

Article

Bioaccumulation and Cadmium Absorption in Cocoa Genotypes Treated with Different Irrigation Volumes

Fanny Rodriguez-Jarama ^{1,*}, Manuel Carrillo-Zenteno ^{2,3}, Roger Pincay-Ganchozo ^{2,*}, Wuellins Durango-Cabanilla ², Karina Peña-Salazar ², Braulio Lahuathe Mendoza ², Bayron Vecilla-Nicola ², Israel Ampuño-Muirragui ² and Katiusca Bermúdez Sotomayor ²

¹ Agronomía, Facultad de Ciencias Agrarias, Universidad Agraria del Ecuador (UAE), Av. 25 de Julio y Pio Jaramillo, Guayaquil 090101, Guayas, Ecuador

² Instituto Nacional de Investigaciones Agropecuarias (INIAP), Estación Experimental Tropical Pichilingue, Departamento de Manejo de Suelos y Agua, Mocache 120313, Los Ríos, Ecuador; manuel.carrillo@iniap.gob.ec (M.C.-Z.); wuellins.durango@iniap.gob.ec (W.D.-C.); karina.pena@iniap.gob.ec (K.P.-S.); braulio.lahuathe@iniap.gob.ec (B.L.M.); bayron.vecilla@iniap.gob.ec (B.V.-N.); israel.ampuño@iniap.gob.ec (I.A.-M.); katiusca.bermudez@iniap.gob.ec (K.B.S.)

³ Universidad Técnica Estatal de Quevedo, Av. Quito km 1.5 vía a Santo Domingo, Quevedo 120501, Los Ríos, Ecuador

* Correspondence: frodriguez@uagraría.edu.ec (F.R.-J.); roger.pincay@iniap.gob.ec (R.P.-G.)

Abstract

Cadmium (Cd) is absorbed by plants and bioaccumulated in their tissues, and its ingestion may cause adverse effects on human health. However, information on the relationship between soil moisture and Cd bioaccumulation in cocoa-growing soils remains limited. The objective of this study was to evaluate the effect of different irrigation volumes (50, 75, 100, and 125%) on Cd bioaccumulation and biomass production in two cocoa genotypes (EET-103 and CCN-51), as well as on soil pH and electrical conductivity (EC), in two soils from El Oro and Guayas, Ecuador. A split-plot design was used with three factors: A (soils), B (genotypes), and C (irrigation). The variables evaluated included plant height, stem diameter, shoot and root dry biomass, Cd absorption, translocation, and bioaccumulation, as well as soil pH and EC. The highest dry matter production and Cd bioaccumulation were observed in genotype EET-103 grown in El Oro soil and in genotype CCN-51 grown in Guayas soil. A higher irrigation volume (125%) increased the pH of El Oro soil and reduced Cd bioaccumulation by 38.48%, whereas pH decreased in Guayas soil under the same irrigation condition. In El Oro soil, pH and EC showed an inversely proportional relationship. These results suggest that irrigation management and the selection of cocoa genotypes with lower affinity for Cd may be useful strategies to reduce Cd bioaccumulation in cocoa plants.

Keywords: heavy metals; rootstocks; soils; clones; irrigation



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1. Introduction

Cocoa is considered a commodity of high socioeconomic importance throughout the tropics of Latin America, generating income through commercialization, exports, and employment; approximately 300,000 farmers benefit in the region, the vast majority being small- and medium-scale producers [1]. America accounts for 21.2% of global production, with Ecuador being the country with the highest productivity of cocoa beans, at 430,000 t year^{−1} [2], and its exports recording an amount of USD 3351.2 million dollars [3].

The main threats facing cocoa production are drought [4] and high concentrations of Cd [5]; these could restrict its commercialization in major export markets since the European Union established 0.80 mg of Cd per kilogram of chocolate as the maximum allowable concentration [6]. Although the maximum limits are set for cocoa derivatives, in Ecuador, Peru, and Colombia, it has been shown that Cd levels in beans ($>1 \text{ mg kg}^{-1}$) exceed the permissible limits for subproducts [7].

In Ecuador, cocoa cultivation covers approximately 609,750 ha, with 76% of the total area concentrated in the Coastal region. The provinces of Los Ríos, Manabí, Esmeraldas, El Oro, and Guayas represent the main cocoa-producing areas due to their extensive cultivated areas and contribution to the export supply chain [8]. In these provinces, cocoa-producing areas with high Cd concentrations in agricultural soils and cocoa beans have been identified; however, elevated Cd levels have not been reported in the province of Los Ríos [9–11]. Particularly, the highest Cd concentrations in cocoa beans have been reported in El Oro (4.08 mg kg^{-1}) and Guayas (3.56 mg kg^{-1}) provinces [12,13], indicating a high transfer of this metal from the soil–plant system to the commercial bean. Furthermore, the coastal region faces water deficit constraints, especially in Guayas, where low water availability becomes more severe during the dry season [4]. Therefore, the interaction between water availability and Cd presence represents a critical factor for the sustainability of cocoa production systems.

