Doi: https://doi.org/10.17584/rcch.2022v16i2.14530

Liming applications and the SPAD chlorophyll index and stomatal conductance in cocoa exposed to cadmium in the soil

Aplicaciones encalántes y el índice de clorofilas SPAD y la conductancia estomática de cacao expuesto a cadmio en el suelo

KAREN VICTORIA SUÁREZ-PARRA[1, 2]
CRISTIAN SANTIAGO CASTAÑEDA-SERRANO[1]
FABIO EMILIO FORERO-ULLOA[1]
PEDRO JOSÉ ALMANZA-MERCHÁN[1]
PABLO CÉSAR SERRANO-AGUDELO[1]

Cacao clon CCN-51.

Photo: C.S. Castañeda-Serrano

ABSTRACT

Cadmium is a heavy metal that affects cell structures, such as walls and membranes, especially in the photosynthetic apparatus (PSII), chlorophylls, chloroplasts and stomata, producing losses in production quantity and quality. In addition, it is harmful to the health of humans and animals. The objective was to analyze the behavior of the relative chlorophyll index (SPAD units) and stomatal conductance in clone CCN-51 cacao plants every 45 days (45, 90, 135 and 180 days) after liming application. Four doses of a dolomite + agricultural gypsum mixture were applied, increasing Ca^{+2} saturation in the soil to 7, 8 and 9 $cmol_c\ kg^{-1}$. The control treatment did not have applications. The results indicated a reduction in SPAD units in the plants without liming, with high cadmium levels in the soil (3.3 $mg\ kg^{-1}$), and there were no statistical differences in the other treatments, possibly because of edaphic factors such as pH, organic matter content and Al^{+3}. The best stomatal conductance was observed with 7 $cmol_c\ kg^{-1}$ in the foliar gas exchange. Supersaturated liming applications efficiently reduce the losses in quality and quantity caused by the accumulation of cadmium in cacao plants.

Additional key words: heavy metals; soil amendments; transpiration; pigments; *Theobroma cacao* L.

[1] Universidad Pedagógica y Tecnológica de Colombia, Facultad de Ciencias Agropecuarias, Grupo de investigación GIPSO, Tunja (Colombia). ORCID Suárez-Parra, K.V.: 0000-0003-2736-9070; ORCID Castañeda-Serrano, C.S.: 0000-0002-1772-5628; ORCID Forero-Ulloa, F.E.: 0000-0001-6815-6579; ORCID Almanza-Merchán, P.J.: 0000-0002-9207-0617; ORCID Serrano-Agudelo, P.C.: 0000-0002-2563-2050

[2] Corresponding author. ksuarez98@unisalle.edu.co

RESUMEN

El cadmio es un metal pesado que causa afectaciones en estructuras celulares como paredes y membrana, especialmente en el aparato fotosintético (PSII), clorofilas, cloroplastos y estomas, produciendo perdidas en cantidad y calidad de la producción. Además, es un agente potencialmente maligno para la salud de humanos y animales. El objetivo de este trabajo consistió en analizar el comportamiento del índice relativo de clorofilas (unidades SPAD) y la conductancia estomática en plantas de cacao clon CCN-51 con la frecuencia de 45 días (45, 90, 135 y 180 días) posterior a la aplicación de encalado. Se aplicaron cuatro dosis de la mezcla dolomita + yeso agrícola aumentando la saturación de Ca^{+2} en el suelo a 7, 8 y 9 $cmol_c$ kg^{-1} y el tratamiento control sin aplicación. Los resultados indican reducción de las unidades SPAD en plantas sin encalado con altos niveles de cadmio en el suelo (3.3 mg kg^{-1}) y no se presentaron diferencias estadísticas con los demás tratamientos, posiblemente a la incidencia de factores edáficos como el pH, los contenidos de materia orgánica y Al^{+3}. Los mejores resultados de conductancia estomática se observaron en 7 $cmol_c$ kg^{-1} en el intercambio gaseoso foliar. Las aplicaciones de materiales encalántes en sobresaturación pueden convertirse en una medida eficiente para disminuir pérdidas en calidad y cantidad ocasionadas por las acumulaciones de cadmio en cacao.

Palabras clave adicionales: metales pesados; enmiendas del suelo; transpiración; pigmentos; *Theobroma cacao* L.

Received: 30-06-2022 Accepted: 19-09-2022 Published: 27-10-2022

INTRODUCTION

Cadmium (Cd) is a heavy element found on the surface of the Earth's crust at low concentrations in the divalent form (Cd^{2+}), with high mobility in the soil and in plants (Santos *et al*., 2020). Currently, cadmium concentrations in agricultural soils are high and present an environmental problem (Meter *et al*., 2019). Notably, some crops are bioaccumulators, creating a threat to human and animal health (Li *et al*., 2022a). Cadmium accumulates in soil in various ways, most related to anthropogenic activities (Argüello *et al*., 2018). Per European regulations, the maximum permitted concentration of cadmium in fruits is 0.05 mg kg^{-1} of fresh weight, a factor for the Latin American market given the current concentrations in the soil and cocoa beans (European Commission, 2021). For the United States, the permissible range is 0.8 to 1.2 mg kg^{-1}; however, high levels in the cocoa market are concerning (Vanderschueren *et al*., 2021).

