Bioagro 35(3): 237-246. 2023 doi: http://www.doi.org/10.51372/bioagro353.7

CADMIUM UPTAKE AND MYCORRHIZATION BY CACAO CLONES IN AGROFORESTRY AND MONOCULTURE SYSTEMS OF PERUVIAN AMAZON

Geomar Vallejos-Torres[1*,2], Nery Gaona-Jimenez[1], Alberto Alva Arevalo[3], Christopher Paredes[4], Andi Lozano[3], Jorge Saavedra-Ramírez[4], Luis A. Arévalo[4], Keneth Reátegui[5], Wilfredo Mendoza-Caballero[6] and César Marín[2,7]

ABSTRACT

The production system influences the cadmium (Cd) content and mycorrhization in cocoa plantations. The objective of this study was to determine the effects of different production systems on Cd uptake and the presence of mycorrhizas in cacao clones in field conditions, in the Peruvian Amazon. Twelve subplots of 108 m^2 were selected in representative cocoa cultivation systems under agroforestry (AF) and monoculture (MON), with the cocoa clones ICS and CCN. Significant differences and data distribution were evaluated using ANOVA, principal component analysis, and Tukey's tests. Mycorrhizal colonization was higher in the AF_ICS system (71.11%) while the length of the extraradical mycelium was higher in the AF_CCN system (17.23%). The highest Cd content in soils was found under the AF_CCN and AF_ICS systems, both with 0.39 $mg \cdot kg^{-1}$. The Cd content in cacao roots, leaves, and beans were higher in the MON_CCN system with 1.87, 2.06, and 1.12 $mg \cdot kg^{-1}$ respectively. Cocoa monocultures (with both clones) generally showed lower levels of mycorrhizal colonization than agroforestry systems, which in turn (also for both clones) presented higher Cd content in beans, even exceeding the limit established by the world health authorities.
Additional keywords: Mycelium, mycorrhizal colonization, Peruvian amazon, *Theobroma cacao*

RESUMEN

Absorción de cadmio y micorrización en clones de cacao bajo agroforestería y monocultivo en la Amazonía peruana

El sistema de producción influye en el contenido de cadmio (Cd) y la micorrización en las plantaciones de cacao. El objetivo de este estudio fue determinar los efectos de diferentes sistemas de producción en la absorción de Cd y la presencia de micorrizas en clones de cacao en condiciones de campo en la Amazonía peruana. Se seleccionaron doce subparcelas de 108 m^2 en sistemas representativos de cultivo de cacao bajo agroforestería (AF) y monocultivo (MON), con los clones de cacao ICS y CCN. Se evaluaron las diferencias significativas y la distribución de datos mediante ANOVA, análisis de componentes principales, y la prueba de Tukey. La colonización micorrícica fue mayor en el sistema AF_ICS (71,11 %) mientras que la longitud del micelio extrarradical fue mayor en el sistema AF_CCN (17,23 %). El mayor contenido de Cd en suelos se encontró bajo los sistemas AF_CCN y AF_ICS, ambos con 0,39 $mg \cdot kg^{-1}$. Los contenidos de Cd en las raíces, hojas, y granos de cacao fueron mayores en el sistema MON_CCN, con 1,87, 2,06 y 1,12 $mg \cdot kg^{-1}$, respectivamente. Los monocultivos de cacao (con ambos clones) mostraron en general menores niveles de colonización micorrícica que los sistemas agroforestales, que a su vez (también para ambos clones) presentaron mayor contenido de Cd en el grano, superando incluso el límite establecido por las autoridades sanitarias mundiales.
Palabras clave: Amazonía peruana, colonización micorrícica, micelio, *Theobroma cacao*

INTRODUCTION

One of the metals with the greatest environmental and human health impacts is cadmium, a metal present in certain crops, particularly in cacao plantations (*Theobroma cacao* L.). This presence has generated alerts and concern on a global scale, and Cd contamination of soil and food crops is considered a critical

Received: January 2, 2023 Acepted: July 31, 2023
[1]Universidad César Vallejo, Maronilla, Cacatachi, San Martín, Perú. Geomar Vallejos-Torres email: gvallejost@gmail.com (corresponding author); gaonaj@ucvvirtual.edu.pe
[2]Centro de Investigación e Innovación para el Cambio Climático (CiiCC), Universidad Santo Tomás. Valdivia, Chile email: cesar.marin@postgrado.uach.cl
[3]Universidad Nacional de San Martín, Tarapoto, San Martín, Perú. email: aalva@unsm.edu.pe; alozanochu@unsm.edu.pe
[4]Universidad Nacional Autónoma de Alto Amazonas (UNAAA), Loreto, Alto Amazonas, Yurimaguas. email: Chrissanchez@unsm.edu.pe; jsaavedrar@unaaa.edu.pe; larevalo@unaaa.edu.pe
[5]Universidad Nacional Intercultural de la Amazonía, Pucallpa, Perú. email: kreateguid@unia.edu.pe
[6]Universidad Católica Sedes Sapientiae, Los Olivos, Lima, Perú email: wmendoza@ucss.edu.pe
[7]Amsterdam Institute for Life and Environment, section Ecology & Evolution, Vrije Universiteit Amsterdam, Netherlands

environmental threat, as it deteriorates soil health and creates a danger to food security and thus to human health (Bali et al., 2020). Cd is relatively mobile in the soil and plant system (Chávez et al., 2015), and consequently, tolerable limits for Cd have been regulated internationally for some commercial crops. It is important to study the innate capacity of cacao to absorb or tolerate certain levels of Cd in the soil, particularly in clones or genotypes developed in specific sites, which could contribute to shortening the trophic transmission of this metal (Maddela et al., 2020).

