

Relationship between soil properties, content and dynamics of cadmium in Creole cocoa genotype grown **organically** in Bocas del Toro – Panama

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Authors' attributions:

This research work was carried out thanks to the collaboration of a group of authors specialized in the area. Author: JAVP was the one who carried out the sampling, laboratory analysis, data analysis and writing of the document. Author JEVN was the collaborator of field sampling and information review. ASP collaborator field sampling and laboratory analyst. Author AMG information review.

Item Information

ABSTRACT SUMMARY

Introduction: Quantifying Cadmium (Cd), helps to know the excess of this element harmful to human health.

Objectives: Determine the concentrations of Cd and its relationship with the management and properties of the soil, leaf tissue, fruit and cocoa beans grown **organically** in small farms of producers in the district of Almirante, and to study the dynamics of this element in a Creole cocoa genotype AS-CP 26-61 (mulatto).

Places and duration of the study: The research was carried out during the years 2019 to 2021, in 16 sites of farms of cocoa producers, organically grown, in Almirante, province of Bocas del Toro and the second, was in a house of vegetation, located in the facilities of the Institute of Agricultural Innovation of Panama, in the town of Divisa, corregimiento of Los Canelos, district of Santa María, province of Herrera, Republic of Panama.

Methodologies: The samples were carried out in the 16 farm sites in a systematic way selecting points at uniform distances in an area of 300 m² with replicas (15 subsamples at depths of 0.30 m), within these areas the samples of leaves and fruits were taken. In the dynamics of the Cd, which was carried out in the creole cocoa genotype AS-CP 26-61 (mulatto), the soil was transferred from a representative area of the Almirante district to a depth of 0.30 m, filling pots with a capacity of 15 kg of dry soil. A completely random design was used, with six (6) treatments and three (3) repetitions. The treatments were (0, 4, 8, 12, 16 and 20 mgCdkg⁻¹) inoculated the soils with cadmium sulfate. Soil samples were taken at two depths (0 to 0.15 m and 0.15 to 0.30 m); followed for plants (roots, stems and leaves), all extracted six (6) months after applying the treatments. Total and bioavailable Cd, Ca, Mg, soil texture, pH, soil organic matter, total foliar Cd, fruits and grains were measured. The analysis of the data was by the Infostat statistician. Soil organic matter was determined by Walkley and Black and total concentrations by USEPA method 3051 A; instead, the bioavailable Cd and K were determined by Mehlich 3; Ca and Mg per KCl; texture by Boyoucos.

Results: As results in the 16 sites, the total and bioavailable Cd in the soil did not exceed the toxic levels for agricultural soils. On the other hand, the average levels of total Cd in the fruits and grains, did not present absorptions greater than 0.6 mgkg⁻¹. While for the dynamics of Cd in the Creole

cocoa genotype AS-CP 26-61 the maximum absorption form is presented by the leaves > roots > stem.

Conclusion: By increasing the organic matter of the soil, it helps to block the concentrations of Cd in the leaves, fruits and grains; the levels of concentrations of Cd in the grain, do not exceed the maximum limits allowed according to the E.U. The use of the Creole genotype AS-CP 26-61 helped us to know in which part of the plant the concentrations of Cd are most concentrated.

Keywords: Cadmium; Total Concentrations; Inceptisols; Soil Organic Matter; cocoa beans

1. INTRODUCTION

Cocoa is a highly marketed and consumed item worldwide, that is why the total content of Cadmium (Cd), allowed in the soil and in the plants, must be quantified to, which can help improve the quality of the beans product. This will determine the value of the effort that each farmer obtains when marketing their grains in the country and in the rest of the world.

Eating food, with high concentrations of cadmium (Cd), has negative implications for human health. This element accumulates mainly in the kidneys, bone and respiratory system; its biological average lifespan in humans is between 10-35 years [1].

Cocoa is grown in warm and humid regions in more than 50 countries located on four continents (Africa, America, Asia and Oceania); 23 of these countries are from the Americas and in them cocoa is produced for commercial purposes, which makes cocoa a crop of great economic, social, environmental and, particularly, cultural importance for the territories where it is produced [2].

In 2014, the European Union (EU) announced maximum permitted levels for cadmium in cocoa and chocolate products sold in the EU. These guidelines were applied on the first (1st) of January 2019 (cocoa farmer in Peru Sci. Total environment. 2017; 605-606 and 792-800), (Meter, Atkinson and Laliberte) [3]; these same authors indicate that many buyers seem to prefer a relatively low cadmium content to ensure that grains can be used in any recipe and buyers are requesting limits for cadmium concentration in grains between 0.5 and 1.1 mgkg⁻¹ [3].

The Codex Alimentarius Commission has established the limits for Cd as follows: i) 0.8 mgkg⁻¹, for chocolate that contains or declares greater than or equal to 50% to less than 70% of total cocoa solids on the dry matter basis; and, ii) 0.9 mgkg⁻¹ for chocolate that contains or declares greater than or equal to 70% of total cocoa solids on a dry matter basis [4].

The cultivation of cocoa in Latin America and the Caribbean (LAC) presents great challenges in the coming decades, especially to position itself in the segment of fine quality and aroma cocoas, challenges in the technological, organizational and institutional order [5].

In the Republic of Panama, cocoa is produced mainly in the provinces of Bocas del Toro, Colon and in the Ngäbe-Buglé indigenous region (Ñokribo District).

For the year 2016 it was estimated that the area with cocoa cultivation was approximately 6000 hectares, of which 4500 hectares belong to members of the Cooperative of Multiples Services Cacao Bocatorenño RL (COCABO) and 1500 hectares to independent farmers, from whom the cooperative buys the dried cocoa [6]. For the Almirante district, it is estimated that there are 71 hectares (78881 plants), of which their production is approximately 0.18 Mgha⁻¹ [7].

The producers of these areas do not maintain adequate organic fertilization, since it is only through harvest waste, decomposing litter and some incinerated field waste and without characterizing the contents and quantity applied, that is how they carry out their cultivation tasks.

The high contents of heavy metals, their mobility and availability depend on many factors, among them are pH, clay content, organic matter, cation exchange capacity and other soil attributes [8]. According to Alloway [9], the concentrations of Cd of geogenic origin in the soil, generally do not

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exceed 1.0 mgkg⁻¹, ~~and being able to find~~ levels of 16.3 mgkg⁻¹ of Cd, ~~which~~ can be associated with weathering processes and type of parental material; with a natural base level ~~that would~~ not exceeding 0.5 mgkg⁻¹ [10,11]. While natural concentrations vary by soil type, higher concentrations of Cd can be expected for soils covering sedimentary rocks (containing the highest concentrations of Cd ranging from 0.3 to 15 mgkg⁻¹) [12].

