Spanish Journal of Agricultural Research
22 (1), e0301, 11 pages (2024)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2024221-20405
INIA-CSIC

RESEARCH ARTICLE

OPEN ACCESS

Cadmium concentration in cocoa beans produced in agroforestry systems of small producers in Panama

Jhon A. Villalaz-Pérez[1], Fernando Casanoves[2,3,4], José E. Villarreal-Núñez[1], Adolfo Santo-Pineda[1], Abiel Gutiérrez-Lezcano[1], and Agustín Merino[5]

[1] Instituto de Innovación Agropecuaria de Panamá (IDIAP). Ciudad del Saber Clayton, Apdo. postal 6-4391, el Dorado 6A, Panama.
[2] CATIE - Centro Agronómico Tropical de Investigación y Enseñanza, 30501 Turrialba, Costa Rica.
[3] Centro de Investigaciones Amazónicas Macagual (CIMAZ). César Augusto Estrada González, Florencia, Colombia.
[4] Grupo de Investigaciones Agroecosistemas y Conservación en Bosques Amazónicos-GAIA, Florencia 180001, Colombia.
[5] Department of Soil Science and Agricultural Chemistry, University of Santiago de Compostela, Lugo, Spain.

***Correspondence** should be addressed to Jhon A. Villalaz-Pérez: jvillalaz14@gmail.com

Abstract

Aim of study: To calculate Cd concentration in cocoa plants and evaluate its relationship with available Cd and other soil properties.

Area of study: Almirante, Bocas del Toro province, Panama, in 2020-2021.

Material and methods: The study was carried out in 21 plots of eight cocoa-producing farms. The total area of each sampled plot was 300 m^2. Soil samples were taken at a depth of 30 cm, and samples of the leaves and fruits of cocoa trees were also taken. Descriptive statistics and correlation analyses were carried out for soil variables and Cd in plants. The relationship between bioavailable Cd and soil physicochemical variables and between soil variables and Cd in plants was evaluated. Multiple linear regression was performed using the backward selection method.

Main results: The pH was acidic (5.1) and the organic matter content of the soil was greater than 3%, suitable for immobilizing Cd from the soil. Total and bioavailable Cd averaged 0.10 mg kg^{-1} and 0.02 mg kg^{-1} respectively. The Cd levels in cocoa leaves exceeded the recommended levels of 0.5 mg kg^{-1}. The Cd concentration in the cocoa bean was low (0.25 mg kg^{-1}).

Research highlights: The levels of bioavailable Cd found do not exceed the United States Environment Agency toxic limits in soil. The level of Cd found in the cocoa bean is below the limit of 0.8 mg kg^{-1} which is taken as a reference for chocolate, with total dry matter content ≥ 50% of the CODEX Alimentarius.

Additional keywords: soil organic carbon; soil contaminants; Inceptisol; soil pH; aluminum saturation; toxicity.

Abbreviations used: Al. Sat. (aluminum saturation); Cp (predictive coefficient); ECEC (effective cation exchange capacity); FAAS (atomic absorption spectrophotometer); LOD (limits of detection); LOQ (limits of quantification); MINAM (Ministry of the Environment); SOC (organic carbon content of the soil); SOM (soil organic matter); VIF (values of inflation of variance).

Citation: Villalaz-Pérez, JA; Casanoves, F; Villarreal-Núñez, JE; Santo-Pineda, A; Gutiérrez-Lezcano, A; Merino, A (2024). Cadmium concentration in cocoa beans produced in agroforestry systems of small producers in Panama. Spanish Journal of Agricultural Research, Volume 22, Issue 1, e0301. https://doi.org/10.5424/sjar/2024221-20405

Received: 12 Apr 2023. **Accepted**: 20 Dec 2023. **Published**: 30 Jan 2024.

Introduction

Cocoa (*Theobroma cacao* L.) is a crop from South America and was brought to Central America in pre-Columbian times (Montemayor et al., 2002). There are an estimated 6 to 7 million cocoa farmers worldwide, and 80-90% are smallholder farmers, with production areas between 2 and 4 ha, from tropical regions of Africa, Asia, Indonesia and Central and South America (Bermúdez et al., 2022).

One of the aspects that affects cocoa cultivation commercialization is the presence of high concentrations of heavy metals (including Cd). This damages cocoa derivative quality, affecting 27% of exports (Huamaní-Yupanqui et al., 2012). Cd is generally adsorbed by roots in the soil and transported to the whole plant through the vascular system by essential cations, including Ca, Fe, and Zn (Huamaní-Yupanqui et al., 2012). High Cd concentrations in beans limit agricultural product marketing (Bravo Realpe et al., 2014).

Total Cd concentrations of geogenic origin in soil generally do not exceed 1 mg kg^{-1}. However, in some cases levels of up to 16.3 mg kg^{-1} Cd have been found, which can be associated with weathering processes and the type of parental material, with a natural base level that would not exceed 0.5 mg kg^{-1} (Kabata-Pendias, 2010).

There are many factors that influence Cd concentration in plants. Soil properties, such as soil starting material, soil organic matter (SOM), pH, carbonates, oxides and hydroxides of Fe and Mn, mineralogy of clays, salinity, anthropogenic contamination and plant genotype, affect the bioaccumulation and transfer of heavy metals to the cocoa bean (Alloway, 2013). In turn, these factors can allow heavy metal dissolution, leaching and solubility (Alloway, 2013).

The concentration of total Cd in the soil is not always a reliable indicator for predicting the concentration of Cd in the cocoa bean, as only one portion is bioavailable to the plant (Shahid et al., 2016). Alternatively, Cd and Zn in the soil present synergy when both metals have high concentrations; however, if one metal has higher concentration than the other, it is not absorbed by the plant (Huamaní-Yapanqui et al., 2012; Alloway, 2013). The total Cd concentration in the soil can be transferred to the plant if the soil pH is very acidic or the SOM is low (Rábago & Aracil, 2011).

The Cd in the plant is mobilized and transferred to the leaves and fruits, then concentrated in the grain, enabling us to verify the correlation between the bioavailable Cd in the soil and Cd in the grain (Argüello et al., 2019; Engbersen et al., 2019).

A high intake of cocoa beans at the maximum concentrations permitted by the Codex Alimentarius (FAO/WHO, 2017) in cocoa bean derivatives may promote the accumulation of Cd in the human body. This may cause serious problems in tissues and organs that are susceptible to it, such as the kidneys and liver (Casteblanco, 2018).