It is well known that different cacao clones respond to cadmium differently. For example, Arévalo et al. (2017) reported differences in accumulation of Cd in farms with different cacao clones, where the clone CCN-51 had lower concentrations of Cd than samples from a combination of clones CCN-51 and ICS-95 together. Different cacao clones also might respond differently under several environmental conditions, for example shade. Cacao (*Theobroma cacao* L.) grows well under the shade of tree canopies. Agroforestry systems use various shade trees useful for wood, fruits, and other non-timber forest products (Schneider et al., 2016). These shade trees, together with cacao trees, have the potential to increase the sustainability of cacao production, due to the various ecosystem services provided (Meylan et al., 2017). Previous studies on cacao plantations in South America have shown a strong correlation between Cd in beans and total Cd in the soil, as well as a considerable influence of soil pH on increasing plant available Cd in the soil (Ramtahal et al., 2019). Soil pH is considered the main factor influencing Cd availability (Chávez et al., 2015).

One adaptation of cacao to adverse environmental conditions, like high Cd content, can be found on its rhizosphere, for example by forming symbiosis with arbuscular mycorrhizal fungi (AMF), which are obligate biotrophs associated with approximately 71% - 78% of terrestrial vascular plants (Brundrett and Tedersoo, 2018). However, the contribution of AMF to the host plant depends on the taxonomic diversity, abundance, and functional diversity of their communities (Schneider et al., 2016). *T. cacao* is considered a highly mycorrhizal plant, highly dependent on these root fungal symbionts reaching values of root colonization of over 70% (Gai et al., 2018), while enhancing plant growth and reducing Cd in soils and stems of Peruvian cacao plants (Vallejos-Torres et al., 2022).

There is evidence of a high Cd content in cacao plantations in many Peruvian regions; such is the case of the cacao farms of Bagua, Amazonas region, where the maximum permissible limits established by Peruvian and European legislation have been exceeded (Oliva et al., 2020). Also, it has been found that the Cd content in cacao plantations located between 600 and 800 masl in the San Martín region (Perú) exceeded the maximum permissible limits indicated by the World Health Organization (Mendoza et al., 2021). Likewise, the average concentration of Cd found in cocoa powder from unfermented seeds in the Huánuco region of Peru was 2.46 mg kg^{-1}, with a range of 0.2 to 12.56 mg kg^{-1}; this maximum is well above the permitted limit of 1.5 mg kg^{-1} (of the World Health Organization), and also the highest reported so far in the literature (Zug et al., 2019). There are very few -if any- studies analyzing the relationships of AMF colonization and Cd and nutrient uptake in the Peruvian Amazon. Furthermore, and in general, most mycorrhizal studies worldwide occur in pots and not in field conditions (Lekberg and Helgason, 2018). A higher number of field-conditions studies are required to test real-world drivers of mycorrhizal colonization.The objective of this study was to determine the influence of different production systems on Cd absorption and mycorrhizal colonization and extraradical mycelium in cacao crops in the Peruvian Amazon, in field conditions. As hypothesis, we proposed that Cd and mycorrhization are influenced by both the cacao clones and the production system of cacao plantations in the Peruvian Amazon.

MATERIALS AND METHODS

Study area. The study was carried out in the San Martín region, Peru, between August 2020 and June 2021. Average annual rainfall and temperature in the region is 1800 mm and 25°C, respectively, though there is high variability associated with altitudinal gradients. The rainy season occurs from November to May. The average altitude of the study area was 608 m (6°21'7.50"S, 76°37'0.20"W).

Cocoa plantations in this region consist mainly of the ICS and CCN clones (Chávez et al., 2022). In this study, four plots of 2500 m^2 each containing three sub-plots were selected; making a total of 12 subplots of 108 m² each. A plot (three subplots) per treatment. The agroforestry system with the clone CCN (AF_CCN) was associated with laurel (*Cordia* sp), capirona (*Calycophyllum spruceanum*), huito (*Genipa americana*), cedar (*Cedrela odorata*), and guava (*Inga* sp.). The agroforestry system with the ICS clone (AF_ICS) was associated with *C. spruceanum*, bolaina (*Guasuna crinita*), *C. odorata*, and *Inga* sp; both agroforestry systems were located in the province of Lamas. The cocoa monoculture system with the CCN clone (MON_CCN) was located in the province of Mariscal Cáceres and the cocoa monoculture system with the ICS clone (MON_ICS) was located in the province of Rioja. All plots had a planting distance of 3 m x 3 m, that is, 1,111 plants per hectare with an age between 9 and 12 years. All plots had a traditional management characterized by a scarce application of compost (organic amendments) prepared by the cocoa producers themselves, from the harvest remains of the same crop.