Several studies on Cd mitigation using mineral and organic amendments have been conducted to address this problem [14–16]. In soils from Santa Elena, Manabí, and El Oro, the application of mineral amendments (calcium carbonate, calcium sulfate, magnesium carbonate, and zeolite) reduces Cd concentrations in the soil and cocoa cotyledons by over 50% [14]. Under field conditions, it was observed that after 24 months of applying organic amendments such as filter cake (cachaza), charcoal, and humus, Cd concentrations in cocoa cotyledons were reduced by 55%, 48%, and 27% in soils from Santa Elena, El Oro, and Manabí, respectively [15]. The application of quinoa biochar (2%) in alkaline and acidic soils reduced Cd availability and leaf concentrations by 71% and 48%, respectively [16]. These results demonstrate that amending agricultural soils has yielded positive effects in reducing Cd presence in cocoa beans by restricting the metal's availability in the soil through adsorption and precipitation mechanisms.

However, the effects of soil water availability on Cd absorption and translocation in cocoa plants are not widely documented. Only one investigation stands out regarding Cd bioaccumulation and extraction in cocoa plants due to soil moisture deficits, reporting an increase in Cd translocation from roots to leaves in plants of the IMC67 $\times$ PA121 and IMC 67 progenies when subjected to 27 and 19 days of water stress [17]. Water is a limiting factor in certain cocoa-growing areas [18], and the presence of Cd contamination hotspots is becoming increasingly common [19].

Therefore, our study focused on evaluating the influence of irrigation volumes in different soil types of the Ecuadorian coastal region on the bioaccumulation and absorption of Cd in two cocoa genotypes. We propose the following hypotheses: (I) there is variability in Cd bioaccumulation and absorption in cocoa soils with different physicochemical characteristics; (II) cocoa genetic materials have different metal extraction capacities; and (III) irrigation volume levels (soil moisture) influence the reduction in Cd bioaccumulation and absorption.

2. Materials and Methods

2.1. Study Location

The experimental work was carried out in the greenhouse and laboratory of the Pichilingue Tropical Experimental Station (EETP) of the National Institute of Agricultural

Research (INIAP), located at km 5 on the Quevedo—El Empalme road (1°04′36.5″ S latitude, 79°29′18.1″ W longitude, 75 masl). The location has an average temperature and relative humidity of 26 °C and 80%, respectively, with 2252 mm of annual rainfall and 1213 sunshine hours year^{−1} [20].

2.2. Physical and Chemical Characteristics of the Soils

The soils used were from cocoa farms in the Ecuadorian coast. One originated from the Río Negro parish, El Oro province, belonging to the Entisol order of the alluvial plain, with a predominantly loam to silty loam texture, very sparse structure development, pH ranging from 3.9 to 6.9, and low organic matter and electrical conductivity contents [21,22]. The other soil was from the Cerecita sector, Guayas Province, belonging to the Vertisol order, with >30% clay, a 25 cm-thick vertic horizon, predominantly 2:1 expandable clays, low organic matter content, but high presence of Ca, Mg, and P [23].

Soil samples were collected from these farms at a depth of 0.10 m and transported to the study site, where they were air-dried and sieved through a 2 mm mesh. Subsequently, their physical and chemical properties were determined (Table 1). The extraction of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn was performed using 2.5 g of soil and 25 mL of modified Olsen extractant, while B and S were extracted from a 10 g soil sample using 25 mL of monobasic calcium phosphate solution. The concentrations of N, P, B, and S were quantified by colorimetry using a Cole-Parmer 1100 spectrophotometer, whereas the concentrations of K, Ca, Mg, Cu, Fe, Mn, and Zn were determined using an atomic absorption spectrophotometer AA-6800 GFA-EX7 (Shimadzu Corporation, Kyoto, Japan). Soil pH and electrical conductivity (EC) were measured using a 1:2.5 soil-to-deionized water ratio. pH measurements were performed with a potentiometer HACH SensION™ MM340 (HACH LANGE GmbH, Düsseldorf, Germany), and EC was determined using a conductivity meter HACH SensION™ EC71, (HACH LANGE GmbH Düsseldorf, Germany). Cadmium extraction from soil was performed using aqua regia (semi-total), consisting of HCl and HNO₃ in a 3:1 ratio. To ensure analytical accuracy, certified reference materials (CRM) were included: NIST 2709 (San Joaquin Soil) [24]. The measurements were carried out at $\lambda = 228.8$ nm using an atomic absorption spectrophotometer (Perkin Elmer, model AAnalyst 800, Yokohama, Japan) equipped with a graphite furnace. Soil organic matter was determined using the Walkley–Black method.

Table 1. Physical and chemical properties of the soils under study.

Parameter	Unit	El Oro	Guayas
OM	dag kg ^{−1}	2.5	3.2
Sand	%	30	8
Silt	%	42	58
Clay	%	28	34
Textural class	–	Clay loam	Silty clay loam
NH ₄	mg kg ^{−1}	13.0	10.0
P	mg kg ^{−1}	16.0	39.0
Zn	mg kg ^{−1}	6.4	2.6
Cu	mg kg ^{−1}	13.4	4.0
Fe	mg kg ^{−1}	106.0	35.0
Mn	mg kg ^{−1}	25.8	5.1
Cd	mg kg ^{−1}	1.5	0.5
B	mg kg ^{−1}	0.3	0.3
S	mg kg ^{−1}	17.0	10.0
Ca	meq 100 mL ^{−1}	13.0	14.0

Table 1. Cont.