Furcal-Beriguete & Torres-Morales (2020) report that the European Food Safety Authority has not established a limit on Cd concentration in the unfermented or raw bean, but there are studies that show that Cd concentrations in the fermented and dried cocoa bean reach levels higher than established. The maximum limit for chocolate and other products with cocoa content between 50 and 70% is 0.6 mg kg^{-1}, while chocolates and products with declared cocoa content above 70% the maximum level is 0.8 mg kg^{-1}.

Cocoa cultivation in Panama presents high Cd concentrations in the bean, reducing its quality, price and marketing possibilities. Unlike other countries such as Peru (Huamaní-Yupanquí et al., 2012), Costa Rica (Florida, 2021) and Ecuador (Barraza et al., 2017), in Panama there are no studies on the relationships between soil Cd and its presence in cocoa beans, despite the fact that moderate concentrations of the metal are usually detected (*personal communication* with members of the Cooperative of multiple services of cacao Bocatoreño R.L).

It is pertinent to note that certain agronomic management such as the application of organic fertilizers, biochar, beneficial microorganisms and liming can also reduce the absorption of Cd and other heavy metals in cocoa (Dávila-Zamora et al., 2020). But cocoa cultivation areas in Panama do not maintain an adequate fertilization program.

This paper evaluates the concentration of Cd in cocoa plants, especially in the bean, and its relationship with the available Cd and other soil properties of small producer's fields in the province of Bocas del Toro, Panama. Our objective was to calculate Cd concentration in cocoa plants and evaluate its relationship with available Cd and other soil properties.

Material and methods

Study site location

The research was conducted during 2020-2021 in the district of Almirante, province of Bocas del Toro, Republic of Panama, on 21 farms cultivated with cocoa of various genotypes within agroforestry systems (Fig.1).

The climate of the province of Bocas del Toro is classified as very humid tropical. Annual precipitation is 2735 mm with an average annual temperature of 24.3 °C (Peel et al., 2007). The soils of the district of Almirante are classified taxonomically within the order of Inceptisol (Soil Survey Staff, 2014).

Field work and sample preparation

The study was carried out in eight producing farms of the district of Almirante, and within each farm two or three sampling plots were selected depending on the area of the plantation (SEMARNAT, 2002) to complete a total of 21 plots. Each plot of 300 m^2 (10 × 30 m) was located at representative sites of the plantation.

In each of these plots, 15 soil subsamples were systematically taken in the form of zig-zags equidistant from

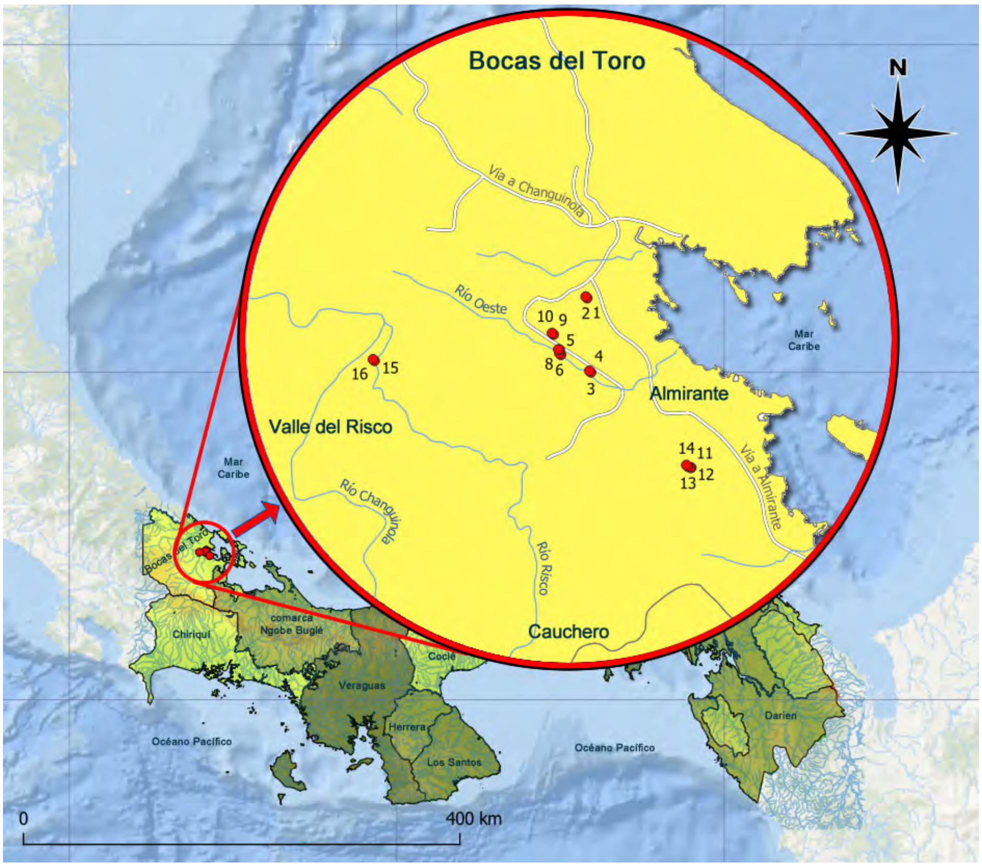

Figure 1. Location of sampled plots of farms in Almirante-Bocas del Toro, Republic of Panama.

each other at a distance of approximately 1.5 m from the trunk of each cocoa tree. The soil samples were taken up to 30 cm deep, and with the 15 subsamples a homogenized composite sample of approximately 2 kg was made.

Leaf and fruit sampling was carried out within 300 m^2 containing 17 cocoa trees established in a planting frame of 3 × 3 m. Fifty-one healthy leaves were collected, three for each tree, located in the middle part of the cocoa tree, taking the fourth leaf from the apex of the selected branch following the methodology of Puentes et al. (2016). Similarly, six fruits of uniform size were collected within the selected area (300 m^2) systematically (1 fruit for every 3 plants).

In the laboratory, rootlets, stones or any material that could alter soil analysis were removed. Then the soil samples were air-dried and crushed in an Agatha mortar and passed through a 2-mm nylon mesh sieve for all analyses except Cd, where another 0.063 mm sieve was also used according to the Soil Sampling Guide of the Ministry of the Environment, Peru (MINAM, 2014).