Determination of mycorrhizal colonization and extraradical mycelium length. In order to analyze AMF root colonization of Cacao and extraradical mycelium in field conditions, roots and rhizospheric soil were collected. AMF inoculants were not added in this study. For the collection of roots, an area around each plant was cleaned of litter and debris within the vertical projection of the crown. In each plot, three samples were taken (one for each sub-plot), mixed, and homogenized (Vallejos et al., 2021). Samples of secondary and tertiary roots were taken from the entire contour of the cacao plant, one meter away from the trunk; the roots were placed in a Ziploc® bag, coded, and taken to the laboratory for their respective treatment. Once in the laboratory, the best rootlets were separated and selected to form the sample.

Roots and AMF were stained using the technique proposed by Phillips and Hayman (1970) and subsequently, radical colonization was quantified by quadrant intersects, i.e. a hundred 1 cm roots fragments were randomly distributed and hypha-line intersections were quantified. Extraradical mycelium was evaluated from 100 g of soil, collected in three different sectors surrounding each cacao plant. To quantify the length of the extraradical mycelium, the samples were placed in Petri dishes and observed in a stereo microscope at 4.5x. The numerical quantity obtained was converted to mycelial length per unit of weight dry soil, using the formula proposed by Newman (1966).

Physical-chemical analysis of soils. For the physical-chemical soil analyses, three sub-samples were taken from each of the four plots (one sample per sub-plot; 12 sub-samples analyzed) as follows: holes 30 cm deep were made in each sub-plot, a cut from 0 cm to 20 cm of soil was extracted, and 2 kg of soil were taken, according to the Technical Guide for Sampling, prepared by the Ministry of the Environment of Peru (MINAM, 2014). The following physical-chemical properties were determined: pH (1:2 H_2O; potentiometer method), available ions (P, K; modified Olsen method), and soil organic matter (OM) by the Walkley and Black method (Table 1).

Table 1: Physical-chemical characteristics of the soil in the production systems

Production system	pH	OM (%)	P (ppm)	K (ppm)
AF _CCN	5.55± 0.12 b	4.31±0.25 a	8.65±1.00 b	142.0±10.97 a
AF_ICS	5.99±0.10 bc	3.85±0.54 b	6.77±1.74 c	143.5 ±8.95 b
MON_CCN	6.01±0.03 b	4.20±0.18 b	19.18±0.65 b	43.0 ±2.31 c
MON_ICS	6.71±0.12 a	4.92±0.23 ab	13.40±0.35 bc	327.0±59.47 a

AF_CCN (agroforestry with CCN clone), AF_ICS (agroforestry with ICS clone), MON_CNN (monoculture with CCN clone), and MON_ICS (monoculture with ICS clone). OM (Soil organic matter). Different lowercase letters indicate significant differences between treatments according to Tukey's test (P≤0.05)

Analysis of cadmium in soils, cacao roots, leaves, and beans. For Cd analyses, 12 soil samples were taken, one for each sub-plot, according to the Technical Guide for Sampling, prepared by the Ministry of the Environment of Peru (MINAM, 2014). For the foliar sampling, in each sub-plot, 4 leaves per plant were collected from the intermediate part of the crown at four cardinal points (4 plants), making a homogeneous composite sample. For bean sampling, 1.5 kg of cocoa beans in slime were collected per sub-plot, from mature fruits from the middle part of healthy and reproductive trees (Sandoval et al., 2020). Cd in soils was determined by the EPA 3050B method by FLAMA (EPA, 1996); in leaves by the digestion method: HNO_3/spectrophotometer and atomic absorption; and Cd in beans by the digestion method: HNO_3:H_2O_2 (2:1)/spectro-photometer and atomic absorption.

Statistical analyses. The data were analyzed using Tukey's mean comparison test with a statistical significance of 95% ($p < 0.05$). Normality and homoscedasticity of the data were verified using the Shapiro Wilk and Levene tests, respectively, and those non-normal variables were transformed by logarithm, square root or arcsine, as appropriate. However, the results are expressed in original units. A Pearson correlation between the average concentrations of Cd in grains and the total concentrations of Cd in the soil was made. A principal components analysis (PCA) was carried out, which includes the five variables measured in the different treatments, using the *Vegan* package (Oksanen et al., 2022) in R Studio.

RESULTS

There were no statistical differences among production systems and clones regarding mycorrhizal colonization, which was slightly higher in the AF_ICS and AF_CCN systems with 71.11% and 64.99%, respectively, with no significant differences between all treatments (Figure 1A). The lowest values were obtained by the MON_CCN and MON_ICS systems, with 60.65% and 58.32%, respectively (Figure 1A). The length of the extraradical mycelium was significantly higher in the AF_CCN and AF_ICS systems with 17.23% and 17.36%, respectively, with no significant differences between both treatments; the lowest values were obtained by the MON_ICS and MON_CCN treatments with 12.51% and 13.58%, respectively, with no significant differences between both treatments (Figure 1B). Table 2 shows that there was no significant correlation between Cd in soils with the Cd in cocoa roots, leaves or beans.