In Latin America, cocoa-producing areas have exceptionally high concentrations that are associated with fertilizer applications with high concentrations of cadmium (0.22-10.8 mg kg^{-1}) and suitable geological traits, especially in young soils, such as Entisols and Inceptisols (Ramtahal *et al*., 2019; Rodríguez *et al*., 2019; Vanderschueren *et al*., 2021).

Once cadmium is in the soil solution, it passes through the cells of the cortical root and moves the apoplastic or symplastic xylem route to the upper parts of the plant, with adverse effects on cell stability that result in oxidative burst and altered cellular functions that reduce photosynthesis and alter concentrations of mineral nutrients, energy transduction, protein synthesis and gas exchange (Castro *et al*., 2015 ; Barraza *et al*., 2017; Kapoor *et al*., 2021).

The accumulation of cadmium (Cd^{2+}) alters the structure of the chloroplast, inhibits the electron transport chain, and decreases the chlorophyll content and photosynthetic rate (Huo *et al*., 2022). According to Bayona-Penagos (2020) and Sánchez-Zepeda *et al*. (2021), these effects result from the replacement of Mg^{2+} in chlorophylls *a* and *b*, and competition in the entry of Ca^{2+} in the protective cells, causing poor stomatal closure, limiting the establishment of intercellular spaces in the leaves, inducing fusion of membranes and the cell wall, and increasing the size and quantity of starch grains, plastoglobules and lipids in the chloroplasts (Rabêlo *et al*., 2022).

Cadmium (Cd^{2+}) is usually found with sulfur (S) at high concentrations in cytoplasmic enzymes that maintain a reduced state through a constant supply of metabolic energy, causing oxidative stress, which damages cells in structures responsible for gas exchange (Herrera, 2000; Qin *et al*., 2020). There is also an increase in stomatal resistance to CO_2 intake, toxic effects initiated at the root where cadmium interferes with the absorption and translocation of water (Moreno *et al*., 2013). However, Fernández (2022) noted the lack of knowledge on how cadmium affects gas exchange processes.

Mitigation techniques for cadmium in tropical cacao soils include applications of lime, which involve calcium and magnesium, to improve the soil exchange complex and benefit plant nutrition, where calcium shares many chemical properties with cadmium, meaning they compete for absorption by roots (Meter *et al*., 2019).

In Latin American soils, the effectiveness of calcium supersaturation has been proven through calcareous amendments such as dolomite and gypsum, generating an oversupply of Ca^{2+} and Mg^{2+} cations that, through a combined effect, neutralize acidity interchangeable and contribute to the soil nutrition, facilitating more efficient absorption of nutritional elements with biochemical functions by plants that improve yield, where cell structures affected by Cd will gradually recover (molecules of chlorophyll and other structures associated with calcium and magnesium) (Castro *et al*., 2015; Suárez-Salazar *et al*., 2017; Huang *et al*., 2020; Zhang *et al*., 2020; Huo *et al*., 2022). Successful cases of Cd mitigation were reported by Tantalean and Huauya (2017), Wong (2017), Pérez *et al*. (2019), Florida *et al*. (2019) and Huaraca-Fernandez *et al*. (2020), where Cd decreased in the cacao zone of central Colombia (Rodríguez *et al*., 2019).

The CCN-51 cacao clone is one of the more commercial, with the highest production and planted area in Latin America (Espinoza, 2019). The objective was to evaluate the application of gypsum and dolomite lime as liming agents to determine the behavior of the relative chlorophyll index (SPAD units) and stomatal conductance in CCN-51 cacao clone exposed to cadmium in western Boyaca (Colombia). Increasing the concentration of calcium in the soil displaces the exchangeable aluminum (5 meq mol kg^{-1} of soil), where the oversupply of calcium means plants absorb it efficiently, and adsorbed cadmium decreases, along with its negative impacts on cellular tissues.

MATERIALS AND METHODS

This experiment was carried out on the Mata de Limón farm in the Palenque village of the municipality of Otanche (5°39”28” N and 74°10”50” W), in the western province of the Department of Boyacá (Colombia), with a 6-year-old cacao crop (clone CCN-51) at 1,100 m a.s.l., monthly average rainfall of 264.55 mm, monthly relative humidity of 80%, and monthly average environmental temperature of 24.03°C, a Tropical Humid Forest life zone according to the Holdridge scale (Motta-Delgado and Ocaña-Martínez, 2018).