In soil, Cd is found in the inorganic form Cd⁺², presenting a low adsorption coefficient and high mobility in the soil-plant system [13].

Moreover, He et al. [14] and Gramlich, et al. [15], mention in different articles when comparing different types of soils ~~that, derivatives of~~ igneous rocks usually contain low amounts of cadmium and soils derived from metamorphic rocks are intermediate. Soils derived from sedimentary rocks such as shales contain high amounts of this toxic metal; while Gramlich et al. [15], found that cadmium levels in cocoa-growing soils varied significantly across different geological substrates in Honduras and was the highest in alluvial soils originating from sedimentary material.

In very acidic and acidic soils, the decrease in pH is the cause of the mobility of Cd [16], this also occurs when the organic matter in the soil decreases [17]; that is, for each unit that increases the pH, the absorption will be reduced ~~by~~ 1.5 times [18]. This heavy metal is absorbed by plants in the form of Cd⁺² [19].

Higher levels of total Cd content in the soil imply a greater potential for cadmium absorption [3]. Root exudates, especially carboxylic acids, increase the absorption of Cd [20].

Total soil cadmium is not always a good indicator of Cd in cocoa beans, as only a portion is available to plants [14, 15, 21, 22, 23, 24].

In the main regions in Ecuador, Argüello et al. [22], ~~they~~ conducted a study ~~in cocoa crops, finding~~ in ~~a total of~~ 159 cocoa farms ~~and found~~ an average concentration of cadmium in the beans ~~to be of~~ 0.90 mgkg⁻¹.

Cd can be absorbed and accumulated in roots, stems, leaves, fruits and seeds [25] ~~with the amount~~; ~~this heavy metal~~ accumulate ~~ds in the seeds~~ [26], ~~whose concentration~~ dependings on age and species [27] ~~the~~ Direct or indirect intake (cereals, chocolates, chewing gums and confectionery) of Cd [28 and 29], causes damage to the kidney, liver, lung, pancreas, testicles and bone [30] ~~leading to, causing~~ kidney deficiencies, osteoporosis, high blood pressure, diabetes, pulmonary emphysema and some cancers of the prostate, lung, bladder and pancreas [31, 32].

~~In the Republic of Panama, province of Bocas del Toro, district of Almirante, cocoa crops are grown organically, this crop being one of the main sustenance's in the economy in this region.~~ The objectives were to determine the concentrations of Cd and its relationship with the management and properties of the soil, leaf tissue, fruit and cocoa beans grown organically in small farms of farmers in the district of Almirante, and to study the dynamics of this element in a Creole cocoa genotype AS-CP 26-61 (mulatto).

2. MATERIALS AND METHODS

2.1 Site description

The research trials were carried out ~~between in the years~~ 2019-2021, in two different locations: The first located in 16 areas ~~comprising eight small farms belonging to producers in the District of Almirante, Province of Bocas del Toro, Republic of Panama (Fig 1); and the second, was in a house of vegetation, located in the facilities of the Institute of Agricultural Innovation of Panama (IDIAP), in the town of Divisa, corregimiento of Los Canelos, district of Santa María, province of Herrera,~~

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Republic of Panama, where the dynamics of the Cd in the genotype of Creole cocoa AS-CP 26-61 (mulatto) was determined, in one-year-old plants (Fig 2).

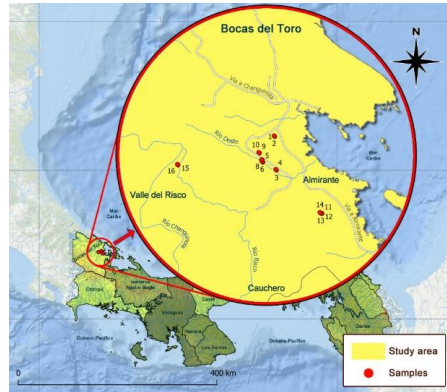


Fig.1. Sampling areas of small farms of cocoa producers. Bocas del Toro, Republic of Panama.

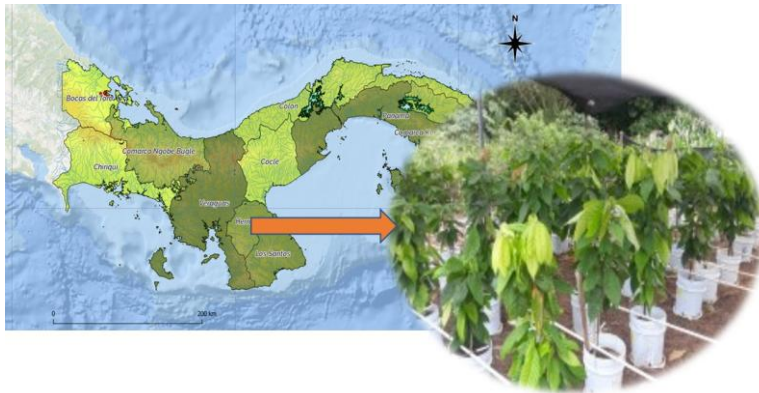


Fig.2. Cd dynamics in Creole cocoa genotype AS-CP 26-61 (mulatto). Divisa, District of Santa Maria , Herrera province, Republic of Panama.

2.2. Edaphoclimatic characteristic of the study areas in the Almirante district

~~Geologically, the province of Bocas del Toro originated from sedimentary rocks; during the Secondary period, it is observed within the Changuinola group with symbol on the map Formation of the Isthmus of Panama by geological eras (K-CHA), with formation composed of limestone rocks, shales, sandstones, ashes, tuffs, lavas, andesitic interspersed; where the first two formations stand out, for being the ones with the highest percentage of sedimentary composition [56].~~

The Climate of Bocas del Toro according to Köppen [33], updated by Peel et al. [34], is classified as very humid tropical climate (Af); every month with rainfall > 60 mm. Average temperature of the coolest month > 18 °C [35].

The taxonomic classification of soils in the area, according to Soil Taxonomy, is within the order Inceptisols, suborder Ochrepts, large groups Dystrochrepts and isohyperthermic temperature regime [36].

Comment [Ma3]: Where is the description for the other district?