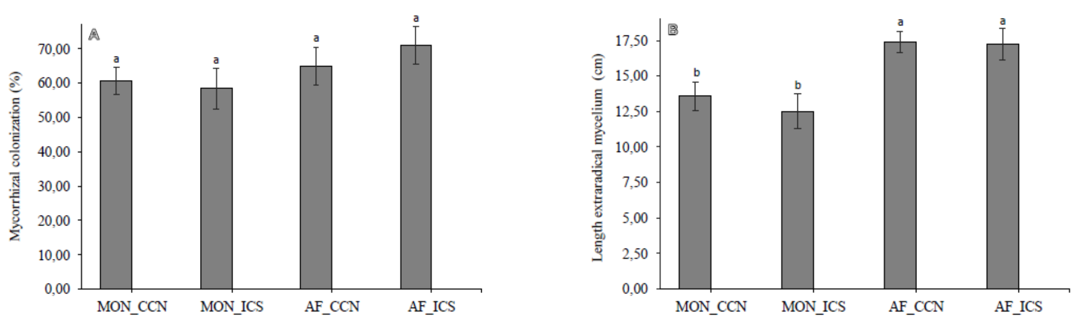

Figure 1. Mycorrhizal colonization (A) and length of extraradical mycelium (B) in the production systems: MON_CNN (monoculture with CCN clone), MON_ICS (monoculture with ICS clone), AF_CCN (agroforestry with CCN clone), and AF_ICS (agroforestry with ICS clone). Different lowercase letters indicate significant differences between treatments according to Tukey's test ($p \leq 0.05$). Vertical bars correspond to the standard deviation

Cd content in soils was only significantly lower in the MON_ICS system (Figure 2A). The highest Cd content in soils was found in the AF_CCN and AF_ICS systems, both with 0.39 mg kg^{-1}, while the lowest values were found in the MON_ICS and MON_CCN systems, with 0.19 mg kg^{-1} and 0.33 mg kg^{-1}, respectively (Figure 2A). Cd content in leaves was significantly higher in the MON_ICSS and MON_ICS systems, with values of 2.06 mg kg^{-1} and 1.81 mg kg^{-1}, respectively, compared to the agroforestry systems. The lowest values were found in the AF_CCN and AF_ICS systems, with 1.12 mg kg^{-1} and 1.28 mg kg^{-1}, respectively (Figure 2B). Cd content in leaves was significantly higher in the MON_ICSS and MON_ICS systems, with values of 2.06 mg kg^{-1} and 1.81 mg kg^{-1}, respectively, compared to the agroforestry systems. The lowest Cd values were found in the AF_CCN and AF_ICS systems, with 1.12 mg kg^{-1} and 1.28 mg kg^{-1}, respectively (Figure 2C). Regarding Cd content in cocoa beans, the highest value was obtained in the MON_CCN and MON_ICS systems, with 1.12 mg kg^{-1} and 0.64 mg kg^{-1}, respectively, with significant differences between treatments; the lowest contents were obtained in the AF_CCN and AF_ICS systems, with 0.35 mg kg^{-1} and 0.44 mg kg^{-1}, respectively, with significant differences between both treatments (Figure 2D).

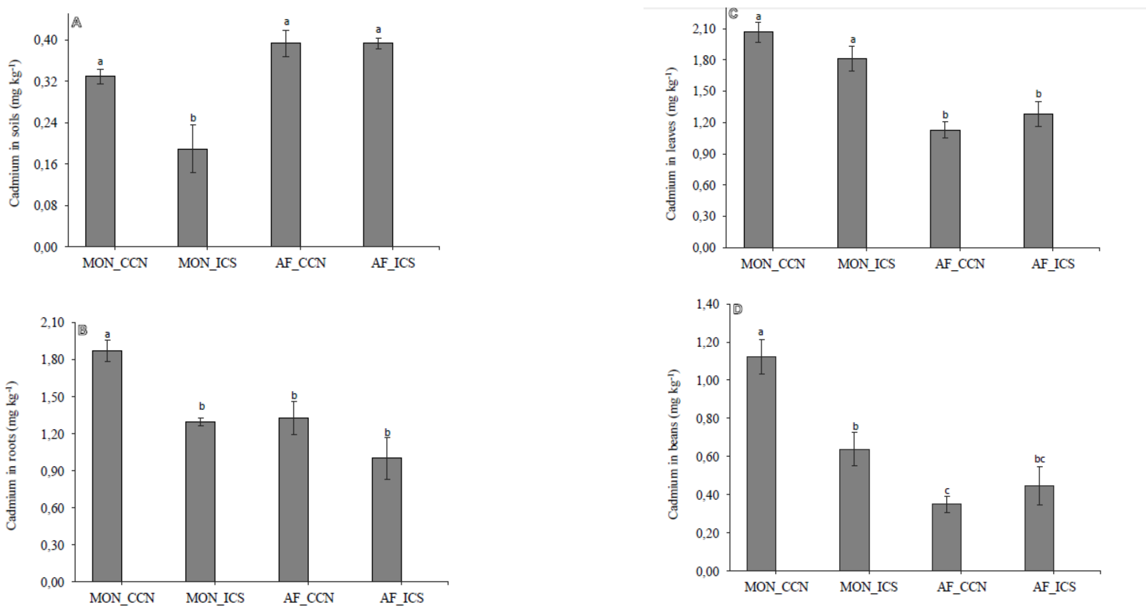

Figure 2. Cd content in soils (A), in root (B), in leaves (C) and in cocoa beans (D) in the production systems. MON_CNN (monoculture with CCN clone), MON_ICS (monoculture with ICS clone), AF_CCN (agroforestry with CCN clone), and AF_ICS (agroforestry with ICS clone). Different lower case letters indicate significant differences between treatments, Tukey test ($P \leq 0.05$). Vertical bars correspond to the standard deviation.