Parameter	Unit	El Oro	Guayas
K	meq 100 mL ⁻¹	0.3	0.6
Mg	meq 100 mL ⁻¹	3.4	5.3
CO ₃	meq L ⁻¹	nd	nd
HCO ₃	meq L ⁻¹	2.9	2.2
Cl	meq L ⁻¹	0.4	0.3
SO ₄	meq L ⁻¹	6.9	6.0
pH	—	6.4	6.9
EC	dS m ⁻¹	0.7	0.8

Note. nd = not determined by the methodology used.

2.3. Fertilization

Nutritional supplementation was provided using conventional fertilizers—urea (46% N), diammonium phosphate DAP (18% N, 46% P₂O₅), potassium chloride KCl (60% K₂O), ammonium sulfate (24% S, 21% N), magnesium sulfate (27% MgO, 16% S), and calcium nitrate (15% N, 26% CaO)—following the nutritional plan by Reyes-Perez et al. [25]. Total N and S doses were split into three fractions applied at 21, 42, and 63 days after sowing (DAS); K, Mg, and Ca in two fractions (42 and 63 DAS); and P in a single application at sowing. Fertilizers were diluted and applied via drench at 20 mL plant⁻¹, except for DAP, which was applied in granular form at sowing (Table 2).

Table 2. Nutritional plan for cocoa plant development in the greenhouse.

Days After Sowing	N	P ₂ O ₅	K ₂ O	S	MgO	CaO	Urea	(NH ₄) ₂ SO ₄	Ca(NO ₃) ₂	DAP	MgSO ₄	KCl
21	1.02	2.58	—	0.41	—	—	—	1.71	—	5.62	—	—
42	0.68	—	0.84	0.41	0.20	—	—	1.24	1.65	—	0.71	1.40
63	1.38	—	0.84	0.41	0.20	0.42	1.48	1.24	1.65	—	0.71	1.40

Note. Values are expressed as g kg soil⁻¹. DAP = diammonium phosphate; KCl = potassium chloride.

2.4. Experimental Design

Three factors were studied using a split-plot block design. The main plot was the soil (El Oro and Guayas), the subplot was the genotype (CCN-51 and EET-103), and the sub-subplot was the water volume (50, 75, 100, and 125%), with four replicates, totaling 16 treatments and 64 experimental units (EU). The research lasted 120 days under greenhouse conditions.

Genotypes were obtained from the INIAP EETP Germplasm Bank; EETP-103 belongs to the “Nacional” genetic group, and CCN-51 to IMC 67 × ICS 95 and F1 × Oriente [26–29].

2.5. Experimental Management

The soils were disaggregated using a glass roller and sieved through a 2 mm mesh. The substrate was prepared in a 3:2 ratio (soil:rice husk), and bags were filled to a 1 kg capacity; these remained unperforated to prevent water and soil loss through runoff. The plants were obtained from seeds resulting from assisted pollination to ensure the purity of the genetic materials. After six months, ripe pods were harvested, the seeds were extracted, the testa was manually removed, and they were soaked in deionized water for 24 h. One seed was sown per pot, with the wider end pointing downwards.

The water volumes of 50, 75, and 125% were quantified based on the weight of the bags containing soil from El Oro (1285 g) and Guayas (1312 g) at field capacity (100% irrigation). To maintain a constant water volume across each treatment, the weight

of the bags was monitored daily, and water was replenished based on the measured weight loss. In the present study, ultrapure water obtained through a deionization system (Aqua-Max Ultra 370, YL Instruments, Anyang, Republic of Korea) was used. Before application, pH (4.15) and electrical conductivity (EC) (0.01 dS m^{-1}) measurements were performed.

2.6. Variables Evaluated

2.6.1. Growth Variables

At 90 DAS, plant height (cm) was measured from the base of the stem to the apical meristem. Stem diameter (mm) was also measured at the collar region using a digital caliper.

2.6.2. Soil pH and EC

The polyethylene bags containing the substrate were dismantled to collect individual soil samples for each plant. Using an analytical balance (A & D Weighing model HR200, Tokyo, Japan), 20 g of the sample were weighed and placed in a plastic beaker; then, 50 mL of deionized water were added (1:2.5 W:V ratio). The mixture was stirred for 5 min and allowed to stand for 1 h [27]. pH readings were performed using a potentiometer (HACH model SensION™ MM340, HACH LANGE GmbH, Düsseldorf, Germany), and EC was measured with a conductivity meter (HACH model SensION™ EC71, HACH LANGE GmbH, Düsseldorf, Germany).

2.6.3. Shoot and Root Dry Biomass

The plants harvested at 90 DAS were rinsed in tap water and subsequently submerged alternately in a hydrochloric acid solution (3%), distilled water, magnesium sulfate (0.001 mol L^{-1}), and finally deionized water. Each plant was sectioned into shoot (leaves + stems) and root; these parts were placed separately in labeled kraft paper bags according to treatment and replicate, and then dried in a forced-air circulation oven (Gmbh 450, Memmert Co. KG, Schwabach, Germany) at 65°C for 72 h. Subsequently, the samples were weighed on an analytical balance to three decimal places.