In Almirante, the soils cultivated with cocoa, present textures ranging from loam, sandy loam and loamy sand, the permeability of the soils oscillates from moderate to very fast, the content of organic carbon found in their horizons of the studied profiles vary from medium to low, decreasing as the soil profile deepens; pH was found with values ranging from very acidic to acidic [36].

2.3. Characteristics of the Creole cocoa genotype AS-CP 26-61 (mulatto):

The plant, can reach a height of 2.5 m, with brown branches, these have leaves of 0.345 m long of light green color with an absence of anthocyanin, for the wet seed expresses a white coloration and a white pigmentation for the flower. The seed has an elliptical shape, while the fruit is rough, with weights greater than 627 grams, ~~having~~ more than 33 seeds per fruit [37].

2.4. Sampling of soil and plants, in small farms producing cocoa in Almirante – Bocas del Toro

Sampling was carried out at 16 farm sites, in varieties of different ages. Sampling was carried out by the systematic or grid method, which consisted of selecting sampling points at uniform distances (zig-zag), depending on the area under study [38].

The rectangular areas consisted of measures 10.0 x 30.0 meters (300 m²) with replicas, on which the 15 subsamples were taken, forming a composite sample taken at ~~soil~~ depths of 0.30 m, homogenizing separately and transferring them to the soil laboratory. of the IDIAP, with quantities of 2.0 kg for each area. With the help of the clinometer instrument, the slopes of each of the studied areas were determined.

~~0 mgCdkg⁻¹; 2~~ Within the areas of 300 m², the sampling of leaves and ~~podseeds~~ were carried out, to measure the content of heavy metals and other elements, leaf tissue sampling was carried out following methodologies of Puentes, Menjivar and Aranzazu [39].

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2.6. Cd dynamics in Creole cocoa genotype AS-CP 26-61 (mulatto):

For the dynamics of the Cd, carried out with the Creole cocoa genotype AS-CP 26-61 (mulatto), the soil used, was transferred from a representative area of the district of Almirante, province of Bocas del Toro, extracted to a depth of 0.30 m, where it was characterized to know its nutritional status and physiochemical and biological properties.

The plants were transplanted into pots with a capacity of 15 kg of dry soil. A completely random design was used, with six (6) treatments and three (3) ~~replications~~ repetitions by treatments.

The soils were inoculated with cadmium sulfate applied to the surface of the soil, using the following treatments: T1: 0 mgCdkg⁻¹; T2: 4 mgCdkg⁻¹; T3: 8 mgCdkg⁻¹; T4: 12 mgCdkg⁻¹; T5: 16 mgCdkg⁻¹; T6: 20 mgCdkg⁻¹.

Two depths of soil sampling were taken (0 to 0.15 m and 0.15 to 0.30 m); for the plants ~~—~~ (roots, stems and leaves) were extracted six (6) months after applying the treatments for their respective analyses.

2.7 Statistical analysis

~~For the evaluation of the data of the variables analyzed at the sites, descriptive statistics (means, coefficients of variation, lower limits and upper limits) and Pearson correlation were used. While, for the variables determined in the Creole cocoa genotype AS-CP 26-61, a comparison of means by Fisher's LSD (p<0.05) was performed, at the same time a linear regression was performed to determine the maximum concentrations by treatments applied. In both trials, a statistical package of the Infostat program, version 2008 [40] was used. After the analysis, it was discriminated through comparative tables, to know the highest concentration of cadmium and later the levels found in soil,~~

leaves, fruits and almonds or cocoa beans were compared with those presented by other countries in the literature and with the standard of the European Union.

2.7.8. Analysis of the samples

The determination of soil organic matter (SOM) was quantified using soil carbon methodology through the method proposed by Walkley and Black [41].

The extraction of the total concentration in soil (elements: Cd, Cu, Fe, Zn, Mn, K, Ca and Mg); leaves, root stems, fruits and grains (elements: Cd, Cu, Fe, Zn, Mn, K, Ca and Mg), were carried out by means of a microwave digester using method EPA 3051 A [42]. For the extraction of bioavailable elements in the soil, the extractive solution Mehlich -3 [43] was used. All quantifications were performed by continuous source flame atomic absorption spectrometry.

To determine the effective cation exchange capacity (ECEC), the sum of interchangeable bases and acidity (Ca + Mg + K + Al) was performed; the pH in water with a ratio of 1:2.5 (soil: water) was determined by potentiometry, using techniques described by Teixeira et al. [44]. The granulometric physical analysis of soil texture was determined using the Bouyoucos methodology [45].

For the determination of the bioavailability of Ca, Mg, K, Al, potassium chloride (KCl) was used, according to Kamprath [46].

Table 1 below, shows the physical, chemical and biological characteristics of the soil used for the Cd dynamics test in Creole cocoa genotype AS-CP 26-61 (mulatto).

Table 1. Soil's properties used in Cd dynamics test in the Creole cocoa genotype AS-CP 26-61 (mulatto).

Variable	Average	Variable	Average
CEC (cmol ₍₊₎ kg ⁻¹)	53.0	Bioavailable-Cd (mgkg ⁻¹)	0.03
P (mgkg ⁻¹)	27.0	Cu (mgkg ⁻¹)	3.22
pH _(water 1:2.5)	6.61	Zn (mgkg ⁻¹)	6.20
% SOM	1.32	Fe (mgkg ⁻¹)	60.40
% Sand	58.67	Mn (mgkg ⁻¹)	48.5
% Silt	28.0	K (cmol ₍₊₎ kg ⁻¹)	0.31
% Clay	13.33	Ca (cmol ₍₊₎ kg ⁻¹)	21.22
Total-Cd	0.52	Mg (cmol ₍₊₎ kg ⁻¹)	6.82

2.8 Statistical analysis

For the evaluation of the data of the variables analyzed at the sites, descriptive statistics (means, coefficients of variation, lower limits and upper limits) and Pearson correlation were used. While, for the variables determined in the Creole cocoa genotype AS-CP 26-61, a comparison of means by Fisher's LSD ($p < 0.05$) was performed, at the same time a linear regression was performed to determine the maximum concentrations by treatments applied. In both trials, a statistical package of the Infostat program, version 2008 [40] was used. After the analysis, it was discriminated through comparative tables, to know the highest concentration of cadmium and later the levels found in soil, leaves, fruits and almonds or cocoa beans were compared with those presented by other countries in the literature and with the standard of the European Union.