Table 2. Correlation between soil Cd and Cd content in cacao roots, leaves, and beans

	Root Cd		Leaf Cd		Bean Cd	
	Rho	*P*	Rho	*P*	Rho	*P*
Soil Cd	-0.101	0.753	-0.545	0.066	-0.259	0.415

Rho: Spearman's Rho correlation coefficient; *P*: statistical probability

There was a low (rho = -0.259) and no significant correlation (P = 0.415) between total Cd in soil and beans. Cd concentration in beans is directly related to Cd concentration in soil (Ramtahal et al., 2016), but this depends on the production system used (Gramlich et al., 2017). Figure 3 shows higher Cd concentration in soils under agroforestry systems for both clones, reducing its content in cacao beans. There was a negative (rho = -0.545) and slightly significant (P = 0.066) relationship between soil Cd and cacao leaf Cd.

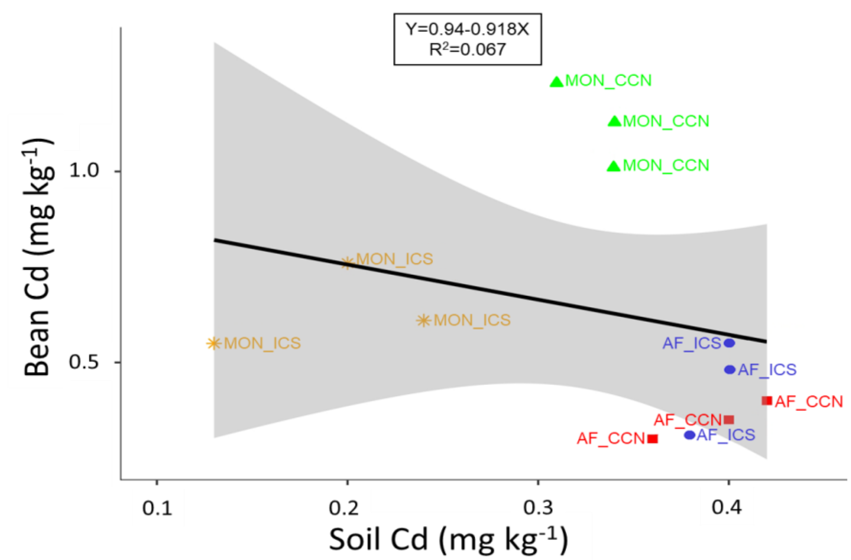

Figure 3: Correlation between the average concentrations of Cd in cacao beans and soil for each production system. The gray line is the 95 % confidence interval for a linear regression

The main component 1 (PC1) had a strong and positive association with cadmium content in leaves, beans, and roots; likewise, it had a strong and negative association with mycorrhizal colonization, extraradical mycelium, and cadmium content in soil. The main component 2 (PC2) had a medium and positive association with the content of cadmium in soil, in beans, and roots, and to a lesser extent with mycorrhizal colonization (Figure 4).

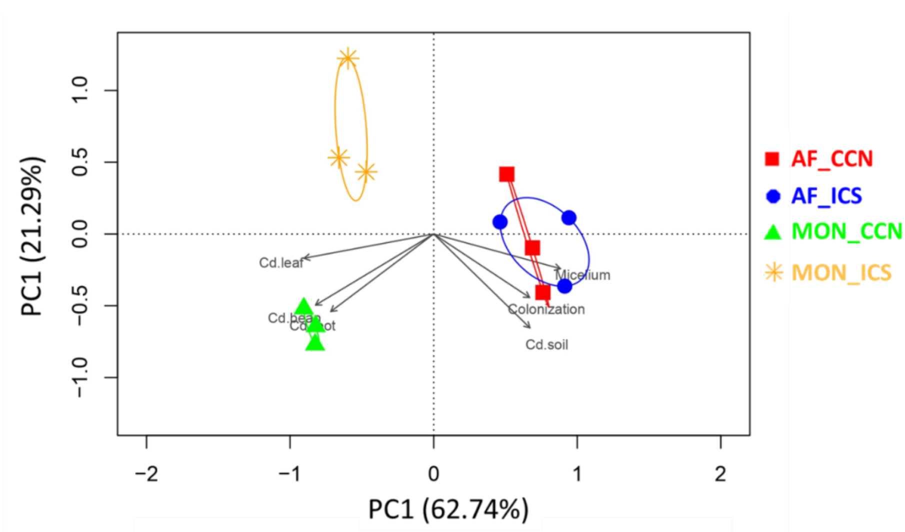

Figure 4: Principal component analysis (PCA) based on the combined data sets of cadmium in soils, roots, leaves, beans, colonization, mycelium, and soil characterization in production systems (as an ecological Eucledian distance). MON_CNN (monoculture with CCN clone), MON_ICS (monoculture with ICS clone), AF_CCN (agroforestry with CCN clone), and AF_ICS (agroforestry with ICS clone)

DISCUSSION

The mycorrhizal colonization values obtained (58.32% to 71.11%) were higher than those obtained by Iglesias et al. (2011) and Gramlich et al. (2017), who reported an average of 38.74% y 28.70% of mycorrhizal colonization in fine roots of cacao trees, respectively. Regarding the length of extraradical mycelium, the highest values were found in agroforestry systems with higher, semi-neutral pH (close to 6.0). This is in agreement with the work of Van Aarle et al. (2002), who found that different species of arbuscular mycorrhizal fungi form more extraradical mycelium at higher pH.