On the other hand, the leaves and fruits were washed with doubly distilled water (type 2) eliminating the soil particles adhered to them. The leaves were taken to the stove for drying at a temperature of 60 °C until a constant weight was maintained. For the fruit, the cocoa beans plus mucilage were separated from the rest of the fruit. Both parts were dried, following the same procedure as leaves. Finally, the leaves, fruits and grains were crushed.

Soil analysis

The pH was determined by potentiometry with a ratio of 1:2.5 (soil-water) using techniques described by Teixeira et al. (2017). Soil organic carbon (SOC) was analyzed using the method of Walkley & Black (1934). Total N was determined by Kjeldahl digestion following the methodologies of Teixeira et al. (2017).

For the extraction of bioavailable elements (Cu, Fe, Zn, Mn, Cd) and cations such as Ca, Mg and K, the Mehlich 3 extractor solution (Mehlich, 1984) was used, taking a soil: water ratio 1:10 (w/v), and the analysis by atomic absorption spectrophotometer of continuous source of high-resolution flame techniques (FAAS) was subsequently performed.

The exchangeable Al was extracted with 1 M KCl extractor solution, using techniques described by Teixeira et al. (2017). The effective cation exchange capacity (ECEC) was determined by the sum of the bases (Ca, Mg, K) + Al. Aluminium's saturation percentage was determined as follows: Al. Sat. (%) = Al / ECEC * 100. The granulometric physical analysis of the soil texture was carried out using the methodology manual (Teixeira et al., 2017).

The total concentrations of Cu, Zn, Fe, Mn, Cd, K, Ca and Mg in soil, leaves, fruits and cocoa beans were evaluated by microwave-assisted extraction using reagents with pure concentrations of HNO_3 and HCl in a 3:1 ratio and subsequently analyzed by high-resolution continuous source atomic absorption spectrophotometry of flame techniques, according to USEPA 3051A methodologies (USEPA, 2007). With this methodology we calculated the limits of detection (LOD), the limits of quantification (LOQ) and the adjusted R^2 for the elements evaluated.

Statistical analysis

Summary statistics and confidence intervals for estimators were used to describe the data. Pearson linear correlation analyses were performed to determine the association between soil variables and Cd in the plant.

To take into account the joint effect of the physicochemical variables of the soil on the concentrations of available Cd, a multiple-linear regression analysis was conducted. This same analysis was performed to evaluate how bioavailable Cd and other physicochemical soil variables relate to Cd in plants (leaves, fruits and grains). In multiple regressions, the backward variable selection method was used, retaining significant regressors ($p<0.05$) and to avoid multicollinearity, values of inflation of variance (VIF) less than five were considered (Balzarini et al., 2008). The model assumptions were evaluated by means of a graphic inspection of the residues. The analyses were performed using the statistical software InfoStat version 2020 (Di Rienzo et al., 2020).

Results

In Table 1, we present the limits of detection and instrumental quantification for the eight elements quantified on the atomic absorption spectrophotometer equipment, which met the requirements for precision and accuracy. By using it, the results were obtained with the necessary level of reliability. Additionally, summary measurements and 95% confidence intervals were used for soil mineral content and physical properties extracted at a depth of 30 cm from 21 evaluated plots (Table 2).

The soils showed high acidity, with a mean pH of 5.1 and a 95% confidence interval of 4.9-5.3. SOM levels were moderate, at 5.1%. There was an average concentration of 0.34 % of total nitrogen, with minimum and maximum values of 0.1 and 0.6 %, respectively. Among the sites sampled for the C: N ratio, the mean value was 9.9, with minimum levels of 4.5 and maximum levels of 26.

A value in the mean of the ECEC of 19.9 $\text{cmol}_{(+)}\ \text{kg}^{-1}$ (Table 2) was observed, this level being high in Inceptisol soils. In the same way, the elements that are part of the ECEC, K, Ca and Mg, obtained high concentrations with means of 0.20, 14.1 and 5.0 $\text{cmol}_{(+)}\ \text{kg}^{-1}$, respectively. The average concentrations of exchangeable Al had a moderate value of 0.7 $\text{cmol}_{(+)}\ \text{kg}^{-1}$, with a low level of Al. Sat. 3.9%, being able to find in the plots, minimum and maximum concentrations of 0.6 and 13.9%, respectively.

The micronutrients in the soil presented, for the concentration of Cu, Zn, Fe and Mn extracted by Mehlich 3, values of 2.5, 3.6, 101.9 and 177.1 mg kg^{-1}, respectively. Soil concentrations of total Cd and bioavailable Cd (Cd_{bd}) yielded mean values of 0.10 and 0.02 mg kg^{-1}, respectively, whose 95% confidence intervals were 0.07-0.14 and 0.02-0.03 mg kg^{-1}, respectively.

With respect to soil texture, the largest fraction corresponded to sand particles (57.8%), followed by silt (28.9%) and clay (13.3%).

A description of the Cd concentration in cocoa leaves, fruits, and beans is presented in Table 3 as well as 95% confidence intervals. A minimum and maximum limit is also provided in Fig. 2. The average concentration of Cd in leaves ($\text{Cd}_{\text{leaves}}$) was found to be 1.04 mg kg^{-1} with a minimum and maximum concentration of 0.13-4.40 mg kg^{-1}. The concentration of Cd in fruits ($\text{Cd}_{\text{fruits}}$) was 0.34 mg kg^{-1} with

Table 1. Determination of the limits of detection (LOD), quantification (LOQ) and adjusted coefficient of determination (R^2) of the atomic absorption spectrophotometer of elements evaluated in the 21 plots.

Element	Unit	LOD	LOQ	R2 (adj)
K	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	0.037	0.112	0.996
Ca	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	0.004	0.012	0.969
Mg	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	0.128	0.383	0.992
Cu	mg kg^{-1}	0.0031	0.093	0.999
Zn	mg kg^{-1}	0.001	0.002	0.996
Fe	mg kg^{-1}	0.007	0.022	0.994
Mn	mg kg^{-1}	0.003	0.008	0.984
Cd	mg kg^{-1}	0.001	0.002	0.999

Table 2. Descriptive statistics of the soil properties up to 30 cm deep in the 21 plots.

