Memories of the Agricultural and Livestock Area of the VI CCIUTM, Ecuador

Morphological Variation of *Theobroma cacao* L. Affected by Increasing Doses of Heavy Metals

Robinson Torres Ronquillo[1], Manuel Carrillo Zenteno[2], Wuellins Durango Cabanilla[2], Diego Franco Ochoa[1], César Quinaluisa Morán[1], Seyed Mehdi Jazayeri[3,4], Gregorio Vásconez Montufar[5], E. Rajasekhar[6], Naga Raju Maddela[7*] and Ronald Villamar-Torres[1,5*]

[1]Instituto Superior Tecnológico, Ciudad de Valencia,. Sector El Pital #1, Predios Universidad Técnica de Babahoyo-Extensión Quevedo, Ecuador; [2]Instituto Nacional de Investigaciones Agropecuarias-INIAP. Estación Experimental Tropical Pichilingue, cantón Mocache, Los Ríos. Ecuador; [3]Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Biología, Bogotá, Colombia; [4]Department of Biology, Faculty of Sciences, University of Tehran, Iran; [5]Universidad Técnica Estatal de Quevedo, Facultad de Ciencias Agropecuarias, Finca Experimental La María, Cantón Quevedo, Los Ríos. Ecuador; [6]Department of Physics, Rayalaseema University, Kurnool-518007, India; [7]Departamento de Ciencias Biológicas, Facultad de Ciencias de la Salud, Universidad Técnica de Manabí, Portoviejo-130105. Ecuador.

Abstract | The objective was to determine the effects on morphological changes in cocoa plants (EET-103 clone) due to the effect of increasing doses of heavy metals (HMs): Mercury (Hg), Lead (Pb), Copper (Cu), Cadmium (Cd). Through seven treatments with four replications, the variations in stem diameter, plant height and chlorophyll activity were determined. The obtained results were analyzed by a completely randomized experimental design, and the comparison of means using the Tukey test ($p < 0.05$). A dose of 1.50 mg kg^{-1} Hg decreased the stem diameter growth rate. Regarding the chlorophyll activity, it was found that both Pb and Cd with the different doses applied to the soil did not exhibit statistically significant differences and without reaching a high degree of toxicity. Regarding Hg, it was found that there is a greater effect in older leaves, observing that at doses greater than 22.5 mg kg^{-1} a decrease in chlorophyll activity was caused. For Cu, it was found that, both at the beginning and at the end, leaf three showed a higher value of R2 (regression analysis) (> 0.6), which can be used to evaluate the effects of soil Cu on chlorophyll activity.

Received | September 22, 2023; **Accepted** | December 5, 2024; **Published** | February 10, 2025
***Correspondence** | Ronald Villamar-Torres and Naga Raju Maddela, Facultad de Ciencias Agropecuarias, Universidad Técnica Estatal de Quevedo, Los Ríos, Ecuador; Departamento de Ciencias Biológicas, Facultad de Ciencias de la Salud, Universidad Técnica de Manabí, Portoviejo-130105. Ecuador; Email: rvillamart@uteq.edu.ec, raju.maddela@utm.edu.ec
Citation | Ronquillo, R.T., M.C. Zenteno, W.D. Cabanilla, D.F. Ochoa, C.Q. Morán, S.M. Jazayeri, G.V. Montufar, E. Rajasekhar, N.R. Maddela and R. Villamar-Torres. 2024. Morphological variation of *Theobroma cacao* L. affected by increasing doses of heavy metals. *Sarhad Journal of Agriculture,* 39(Special issue 2): 139-144.
DOI | https://dx.doi.org/10.17582/journal.sja/2023/39/s2.139.144
Keywords | Heavy elements, Cocoa, Toxicity, Chlorophyll