The cocoa cultivation systems in both the ICS and CCN clones associated with shade trees showed the highest percentages of root mycorrhizal colonization as well as the length of extraradical mycelium. This might due to the fact that agroforestry systems present a greater biodiversity of plant species, favoring a greater root system and providing better habitat conditions for AMF spores. Our results support the existence of a protective effect of AMF against Cd phytotoxicity, since plants with a very high mycorrhizal colonization reduce the translocation of Cd to the shoots, as reported by several studies (Tan et al., 2015; Zhang et al., 2018; Rask et al., 2019). Such protective effect means an advantage over less-mycorrhized plants, because toxic elements are immobilized within the mycelium (including the extraradical mycelium) or in the root system (Hildebrandt et al., 2007; Jiang et al., 2016).

According to He et al. (2015), there is a direct relationship between soil pH and Cd phytotoxicity in soils. This study shows that Cd contents in soils are below the permissible limits, as they do not exceed the 1.4 ppm permitted by the World Health Organization (WHO) (Figure 2A); i.e. the highest value was 0.39 mg kg^{-1} in the AF_CCN and AF_ICS systems. These results are probably due to the much higher tree density in the agroforestry system as compared to monocultures. The pH in the study plots was slightly-acid to neutral (between 5.55 and 6.71), an important factor since the mobility and availability of Cd in the soil is mainly controlled by soil pH (Alloway, 2013). The overall low Cd content in our study could also be explained by the use of compost, as this material has been shown to reduce or immobilize Cd (Vallejos et al., 2022). Regarding Cd content in leaves (higher in monoculture systems; Figure 2c), our results are consistent with those of Gramlich et al. (2017), who reported lower Cd contents in cacao leaves from agroforestry systems in the Alto Beni region, Bolivia, compared to leaves from monocultures.

Cd content in beans was higher in the MON_CCN system with 1.12 mg kg^{-1}, while the lowest value was shown by the AF_CCN system with 0.35 mg kg^{-1}. Likewise, Mendoza et al. (2021) found 0.887 mg kg^{-1} in cocoa beans in the San Martín region, Peru, surpassing the limits established by the WHO. Monoculture systems present Cd concentrations above the critical threshold for cacao beans; likewise, in Honduras, Gramlich et al. (2018) reported high concentrations of Cd in cocoa beans, with an average of 2.60 ± 0.40 mg kg^{-1}, exceeding the maximum permissible limit. Another study also reports much higher concentrations of Cd in cocoa beans than in the soil (Ramtahal et al., 2015). We attribute the differences between these latest studies and our study to differences in clones and site conditions. Accumulation of Cd in the soil favors its uptake by plants (Ramtahal et al., 2016; Gramlich et al., 2017), however, other authors have found low to moderate Cd mobilization from the soil to beans (Engbersen et al., 2019; Argüello et al., 2019). The high concentrations of this heavy metal in beans and leaves exceed the maximum permissible limit of 0.50 ppm established by the WHO, a fact that leads to a serious problem for cacao-producing areas (Furcal and Torres, 2020).

The average Cd content in agroforestry system beans (0.39 mg kg^{-1}) did not surpass the UE permissible limits of 0.80 mg kg^{-1} (European Commission, 2014), while the monoculture systems did surpass such limit, with a Cd bean concentration of 0.88 mg kg^{-1}. This could be caused by several factors, for example tree density and biodiversity. Although it is true that cacao density was the same in both cultivation systems, total tree density was much higher in the agroforestry system. Direct competition for available Cd in the soil would also explain the lower Cd uptake in agroforestry systems at the same available Cd concentrations (Gramlich et al., 2017). These results indicate that agroforestry has a significant potential for Cd phytoextraction from the soil as reported by Fan et al. (2011), for the species *Leucaena leucocephala* and *Swietenia macrophylla*, respectively. Trees have been traditionally considered as phytoremediation tools due to their considerable production of aerial biomass (Raza et al., 2020; Silva et al., 2020). The lower Cd content in agroforestry systems found in this study agree with Gramlich et al. (2017), who found that cocoa trees in agroforestry systems accumulated less foliar Cd than trees in monocultures.

CONCLUSIONS

The production system influenced extraradical mycelial length, with lower levels found in the monoculture system as compared to the agroforestry systems, for the two cacao clones included in the study. Likewise, monoculture systems showed higher Cd content in leaves and beans as compared to agroforestry systems. Cocoa under agroforestry systems showed an overall lower Cd presence compared to monocultures, demonstrating that the trees associated with cocoa cultivation could be a potential tool for the phytoextraction of Cd. Such high contents of cadmium found in cacao leaves and beans in MON_CCN and MON_ICS exceeded the maximum permissible limit allowed by the WHO, a fact that generates concerns in the cacao-producing areas of the Peruvian Amazon.

ACKNOWLEDGEMENTS

To Cesar Vallejo University and the Catholic University Sedes Sapientiae. Contract 105-2018 - FONDECYT - WB - IADT – AV. CM. thanks the funding from Convocatoria Nacional Subvención a Instalación Academia Convocatoria Año 2021+Folio SA77210019 (ANID—Chile).