3 RESULTS AND DISCUSSIONS

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3.1 pH and SOM content at 16 small farm sites

In the soils of the areas studied (Fig. 3), the pH indices ranged from 4.60 to 5.85. Most of these soils are within the range of 5.0 to 7.5 which are optimal for the cocoa crop sector [47]. The increase in the pH index in acidic soils can lead to a low absorption of Cd by plants [21 and 8]. Contrary to the reported by Porta and Lopez [48], the optimal pH values for this crop are between ranges of 6.0 to 7.0 and the tolerance for a satisfactory yield is at a level of 4.5 to 8.0. Acevedo et al. [49] found that the slight contamination with Cd occurs between pH of 5.0-6.0 and as the pH decreases the contamination increases.

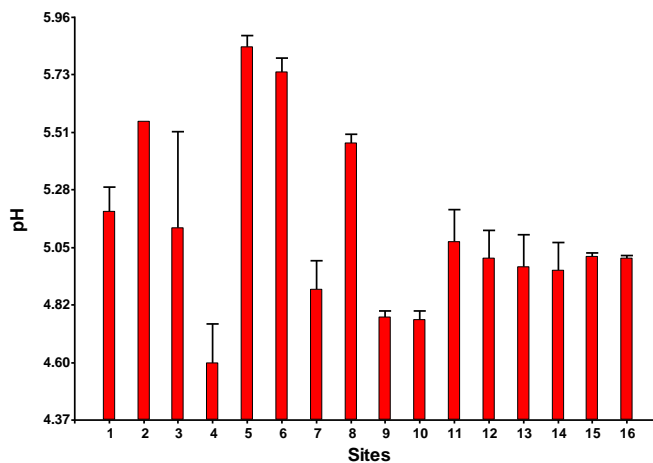


Fig. 3. Average pH indices, Almirante – Bocas del Toro, Republic of Panama

Figure 4 indicates For the average values of organic matter in the soils of the study áreas which were (SOM) (Fig 4), characterized in the areas of the farms with cocoa crops grown organically, it can be observed that they were found to range from 3.08 % to 7.37 % , these values are being acceptable for this crop; since organic amendments help the retention of heavy metals in the soil contributing to decrease in the mobility of metals these by producing an increase in exchange sites, forming stable complexes, mineral precipitation and ion exchange and their absorption by plants [50, 51, 52, 53]. Values greater than or equal to 5 % of SOM in soils that are not of volcanic origin are considered good [54].

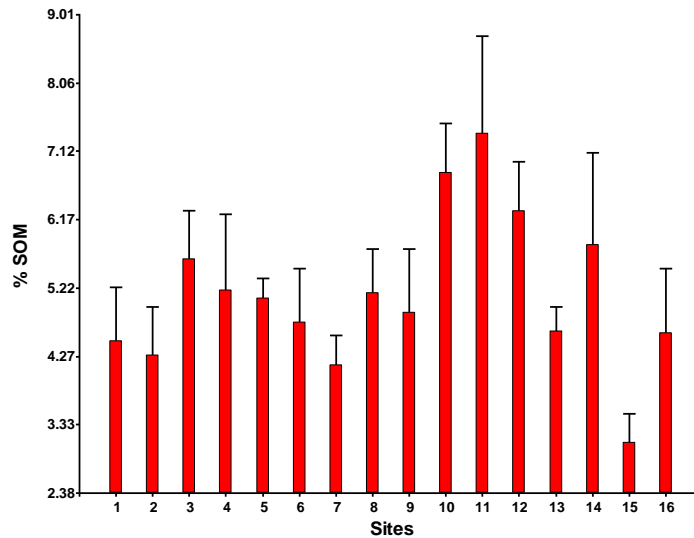
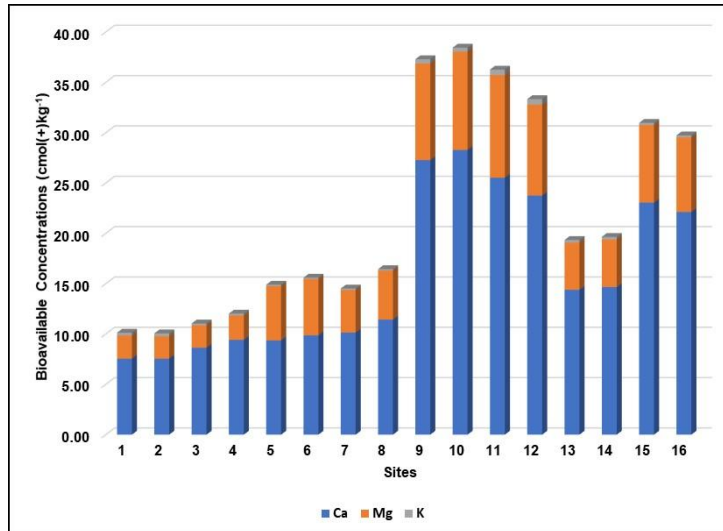


Fig. 4. Average values of SOM, Almirante – Bocas del Toro, Republic of Panama

3.2. Nutrient bioavailability at 16 sites with cocoa crops growing organically, Almirante – Bocas del Toro, Republic of Panama

For the elements of The bioavailable concentrations concentrations of Ca, Mg, and K in the study areas are presented in (Fig. 5); means Ca concentrations were found to ranged from 27.78 $\text{cmol}(+)\text{kg}^{-1}$ to 7.55 $\text{cmol}(+)\text{kg}^{-1}$; mean concentrations of Mg ranged were measured between 9.69 $\text{cmol}(+)\text{kg}^{-1}$ and 1.60 $\text{cmol}(+)\text{kg}^{-1}$, presenting these elements with high levels; while the average concentrations of K, levels ranging from 0.74 $\text{cmol}(+)\text{kg}^{-1}$ to 8.0 $\text{cmol}(+)\text{kg}^{-1}$ were considered as found, presenting levels ranging from medium to high values [55]. These elements influence adsorption, being able to retain Cd in the soil. Studies carried out by other researchers showed that, as the concentrations of Ca and Mg increased with lime applications, they had high correlation and influence on Cd adsorption [52].