A completely randomized design with four treatments was carried out and replicated three times. The treatments corresponded to the mixture of calcareous amendments, dolomite (Do) and gypsum (y) at 7, 8 and 9 $cmol_c\ kg^{-1}$ of Ca^{2+} in the soil. The control did not have applications of calcareous amendments. The concentrations of the liming material were calculated using equation (1) of Cochrane *et al*. (1980) and the information of soil analysis (Tab. 1).

$$\text{Lime required } (CaCO_3 \text{ equivalent, t ha}^{-1}) = 1.5([Al\text{-}RAS] \ x\ ECEC)/100 \quad (1)$$

Table 1. Physicochemical analyses of the soil before applications of the amendment.

Texture	Sand (%)	Lime (%)	Clay (%)	pH	OM (%)	EA	Al^{3+} ($cmol_c\ kg^{-1}$)	H^+ ($cmol_c\ kg^{-1}$)	Cd ($cmol_c\ kg^{-1}$)
Clayey	29	24	47	4.75	2.49	5.60	5.0	0.60	3.3

Ca^{2+} ($cmol_c\ kg^{-1}$)	Mg^{2+} ($cmol_c\ kg^{-1}$)	K^+ ($cmol_c\ kg^{-1}$)	Na^+ ($cmol_c\ kg^{-1}$)	P (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	B (mg kg^{-1})
1.84	0.57	0.15	0.01	10.23	131.86	2.20	1.53	0.13

Texture (Bouyucos), pH (1:1 ratio), OM-organic material (Walkey-Black), EA-exchangeable acidity ($Al^{3+} + H^+$) (KCl 1N), Cd (P-PAS-21 procedure for the determination of cadmium in soils [NTC 3888 – Incontec, 2014]), Ca-Mg-k-Na (atomic absorption), P (Bray II - colorimetry), Fe-Mn-Zn (DTPA extract – atomic absorption), and boron (hot water).

Where, *Al* is the current aluminum percentage ($cmol_c$ kg^{-1}), *RAS* is required percentage Al saturation, and ECEC is the effective cation exchange capacity.

The calcareous materials were selected by taking into account the percentage of participation of the elements calcium and magnesium, the relative power of total neutralization of acidity of the amendment, and references in the literature that an amendment reduces cadmium concentrations in tropical cacao soils.

Healthy 6-year-old plants of a similar size were selected. The amendments were applied in a crescent on the upper part of the trunk. The selected plants had floral differentiation and fruit set, some in the gherkin state (according to the BBCH scale), with a length not exceeding 5 cm.

After the application of the treatments, samples of the chlorophyll index variables (SPAD units) and stomatal conductance were carried out at four intervals of 45 days after application (daa). The samplings were carried out at the same hour. Four leaves that were exposed directly to the sun were selected, from productive branches of the middle-third of 10 plants. Three samples per leaf were evaluated to calculate the average for the data per experiment unit. A SPAD-502Plus chlorophyll meter (Konica Minolta, Osaka, Japan) and SC-1 Leaf porometer (Meter, Decagon Devices Inc., Pullman, WA) were used. The samples for the soil Cd analysis were taken at 0-10 and 10-20 cm depths and were determined with the P-PAS-21 procedure following the NTC 3888 standard (Incontec, 2014).

Statistical analysis

The data were subjected to an analysis of variance after verifying the assumptions of normal and homogeneity with the Shapiro-Wills and Bartlett tests ($P<0.05$), respectively. Subsequently, Tukey's multiple comparison test of means ($P<0.05$) was performed for each measurement interval. The statistical software Infostat was used.

RESULTS AND DISCUSSION

Relative chlorophyll index

During the four evaluation periods, there were no statistically significant differences between the treatments (Tab. 2).

For the treatment without amendment applications (control), the SPAD units were reduced throughout the four evaluation intervals. The first interval (45 daa) had the highest value with 65.15 SPAD, and 35.73 SPAD units were recorded at 180 daa. The behavior of the chlorophyll index in the control treatment confirmed the reports by Jácome (2017) and Fernández (2022), who stated that SPAD units decrease as plants adsorb more cadmium. Hakeem *et al*. (2022) reported that the degradation of photosynthetic pigments in plants can decrease by up to 80% under cadmium stress, which can be corrected with calcium applications that reduce the degradation of the chlorophyll molecule.

At 90 daa, the lowest SPAD chlorophyll index was observed, which was related to high concentrations of cadmium, especially in the control treatment with an average value of 3.3 mg kg^{-1} in the soil, accompanied by low pH values (4.2 - 4.7), coinciding with the highest rainfall (552 mm) and concentrations of Al^{+3} (7 meq mol kg^{-1}). These conditions created a greater expression of the stressful action of cadmium, affecting the chlorophyll synthesis, hindering photosynthetic electron transfer, and resulting in excess electrons in the photosynthetic electron transfer chain with a rapid increase in ROS (reactive oxygen species) and feedback inhibition of plant photosynthesis, which

Table 2. SPAD units in *T. cacao* clone CCN-51 subjected to calcium supersaturation under cadmium stress.