LITERATURE CITED

1. Alloway, B.J. 2013. Sources of Heavy Metals and Metalloids in Soils. Heavy Metals in Soils. Dordrecht. 22: 11-50.
2. Arévalo-Gardini, E., C.O. Arévalo-Hernández., V.C. Baligar and Z.L. He. 2017. Heavy metal accumulation in leaves and beans of cacao (*Theobroma cacao* L.) in major cacao growing regions in Peru. Sci. Total Environ. 605-606: 792-800.
3. Argüello, D., E. Chávez, F. Lauryssen, R. Vanderschueren, E. Smolders and D. Montalvo. 2019. Propiedades del suelo y factores agronómicos que afectan las concentraciones de cadmio en los granos de cacao: una encuesta nacional en Ecuador. Sci. Medio Ambiente Total. 649: 120-127.
4. Bali, A.S., GP. Sidhu and V. Kumar. 2020. Root exudates ameliorate cadmium tolerance in plants: A review. Environ. Chem. Lett. 18: 1243-1275.
5. Brundrett, M.C. and L. Tedersoo. 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220(4): 1108-1115.
6. Chávez, E., Z.I. He., P.J. Stoffella, R.S. Mylavarapu., Y.C. Li., B. Moyano and V.C. Baliga. 2015. Concentration of cadmium in cacao beans and its relationship with soil cadmium in southern Ecuador. Science of the Total Environment 533: 205–214.
7. Chávez-Salazar, A., A. Cueva-Benavides., V. Muñoz-Delgado., K. Documet-Petrlik and P. Vidaurre-Rojas. 2022. Beneficio del cacao clones CCN-51, ICS-39 y cacao Nativo (*Theobroma cacao* L.). Revista Agrotecnológica Amazónica, 2(1): e255-e255.
8. EPA, U.S. 1996. Method 3050B: Acid Digestion of Sediments, Sludges, and Soils", Revision 2. Washington, DC.
9. Engbersen, N., A. Gramlich, M. Lopez, G. Schwarz, B. Hattendorf, O. Gutierrez and R. Schulin. 2019. Acumulación y asignación de cadmio en diferentes cultivares de cacao. Sci. Medio Ambiente Total 678: 660-670.
10. European Commission. 2014. Reglamento No 488/2014 de la comisión de 12 de mayo de 2014. Diario Oficial de La Unión Europea. https://eur-lex.europa.eu/legal-content/es/txt/pdf (retrieved June 2023)
11. Fan K.C., H.C. Hsi, C.W. Chen, H.L. Lee and Z.Y. Hseu. 2011. Cadmium accumulation and tolerance of mahogany (*Swietenia macrophylla*) seedlings for

phytoextraction applications. J. Environ. Manag. 92: 2818-2822.

12. Furcal-Beriguete, P. and J.L. Torres-Morales. 2020. Determinación de concentraciones de cadmio en plantaciones de *Theobroma cacao* L. en Costa Rica. Tecnología en Marcha, 33(1): 122-137.
13. Gai, J.P., J.Q. Fan, S.B. Zhang, NN. Mi., P. Christi, XL. Li and G. Feng. 2018. Direct effects of soil cadmium on the growth and activity of arbuscular mycorrhizal fungi. Rhizosphere 7: 43-48.
14. Gramlich, A., S. Tandy, C. Andres, J. Chincheros-Paniagua, L. Armengot, M. Schneider and R. Schulin. 2017. Cadmium uptake by cocoa trees in agroforestry and monoculture systems under conventional and organic management. Science of The Total Environment, 580: 677-686.
15. Gramlich, A., S. Tandy, C. Gauggel, M. López, D. Perla, V. Gonzalez and R. Schulin. 2018. Soil cadmium uptake by cocoa in Honduras. Sci. Total Environ. 612: 370-378.
16. He, S., Z. He, X. Yang, P.J. Stoffella and V.C. Baligar. 2015. Soil Biogeochemistry, Plant Physiology, and Phytoremediation of Cadmium-Contaminated Soils 134: 135-225.
17. Hildebrandt, U., M. Regvar, and H. Bothe. 2007. Arbuscular mycorrhiza and heavy metal tolerance, Phytochemistry 68(1): 139-146.
18. Iglesias, L., E. Salas, H.A. Leblanc and P. Nygren. 2011. Response of *Theobroma cacao* and *Inga edulis* seedlings to cross-inoculated populations of arbuscular mycorrhizal fungi. Agroforest Syst. 83: 63.
19. Jiang, Q.Y., F. Zhuo, S.H. Long, H.D. Zhao, D.J. Yang, Z.H. Ye et al. 2016. Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of *Lonicera japonica* grown in Cd-added soils?. Sci. Rep. 6: 21805.
20. Kaur, B., B. Singh., N. Kaur, et al. 2018. Fitorremediación de suelos contaminados con cadmio a través de especies arbóreas multipropósito. Sistema Agroforestal 92: 473-483.
21. Lekberg, Y. and T. Helgason 2018. In situ mycorrhizal function–knowledge gaps and future directions. New Phytologist 220(4): 957-962.
22. Maddela, N.R., L.C. García, S. Chakraborty, K. Venkateswarlu and M. Megharaj. 2020. Cocoa-laden cadmium threatens human health and cacao economy: A critical view. Science of the Total Environment 720: 137645.
23. Mendoza-López, K.L., J. Mostacero-León, S.E. López-Medina, A.E. Gil-Rivero, A. Cruz-Castillo and L. Villena-Zapata. 2021. Cadmio en plantaciones de *Theobroma cacao* L. "cacao" en la región San Martín (Lamas), Perú. Manglar 18(2): 169-173.
24. Meylan, L., C. Gary, C. Allinne, J. Ortiz, L. Jackson and B. Rapidel. 2017. Evaluating the effect of shade trees on provision of ecosystem services in intensively managed coffee plantations. Agriculture, Ecosystems & Environment 245: 32-42.
25. MINAM (Ministerio del Ambiente). 2014. Guía para muestreo de suelos. https://n9.cl/ihjkn (retrieved June 2023).
26. Newman, E.I. 1966. A Method of estimating the total length of root in a sample. Journal of Applied Ecology 3(1): 139-145.
27. Oksanen, J., G.L. Simpson, F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, et al. 2022. Vegan: Community Ecology Package. R package version 2.6-2. https://CRAN.R-project.org/package=vegan
28. Oliva, M., K. Rubio, M. Epquin, G. Marlo and S. Leiva. 2020. Cadmium uptake in native cacao trees in agricultural lands of Bagua, Peru. Agronomy 10(10): 1551.
29. Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55(1): 158-161.
30. 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. Science of The Total Environment 693(25): 133563.