2.6.4. Cd Determination in Plant Tissues

Dry plant tissue samples were ground using a “Willey”-type mill (IKA, model A11 basic, Wilmington, NC, USA). To prevent cross-contamination, the equipment was cleaned before each grinding process using absorbent towels, 10% diluted aqua regia, and deionized water. Cadmium extraction was performed through nitric–perchloric mineralization ($8 \text{ mL HNO}_3 + 2 \text{ mL HClO}_4$) according to [28]. To ensure analytical precision, the certified reference material (CRM) NIST 1575a (Pine Needles) was included. Cadmium concentrations were determined using an atomic absorption spectrophotometer (Perkin Elmer, model AAnalyst 800, Yokohama, Japan) equipped with a graphite furnace at a wavelength of $\lambda = 228.8 \text{ nm}$.

2.6.5. Cd Content in Roots and Shoots

$$\text{Cd content } (\mu\text{g}) = \text{Biomass (g)} \times \text{Cd concentration } (\mu\text{g g}^{-1})$$

2.6.6. Cd Uptake Capacity

Following the equations described by Wang et al. [30], the Cd absorption (1) and translocation (2) efficiencies were quantified.

$$\begin{aligned} (1) \text{ Absorption efficiency } (\mu\text{g g}^{-1}) &= \frac{\text{Total plant Cd content}}{\text{Root dry mass}} \\ (2) \text{ Translocation efficiency} &= \frac{\text{Cd content in the shoot (stem and leaves)}}{\text{Cd content in the root}} \end{aligned}$$

2.6.7. Statistical Analysis

The data were subjected to Shapiro–Wilk and Bartlett tests to examine normality and homoscedasticity, respectively. Once these assumptions were met, a Multivariate Analysis of Variance (MANOVA) was performed using a split-split-plot design. For interactions with statistical significance, Tukey’s post hoc test ($p < 0.05$) was applied; additionally, the dependent variables were subjected to Pearson correlation analysis. Statistical analyses were performed using RStudio software version 4.5.1, and the graphs were created with SigmaPlot version 15 (Systat Software, San José, CA, USA).

3. Results

3.1. Growth Indicators

Regarding the growth variables, the interaction (soils $\times$ clones $\times$ irrigation volumes) showed no significant effects (Table 3), except for plant height. In this case, the CCN-51 clone exhibited the highest averages when treated with 75% and 125% irrigation volumes (28.37 ± 1.11 and 28.50 ± 2.86 cm) in El Oro and Guayas soils, respectively.

Table 3. Plant height of cocoa clones grown in tropical soils with different irrigation depths under greenhouse conditions.

Treatment		Plant Height (cm)	
Soil	Genotype		Volume of Water
El Oro	CCN-51	50%	23.63 ± 1.70 abc
		75%	28.37 ± 1.11 a
		100%	23.13 ± 2.53 bc
		125%	25.01 ± 1.69 abc
	EET-103	50%	26.54 ± 1.75 ab
		75%	24.13 ± 0.85 abc
		100%	20.17 ± 1.29 c
		125%	22.68 ± 2.17 bc
Guayas	CCN-51	50%	24.12 ± 1.31 abc
		75%	24.75 ± 3.28 abc
		100%	24.47 ± 0.41 abc
		125%	28.50 ± 2.86 a
	EET-103	50%	21.00 ± 1.87 c
		75%	23.87 ± 2.98 abc
		100%	22.00 ± 4.45 bc
		125%	25.00 ± 1.47 abc
P (Soil × Clone × Volume of water)			0.0129

Different letters above the bars indicate significant differences between treatments according to Tukey’s test ($p < 0.05$), $\pm$ SD ($n = 4$).

In contrast, stem diameter only had significant effects ($p = 0.0170$) in the soils $\times$ clones interaction, evidencing that CCN-51 obtained the greatest stem width in Guayas soil, with 5.97 ± 0.42 mm, while EET-103 showed the best average in El Oro soil, with 5.62 ± 0.42 mm. Likewise, shoot dry biomass showed a highly significant interaction between soils and clones ($p < 0.0001$), observing that CCN-51 obtained the highest average in Guayas soil, with 4.88 ± 0.72 g, while EET-103 recorded its best response in El Oro soil, with 3.36 ± 0.81 g (Table 4).

Table 4. Growth indicators: stem diameter (SD), shoot dry biomass (SDB), and root dry biomass (RDB) of CCN-51 and EET-103 genotypes grown in El Oro and Guayas soils under greenhouse conditions.

Soil	Genotype	SD (mm)	SDB (g)	RDB (g)
El Oro	CCN-51	5.58 ± 0.41 ab	2.95 ± 0.61 c	0.75 ± 0.21
	EET-103	5.62 ± 0.42 ab	3.36 ± 0.81 bc	0.81 ± 0.17
Guayas	CCN-51	5.97 ± 0.42 a	4.88 ± 0.72 a	1.21 ± 0.20
	EET-103	5.40 ± 0.41 b	3.49 ± 0.55 b	1.10 ± 0.20
<i>P</i> (Soils × Clones)		0.0170	<0.0001	0.0585

Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$), $\pm$ SD ($n = 16$).