Variable	**Unit**	**Mean**	**SE**	**CV**	**Min**	**Max**	**Median**	**LI**	**UL**
								95%	
pH (H_2O)		5.10	0.06	7.60	4.40	6.00	5.10	4.90	5.30
SOM	%	5.10	0.25	31.70	1.80	10.10	4.90	4.50	5.80
SOC	%	2.90	0.15	31.70	1.10	5.90	2.90	2.60	3.40
Total N	%	0.34	0.02	37.10	0.10	0.60	0.40	0.30	0.40
Ratio (C:N)		9.90	0.64	41.80	4.50	26.10	9.10	8.20	11.60
ECEC	$cmol_{(+)}\ kg^{-1}$	19.90	1.33	43.30	6.40	38.70	17.60	15.90	23.90
K	$cmol_{(+)}\ kg^{-1}$	0.20	0.02	69.90	0.04	0.60	0.14	0.14	0.30
Ca	$cmol_{(+)}\ kg^{-1}$	14.10	0.97	44.70	3.20	28.30	11.40	11.20	16.90
Mg	$cmol_{(+)}\ kg^{-1}$	5.00	0.41	52.60	1.50	11.10	4.60	3.80	6.20
Al	$cmol_{(+)}\ kg^{-1}$	0.70	0.10	97.20	0.10	2.50	0.40	0.40	0.90
Al. Sat.	%	3.90	0.56	96.00	0.60	13.90	2.90	2.10	5.50
Cu	$mg\ kg^{-1}$	2.50	0.26	68.60	0.00	5.90	2.50	1.70	3.30
Zn	$mg\ kg^{-1}$	3.60	0.40	70.70	0.60	10.90	2.40	2.50	4.80
Fe	$mg\ kg^{-1}$	101.90	8.20	52.30	49.50	250.20	86.40	77.80	12.10
Mn	$mg\ kg^{-1}$	177.10	16.40	59.90	50.00	491.30	147.90	128.40	225.80
Total Cd	$mg\ kg^{-1}$	0.10	0.02	36.20	0.10	0.12	0.10	0.10	0.14
Cd_{bd}	$mg\ kg^{-1}$	0.02	0.01	32.20	0.02	0.03	0.02	0.02	0.03
Sand	%	57.80	2.04	22.80	36.00	76.00	60.00	52.00	63.60
Silt	%	28.90	1.72	38.40	12.00	48.00	27.50	24.10	33.90
Clay	%	13.30	1.01	49.00	1.00	24.00	13.50	10.60	16.30

SE: standard error. CV: coefficient of variation. LI: lower limit. UL: upper limit. SOM: soil organic matter. SOC: soil organic carbon. ECEC: effective cation exchange capacity. Al. Sat.: aluminum saturation. Cd_{bd}: bioavailable cadmium.

Table 3. Descriptive statistics of cadmium concentrations ($mg\ kg^{-1}$) in plants in the 21 plots using EPA 3051 A method (USEPA, 2007).

Variable[1]	**Mean**	**SE**	**CV**	**Min**	**Max**	**Median**	**LI**	**UL**
							95%	
Cd_{leaves}	1.04	0.15	90.9	0.13	4.40	0.74	0.60	1.40
Cd_{fruits}	0.34	0.03	52.3	0.09	0.76	0.30	0.30	0.40
Cd_{beans}	0.25	0.03	90.7	0.01	0.95	0.14	0.20	0.30

SE: standard error. CV: coefficient of variation. LI: lower limit. UL: upper limit. Cd_{leaves}: cadmium in leaves. Cd_{fruits}: cadmium in fruits. Cd_{beans}: cadmium in beans.

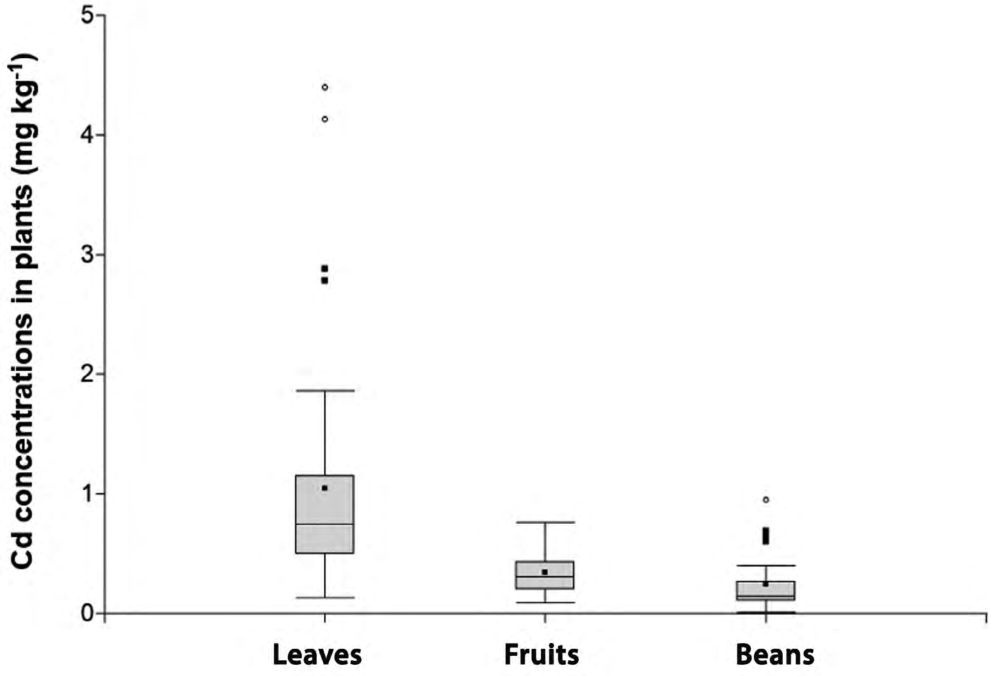

Figure 2. Cd concentration in cocoa plants in 21 plots evaluated from eight farms in Almirante-Bocas del Toro, Republic of Panama.

a minimum concentration of 0.09 mg kg^{-1} and maximum of 0.76 mg kg^{-1}. The concentration of Cd in cocoa beans (Cd$_{beans}$) was 0.25 mg kg^{-1}, with minimum and maximum levels of 0.01-0.95 mg kg^{-1} respectively with lower limits of 0.15 mg kg^{-1} and upper limits of 0.34 mg kg^{-1}.

Pearson's correlations were calculated to evaluate the relationship between Cd$_{beans}$ concentration and soil variables (Table 4). Pearson's correlations between the concentration variables of Cd$_{bd}$, Cd$_{leaves}$ and Cd$_{fruits}$ were also calculated (Table 5).