31. Ramtahal, G., I. Yen, I. Bekele, F. Bekele, L. Wilson, K. Maharaj, and L. Harrynanan. 2016. Relationships between cadmium in tissues of cacao trees and soils in plantations of Trinidad and Tobago. Food and Nutrition Sciences **7**: 37-43.
32. Ramtahal, G., I. Chang, N. Ahmad, I. Bekele, F. Bekele, K. Maharaj, E. Lawrence and L. Harrynanan. 2015. Prediction of soil cadmium bioavailability to cacao (*Theobroma cacao* L.) using Single-Step Extraction Procedures. Communications in Soil Science and Plant Analysis 46(20): 2585-2594.
33. Rask, K.A., J.L. Johansen, R. Kjoller, and F. Ekelund. 2019. Differences in arbuscular mycorrhizal colonization influence cadmium uptake in plants, Environmental and Experimental Botany 162: 223-229.
34. Raza, A., M. Habib, S.N. Kakavand., Z. Zahid., N. Zahra., R. Sharif and M. Hasanuzzaman. 2020. Phytoremediation of cadmium: physiological, biochemical, and molecular mechanisms. Biology 9(7): 177.
35. Sandoval-Pineda, J.F., U.A. Pérez-Moncada, A. Rodriguez and E. Torres. 2020. Alta presencia de cadmio resulta en baja diversidad de hongos formadores de micorrizas arbusculares asociados a cacao (*Theobroma cacao* L.). Acta Biol Colomb. 25(3): 333-344.
36. Schneider, J., J. Bundschuh and C.W.A. Nascimento. 2016. Arbuscular mycorrhizal fungi-assisted phytoremediation of a lead-contaminated site. Sci. Total Environ. 572: 86-97.
37. Silva, S., F.J. Rodrigues, E.U. Lima, V.U. Lima, J.S. Silva Teixeira, D.J. Palheta et al. 2020. Cadmium toxicity and phyto-remediation in trees A review. Australian Journal of Crop Science 14(5): 857-870.
38. Tan, S.Y., Q.Y. Jiang, F. Zhuo, H. Liu, Y.T. Wang, S.S. Li, Z.H. Ye and Y.X Jing. 2015. Effect of inoculation with *Glomus versiforme* on cadmium accumulation, antioxidant activities and phytochelatins of *Solanum photeinocarpum*. PLoS One 10(7): e0132347.
39. Vallejos-Torres, G., R. Ruíz-Valles, CE. Chappa-Santa María, N. Gaona-Jiménez and C. Marín. 2022. High genetic diversity in arbuscular mycorrhizal fungi influence cadmium uptake and growth of cocoa plants. Bioagro 34(1): 75-84.
40. Vallejos-Torres, G., E. Espinoza, J. Marín-Díaz, R. Solis and L.A. Arévalo-López. 2021. The role of arbuscular mycorrhizal fungi against root-knot nematode infections in coffee plants. J. Soil Sci. Plant Nutr. 21**:** 364-373.
41. Van Aarle, I.M., P.A. Olsson and M.B. Soderstrom. 2002. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. New Phytologist 155: 173-182.
42. Zhang, Y., X. Guo, X. Yan, M. Ren, C. Jiang Y. Cheng et al. 2018. Identification of stably expressed QTL for resistance to black shank disease in tobacco (*Nicotiana tabacum* L.) line Beinhart 1000-1. The Crop Journal 6(3): 282-290.
43. Zug, K.L.M., H.A. Huamaní-Yupanqui, F. Meyberg, J.S. Cierjacks and A. Cierjacks. 2019. Cadmium accumulation in peruvian cacao (*Theobroma cacao* L.) and Opportunities for Mitigation. Water Air Soil Pollut. 230: 72.