Introduction

The cultivation of cocoa (*Theobroma cacao* L.) is distributed in the latitudes of the various tropical countries between 20° north latitude and 20° south latitude; up to an altitude of 1,300 meters above sea level (Intriago *et al*., 2019). According to Casteblanco (2018), it is a food product that has had quite a marked growth in producing countries due to its easy handling and the excellent economic benefits it presents. Ecuador is the world's leading producer of fine and aroma cocoa (it produces more than 60% of world production) (Díaz *et al*., 2018). According to Arévalo-Gardini *et al*. (2016), this increase in the areas planted with cocoa is the result of the growth in demand for this product in Latin America, in addition to higher quality and safety of the beans, especially in the content of important heavy metals (HMs) in the main products derived from cocoa. Cocoa is considered one of the most important perennial crops in the world (Maddela *et al*., 2020), and it is exploited commercially for the production of beans mainly for the manufacture of chocolate, in addition to its great potential in the food, cosmetic and pharmaceutical industries (Arguello-Navarro and Moreno-Rozo, 2014).

The presence of HMs in agricultural products above the maximum permissible limits is generating great concern for farmers, the accumulation of HMs in the soil is a serious risk for all living beings (Sánchez and Rengifo, 2017; Ramakrishnan *et al*., 2021). The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous in even very low concentrations (Prieto *et al*., 2009), are defined as elements with a specific gravity equal to or greater than 5 g cm^{-3} (Arévalo-Hernández *et al*., 2017). Tolerant values in the presence of HMs vary from a few milligrams to only micrograms in the final commercial product, these extremely low ranges make it even more complicated as per International Plant Nutrition Institute (IPNI, 2015).

HMs are a group of chemical elements that are present in nature, making part of the earth's crust in small quantities (Beltrán and Gómez, 2015). For Apraez and Furtado (2019) whose balances of biogeochemical cycles have been drastically altered by human activity, causing serious environmental problems and putting their health at risk. Some plant species have the ability to grow in soils and waters contaminated with HMs; in addition, they present the ability to accumulate a high amount of these substances through physiological adaptations (Maiti *et al*., 2004). According to Arévalo-Gardini *et al*. (2016), HMs from the soil, once available, can be absorbed by the cacao plant; however, its distribution in the plant and its accumulation is variable.

Cultivated plants represent an important pathway for the movement of potentially toxic HMs from the soil to humans, accumulating them in different amounts and in their different plant and reproductive organs, resulting in poisonous contributions to diets (Rodríguez, 2017). Cocoa cultivation is currently a serious problem for farmers, because the plant absorbs HMs from the soil and concentrates them in the seeds (Huamani-Yupanqui *et al*., 2012). The European Union approved the new regulation on maximum limits for cadmium in cocoa and chocolate, EU Commission Regulation No. 488/2014 that modifies EC Regulation No. 1881/2006 and establishes that as of January 1, 2019, it will enter into force for the EU, the standard considers the maximum cadmium content in various cocoa products in a range of 0.10 to 0.80 mg kg^{-1} (Florida *et al*., 2018). The sale of cocoa from contaminated places could jeopardize the acquisition of this product in international markets. It is known that there are increasing demands on the safety of products that come from areas where there are no greater quality controls (Díaz *et al*., 2018). From the aforementioned, this work aimed to determine the morphological variations of the seedlings and their chlorophyll activity in cocoa seedlings subjected to variable doses of heavy elements.

Materials and Methods

This study was carried out between August and October 2018, in the greenhouse of the National Department of Soil and Water Management (DMSA) of the Tropical Pichilingue Experimental Station (EETP) of the National Agricultural Research Institute (INIAP), located at km 5 via Quevedo - El Empalme, Mocache canton, Los Ríos Province, Ecuador (Supplementary Figures S1, S2). Its geographical coordinates are Latitude 1° 20' South and Longitude of 79° 45' West, at 75 m above sea level. The region is characterized by a warm monsoon tropical climate, with a dry season between June and November. According to the Instituto Nacional de Meteorología e Hidrología (INAMHI), the average temperature ranges from 22°C to 33°C (INAMHI, 2017). The materials such as EET-103 Nacional Cocoa plantlets (180 days) were planted in soils contaminated with the chemical elements Mercury (Hg), Lead (Pb), Copper (Cu), Cadmium (Cd). For this study, the effects of six doses and a control (Supplementary Table S1), were evaluated, each one replicated four times giving a total of 112 experimental units.