Cd$_{beans}$ (Table 4) was positively correlated with total Cd and Cd$_{bd}$ ($r = 0.66$ and $r = 0.50$ respectively; $p<0.05$). A positive correlation was also found with the percentage of slime and the content of Fe ($r = 0.47$ in both; $p<0.05$). The content of organic matter and Zn maintained a negative correlation with Cd in the cocoa bean with values of ($r = -0.50$; $p<0.05$) and ($r = -0.38$; $p<0.10$) respectively.

There was no significant correlation between Cd$_{leaves}$ and C$_{bd}$ (p=0.1169, Table 5). The concentration of Cd$_{leaves}$ presented a positive correlation with Cd$_{beans}$ ($r = 0.84$, $p<0.0001$) and with Cd$_{fruits}$ ($r = 0.57$, $p = 0.0075$). In turn, the Cd$_{fruits}$ correlate positively with the Cd$_{beans}$ ($r = 0.64$, p=0.0016).

The relationship between the concentration of bioavailable Cd (Cd$_{bd}$) and the physicochemical characteristics of the soil could be modeled with five variables according to the backward selection method (Table 6a), where the adjusted R^2 of the multiple regression model was 0.54. The resulting model was: $Cd_{bd} = 0.16 + 5.4 \times 10^{-4}$ Silt (%) $- 8.9 \times 10^{-4}$ Clay (%) $- 0.03$ pH $+ 1.9 \times 10^{-3}$ Zn $+ 1.2 \times 10^{-4}$ Fe.

The concentration of Cd$_{bd}$ is mostly influenced by soil pH, due to the high predictive coefficient (Cp) of Mallows

Table 4. Pearson's correlation for the variable concentration of Cd$_{beans}$ cocoa in the 21 plots evaluated.

Variables		R	p-value
Cd$_{beans}$	Cd$_{bd}$	**0.50**	**0.0243**
Cd$_{beans}$	Total Cd	**0.66**	**0.0010**
Cd$_{beans}$	SOM (%)	**-0.50**	**0.0207**
Cd$_{beans}$	Ratio (C:N)	0.05	0.8432
Cd$_{beans}$	Silt (%)	**0.47**	**0.0316**
Cd$_{beans}$	Clay (%)	0.37	0.1005
Cd$_{beans}$	pH	-0.04	0.8773
Cd$_{beans}$	Al	0.36	0.1138
Cd$_{beans}$	Ca$_{soil}$	-0.06	0.8114
Cd$_{beans}$	Mg$_{soil}$	-0.12	0.5947
Cd$_{beans}$	K$_{soil}$	-0.23	0.3052
Cd$_{beans}$	ECEC	-0.05	0.8138
Cd$_{beans}$	Cu$_{soil}$	-0.17	0.4634
Cd$_{beans}$	Zn$_{soil}$	-0.38	0.0929
Cd$_{beans}$	Fe$_{soil}$	**0.47**	**0.0317**
Cd$_{beans}$	Mn$_{soil}$	-0.14	0.5391

Cd$_{db}$: bioavailable cadmium. SOM: soil organic matter. ECEC: effective cation exchange capacity. Bold probabilities indicate significant correlation ($p<0.05$).

Table 5. Pearson's correlation for the Cd (bioavailable, cocoa leaves and fruits) concentration variables in the 21 evaluated plots.

Variables		R	p-value
Cd_{bd}	Cd_{leaves}	0.35	0.1169
Cd_{bd}	Cd_{fruits}	**0.49**	**0.0234**
Cd_{leaves}	Cd_{fruits}	**0.57**	**0.0075**
Cd_{leaves}	Cd_{beans}	**0.84**	**<0.0001**
Cd_{fruits}	Cd_{beans}	**0.64**	**0.0016**

Cd_{bd}: bioavailable cadmium. Bold probabilities indicate significant correlation ($p<0.05$).

(24.53); the lower the soil pH there is more Cd_{bd}. The percentage content of clay follows in predictive value to the pH; the lower the percentage of clay, the higher the concentration of Cd_{bd}. The opposite occurs with the percentage of silt, the higher the percentage more Cd_{bd}. Zn and Fe complete the set of significant variables to explain Cd_{bd}; in this case the higher the Fe and Zn, the higher the content of $Cd_{bd.}$

The relationship between cadmium in leaves (Cd_{leaves}) and the physicochemical characteristics of the soil could be modeled with five variables according to the backward method (Table 6b). The adjusted R^2 of the model was 0.47.

The estimated multiple regression model was: $Cd_{leaves} = -0.26 + 0.50\ Al + 0.02\ Silt\ (\%) + 0.04\ Clay\ (\%) - 0.15\ SOM\ (\%) + 22.37\ Cd_{bd}$, interpreting that the variables that better predict according to Mallows' Cp were exchangeable Al (11.74) and silt content (9.40), while Cd_{bd} had a positive relationship with Cd_{leaves}.

The relationship between Cd_{fruits} and the physicochemical characteristics of the soil could be modeled with fiur variables according to the backward method (Table 6c). The adjusted R^2 of the model was 0.44. The resulting model for Cd in fruits was: $Cd_{fruits} = 0.36 + 11.40\ Cd_{bd} - 0.09\ Al -0.47\ K - 0.03\ Zn$.

According to Mallows' Cp, the variables that better predict Cd concentration in fruits were Cd_{bd} (15.75) and Zn (8.97). Except for Cd_{bd}, which had a positive relationship with Cd_{fruits}, the other variables affected it negatively.

The relationship between the concentration of Cd_{beans} and the physicochemical characteristics of the soil was modeled with four variables according to the backward method (Table 6d). The adjusted R^2 of the model was 0.68. The resulting model was: $Cd_{beans} = -0.64 + 20.44\ Cd_{bd} - 0.03\ Zn + 0.03\ Clay\ (\%) + 0.04\ Mg$.

As in the case of Cd_{fruits}, the variable of greater intervention for the concentration of Cd_{beans} was the Cd_{bd} with a Cp of Mallows of 35.23 and with a positive association, as well as Mg and Clay. The only variable negatively affecting the amount of Cd_{beans} was the Zn content.

Discussion

In soil, the concentration of heavy metals (e.g., Cd) is closely linked to the content of SOM, which forms organometallic complexes, immobilizing their absorption by plants (Arévalo-Gardini et al., 2016). Researchers such as Cameselle & Gouveia (2019) mention that bioavailable Cd in soil can vary due to interactions with other metals. In turn, the concentration of Cd in the soil depends on the depth at which the sample is taken (Goebes et al., 2019).