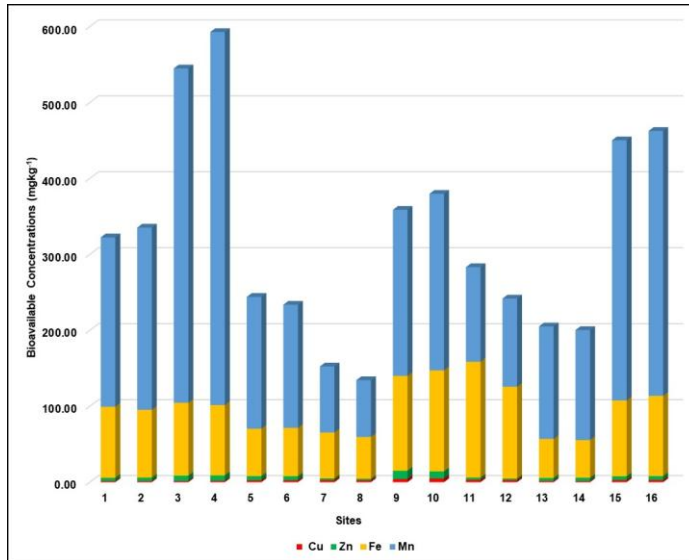


Comment [Ma4]: The Y-axis should read as: 'Bioavailable soil concentrations of Ca, Mg and K (cmol+kg⁻¹)'

Fig. 5. Bioavailable soil concentrations of Ca, Mg and K Average Ca-Mg-K concentrations in soils, Almirante – Bocas del Toro, Republic of Panama.

On the other hand, in soils of the district of Almirante, a nutritional imbalance was found in these elements, due to the high content of Ca and Mg, with respect to K [36]. These values may be due to geological formation, coming from primary minerals of limestone rocks, shales and sandstones [56].

For bioavailable concentrations of Cu, Zn, Fe and Mn (Fig.6) the following results were found: for Cu, the mean values ranged from 0.59 mgkg⁻¹ to 3.71 mgkg⁻¹; for Zn, the mean values were between 2.10 mgkg⁻¹ to 15.65 mgkg⁻¹; while for Fe and Mn, the concentrations presented mean values of 55.9 mgkg⁻¹ to 84.59 mgkg⁻¹ and 127 mgkg⁻¹ to 581 mgkg⁻¹; respectively. These critical levels are those used in the IDIAP Soil Laboratory for the interpretation of soil analyses [57].



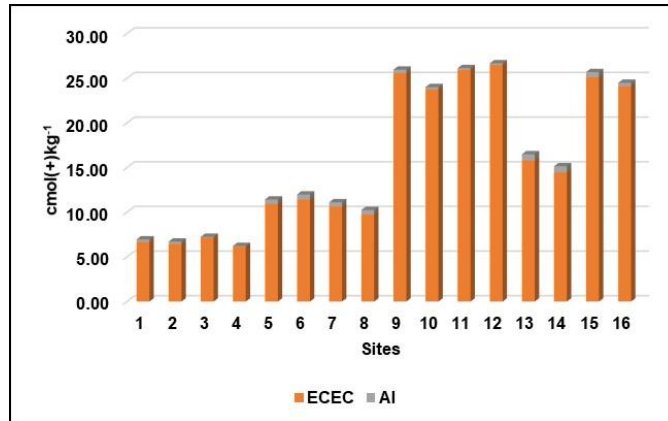
Comment [Ma5]: The Y-axis should read as: 'Bioavailable soil concentrations of Cu, Zn, Fe and Mn (mg kg⁻¹)'

Fig. 6. Bioavailable soil concentrations of Cu, Zn, Fe and Mn~~Average concentrations of Cu-Zn-Fe-Mn in soils, Almirante – Bocas del Toro, Republic of Panama.~~

For the effective cation exchange capacity (ECEC) and Aluminum (Al) variables (Fig. 7), the highest mean concentration was 26.0 cmol(+) kg^{-1} and 2.42 cmol(+) kg^{-1} ; respectively. Soils with high SOM contents or with applications of organic amendments, can help improve the chemical properties of the soil and lower the concentrations of Al thus improving the pH, leading to significantly reduction in lowering the concentrations of Cd concentration in cocoa beans [53].

Only three sites studied recorded present the ECEC with average values and two sites with high values of aluminum concentration, according to the table of critical levels described by Name and Cordero [55].

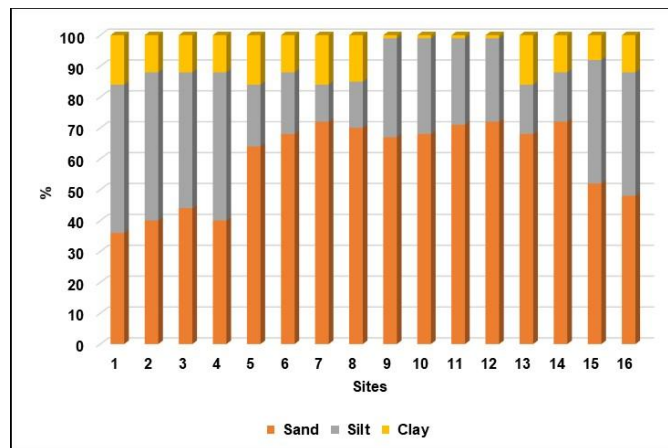
The high bioavailability of Al, can affect the absorption of Cd in the plant, facilitating that it is translocated towards the leaves, fruits and cocoa beans. Toxicity in soils is increased by increasing Cd mobility, when pH decreases [16].



Comment [Ma6]: The Y-axis should read as: 'ECEC and Al levels (cmol⁺kg⁻¹)'

Fig.7. Effective cation exchange capacity (ECEC) and Aluminum (Al) levels in the soil of 16 areas in Almirante- Bocas del Toro, Republic of Panama.

The average content of sand, silt and clay in percentage is shown in Figure 8. All ~~sites~~ ~~these farms~~ are found in the textural classes such as sandy loam, loamy sand, loam and sandy clay loam [58]. Soil organic matter and texture influence the availability of Cd in the soil for plants [59]. Likewise, Cargua [60] found that soils with loam-clay textures contribute to ~~the adsorption of~~ ~~adsorbing~~ heavy metals and in turn decrease accumulation in crops.



Comment [Ma7]: The Y-axis should read as: 'Sand, Silt and Clay (%)'

Fig 8. Percentages of Sand-Silt-Clay in the soil of 16 sites in Almirante-Bocas del Toro, Republic of Panama.