The obtained results were analyzed by a completely randomized experimental design. To identify difference and similarities between averages, the Tukey Comparison Test (P<0.05) and Regression analysis (R2) were used. Fertilization took place 70 and 100 days after planting. Nutrient solution composed of Nitrogen (52 mg L^{-1}), Phosphorus (37 mg L^{-1} P_2O_5), Potassium (49 mg L^{-1} K_2O), Calcium oxide (50 mg L^{-1} CaO) Magnesium oxide (12 mg L^{-1} MgO) and Sulfate (16 mg L^{-1} SO_4). Irrigation was also carried out while keeping soil moisture at field capacity, using ultrapure water. The variables evaluated were collected weekly, the plant height being determined with the help of a graduated ruler, considering the distance from the base of the stem to the point of apical growth. The stem diameter, evaluated in the first between nodes, using a digital caliper and for chlorophyll activity, the SPAD-502 PLUS chlorophyllometer was used, recording in the four youngest leaves of the seedling and the averages are expressed in units of SPAD.

Results and Discussion

Mercury

With the data presented in Figure 1a, it is observed that the stem diameter showed an effect due to the presence of Hg in the soil, decreasing the growth rate from the dose 7.50 mg kg^{-1}, resulting less than the control treatment. It seems not occur with the height of the plant, where the initial and final values have a parallel growth. The trends observed in these two variables presented R^2 values that varied between 0.17 and 0.28, indicating that there was no effect for the doses of Hg evaluated. The greatest effects of Hg on chlorophyll activity are evidenced in the leaves evaluated at the end of the work, presenting R^2 values higher than those obtained in the initial evaluations (Figure 1b). The trend presented by this variable indicates that there is an increase in chlorophyll activity in the presence of Hg up to a dose of 7.5 mg kg^{-1}, with values greater than 22.50 mg kg^{-1} of Hg in the soil, causing a decreasing in this activity when compared to the control treatment (Figure 1b). Studies carried out in the world on photosynthesis in cocoa are relatively recent, not very abundant, and have been concentrated in few countries such as Brazil, Colombia, Ghana, Nigeria, Great Britain and Venezuela. As for studies of this type in Ecuador, they have not been carried out. In general, the knowledge about the physiology of photosynthesis in this species is limited, except its close relationship with the availability of water, the concentration of nitrogen and the stomatal conductance of the leaves, especially in younger plants, but not of HMs (Héctor *et al*., 2018). Photosynthetic activity is correlated with foliar nitrogen content (Daymond *et al*., 2011) and the concentration of this element -component of the chlorophyll molecule- is lower in cocoa leaves during the dry season (Ávila-Lovera *et al*., 2015).

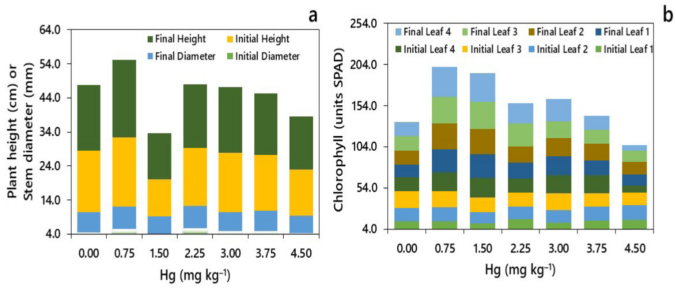

Figure 1: *Cacao plant stem diameter (mm) and plant height (cm) (a) and chlorophyl content (b) as affected by different concentrations of mercury (Hg).*