3.2. Cd Concentration, Absorption, and Content

Regarding the cadmium bioaccumulation and extraction variables, significant effects were found in the soil × genotype interaction (Table 5). In terms of shoot Cd concentrations, it was evidenced that in El Oro soil, the EET-103 genotype ($2.81 \pm 0.39 \text{ mg kg}^{-1}$) had a higher Cd concentration than CCN-51 ($2.58 \pm 0.26 \text{ mg kg}^{-1}$). Conversely, in Guayas soil, the opposite effect occurred; the CCN-51 clone showed the highest Cd concentration ($2.48 \pm 0.23 \text{ mg kg}^{-1}$).

Table 5. Cd concentration in shoot (Cd CS), in root (Cd CR), Cd content in shoot (Cd CtS), in root (Cd CtR), and Cd absorption efficiency (Cd AE) in cocoa genotypes grown in El Oro and Guayas soils under greenhouse conditions.

Soil	Genotype	Cd CS	Cd CR	Cd CtS	Cd CtR	Cd AE
		mg kg^{-1}	mg kg^{-1}	$\mu\text{g Shoot}^{-1}$	$\mu\text{g Root}^{-1}$	$\mu\text{g g}^{-1}$
El Oro	CCN-51	2.58 ± 0.26 b	1.55 ± 0.26 b	7.63 ± 1.86 c	1.14 ± 0.38 c	12.92 ± 5.48 a
	EET-103	2.81 ± 0.39 a	1.75 ± 0.39 a	9.58 ± 2.05 b	1.41 ± 0.31 ab	14.24 ± 5.31 a
Guayas	CCN-51	2.48 ± 0.23 b	1.31 ± 0.14 c	11.95 ± 1.14 a	1.56 ± 0.15 a	11.42 ± 1.74 ab
	EET-103	2.34 ± 0.17 c	1.10 ± 0.17 d	8.10 ± 1.11 bc	1.21 ± 0.23 bc	8.63 ± 1.50 b
<i>P</i> (Soil × Clone)		<0.0001	<0.0001	<0.0001	<0.0001	0.0423

Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$), $\pm$ SD ($n = 16$).

In the root Cd concentration variable, the EET-103 clone in El Oro soil had the highest average ($1.75 \pm 0.39 \text{ mg kg}^{-1}$). In contrast, CCN-51 presented the highest concentration values ($1.31 \pm 0.14 \text{ mg kg}^{-1}$) in Guayas soil (Table 4).

Regarding shoot content in cocoa plants (Table 4), it was observed that in El Oro soil, EET-103 had the highest shoot Cd content ($9.58 \pm 2.05 \mu\text{g shoot}^{-1}$), while the opposite effect was found in Guayas soil, where CCN-51 obtained the highest average content ($11.95 \pm 1.14 \mu\text{g shoot}^{-1}$) (Table 4).

Furthermore, a significant effect was found in the interaction (soils × water volumes); as each irrigation volume increased in El Oro soil, shoot Cd contents were reduced, reaching up to 38.48% less Cd with the 125% water volume (Figure 1). In Guayas soil, increasing the irrigation volume to 75% raised the Cd contents (10.09%), but they remained constant up to 125% water.

In root Cd content, statistical differences were presented among the soil × clone interactions. The EET-103 clone had the highest Cd contents ($1.41 \pm 0.31 \mu\text{g root}^{-1}$) in El Oro soil. Meanwhile, the CCN-51 clone presented the highest averages ($1.56 \pm 0.15 \mu\text{g root}^{-1}$) in Guayas soil.

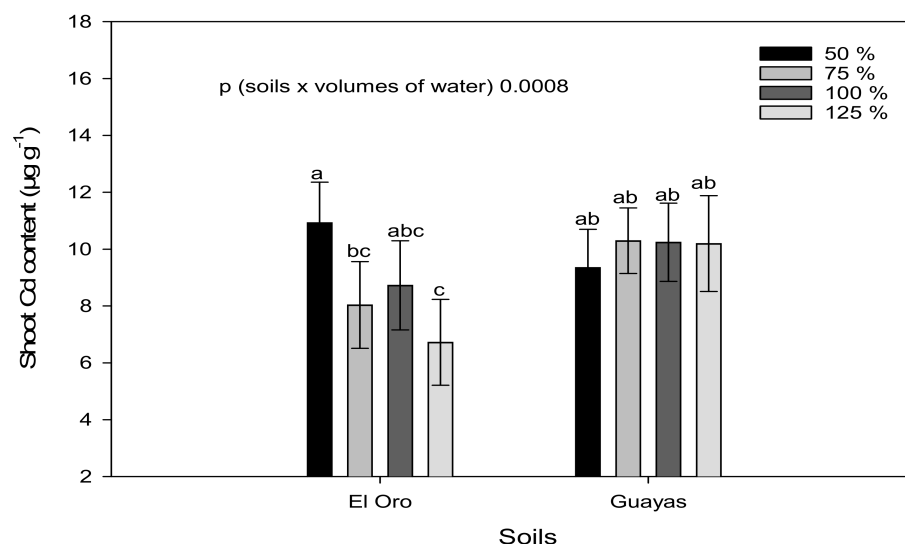


Figure 1. Cd content in shoots from El Oro and Guayas soils treated with 50, 75, 100, and 125% irrigation volumes under greenhouse conditions. Vertical bars represent the mean $\pm$ SD ($n = 8$). Different letters above the bars indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

Cd translocation efficiency showed no significant effects in the interactions of the factors under study. However, in Cd absorption efficiency, significant effects were found in the soil $\times$ clone interaction, observing that between the two, EET-103 had 10.22% more Cd absorption in El Oro soil. In contrast, in Guayas soil, the CCN-51 genotype had 33.32% more Cd extraction than the EET-103 clone.