The average pH value found in this study is considered very acidic (Snoeck et al., 2016). Low pH values allow for the highest solubility of toxic elements such as Al (0.7 $cmol_{(+)}\ kg^{-1}$ on average in this study) and Cd. This is significant in cocoa cultivation since the absorption processes of the Cd concentration are favored when the pH is very acid. In the same way, when the pH in the soil increases, Cd is adsorbed by colloids, blocking its bioavailability (Liu et al., 2020).

This could be verified in the regression model (Table 6a) where the pH of the soil was presented with a p-value < 0.05 and a high prediction value, indicating that is the variable that most interferes in the concentration of Cd_{bd}; the lower the pH, the more Cd_{bd}. Several studies affirm that the bioavailability of Cd is closely related to pH values (Arévalo-Gardini et al., 2016; Florida, 2021).

Generally, the average SOM content is greater than 3% (Table 2) which is adequate for cocoa cultivation (Snoeck et al., 2016) while helping the nutrition of the crop and immobilization of bioavailable Cd in the soil (Fernández-Nieto & Betancourt-González, 2018). The SOM at the levels found could have contributed to the concentration of total N in the soil maintaining high values (SEMARNAT, 2002), which is an important element in the inorganic nutrition of plants and improving product quality.

On the other hand, the ECEC (19.9 $cmol_{(+)}\ kg^{-1}$) is considered high (Cabalceta & Molina, 2006), while the exchangeable K is within the critical (optimal) level and the exchangeable cations Ca and Mg are sufficient in a soil classified as Inceptisol (Cabalceta & Molina, 2006). The microelements (Cu, Zn, Fe

Table 6. Multiple regression coefficients for the relationship between bioavailable cadmium, cadmium in leaves, fruit, cocoa beans and physical and chemical properties in the soil.

Variable	Unit	Estimate	SE	LI	UL	T	p-value	CP Mallows	FIV
				95%					
a) Bioavailable Cd									
Constant	--	0.16	0.03	0.09	0.22	4.97	0.0002		
Silt	%	$5.4\cdot10^{-4}$	$1.60\cdot10^{-4}$	$2\cdot10^{-4}$	$8.8\cdot10^{-4}$	3.35	0.0044	15.24	1.64
Clay	%	$-8.9\cdot10^{-4}$	$2.60\cdot10^{-4}$	$-1.4\cdot10^{-3}$	$-3.4\cdot10^{-4}$	-3.44	0.0036	15.84	1.44
pH	--	-0.03	0.01	-0.05	-0.02	-4.53	0.0004	24.53	3.56
Zn	mg kg^{-1}	$1.9\cdot10^{-3}$	$6.7\cdot10^{-4}$	$4.7\cdot10^{-4}$	$3.3\cdot10^{-4}$	2.84	0.0124	12.06	1.64
Fe	mg kg^{-1}	$1.2\cdot10^{-4}$	$4.3\cdot10^{-5}$	$3\cdot10^{-4}$	$2.1\cdot10^{-4}$	2.83	0.0126	12.02	2.81
b) Cd in cocoa leaves									
Constant	--	-0.26	0.62	-1.51	1.00	-0.41	0.6810		
Al	cmol $_{(+)}$ kg^{-1}	0.50	0.18	0.13	0.86	2.78	0.0085	11.74	1.15
Silt	%	0.02	0.01	$3.2\cdot10^{-3}$	0.05	2.32	0.0259	9.40	1.25
Clay	%	0.04	0.02	$3.2\cdot10^{-3}$	0.07	2.21	0.0337	8.87	1.17
SOM	%	-0.15	0.07	-0.29	$-2.7\cdot10^{-3}$	-2.07	0.0461	8.27	1.15
Cd$_{bd}$	mg kg^{-1}	22.37	10.62	0.82	43.91	2.11	0.0423	8.43	1.11
c) Cd in cocoa fruits									
Constant	--	0.36	0.10	0.15	0.57	3.67	0.0021		
Cd$_{bd}$	mg kg^{-1}	11.4	3.19	4.63	18.2	3.57	0.0026	15.75	1.06
Al	cmol $_{(+)}$ kg^{-1}	-0.09	0.05	-0.20	0.01	-1.97	0.0669	6.86	1.27
K	cmol $_{(+)}$ kg^{-1}	-0.47	0.21	0.92	-0.02	-2.21	0.0423	7.87	1.17
Zn	mg kg^{-1}	-0.03	0.01	-0.05	$-3.8\cdot10^{-3}$	-2.44	0.0265	8.97	1.17
d) Cd in cocoa beans									
Constant	--	-0.64	0.2	-1.06	-0.21	-3.19	0.0057		
Cd$_{bd}$	mg kg^{-1}	20.44	3.6	12.81	28.07	5.68	>0.0001	35.23	1.39
Zn	mg kg^{-1}	-0.03	0.01	-0.05	$-2.7\cdot10^{-3}$	-2.36	0.0313	8.57	1.11
Clay	%	0.03	0.01	0.01	0.04	4.45	0.0006	21.01	1.87
Mg	cmol $_{(+)}$ kg^{-1}	0.04	0.01	0.01	0.07	2.91	0.0103	11.46	1.89

SE: standard error. LI: lower limit. UL: upper limit. T: T-statistic. CP: predictive coefficient. FIV: values of inflation of variance. SOM: soil organic matter. Cd$_{bd}$: bioavailable cadmium.

and Mn) presented high concentrations exceeding the critical levels described for each of them by Calbacete & Molina (2006).

The Zn content in the soil can interfere with the adsorption and absorption of Cd due to its chemical similarity (Sikora & Wolt, 1986), since they are competing elements and therefore the Zn content negatively affects the uptake of bioavailable Cd by the plant. It could be verified in this work (Table 6a), where Mallows' Cp demonstrated that Zn is among the variables that predict the bioavailability of soil Cd (Huamaní-Yapanqui et al., 2012; Alloway, 2013).

The plots evaluated showed sandy loam, loam and sandy clay loam textures (Soil Survey Staff, 2014). According to the granulometry found of sand, silt and clay (Table 2) the study did not present textures with a high percentage of clay, but abundant in sand, similar to those found in studies carried out in Ecuador by Moreno et al. (2018) representing a very unfavorable factor because there may be a high mobilization of Cd moving by leaching to underlying surfaces at the depth of 30 cm (Kruszewski et al., 2018).