Treatment	Days after the application			
	45	90	135	180
Control	65.15±1.59	48.05±1.24	43.85±1.99	35.73±1.99
Do+y7	55.2±1.36	41.4±7.44	62.55±9.90	42.93±6.98
Do+y8	55.87±1.65	52.4±1.38	43.5±4.7	62.3±17.1
Do+y9	56.3±4.01	46±1.67	48.4±1.84	45.33±13.01

Control: no application of amendment; Do+y7: dolomite + gypsum at 7 $cmol_c$ kg^{-1} of Ca; Do+y8: dolomite + gypsum at 8 $cmol_c$ kg^{-1} of Ca; Do+y9: dolomite + gypsum at 9 $cmol_c$ kg^{-1} of Ca. Means±standard error.

impairs the maintenance of the redox balance in the chloroplast (Huang *et al*., 2020; Zhang *et al*., 2020; He *et al*., 2021; Huo *et al*., 2022).

Cadmium inhibits root growth, decreases chlorophyll content, and suppresses photoactivation of plant photosystem II (PSII) by preventing electron transport. In addition, it interferes with carbon metabolism and contributes to water stress in plants and the absorption of nutrients, affecting the respiration process and accumulation of cadmium in organs, such as leaves in cacao plants (Huang *et al*., 2017; Kapoor *et al*., 2021).

Reducing the quantity and quality of chlorophylls in leaf blades in turn decreases photosynthetic activity, considerably limiting plant growth and development, leading to greater susceptibility to diseases and low-quality production, and resulting in definitive loss of the plant because of a deficient conversion of photo-assimilates and nutritional elements (Choudhury *et al*., 2022).

Among the treatments of calcareous amendments, Do+y8 stood out (Tab. 3) with fewer variations in the relative chlorophyll index, where, 45 daa, values of 55.87 SPAD units were recorded that slowly decreased to 52.4 SPAD on day 90. The lowest value occurred at 135 d (43.5 SPAD), until reaching 62.3 SPAD units at 180 daa of the liming agents.

The trend in treatments Do+y7, Do+y8 and Do+y9 for the relative chlorophyll index (SPAD) may have been related to the action of the applied amendment as a result of a significant increase in soil pH, which started at 4.75 and reached 5.2 on average in all treatments, possibly decreasing the mobility and concentration of cadmium (average of 0.3 mg kg^{-1} in soil for all treatments, except the sampling at 45 daa with 0.1 mg kg^{-1} of Cd). Hu *et al*. (2016), Sun *et al*. (2016), Huang *et al*. (2017), Huaraca-Fernandez *et al*. (2020) and Hakeem *et al*. (2022) stated that Cd is found in complexes that can precipitate more easily, such as the formation of cadmium sulfate ($CdSO_4$) because the reaction with calcium sulfate ($CaSO_4$. $2H_2O$) or agricultural gypsum in the redox process can lead to dissolution or precipitation of cadmium. The interaction of S within plants promotes the precipitation or immobilization of cadmium in the cells and facilitates contractions of calcium and magnesium, reducing the absorption of cadmium and generating less sensitive cell walls (Cao *et al*., 2018; Guan *et al*., 2018; Yao *et al*., 2021).

By fixing calcium ions on the negative charges of the colloidal fraction in the soil, calcium enters the plant more efficiently and facilitates a recovery of metabolic activity, especially in the reconstruction of chlorophyll molecules where significant concentrations of calcium and magnesium are required, improving photosynthetic efficiency (Choudhury *et al*., 2022; Li *et al*., 2022b). Likewise, soil moisture plays an essential role in the release of calcium in the soil solution, as well as the precipitation of cadmium in the form of cadmium sulfate ($CdSO_4$), because the correct activation of amendments requires a sufficient amount of water; the average rainfall in the area is sufficient (262.53 mm) and does not require additional water applications, which is why, at 45 daa, the cadmium concentrations were 0.1 mg kg^{-1}.

Stomatal conductance

The stomatal conductance presented statistically significant differences ($P<0.05$) between treatments at

Table 3. Stomatal conductance of *T. cacao* clone CCN-51 subjected to different doses of calcium supersaturation under cadmium stress.

Treatments	Days after the application			
	45	90	135	180
Control	267±6.95 ab	471.3±16.16 a	62.13±8.23 b	418.53±3.57 a
Do+y7	184.5±0.57 b	379.15±4.64 b	415.03±9.5 a	500.03±2.41 a
Do+y8	329±1.70 a	504.15±3.17 a	165.07±4.65 ab	483.33±1.06 a
Do+y9	321±3.51 a	472.55±1.58 a	173.87±1.86 ab	337.60±1.09 b

Control: no application of amendment; Do+y7: dolomite + gypsum at 7 cmolc kg^{-1} of Ca; Do+y8: dolomite + gypsum at 8 cmolc kg^{-1} of Ca; Do+y9: dolomite + gypsum at 9 cmolc kg^{-1} of Ca.