Lead

Results of cacao seedling stem diameter as influenced by different Pb doses were presented in the Figure 2a, at the dose of 10 mg kg^{-1} of Pb the smallest stem diameter was observed. Chupillon (2017), did not observe significant statistical differences for Pb in the variables of plant height and stem diameter, in the six commercial clones studied in five evaluation periods. The trends observed in these two variables present values of R^2 ranging from 0.5027 to 0.8455, indicating that there is a relationship between the Pb present in soil and the cacao plant height and stem diameter. Prieto *et al*. (2009), mention that the chemical immobilization processes in the soil have been reported or limited the growth of the plant before the absorbed Pb reaches values that may be harmful to humans. Regarding the chlorophyll activity, the results obtained by the effect of Pb indicate that, with the different doses applied to the soil, and results were presented in Figure 2b. Leaves 1, 2 and 3 both in final state, reached R2 values greater than 0.6, indicating that they could be used to evaluate effects of Pb on cocoa plants.

Copper

The stem diameter tends to increase from the dose of 7.50 mg kg^{-1} of Cu, while the plant height, has a tendency to decrease from the dose of 12 mg kg^{-1}

of Cu (Figure 3a). Data on the determination of chlorophyll activity in cocoa leaves were presented in Figure 3b, which shows that leaf 3, in final state, showed the highest value of R^2 (i.e. >0.6), which could indicate that this leaf could serve to assess the effects of Cu's presence in the soil on chlorophyll activity.

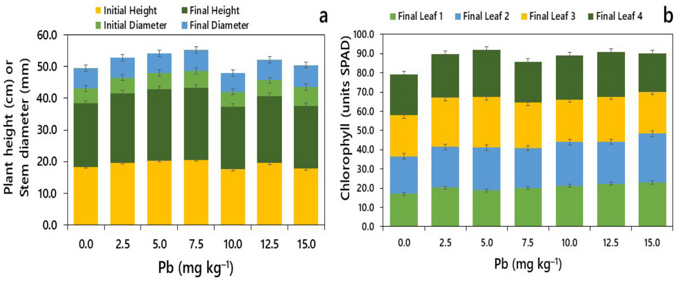

Figure 2: *Effects of different concentrations of lead (Pb) on cacao plant stem diameter (mm) and plant height (cm) (a) and chlorophyl content (b).*

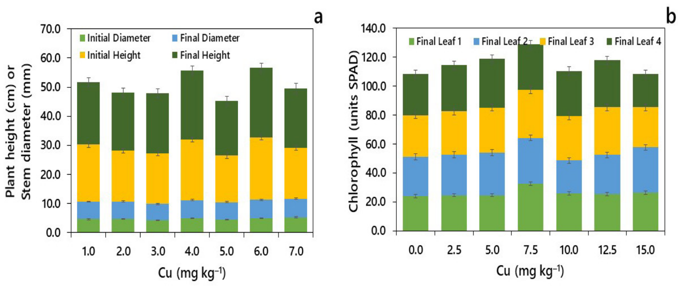

Figure 3: *Cacao plant stem diameter (mm) and plant height (cm) (a) and chlorophyl content (b) as affected by different concentrations of copper (Cu).*

Cadmium

Cocoa seedlings did not have significant statistical differences in plant diameter and height, as an effect of Cd doses, without reaching a high degree of toxicity. However, at the dose of 2.5 mg kg^{-1} Cd the smallest diameter and the lowest plant height were observed (Figure 4a). The results in this study, consistent with Acosta (2013), who reported that this response could explain the way of translocation of metals to the organs of the plant, differing from an initial stage of germination to a final stage of growth. The highest values of these two variables were observed in control treatment, indicated that the presence of Cd in the soil, starting at the dose of 0.5 mg kg^{-1}, cause delay in plant development. These values are below that found in soils of the Province of El-Oro, which are contaminated by this metal with contents of 2.53 and 2.23 mg kg^{-1} in the surface layer and 2.40 mg kg^{-1} to 20 centimeters, values that exceed 2.00 mg kg^{-1}, of critical contamination (Mite *et al*., 2010). As expressed by He *et al*. (2017), plants can show signs of toxicity when the total concentration of Cd in the soil exceeds 8 mg kg^{-1}, the concentration of bioavailable Cd in the soil is >0.001 mg kg^{-1}, or the concentration of Cd in plant tissue reaches 3-30 mg kg^{-1}. The age of a tree can influence its absorption of Cd; for instance, results from Meter *et al*. (2019), indicated that young cacao plants absorb more Cd than adults.