A high level of tolerance was observed for Cd_{leaves} (Table 3), according to Kabata-Pendias (2010) and Alloway (2013), who recommend a concentration between 0.05 and 0.5 mg kg^{-1}. According to Barraza et al. (2017) and Arguello et al. (2019), the Cd concentration values in leaves can be used as an approximate diagnosis, when the cocoa crop does not present its fruits. This may mean that there is a 3:1 ratio (cocoa leaves: beans).

A statistical correlation was found between the total Cd in the soil and the Cd_{grains} (Table 4). Ramtahal et al. (2016) reported similar results on cocoa plantations in Trinidad and Tobago. The results indicate that we can predict the amount of Cd in fruits and grains based on the amount of bioavailable Cd.

Cd_{leaves} did not show a significant correlation with Cd_{bd} in the soil, but when combined with other variables, it was significant (Table 6b). This may be because other metals, such as Al, can interact with the adsorption of this metal by the plant (Cameselle & Gouveia, 2019). Other authors, such as Huaraca-Fernández et al. (2020) refer to the fact that Cd_{bd} in soil is associated with ions (e.g., carboxyls), and these are related to SOM, which by increasing its contents decreases the concentration of bioavailable Cd and affects Cd_{leaves} (Table 6b).

It is worth mentioning that according to the European Union there are still no tolerance levels for Cd in cocoa beans (Barraza et al., 2017; Furcal-Beriguete & Torres-Morales, 2020) so the Cd level is taken as a reference for chocolate with total dry matter content ≥ 50% of the Codex Alimentarius. This should not exceed 0.80 mg kg^{-1} (FAO/WHO, 2017). According to the European Union in its regulation No. 488 (EC, 2014) this level may also apply to cocoa fruit. In this study the Cd_{grain} was well below this limit, with an average of 0.25 mg kg^{-1}.

Similarly, it could be found in Table 2 that the concentration of total and bioavailable Cd in the soil could be related to the average concentration of Cd found in the leaves, fruits and cocoa beans (Table 3), since The Cd transfer coefficient of the soil and plant is 1.0-10.0 (Alloway, 2013).

Conclusions

The concentrations of bioavailable cadmium are within the levels of precision and accuracy of the analytical equipment used, for the 21 Inceptisol soil plots extracted at a depth of 30 cm and are relatively low despite the fact that the average pH level is very acid.

The content of soil organic matter presented medium levels, favoring the non-solubility of interchangeable Al and bioavailable Cd.

The models developed for bioavailable Cd, Cd in cocoa leaves, fruits and beans can be used to correct some chemical and biological properties of the soil that can be managed at the farm level, including liming to increase pH and adding organic material to increase the SOM content.

Data availability: Not applicable

Acknowledgements: We thank the support provided in this research study to: the National Secretariat of Science, Technology and Innovation of the Republic of Panama (SENACYT); the Agricultural Innovation Institute of Panama (IDIAP); and the owners of the cocoa farms in the Almirante district in the province of Bocas del Toro.

Competing interests: The authors have declared that no competing interests exist.

Authors' contributions: Jhon A. Villalaz-Pérez: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Fernando Casanoves:** Formal analysis, Methodology, Resources, Software, Validation, Writing – original draft, Writing – review & editing. **José E. Villarreal-Núñez:** Conceptualization, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Adolfo Santo-Pineda:** Investigation. **Abiel Gutiérrez-Lezcano:** Investigation, Project administration. **Agustín Merino:** Conceptualization, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding: The authors received no specific funding for this work.

References

Alloway BJ, 2013. Sources of heavy metals and metalloids in soils. In: Heavy metals in soil; Alloway BJ (Ed.), pp. 11-50. Dordrecht: Springer. https://doi.org/10.1007/978-94-007-4470-7_2

Arévalo-Gardini E, Obando-Cerpa ME, Zúñiga-Cernades LB, Arévalo-Hernández CO, Baligar VC, He ZL, 2016. Heavy metals in soils of cocoa plantations (*Theobroma cacao* L.) in three regions of Peru. Appl Ecol 15(2): 81-89. https://doi.org/10.21704/rea.v15i2.747

Argüello D, Chavez E, Lauryssen F, Vanderschueren R, Smolders E, Montalvo D, 2019. Soil properties and agronomic factors affecting cadmium concentrations in cacao beans: A nationwide survey in Ecuador. Sci Total Environ 649: 120-127. https://doi.org/10.1016/j.scitotenv.2018.08.292

Balzarini MG, González L, Tablada M, Casanoves F, Di Rienzo JA, Robledo CW, 2008. Infostat. User Manual, Editorial Brujas, Córdoba, Argentina. 331 pp.

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

Bermúdez S, Voora V, Larrea C, Luna E, 2022. Global Market Report. Cocoa prices and sustainability. https://www.iisd.org/publications?viewsreference[parent_entity_type]=node&viewsreference[parent_entity_id]=36&viewsreference[parent_field_name]=field_listing_type&page=10 [Jun 20, 2023].

Bravo Realpe IDS, Arboleda Pardo CA, Martín Peinado FJ, 2014. Efecto de la calidad de la materia orgánica asociada con el uso y manejo de suelos en retención de cadmio, en sistemas altoandinos de Colombia. Acta Agron 63(2): 164-174. https://doi.org/10.15446/acag.v63n2.39569

Cabalceta G, Molina E, 2006. Niveles críticos de nutrimentos en suelos de Costa Rica utilizando la solución extractora Mehlich 3. Agronomía Costarricense 30(2): 31-44.

Cameselle C, Gouveia S, 2019. Phytoremediation of mixed contaminated soil enhanced with electric current. J Hazard Mater 361: 95-102. https://doi.org/10.1016/j.jhazmat.2018.08.062

Casteblanco JA, 2018. Heavy metals remediation techniques with potential application in cocoa cultivation. La Granja 27(1): 21-35. https://doi.org/10.17163/lgr.n27.2018.02

Dávila-Zamora EC, Reyes-Evangelista LA, Aldoradin-Puza E, Londoñe-Bailon P, Aleman-Polo M, 2020. Cd and Pb reduction in cocoa (*Theobroma cacao* L.) nib using two organic amendments. Revista Colombiana de Investigaciones Agroindustriales 7(1): 20-29. https://doi.org/10.23850/24220582.2776

Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo CW, 2020. InfoStat vers 2020. Centro de Transferencia InfoStad, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar.