Means with different letters in the column show significant differences according to the Tukey test ($P<0.05$). Means±Standard error.

45, 135 and 180 DAA, with the Do+y7 and Do+y8 treatments showing the best responses (Tab. 4).

The lowest stomatal conductance (Tab. 3) was observed at 135 DAA in the control treatment (62.13 mmol m^2 s^{-1}), where a greater deterioration of cellular structures was suggested where cadmium replaced calcium, as reported by Kapoor *et al*. (2021), entering plant as a result of the effect of physiological sensitivity.

Treatments Do+y7 and Do+y8 had higher measurements (greater than 500 mmol m^2 s^{-1}, Tab. 3). The control treatment had values between 267 mmol m^2 s^{-1} (45 daa) and 418.53 mmol m^2 s^{-1} (180 daa), and the Do+y7 treatment sustained an increase throughout the experiment, from 184.5 mmol m^2 s^{-1} (45 daa) to 481 mmol m^2 s^{-1} (180 daa), considered the best treatment, improving the opening and closing capacity of the stomata, providing a better respiratory process, and achieving better efficiency in gas exchange (Suárez-Salazar *et al*., 2017). Considerable concentrations of cadmium in cellular structures increase susceptibility to water stress because, while calcium has the biological function of opening and closing stomata and allowing adequate gas exchange, stomata with cadmium cannot close normally, affecting the photosynthetic process through an inefficient CO_2 exchange, increasing damage to the photosystem by stress (Dell'Amico and Morales-Guevara, 2017; Ramtahal *et al*., 2019; Choudhury *et al*., 2022; Li *et al*., 2022a).

Although little is known about the dynamics of cadmium in affecting stomatal conductance, it can be assumed that the insufficiency in the respiratory process caused by cadmium is related to a calcium deficiency in the system, which can be corrected with calcium applications. Li *et al*. (2022b) stated that increasing extracellular calcium and cytosolic calcium leads to efficient stomatal closure, allowing better performance of stomatal conductance, giving better conditions for their closing and opening, and increasing stomatal density. In addition, there is a better and greater production of dibromothymoquinone (DBMIB), an enzyme that allows stomatal closure, where H_2O_2 generated by chloroplast in the mesophyll is necessary to avoid losses from stress. This explains the behavior of the variable in the Do+y7 treatment, which had an increasing trend.

Although no consistent data were found, multiple factors affected the stomatal opening of the plants that were related to stomatal conductance, CO_2 assimilation, air humidity and leaf temperature, along with soil moisture and efficiency of plants to transport water under stress from biotic or abiotic factors, which must be taken into account when evaluating the variable (Pino *et al*., 2019).

CONCLUSIONS

The relative chlorophyll index in the treatments with calcareous amendments did not have significant statistical differences; however, it increased throughout the experiment. The Do+y8 treatment presented the best response with 62.3 SPAD units at 180 days after the application of the calcareous amendment.

The stomatal conductance presented significant statistical differences between treatments, where treatment Do+y7 had an increase at each evaluation interval, which could indicate a recovery of stomatal activity, improving the physiological process of gas exchange and decreasing cadmium affectation.

Applications of amendments or supersaturated applications of calcium in the soil can be a useful tool for reducing losses caused by the accumulation of cadmium in plants; however, these applications must be made consecutively and must complement each other to achieve long-term results.

Conflict of interests: This manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

Acknowledgments

The authors thank the Gobernación de Boyacá, the Ministerio de Ciencia y Tecnología, and the Cooperativa Integral de productores agropecuarios de Otanche – CIPAOTANCHE, for the financing and support provided via Contrato No. 80740-691-2020 convocatoria 865 de 2019 Cierre de Brechas for the Departament of Boyacá.

BIBLIOGRAPHIC REFERENCES

Argüello, D., E. Chavez, F. Lauryssen, R. Vanderschueren, E. Smolders, and D. Montalvo. 2018. Soil properties and agronomic factors affecting cadmium concentrations in cacao beans: A nationwide survey in Ecuador. Sci. Total Environ. 649, 120-127. Doi: https://doi.org/10.1016/j.scitotenv.2018.08.292

Barraza, F., E. Schreck, T. Lévêque, G. Uzu, F. López, J. Ruales, J. Prunier, A. Marquet, and L. Maurice. 2017. Cadmium bioaccumulation and gastric bioaccessibility in cacao: A field study in areas impacted by oil activities in Ecuador. Environ. Pollut. 229, 950-963. Doi: https://doi.org/10.1016/j.envpol.2017.07.080

Bayona-Penagos, L.V. 2020. Efecto y mitigación de la toxicidad por arsénico y cadmio en cultivo de arroz. Rev. Cienc. Agropec. 6(2), 49-70.