The areas studied in the district of Almirante, province of Bocas de Toro, Republic of Panama, did not ~~observe any~~ ~~present~~ toxicity of ~~for both the heavy metal~~ ~~the total and bioavailability~~ Cd; ~~the total and bioavailability~~, minimum mean critical values were found ~~that oscillated~~ between 0.01 mgkg⁻¹ to 0.24 mgkg⁻¹ and 0.02 mgkg⁻¹ ~~to~~ 0.05 mgkg⁻¹ respectively (Fig. 9); these concentrations are within the critical levels in agricultural soils of 0.43 mgkg⁻¹ [61]. Alloway [9], mentions that soil's concentrations of geogenic origin generally do not exceed 1.0 mgkg⁻¹ of Cd. ~~It could be owed to the concentrations of bases (Ca, Mg and K) in soils belonging to the order Inceptisols [36].~~

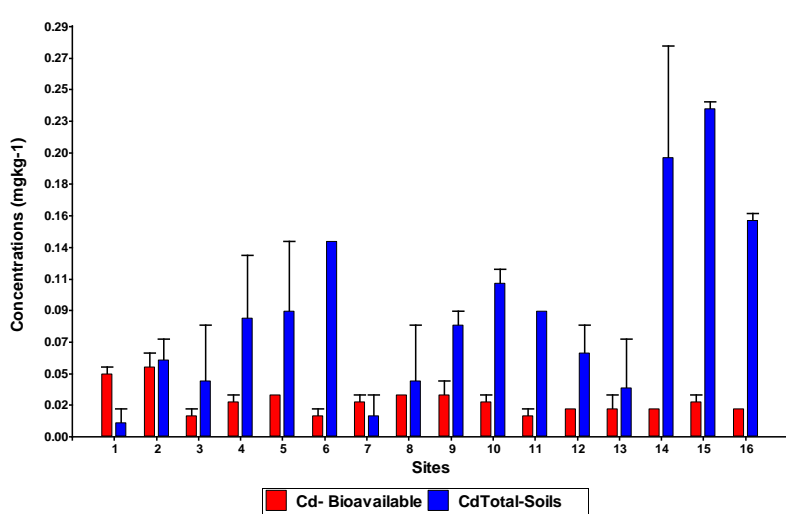


Fig 9. Total and bioavailable Cd concentrations in the soil of 16 farms in Almirante-Bocas del Toro, Republic of Panama.

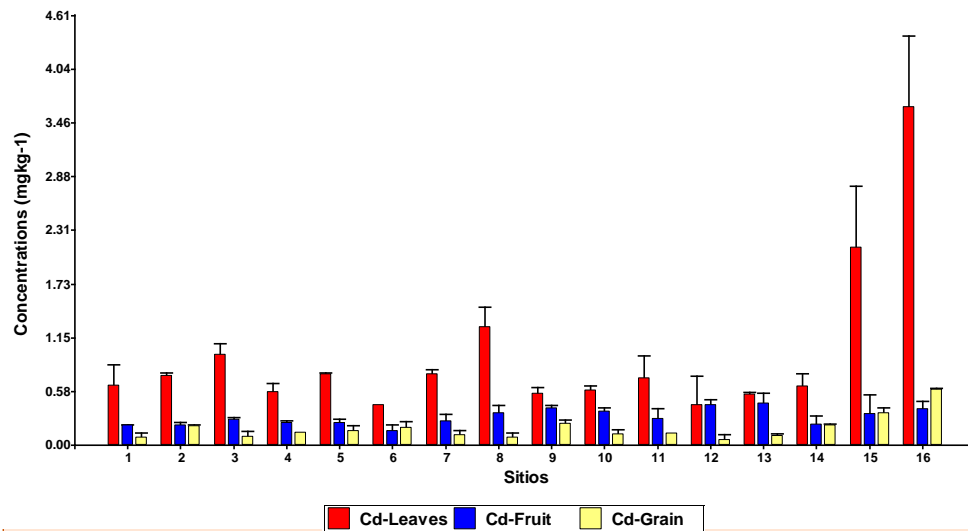
Comment [Ma8]: The Y-axis should read as: 'Total and bioavailable soil concentrations of Cd (mg kg⁻¹)'

The concentrations of Cd in leaves, fruits and cocoa beans are presented in Figure 10. The with mean values in leaves ranged from 0.45 mgkg⁻¹ to 4.41 mgkg⁻¹, and these concentrations found at exceed 0.2 mgkg⁻¹ [62]. Data similar to this study were found by Cardenas [63] and Huamani-Yupanqui [26]. Arevalo [64] reported concentrations in farms with average values less than 3 mgkg⁻¹. On the other hand, Wong [65] reported studies on the CCN-51 clone, finding values ranging from 0.37 to 0.43 mgkg⁻¹ of Cd. Ramtaha et al. [66], they found that one way to diagnose Cd contents in almonds is from the leaves, since cocoa plants are hyper accumulators of this element. Shahid [21], suggest that for some species foliar absorption of cadmium might be important, in cocoa this appears to be insignificant. Barraza et al [67] for the fruits were found fruit levels of Cd to be average concentrations that are between 0.16 mgkg⁻¹ to 0.46 mgkg⁻¹. In the beansgrains, their average concentrations ranged from 0.06 mgkg⁻¹ to 0.61 mgkg⁻¹.

For the concentrations of Cd characterized in the fruits, the mean levels report minimum values of 0.16 mgkg⁻¹ and maximum values of 0.46 mgkg⁻¹ were recorded; on the other hand, I the average concentrations of Cd in the bean grain ranged from 0.06 mgkg⁻¹ to 0.61 mgkg⁻¹. These values do not exceed the maximum permitted levels of 0.80 mgkg⁻¹ of Cd for chocolate whose cocoa dry matter content is more than half its weight, according to European Union in its regulation No 488/2014 [68], this level can also be applicable to cocoa fruit. Cadmium accumulation between different cocoa genotypic materials has been found in several studies [69, 2, 15, 67, 22, and 70].

The accumulation of Cd found in the leaf could have transferred the concentrations to the fruit and therefore to the grain.

Comment [Ma9]: 1. 'Cocoa Beans' not 'Cocoa grains'
2. Are measuring the concentrations in the fruits or in the beans?
Be careful of your analyses to avoid errors!!!