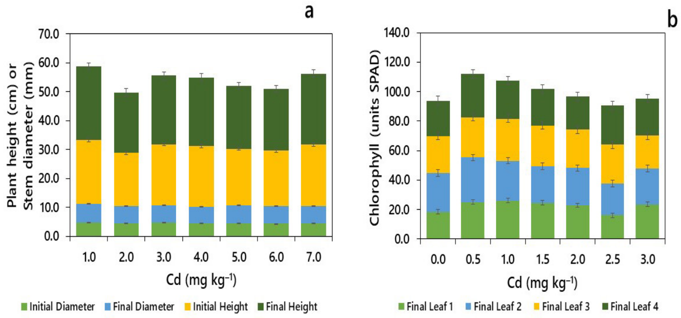

Figure 4: *Cacao plant stem diameter (mm) and plant height (cm) (a) and chlorophyl content (b) as affected by different concentrations of cadmium (Cd).*

In assessing the effect of the Cd on chlorophyll activity, according to the data presented in Figure 4b. The leaves evaluated at the end of the study (H1, H2 and H3), would be the ones that best demonstrate the changes, presenting values of R^2 > 0.7. Plants exposed to high levels of Cd showed a reduction in photosynthesis, water absorption and nutrient absorption and, consequently, chlorosis, growth inhibition, browning of the root tips and finally death were observed (Jiménez, 2015).

Conclusions and Recommendations

HMs induced morphological alterations in cocoa seedlings. For instance, Hg at 7.50 mg kg^{-1} decreased the diameter of seedlings. Chlorophyll content was badly affected at 7.5-22.5 mg kg^{-1} of Hg. Stem diameter decreased by Pb at 10 mg kg^{-1}. Inverse relation was observed between stem diameter and Cu content. The presence of Cu in the soil, from 6 mg kg^{-1}, caused an increase in stem diameter and with 12 mg kg^{-1}, a decrease in plant height. Cd was found to be more phytotoxic than other studied HMs. The older of the cocoa leaves, the better the effects of the elements in the soil are evident on the chlorophyll activity. The above results clearly suggest that different metals at different concentrations have significant toxic effects on cacao seedling. Therefore, HMs present in the cacao cultivating soils have significant negative impact on the cacao seedlings.

Acknowledgements

The authors wish to acknowledge the institutions that made this study possible. To the Equinoctial Technical University (UTE), Soil and Water Department of the National Institute of Agricultural Research (INIAP), Higher Technological Institute "*Ciudad de Valencia*" (ISTCV), and the State Technical University of Quevedo (UTEQ) for financial and scientific support provided in each phase within the framework of this project that is part of a multidisciplinary research program linked to the monitoring of the presence of cadmium in cocoa beans, soils and waters in Ecuador.

Novelty Statement

Hallmark of this study was the analysis of effects of seven different increasing doses of 4 heavy metals on morphological properties of cacao plants.

Author's Contribution

Robinson Torres Ronquillo, Manuel Carrillo Zenteno, Wuellins Durango Cabanilla, Diego Franco Ochoa and César Quinaluisa Morán: Carried out the field work and data analysis.
Wuellins Durango Cabanilla, Seyed Mehdi Jazayeri, Gregorio Vásconez Montufar, E. Rajasekhar, Naga Raju Maddela and Ronald Villamar-Torres: Planned the research, reviewed and approved all the version of the manuscript.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.sja/2023/39/s2.139.144

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Acosta, A., 2013. Efecto del sulfato de cadmio en la germinacion y el crecimiento de plantulas de cacao (*Theobroma cacao* L). Invest. Amazon. Peru, 3(1): 20-29.