Cao, Z.-Z., M.L. Qin, X.Y. Lin, Z.-W. Zhu, and M.-X. Chen. 2018. Sulfur supply reduces cadmium uptake and translocation in rice grains (*Oryza sativa* L.) by enhancing iron plaque formation, cadmium chelation and vacuolar sequestration. Environ. Pollut. 238, 76-84. Doi: https://doi.org/10.1016/j.envpol.2018.02.083

Castro, A.V., A.-A.F. Almeida, C.P. Pirovani, G.S.M. Reis, N.M. Almeida, and P.A.O. Mangabeira. 2015. Morphological, biochemical, molecular and ultrastructural changes induced by Cd toxicity in seedlings of *Theobroma cacao* L. Ecotoxicol. Environ. Saf. 115, 174-186. Doi: https://doi.org/10.1016/j.ecoenv.2015.02.003

Choudhury, M.R., J. Christopher, S. Das, A. Apan, N.W. Menzies, S. Chapman, V. Mellor, and Y.P. Dang. 2022. Detection of calcium, magnesium, and chlorophyll variations of wheat genotypes on sodic soils using hyperspectral red edge parameters. Environ. Technol. Innov. 27, 102469. Doi: https://doi.org/10.1016/j.eti.2022.102469

Cochrane, T.T., J.G. *Salinas*, and P.A. *Sánchez. 1980*. An equation for liming acid mineral soils to compensate crop aluminum tolerance. Trop. Agric. 57(2), 133-140.

Dell'Amico-Rodriguez, J.M. and D.M. Morales-Guevara. 2017. Comportamiento de la conductancia estomática de dos variedades de tomate cubanas en condiciones de campo y riego limitado. Cult. Trop. 38(2), 137-144.

Espinoza Principe, B.J. 2019. Efecto del compost, dolomita y magnocal en el contenido de cadmio del suelo y los granos de cacao (*Theobroma cacao* L.) del clon ccn-51. Undergraduate thesis. Facultad de Agronomía, Universidad Nacional Agraria de La Selva, Tingo Maria, Peru. http://repositorio.unas.edu.pe/handle/UNAS/1492

European Commission. 2021. Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in certain foodstuffs. OJEU L288/13. Brussels.

Fernández Paz, J.A. 2022. Efecto fisiológico de la absorción de cadmio (Cd^{2+}) sobre accesiones de cacao (*Theobroma cacao* L.). MSc thesis. Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Palmira, Colombia.

Florida Rofner, N., H. Juan, P. García, S.S. Jacobo Salinas, F. Escobar Mamani, and J. Torres García. 2019. Efecto de compost y NPK sobre los niveles de microorganismos y cadmio en suelo y almendra de cacao. Rev. Investig. Altoandin. 21(4), 264-273. Doi: https://doi.org/10.18271/RIA.2019.503

Guan, M.Y., H.H. Zhang, W. Pan, C.W. Jin, and X.Y. Lin. 2018. Sulfide alleviates cadmium toxicity in *Arabidopsis* plants by altering the chemical form and the subcellular distribution of cadmium. Sci. Total Environ. 627, 663-670. Doi: https://doi.org/10.1016/J.SCITOTENV.2018.01.245

Hakeem, K.R., H.F. Alharby, and T.B. Pirzadah. 2022. Exogenously applied calcium regulates antioxidative system and reduces cadmium-uptake in *Fagopyrum esculentum*. Plant Physiol. Biochem. 180, 17-26. Doi: https://doi.org/10.1016/J.PLAPHY.2022.03.011

He, L.-L., D.Y. Huang, Q. Zhang, H.-H. Zhu, C. Xu, B. Li, and Q.-H. Zhu. 2021. Meta-analysis of the effects of liming on soil pH and cadmium accumulation in crops. Ecotoxicol. Environ. Saf. 223, 112621. Doi: https://doi.org/10.1016/j.ecoenv.2021.112621

Herrera Marcano, T. 2000. La contaminación con cadmio en suelos agrícolas. Venesuelos 8(1-2), 42-47.