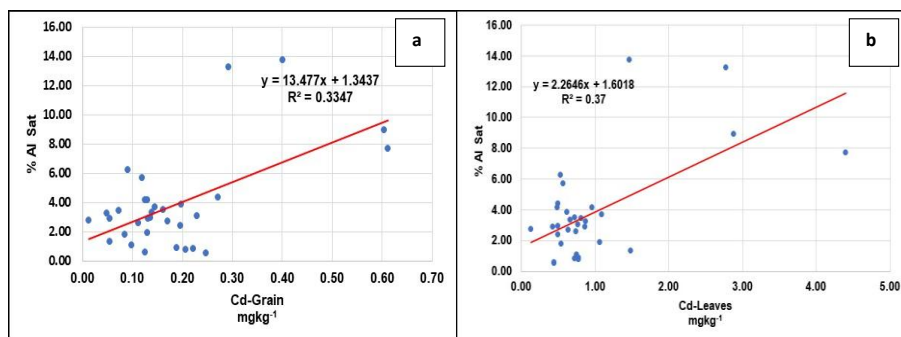
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The Key should be as the followig: Cd-Leaf Cd-Fruit Cd- Bean

Comment [Ma11]: The Y-axis should read as: 'Cd levels in Cocoa leaves, fruits and beans (mg kg⁻¹)'

Fig. 10. Cd levels in Cocoa leaves, fruits and beans~~Levels of average concentrations of Cd found in leaves-fruits and cocoa beans~~ from the 16 farms of Almirantes- Bocas del Toro, Republic of Panama.

It can be said that, the concentrations presented by the beansgrain and leaves (Fig.11a and b), could be due to the fact that the Aluminum saturation content in the soil has a directly proportional influences, since by increasing the concentrations of this toxic element, the Cd in the beansgrain also increases. ~~This also affects W~~when the SOM-MOS content decreases, increasing the concentrations of Cd in the leaves increase(Fig. 11c). Bot ~~and~~& Benites [71] and Alloway [9] ~~they~~ reported that MOSOM has an impact on the bioavailability of some nutrients in the soil, and also on heavy metals such as Cd. ~~On the other hand, the nutritional imbalance presented by the soil (Fig. 11d), it is observed that the relationship in the soil K/Mg, As~~ as magnesium increases, with respect to potassium, ~~increases~~the bioavailability of Cd also increases(Fig. 11d) in these areas. Cations, such as Ca, Mg and K, have an effect on the form of entry of Cd into the system in the plant, since this toxic element (Cd), follows the same absorption channels of the aforementioned cations. In the case of cations, the relationship is explained, because the absorption of Cd in the roots of plants enters or is absorbed in the same way like Ca, Mg and K [11].



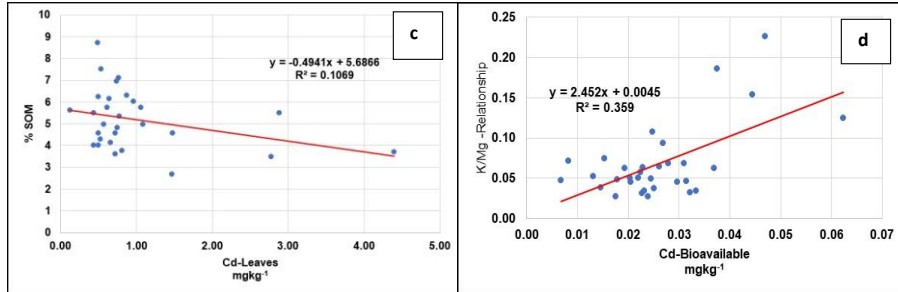


Fig 11. Linear regression curve (a) % of Aluminum Saturation vs Cd in grain; (b) % Aluminum Saturation vs Cd in leaves; (c) % of SOM vs Cd in the leaves; (d) K/Mg ratio vs bioavailable Cd ratio in soil

3.3. Total cadmium and Bioavailable at two depths (0 - 0.15 m and 0.15 - 0.30 m) in Creole cocoa genotype AS-CP 26-61 (mulatto)

At the depth of 0 to 0.15 m, the mean values of total Cd were found in total (Fig.12a), ranging from 0.28 mgkg^{-1} to 16.4 mgkg^{-1} ; and for depths from 0.15 to 0.30 m the values were finding higher (Fig. 12a). concentration of total Cd at greater depth. On the other hand, mean values were obtained (Fig.12b) of The bioavailable Cd concentrations ranging from 0.03 mgkg^{-1} to 6.19 mgkg^{-1} at depth of 0.15 to 0.30 m, while the mean values at 0.15 to 0.30 m found present concentrations ranging from 0.02 mgkg^{-1} to 7.18 mgkg^{-1} (Fig. 12b); The European Union (EU), Regulation (EC) No 488/2014 [68], indicated that the typical values in soils not contaminated with Cd are between 0.0 and 1.0 mgkg^{-1} [49]; finding this study to exceed the values the total Cd applied in T treatments applied above, when increased from 4.0 mgCdkg^{-1} gave results that exceeded the EU standard.

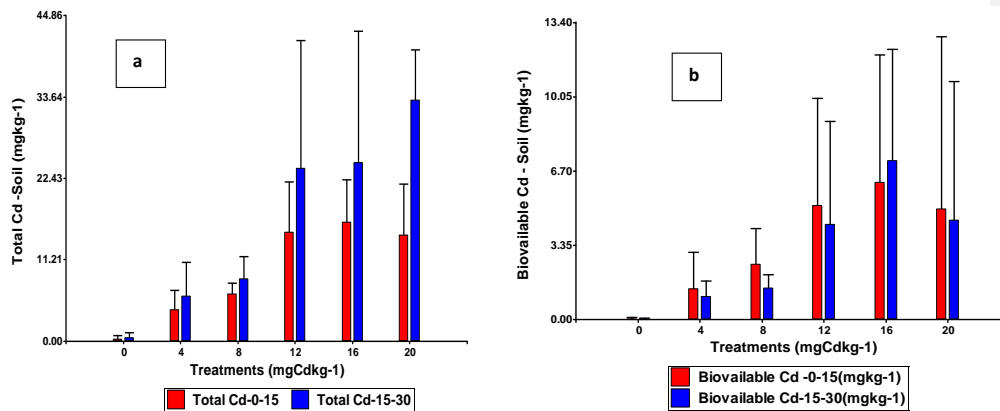


Fig. 12. Dynamics of the total Cd (a) and bioavailable (b) at two depths (0 to 0.15 m) and (0.15 to 0.30 m), for the Creole cocoa genotype AS-CP 26-61 (mulatto)

3.4. Regression curve of Cadmium in the roots, stems and leaves, according to treatments applied in the soil in Creole cocoa genotype AS-CP 26-61 (mulatto)

Cd concentrations apply or exceed Soil treatment of Cd at 12.0 mgkg⁻¹ gave peak concentrations of Cd in the cocoa roots(9.0 mgkg⁻¹), stems(6.0 mgkg⁻¹) and leaves(11.0 mgkg⁻¹) (Fig.13a, 13b and 13c), in the roots, stem and leaf, reaching an inflection in the curve, when absorbed values were higher of 9.0 mgkg⁻¹ for the roots, 6.0 mgkg⁻¹ for the stems and 11.0 mgkg⁻¹ for the leaves. Plants may show symptoms of toxicity when the concentration of total Cd in the soil exceeds 8 mgkg⁻¹ [72]; the concentration of bioavailable cadmium in the soil is > 0.001 mgkg⁻¹ or the concentration of cadmium in the plant tissue reaches 3.0-30.0 mgkg⁻¹ [73, 74]. According to Alloway [9]; Kabata-Pendias and Pendias [75], permissible limit of cadmium in leaf tissue is 0.5 mgkg⁻¹.