3.3. Soil pH and Electrical Conductivity

The soil pH variable showed significant effects in the interaction (soil $\times$ irrigation water volume). It was observed that in El Oro soil, increasing the irrigation volume raised the pH from 5.70 (50% irrigation) to 6.10 (125% irrigation). Conversely, in Guayas soil, increasing the irrigation percentage reduced the pH, descending from 6.19 at 50% irrigation to 5.44 at 125% irrigation (Figure 2).

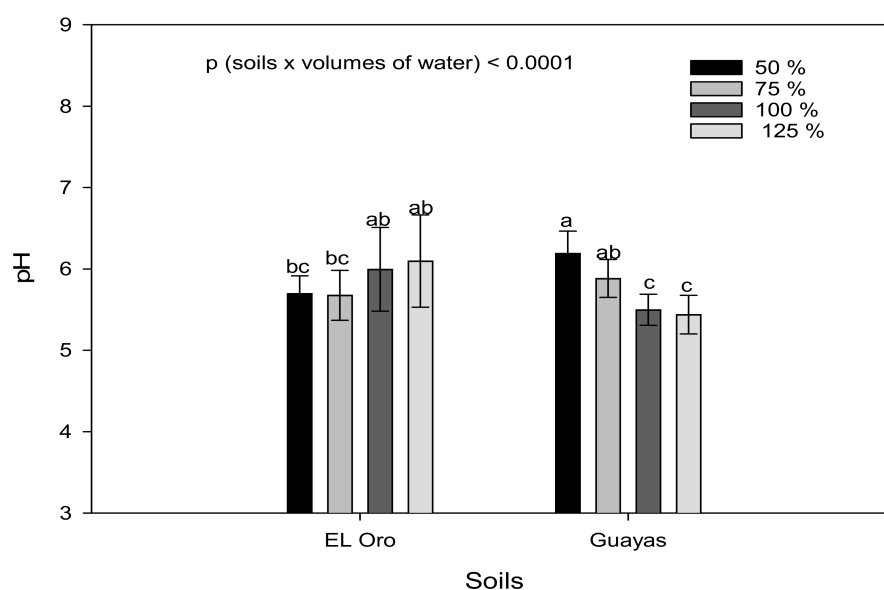


Figure 2. Soil pH from El Oro and Guayas soils with 50, 75, 100, and 125% irrigation under greenhouse conditions. Vertical bars represent the mean $\pm$ SD ($n = 8$). Different letters above the bars indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

Meanwhile, EC only showed statistical differences between soils, being higher in Guayas soil ($3.07 \pm 0.29 \text{ dS m}^{-1}$) compared to El Oro province soil, which recorded $2.48 \pm 0.48 \text{ dS m}^{-1}$.

3.4. Pearson Correlation

Shoot Cd concentration showed positive associations with root Cd concentration, shoot Cd content, and Cd absorption efficiency. Likewise, root Cd concentration had positive associations with shoot Cd content, root Cd content, and Cd absorption efficiency (Table 5 A). Directly proportional relationships were also found between shoot Cd content and shoot dry weight, as well as Cd absorption and translocation efficiencies. Root Cd content showed an inversely proportional relationship with Cd translocation efficiency and was positively associated with root dry weight. It was found that Cd absorption and translocation are strongly and positively associated. However, inversely proportional relationships were observed between soil pH and EC (Figure 3).

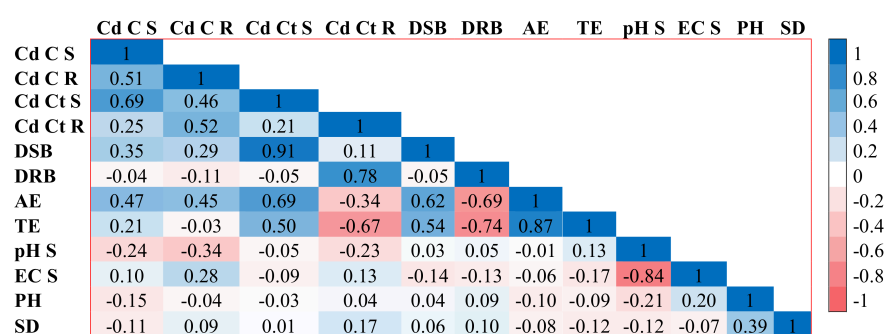


Figure 3. Pearson correlation matrix between variables analyzed in El Oro soil ($n = 32$). C: concentration, S: shoot, Ct: content, AE: absorption efficiency, TE: translocation efficiency, DSB: dry shoot biomass, DRB: dry root biomass, pH: hydrogen potential, pH S: soil pH, EC: electrical conductivity, PH: plant height, SD: stem diameter, Cd: cadmium.

In Guayas soil, shoot Cd concentration was found to have directly proportional relationships with soil pH, Cd absorption efficiency, and root Cd concentration (Figure 4). Likewise, shoot and root Cd contents, as well as Cd absorption efficiency, showed positive associations with root Cd concentration. Shoot Cd content had an inversely proportional relationship with soil pH, but also showed positive associations with root Cd content, shoot dry weight, stem diameter, plant height, and both Cd absorption and translocation efficiencies. Meanwhile, root Cd content presented significant positive associations with shoot and root dry biomass.