EC, 2014. Regulation (EC) No 488/2014 amending Regulation (EC) No 1881/2006 as regards the maximum level for cadmium in foodstuffs. European Community Regulation, Belgium, 138/78.

Engbersen N, Gramlich A, López M, Schwarz G, Hattendorf B, Gutiérrez O, et al., 2019. Cadmium accumulation and allocation in different cacao cultivars. Sci Total Environ 678: 660-670. https://doi.org/10.1016/j.scitotenv.2019.05.001

FAO/WHO, 2017. Codex Alimentarius (Codex). Report of the 11th meeting of the Codex Committee on Food Contaminants. http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/. [May 29, 2021].

Fernández-Nieto A, Betancourt-González AR, 2018. Destino sostenible de los residuos generados en las plantas de beneficio avícola. AiBi Revista de investigación, administración e ingenieria 6(1): 11-22. https://doi.org/10.15649/2346030X.473

Florida Rofner N, 2021. Review of maximum limits for cadmium in cocoa (*Theobroma cacao* L.). La Granja 34(2): 117-130. https://doi.org/10.17163/lgr.n34.2021.08

Furcal-Beriguete P, Torres-Morales JL, 2020. Determinación de concentraciones de cadmio en plantaciones de *Theobroma cacao* L. en Costa Rica. Revista Tecnología En Marcha 33(1): 122-137. https://doi.org/10.18845/tm.v33i1.5027

Goebes P, Schmidt K, Seitz S, Both S, Bruelheide H, Erfmeier A, et al., 2019. The strength of soil-plant interactions under forest is related to a critical soil depth. Sci Rep 9: 8635. https://doi.org/10.1038/s41598-019-45156-5

Huamaní-Yupanqui HA, Mansilla-Minaya LG, Florida-Rofner N, Neira-Trujillo GM, 2012. Presencia de metales pesados en cultivo de cacao (*Theobroma cacao* L.) orgánico. Acta Agronómica 61(4): 339-344.

Huaraca-Fernández J, Pérez-Sosa L, Bustinza-Cabala L, Pampa-Quispe N, 2020. Organic amendments on the immobilization of cadmium in contaminated agricultural soils: a review. Technol Inform 31(4): 139-152. https://doi.org/10.4067/S0718-07642020000400139

Kabata-Pendias A, 2010. Trace elements in soils and plants (4th ed). CRC PRESS, 548 pp. https://doi.org/10.1201/b10158

Kruszewski B, Wiesław Obiedziński M, Kowalska J, 2018. Nickel, cadmium and lead levels in raw cocoa and processed chocolate mass materials from three different manufacturers. J Food Compos Anal 66: 127-135. https://doi.org/10.1016/j.jfca.2017.12.012

Liu N, Jiang Z, Li X, Liu H, Li N, Wei S, 2020. Mitigation of cadmium (Cd) accumulation in rice through the joint application of organic amendments and selenium (Se) in contaminated soils with high Cd content. Chemosphere 241: 125106. https://doi.org/10.1016/j.chemosphere.2019.125106

Mehlich A, 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun Soil Sci Plant Anal 15(12): 1409-1416. https://doi.org/10.1080/00103628409367568

MINAM, 2014. Guía para el muestreo de suelo. En el marco del Decreto Supremo Nº.002-2013-MINAM, Estándares de Calidad Ambiental (ECA) para suelo. Ministerio del Ambiente, Perú.

Montemayor JC, Risterucci AM, López PA, Ortiz CF, Moreno A, Lanaud C, 2002. Cacao domestication I: The origin of the cacao cultivated by the Mayas. Heredity 89(5): 380-386. https://doi.org/10.1038/sj.hdy.6800156

Moreno J, Sevillano G, Valverde O, Loayza V, Haro R, Zambrano J, 2018. Soil from the coastal plane. In: The soils of Ecuador, pp: 27-77. Springer, Cham. https://doi.org/10.1007/978-3-319-25319-0_2

Peel MC, Finlayson BL, McMahon TA, 2007. Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sci 11(5): 1633-1644. https://doi.org/10.5194/hess-11-1633-2007

Puentes PYJ, Menjivar JC, Aranzazu F, 2016. Concentration of nutrients in leaves, a tool for nutritional diagnosis in cocoa. Agron Mesoam 27(2): 329-336. https://doi.org/10.15517/am.v27i2.19728

Rábago I, Aracil J, 2011. Buffer capacity for lead and cadmium contamination in soils in the Community of Madrid. PhD Thesis. Universidad Complutense, Madrid, Spain, 285 pp.

Ramtahal G, Yen IC, Bekele I, Bekele F, Wilson L, Maharaj K, et al., 2016. Relationships between cadmium in tissues of cacao trees and soils in plantations of Trinidad and Tobago. Food Nutr Sci 07(01): 37-43. https://doi.org/10.4236/fns.2016.71005

SEMARNAT, 2002. Official Mexican Standard NOM-021-RECNAT-2000 that establishes the specifications of fertility, salinity and classification of soils, study, sampling and analysis. Secretary of Environment and Natural Resources, Mexico, 85 pp.

Shahid M, Dumat C, Khalid S, Niazi NK, Antunes PMC, 2016. Cadmium bioavailability, uptake, toxicity and detoxification in soil plant system. Rev Environ Contam Toxicol 241: 73-137. https://doi.org/10.1007/398_2016_8

Sikora FJ, Wolt J, 1986. Effect of cadmium and zinc treated sludge on yield and cadmium-zinc uptake of corn. J Environ Qual 15: 340-345. https://doi.org/10.2134/jeq1986.00472425001500040005x

Snoeck D, Koko L, Joffre J, Bastide P, Jagoret P, 2016. Cocoa nutrition and fertilization. In: Sustainable agriculture reviews, vol 19; Lichtfouse E (ed). Springer International Publishing, Cham. pp: 155-202. https://doi.org/10.1007/978-3-319-26777-7_4

Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th Edition, USDA-Natural Resources Conservation Service, Washington DC.

Teixeira PC, Donagemma GK, Fontana To, Teixeira WG, 2017. Manual de métodos de análise do solo, 3rd ed. rev. Brasília, DF: Embrapa, 573 pp.

USEPA, 2007. Method 3051A (SW-846): Microwave assisted acid digestion of sediments, sludges, and oils. Revision 1. Washington, DC.

Walkley A, Black AI, 1934. An examination of the method for determination soil organic matter, and a proposed codification of the chromic acid titration method. Soil Sci 37: 29-38. https://doi.org/10.1097/00010694-193401000-00003