In the plant, cadmium preferentially accumulates in the sequestered root in the vacuole of the cells, and only a small part is transported to the aerial part of the plant, concentrating in decreasing order on stems, leaves, fruits and seeds [76].

Cd can be absorbed via foliar by the plant [75], it is, not necessary that all the Cd found within the plant has to originate from the soil.

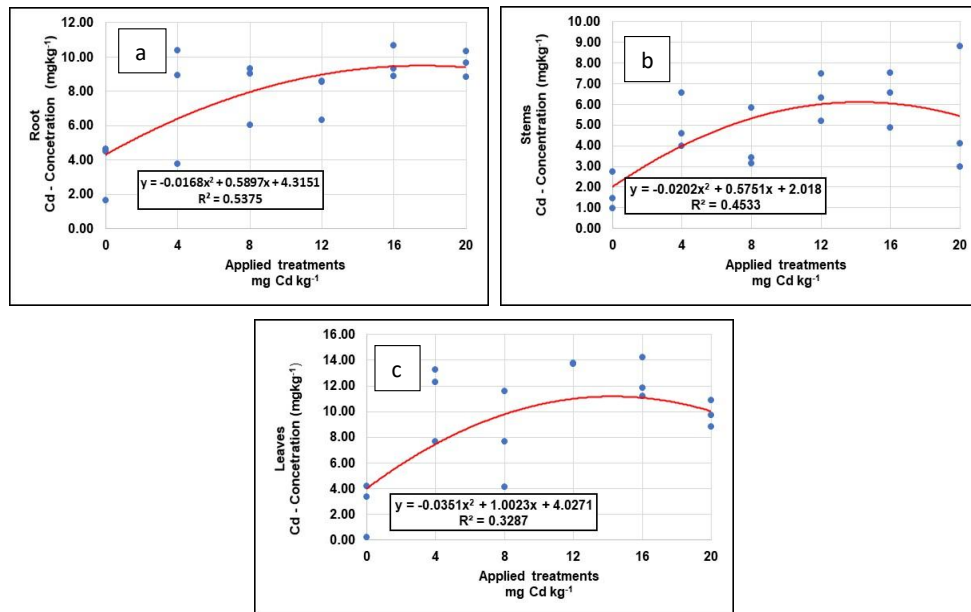


Fig. 13. Behavior of Cd in the roots (a), stems (b) and leaves (c), according to treatments applied in the soil in Creole cocoa genotype AS-CP 26-61 (mulatto).

In order to evaluate the future behavior of Cd and make a forecast using a regression plot between the Cd absorbed in the grain and Cd in leaves (Fig. 14), with the data found in the 16 sites of the areas studied in the district of Almirante and the application of different concentrations of Cd of the treatments with the Creole cocoa genotype AS-CP 26-61 of one year old, it was observed that as

the concentration of Cd in the leaves increased, the absorption of Cd in the cocoa bean increased. This does not lead to the fact that it can be a constant behavior, since the absorption of Cd in the plant has a direct relationship with the age of the tree [77].

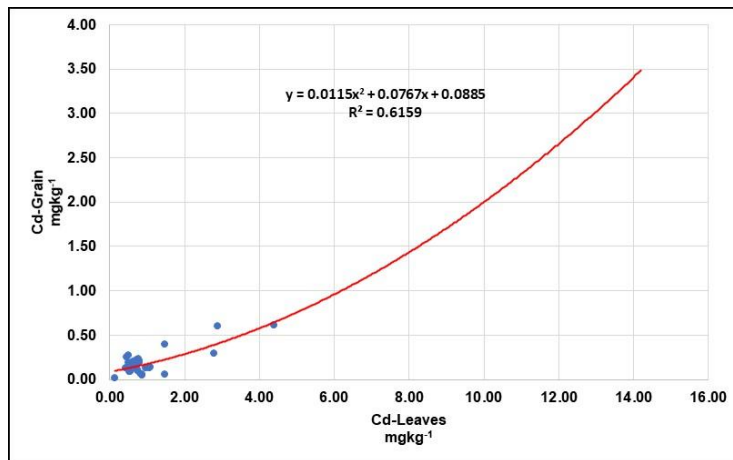


Fig. 14. Forecast of the behavior of the translocation of Cd in leaves and Cd in beansgrains, according to sampling in 16 sites and doses of Cd applied to the soil in Creole cocoa genotype AS-CP 26-61 (mulatto).

4. CONCLUSION

1. In the 16 sites sampled in the district of Almirante, was found pH levels from very acidic to acidic, this being an alert so that the plants can have greater absorption of Cd.
2. Maintaining levels (medium and high) in SOM, has helped block high of Cd by the plant at these 16 sites.
3. The levels of Cd bioavailable and total in the soil do not exceed the maximum levels required for agricultural soils.
4. The concentrations of Cd found in fruits and grains are below the maximum levels allowed by European Union and these may be influenced by the good level of organic matter in the soil.
5. By increasing the organic matter in the soil with the application of organic amendments, this will help the concentration of aluminum in the soils decrease and would not allow the levels of Cd in the leaves to increase and be translocated to the fruit and cocoa bean.
6. With the application of Cd treatments to the soil, the bioavailability of this metal is higher after 15 cm deep, and can affect older plants.
7. For the Creole cocoa genotype AS-CP 26-61, the greatest absorption of Cd occurred in the leaves, followed by the roots and finally the stem.

5. RECOMMENDATIONS

1. Increasing the amount of organic matter in the soil with applications of organic amendments, previously characterized would help increase the pH of the soil, being able to form complexes and decreases the absorption of Cd.

Comment [Ma12]: Summarise – Base the conclusion strictly on the study objectives. Check the grammer – It is not clear!

Comment [Ma13]: Delete this section

2. Renew the planting of these farms with genotypes that absorb less content of this heavy metal.
3. Conduct remediation investigations, to decrease the concentration of Cd.

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Comment [Ma14]: Revise this section based on the in-text corrections.

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