Apraez, M.J. and D.A. Furtado. 2019. Evaluacion preliminar de metales pesados en diferentes plantas clonales de cacao. Rev. Colomb. Zoot., pp. 38-39.

Arévalo-Gardini, E., M.E. Obando-Cerpa, L.B. Zúñiga-Cernades, C.O. Arévalo-Hernández and V.B. He. 2016. Metales pesados en suelos de plantaciones de cacao (*Theobroma cacao* L.) en tres regiones del Perú. Ecol. Aplicada, 15(2): 6. https://doi.org/10.21704/rea.v15i2.747

Arévalo-Hernández, C., E. Arévalo-Gardini and A.V.B. Farfán-Pinedo. 2017. Metales pesados en suelos, hojas y granos de zonas cacaoteras del Perú. 2017 Int. Symp. Cocoa Res. Lima, Peru, pp. 6. Lima, Perú .

Arguello-Navarro, A.Z. and L.Y. Moreno-Rozo. 2014. Evaluación del potencial biofertilizante de bacterias diazótrofas aisladas de suelos con cultivo de cacao (*Theobroma cacao* L.). Acta Agron., 63: 1-12. http://www.redalyc.org/articulo.oa?id=169931322006, https://doi.org/10.15446/acag.v63n3.41033

Avila-Lovera, E., I. Coronel, R. Jaimez, R. Urich, G. Pereyra, O. Araques and I.A. Chacon. 2015. Ecophysiological traits of adult trees of criollo cocoa cultivars (*Theobroma cacao* L.) from a Germplasm bank in Venezuela. Exp. Agric., 52(1): 137-153. https://doi.org/10.1017/S0014479714000593

Beltrán, P.M. and R.A. Gómez. 2015. Metales pesados (Cd, Cr y Hg): Su impacto en el ambiente y posibles estrategias biotecnológicas para su remediación. Invest. Innov. Ingenieria I3+, 2(2): 82-112. https://doi.org/10.24267/23462329.113

Casteblanco, J.A., 2018. Técnicas de remediación de metales pesados con potencial aplicación en el cultivo de cacao. LA Granja: Revista Ciencias de la Vida, 27(1): 21-35. https://doi.org/10.17163/lgr.n27.2018.02

Chupillon, C.J., 2017. Determinacion de la absorcion de cadmio y plomo en genotipos de cacao (*Theobroma cacao* L), para el establecimiento de plantaciones comerciales. Universidad Nacional de San Martin-Tarapoto; Facultad de ciencias Agrarias; Tesis de grado para la Obtencion del titulo de Ingeniero Agronomo, pp. 1-75.

Daymond, A.J., P.J. Tricker and P. Wadley. 2011. Genotypic variation in Photosynthesis in cacao is correlated whit stomatal conductance and leaf nitrogen. Biol. Plant., 55(1): 99-104. https://doi.org/10.1007/s10535-011-0013-y

Díaz, U.L., H.E. Mendoza, B.M. Bravo and V.N.

Dominguez. 2018. Determinación de Cadmio y Plomo en almendras de cacao (*Theobroma cacao*), proveniente de fincas de productores orgánicos del cantón Vinces. Espirales Rev. Multidis. Invest., 2(15): 1-6.

