was used for data analysis. The regression equation model Y (X1, X2) = B0 + B1X1 + B2 X12 + B3 X2 + B4X22 + B5X1 * X2 was generated by procedure PROC Rs reg. The F test was performed to significance ($p<0.05$) of the interaction between cadmium and silicon concentrations. Then, response surface analysis was performed.

Conclusion

This study presents unedited evidence about the effect of cadmium and silicon on *Khaya ivorensis* because were founds toleration of cadmium toxicity up to 25 mg L^{-1} Cd without considerable growth reduction. Furthermore, silicon has shown a positive modulation in tissue thickness, which is important to water use efficiency and CO_2 carboxylation with positive impacts on the vegetative growth of *Khaya ivorensis*. Therefore, this study indicates that *Khaya ivorensis* tolerates cadmium toxicity up to 25 mg L^{-1} Cd and positively responds to anatomical modulations induced by silicon.

Acknowledgment

This work was supported by the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq/Brasil). In addition, we thank the Museu Paraense Emílio Goeldi (MPEG/Brazil) for the use of infrastructure for anatomical analysis.

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