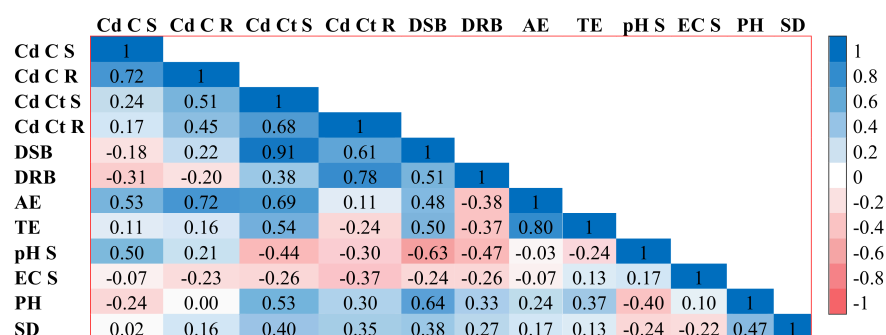


Figure 4. Pearson correlation matrix between variables evaluated in Guayas soil ($n = 32$). C: concentration, S: shoot, Ct: content, AE: absorption efficiency, TE: translocation efficiency, DSB: dry shoot biomass, DRB: dry root biomass, pH: hydrogen potential, pH S: soil pH, EC: electrical conductivity, PH: plant height, SD: stem diameter, Cd: cadmium.

4. Discussion

The results obtained in this study showed different Cd contents in the vegetative parts of cocoa plants in El Oro and Guayas soils, supporting hypothesis (I). The CCN-51 and EET-103 cocoa genetic materials had different cadmium extraction capacities; the highest Cd bioaccumulation was obtained by the EET-103 genotype in El Oro soil and CCN-51 in Guayas soil, meaning that a genetic alternative may exist to reduce Cd bioaccumulation in cocoa, thus accepting hypothesis (II). A higher irrigation volume (125% irrigation) raised the soil pH in El Oro and reduced Cd bioaccumulation by 38.48%, supporting hypothesis (III).

The CCN-51 genotype had the best growth with 75 and 125% of the applied water volume in El Oro and Guayas soils, respectively. These are soils with different textures and OM concentrations, physicochemical properties that influence water infiltration and retention [31,32], as well as turgor and cellular expansion of plant tissues [31,33], which is directly related to water availability in the soil.

In Guayas soil, it was evidenced that the CCN-51 clone had the highest dry matter production, and in El Oro, EET-103 presented the highest production of shoot and root dry biomass. This was because the fertility of each soil affected each cocoa genotype differently; in Guayas soil, the concentrations of P, K, Ca, Mg, and OM are higher, and in El Oro, sulfur was higher. Authors such as Araújo et al. [34] and Tamayo-Ramírez et al. [35] maintain that increases in dry mass production are influenced by soil fertility, and it has been found that CCN-51, unlike other clones, is more demanding and has a greater capacity to extract N and P [36,37]. However, in El Oro soil, contrasting results were obtained to those described by Reyes-Pérez et al. [25], who found effects among cocoa genetic materials, evidencing that CCN-51 had a higher N concentration in shoots and produced more biomass than EET-103 in El Oro soil.

In cocoa, there is no information describing the metabolic or molecular mechanism underlying the difference in metal absorption and translocation between genetic materials [7]. However, in the present work, it was identified in El Oro soil that EET-103 bioaccumulates more Cd in the shoot, while the opposite was observed in Guayas soil, where CCN-51 stood out. These results differ from those found by Reyes-Perez et al. [25], who reported that in El Oro soil, EET-103 bioaccumulates less Cd than the CCN-51 clone; however, these authors also indicated that the less dry matter a clone produces, the less Cd it accumulates, which was also detected in the present work. Likewise, Ortiz-Álvarez et al. [17] observed that Cd accumulation is influenced by biomass reduction, finding in the IMC67 X PA121 progeny lower dry biomass and Cd accumulation when subjected to water stress for 19 and 27 days. Nevertheless, in a soil contaminated with different Cd concentrations (0, 50, 100, and 150 mg kg⁻¹), Borjas et al. [38] detected that POUND-7 produces more dry biomass and contains lower Cd concentrations in leaves compared to the ICS-60, ICS-95, and VRAE-99 genotypes.

The soil pH in El Oro increased and that in Guayas decreased with an increase in irrigation volume. A similar phenomenon was reported by Ding et al. [39], who evidenced that flooded soils with an initial pH < 6.5 increase their pH, while those with a pH > 6.5 decrease their pH. A high moisture regime can produce a reduced environment in soils due to the absence of O₂ [40]; this influences the increase in pH [41] because OH is dissociated and released from Fe(OH)₃ [42]. El Oro soil had high Fe concentrations and an initial pH of 6.4; however, it has also been described that increased irrigation or precipitation produces leaching of bases and generates a reduction in pH [43,44]. According to the initial analysis of Guayas soil, it had a pH of 6.9 and high amounts of K, Mg, and Ca. These results confirm what was reported by Mohanty et al. [45], who described that redox is different in

each soil despite being under the same moisture level, due to the high physicochemical heterogeneity of soils.