Florida, R.N., L.C. Shilthon, Gómez and B. Raúl. 2018. El pH y la absorcion del cadmio en almendras de cacao organico (*Theobroma cacao* L) en Leoncio Prado, Huanuco, Perú. Folia Amaz. Rev. Inst. Invest. Amaz. Peruana, 27(1): 1-8. https://doi.org/10.24841/fa.v27i1.438

He, S., Z. Yang, V. He and C. Baligar. 2017. Morphological and physiological responses of plants to cadmium toxicity: A review. Pedosphere, 27(3): 421-438. https://doi.org/10.1016/S1002-0160(17)60339-4

Hector, A.E., G.A. Torres, T.O. Fosado, P.G. Sancan and A.R. Leon. 2018. Contenido de clorofilas totales en doce clones de cacao (*Theobroma cacao* L.). La Tecnica, Rev. AgroCiencias, 20: 11-18. https://doi.org/10.33936/la_tecnica.v0i20.1324

Huamani-Yupanqui, H.A., M.A. Huauya-Rojas, L.G. Mansilla-Manaya, N. Florida-Rofiner, Neira-Trujillo and Gilmer. 2012. Presencia de metales pesados en cultivo de cacao (*Theobroma cacao* L.) orgánico. Acta Agron., 61(4): 339-344.

INAMHI (Instituto Nacional de Meteorología e Hidrología). 2017. Servicio meteriologico.

Intriago, F.F., S.M. Talledo, B.J. Macias, N.G. Cuebca and F.J. Menjivar. 2019. Evaluación del contenido de metales pesados en almendras d cacao (*Theobroma cacao* L.) durante el procso de beneficiado. Pro-Sci. Rev. Prod. Ciencia Invest., 3(26): 17-23. https://doi.org/10.29018/issn.2588-1000vol3iss26.2019pp17-23

IPNI (International Plant Nutrition Institute) (7 de Julio de) 2015. Metales pesados en cacao, Perpertivas y posible manejo. Obtenido de http://www.ipni.net/

Jiménez, T.C., 2015. Estado legal mundial del cadmio en cacao (*Theobroma cacao*): Fantasía o realidad. Rev. Prod. + Limpia, 10(1): 89-104. https://doi.org/10.22507/pml.v10n1a8

Maddela, N.R., D. Kakarla, L.C. García, S. Chakraborty, K. Venkateswarlu and M. Megharaj. 2020. Cocoa-laden cadmium threatens human health and cacao economy: A critical view. Sci. Total Environ., 720: 137645. https://doi.org/10.1016/j.scitotenv.2020.137645

Maiti, R., J. Hernández and J.Y. González. 2004. Plant based biorremediation and mechanims of heavy metals tolerance of plants: A review. Proc Indian Nat. Sci. Acad., B70, 1: 1-12.

Meter, A., R.J. Atkinson and B. Laliberte. 2019. Cambio en el cacao de America Latina y el Caibe- Analisis de la investigacion y soluciones potenciales para la mitigación. Bioversity Int., pp. 83.

Mite, F., M. Carrillo and W. Durango. 2010. Avances del monitoreo de presencia del cadmio en almendras de cacao, suelo y aguas en el ecuador. XII Congreso Ecuatoriano de la Ciencia del Suelo, Santo Domingo. pp. 1-21.

Prieto, M., R.C. González, G. Román and G. Prieto. 2009. contaminación y fitoxicidad en plantas por metales pesados provenientes de suelos y agua. Trop. Subtrop. Agroecosyst., 10(1): 29-44.

Ramakrishnan, B., N.R. Maddela, K. Venkateswarlu and M. Megharaj. 2021. Organic farming: Does it contribute to contaminant-free produce and ensure food safety? Sci. Total Environ., pp. 145079. https://doi.org/10.1016/j.scitotenv.2021.145079

Rodríguez, A.H., 2017. Dinámica del cadmio en suelos con niveles altos del elemento, en zonas productoras de cacao de Nilo y Yacopí, Cundinamarca. Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Bogota-Colombia, pp. 133.

Sánchez, R.M. and T.J. Rengifo. 2017. Evaluación del contenido de metales pesados (Cd y Pb) en diferentes edades y etapas fenologicas del cultivo de cacao en dos zonas del Alto Huallaga, Huanuco (Peru). Rev. Invest. Agroprod. Susten., 1(1): 87-94. https://doi.org/10.25127/aps.20171.356