Hu, Y., H. Cheng, and S. Tao. 2016. The challenges and solutions for cadmium-contaminated rice in China: a critical review. Environ. Int. 92-93, 515-532. Doi: https://doi.org/10.1016/j.envint.2016.04.042

Huang, Y., H. Sheng, P. Zhou, and Y. Zhang. 2020. Remediation of Cd-contaminated acidic paddy fields with four-year consecutive liming. Ecotoxicol. Environ. Saf. 188, 109903. Doi: https://doi.org/10.1016/j.ecoenv.2019.109903

Huang, M., H. Zhu, J. Zhang, D. Tang, X. Han, L. Chen, D. Du, J. Yao, K. Chen, and J. Sun. 2017. Toxic effects of cadmium on tall fescue and different responses of the photosynthetic activities in the photosystem electron donor and acceptor sides. Sci. Rep. 7, 14387. Doi: https://doi.org/10.1038/s41598-017-14718-w

Huaraca-Fernandez, J.N., L. Pérez-Sosa, L.S. Bustinza-Cabala, and N.B. Pampa-Quispe. 2020. Enmiendas orgánicas en la inmovilización de cadmio en suelos agrícolas contaminados: una revisión. Inf. Tecnol. 31(4), 139-152. Doi: https://doi.org/10.4067/S0718-07642020000400139

Huo, L., Z. Guo, Q. Wang, X. Jia, X. Sun, and F. Ma. 2022. The protective role of *MdATG10*-mediated autophagy in apple plant under cadmium stress. Ecotoxicol. Environ. Saf. 234, 113398. Doi: https://doi.org/10.1016/j.ecoenv.2022.113398

Incontec, Instituto Colombiano de Normas Técnicas y Certificación. 2014. NTC 3888. Gestión ambiental. Calidad del suelo extracción de elementos traza solubles en agua regia. Bogota.

Jácome Molina, D.M. 2017. Efecto de la inoculación de hongos formadores de micorrizas arbusculares (HFMA) sobre un sistema suelo-planta de cacao en suelos contaminados con cadmio en etapa de vivero. MSc thesis. Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota. https://repositorio.unal.edu.co/handle/unal/62938

Kapoor, D., S. Singh, P.C. Ramamurthy, S. Jan, S. Bhardwaj, S.S. Gill, R. Prasad, and J. Singh. 2021. Molecular consequences of cadmium toxicity and its regulatory networks in plants. Plant Gene 28, 100342. Doi: https://doi.org/10.1016/j.plgene.2021.100342

Li, B., L. Hou, C. Song, Z. Wang, Q. Xue, Y. Li, J. Qin, N. Cao, C. Jia, Y. Zhang, and W. Shi. 2022a. Biological function of calcium-sensing receptor (CAS) and its coupling calcium signaling in plants. Plant Physiol. Biochem. 180, 74-80. Doi: https://doi.org/10.1016/j.plaphy.2022.03.032

Li, J., S. Zhang, and X. Ding. 2022b. The combined application of biochar and high phosphate fertilizer promoted the mobilization and redistribution of cadmium in rhizosphere soil. J. Environ. Chem. Eng. 10(3), 107482. Doi: https://doi.org/10.1016/j.jece.2022.107482

Meter, A., R.J. Atkinson, and B. Laliberte. 2019. Cadmio en el cacao de América Latina y el Caribe. Análisis de la investigación y soluciones potenciales para la mitigación. Bioversity International, Roma.

Moreno Chacón, A.L., M. Cruz Aguilar, and L.M. Melgarejo. 2013. Respuesta fisiológica de plántulas de *Avicennia germinans* y *Rhizophora mangle* frente al cadmio. pp. 153-174. In: Melgarejo, L.M. and C.B. García Ramírez (eds.). Investigación en ciencias del mar: aportes de la Universidad Nacional de Colombia. Universidad Nacional de Colombia, Bogota.

Motta-Delgado, P.A. and H.E. Ocaña-Martinez. 2018. Caracterización de subsistemas de pasturas braquiarias en hatos de trópico húmedo, Caquetá, Colombia. Cienc. Agric. 15(1), 81-92. Doi: https://doi.org/10.19053/01228420.v15.n1.2018.7759

Pérez Moncada, U.A., M. Ramìrez Gómez, D.P. Serralde Ordoñez, A.M. Peñaranda Rolón, W.A. Wilches Ortiz, L. Ramírez, and G.A. Rengifo Estrada. 2019. Hongos formadores de micorrizas arbusculares (HFMA) como estrategia para reducir la absorción de cadmio en plantas de cacao (*Theobroma cacao*). Terra Latinoam. 37(2), 121-130. Doi: https://doi.org/10.28940/terra.v37i2.479

Pino V., E., I. Montalván D., A. Vera M., L. Ramos F. 2019. La conductancia estomática y su relación con la temperatura foliar y humedad del suelo en el cultivo del olivo (*Olea europaea* L.), en periodo de maduración de frutos, en zonas áridas. La Yarada, Tacna, Perú. Idesia 37(4), 55-64. Doi: https://doi.org/10.4067/S0718-34292019000400055

Qin, S., H. Liu, Z. Nie, Z. Rengel, W. Gao, C. Li, P. Zhao. 2020. Toxicity of cadmium and its competition with mineral nutrients for uptake by plants: a review. Pedosphere 30(2), 168-180. Doi: https://doi.org/10.1016/S1002-0160(20)60002-9