The reduction in soil pH in Guayas increased Cd bioaccumulation by up to 10.09%. These findings are similar to Cd mitigation studies using soil amendments that modify (raise) pH and reduce Cd accumulation, such as the study by López et al. [16], who used quinoa biochar in alkaline and acid soils and reduced Cd bioaccumulation in CCN-51 clone leaves by 48%. Meanwhile, Ramtahal et al. [46] found that hydrated lime and biochar decreased cadmium in the leaves of clone TSH 1348 by 37% and 28%, respectively. It has been described that the reduction of cadmium in plant tissues due to soil amendment applications occurs because they immobilize Cd in the soil by forming molecules or precipitates; they also raise soil pH, which allows for greater Cd adsorption in the solid fraction of the soil and decreases its availability [47].

Only in El Oro soil was it observed that pH and EC had an inversely proportional relationship ($r = -0.84$). Likewise, Reyes-Pérez et al. [25] found a similar behavior between these variables (pH/EC) in the same soil ($r = -0.83$). In both studies, ammonium sulfate and magnesium sulfate were applied via drenching to meet the plants' nutritional needs. Authors such as Vargas & Bryla [48] detected that ammonium sulfate applied in granular form and through fertigation increases the EC of the soil solution. Furthermore, Martikainen [49] evidenced that the application of ammonium sulfate and potassium sulfate fertilizers reduces soil pH and increases EC; similarly, Hashemimajd et al. [50] found that elemental sulfur, alone or combined with organic matter, reduces pH and increases soil EC. Likewise, Pourbabaee et al. [51], when applying elemental sulfur at 50%, 75%, and 100% soil moisture, detected that sulfur oxidation produces sulfuric acid, leading to a decrease in pH and an increase in the EC of the soil solution.

In both soils, it was evidenced that as dry biomass increased, Cd bioaccumulation in cocoa plants also increased. When Ortiz-Álvarez et al. [17] examined the associations between variables independently in the cocoa progenies IMC-67 X PA-121, SCA-6 X PA-121, and IMC-67, and also observed directly proportional relationships between dry biomass and Cd bioaccumulation in stems, leaves, and roots. In *Parthenium hysterophorus* plants, Ali & Hadi [52] found no association between cadmium accumulation and dry biomass, but they did obtain negative associations between dry biomass and Cd concentration in stems, leaves, and roots—a finding also detected in this work and that of Khan et al. [53] in *Triticum aestivum* plants.

In Guayas soil with a pH of 6.9, it was observed that when the pH was raised, dry matter production was reduced and Cd concentration increased. Ortiz-Álvarez et al. [17] also found an increase in Cd concentrations in the stems of the SCA6 × PA121 progeny when leaf and root dry mass was reduced. In tobacco cultivars, Vasiliadou & Dordas [54] likewise observed this type of correlation, where Cd concentration decreases as dry matter increases. Contrary to this, López et al. [16], in greenhouse-grown cocoa plants, found in the Pearson correlation analysis that Cd concentrations in leaves increased when soil pH was reduced.

5. Conclusions

The CCN-51 genotype exhibited the highest growth with 75% and 125% irrigation in El Oro and Guayas soils, respectively. Different genetic effects were observed in each soil: the EET-103 genotype had the highest dry matter production and Cd accumulation in El Oro soil, while the CCN-51 genotype showed the highest dry biomass bioaccumulation and Cd content in Guayas soil. Soil moisture levels influence Cd bioaccumulation by inducing modifications in soil pH; specifically, an irrigation volume of 125% in El Oro soil reduces Cd bioaccumulation in cocoa shoots by up to 38.48%. However, soil pH in Guayas decreased

due to irrigation, leading to a 10.09% increase in shoot Cd bioaccumulation. These results demonstrated that water management can differentially modify Cd accumulation in cacao depending on the specific soil conditions. Therefore, Cd mitigation in cacao should be addressed through an integrated management approach that combines the use of low Cd-accumulating genotypes, irrigation strategies adjusted to soil properties, and continuous monitoring of edaphic parameters such as pH, in order to optimize metal availability and reduce its transfer to the plant.

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Abbreviations

The following abbreviations are used in this manuscript:

AE	Absorption efficiency
CaO	Calcium oxide
CCN-51	Coleccion Castro Naranjal 51
Cd	Cadmium
Cd AE	Cadmium absorption efficiency
DAS	Days after sowing
DAP	Diammonium phosphate
EC	Electrical conductivity
EETP	Pichilingue Tropical Experimental Station
HClO ₄	Perchloric acid
HNO ₃	Nitric acid
INIAP	National Institute of Agricultural Research
KCl	Potassium chloride
K ₂ O	Potassium oxide
MgO	Magnesium oxide
MgSO ₄	Magnesium sulfate
N	Nitrogen
NH ₄	Ammonium
OM	Organic matter
P	Phosphorus
P ₂ O ₅	Phosphorus pentoxide
PH	Plant height
RC Cd	Root Cd concentration
RCt Cd	Root Cd content
RDB	Root dry biomass
S	Sulfur

SC Cd	Shoot Cd concentration
SCt Cd	Shoot Cd content
SD	Stem diameter
SDB	Shoot dry biomass
TE	Translocation efficiency

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