Rabêlo, F.H.S., G.S. Daneluzzi, F.H. Santos, M. Colzato, G.S. Montanha, L.R. Nakamura, H.W.P. Carvalho, J. Lavres, and L.R.F. Alleoni. 2022. Role of nodes in accumulation and distribution of cadmium and its relationship with nutrient distribution and photosynthesis in the growth and regrowth of *Brachiaria decumbens*. Environ. Exp. Bot. 195, 104794. Doi: https://doi.org/10.1016/j.envexpbot.2022.104794

Ramtahal, G., P. Umaharan, A. Hanuman, C. Davis, and L. Ali. 2019. The effectiveness of soil amendments, biochar and lime, in mitigating cadmium bioaccumulation in *Theobroma cacao* L. Sci. Total Environ. 693, 133563. Doi: https://doi.org/10.1016/j.scitotenv.2019.07.369

Rodríguez Albarracín, H.S., A.E. Darghan Contreras, and M.C. Henao. 2019. Spatial regression modeling of soils with high cadmium content in a cocoa producing area of Central Colombia. Geoderma Reg. 16, e00214. Doi: https://doi.org/10.1016/J.GEODRS.2019.E00214

Sánchez-Zepeda, M.Y., M. López-Herrera, and L. Romero-Bautista. 2021. Determinación de la capacidad de biacumulación de cadmio en *Vicia faba* L. y su efecto en la raíz y el crecimiento vegetativo. Rev. Biol. Agrop. Tuxpan 9(2), 46-60. Doi: https://doi.org/10.47808/revistabioagro.v9i2.358

Santos, M.L.S., A.-A.F. Almeida, N.M. Silva, B.R.M. Oliveira, J.V.S. Silva, J.O. Souza Junior, D. Ahnert, and V.C. Baligar. 2020. Mitigation of cadmium toxicity by zinc in juvenile cacao: physiological, biochemical, molecular and micromorphological responses. Environ. Exp. Bot. 179, 104201. Doi: https://doi.org/10.1016/J.ENVEXPBOT.2020.104201

Suárez-Salazar, J.C., E.H. Duran-Bautista, J.A. Rojas-Castillo, and N. Ortiz-Cifuentes. 2017. Pigmentos fotosintéticos y conductancia estomática en ecotipos de copoazú (*Theobroma grandiflorum* Willd. Ex. Spreng K. Schum.). Agron. Mesoam. 28(1), 199-206. Doi: https://doi.org/10.15517/AM.V28I1.20814

Sun, Y., Y. Xu, Y. Xu, L. Wang, X. Liang, and Y. Li. 2016. Reliability and stability of immobilization remediation of Cd polluted soils using sepiolite under pot and field trials. Environ. Pollut. 208 Part B, 739-746. Doi: https://doi.org/10.1016/j.envpol.2015.10.054

Tantalean Pedraza, E. and M.Á. Huauya Rojas. 2017. Distribución del contenido de cadmio en los diferentes órganos del cacao CCN-51 en suelo aluvial y residual en las localidades de Jacintillo y Ramal de Aspuzana. Rev. Investig. Agroprod. Sustent. 1(2), 69-78. Doi: https://doi.org/10.25127/aps.20172.365

Vanderschueren, R., D. Argüello, H. Blommaert, D. Montalvo, F. Barraza, L. Maurice, E. Schreck, R. Schulin, C. Lewis, J.L. Vazquez, P. Umaharan, E. Chavez, G. Sarret, and E. Smolders. 2021. Mitigating the level of cadmium in cacao products: Reviewing the transfer of cadmium from soil to chocolate bar. Sci. Total Environ. 781, 146779. Doi: https://doi.org/10.1016/j.scitotenv.2021.146779

Wong Rivera, A.F. 2017. Determinación de cadmio (Cd) en suelo de cultivo para cacao CCN-51 mediante análisis de espectroscopía de absorción atómica. Undergraduate thesis. Facultad de Ciencias Naturales, Universidad de Guayaquil, Guayaquil.

Yao, A., Y. Liu, X. Luo, C. Liu, Y. Tang, S. Wang, X. Huang, and R. Qiu. 2021. Mediation effects of different sulfur forms on solubility, uptake and accumulation of Cd in soil-paddy rice system induced by organic carbon and liming. Environ. Pollut. 279, 116862. Doi: https://doi.org/10.1016/j.envpol.2021.116862

Zhang, H., Z. Xu, Y. Huo, K. Guo, Y. Wang, G. He, H. Sun, M. Li, X. Li, N. Xu, and G. Sun. 2020. Overexpression of *Trx CDSP32* gene promotes chlorophyll synthesis and photosynthetic electron transfer and alleviates cadmium-induced photoinhibition of PSII and PSI in tobacco leaves. J. Hazard. Mater. 398, 122899. Doi: https://doi.org/10.1016/J.JHAZMAT.2020.122899