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A Sunflower and Dolomitic Lime Experiment to Modify Cadmium Concentration in Cacao*

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Abstract: Cadmium (Cd) is a toxic heavy metal naturally present in soils and is readily absorbed by edible plants, including cacao, the primary ingredient in chocolate products. Elevated Cd levels in cacao beans pose a public health risk, leading to strict regulations—particularly in the European Union—that negatively impact small-scale cacao farmers. This study evaluated the effects of sunflower (Helianthus annuus) and dolomitic lime on Cd levels in one-month-old cacao plants (Theobroma cacao) and the soils in which they were grown. A greenhouse experiment was conducted in which sunflower and cacao were cultivated together under two levels of soil Cd exposure, with dolomitic lime applied to some treatments. The results showed that dolomitic lime significantly reduced ($P < 0.05$) Cd concentrations in cacao. In contrast, the presence of sunflower increased Cd accumulation in cacao, possibly due to the acidic conditions created by sunflower roots. A negative correlation was also observed between soil pH and Cd concentrations in the soil. These findings suggest that dolomitic lime may be an effective strategy to mitigate Cd uptake in cacao under conditions of low to moderate soil Cd contamination.

Keywords: Cadmium; Dolomitic Lime; Phytoextraction; Sunflower

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Experimento con girasol y cal dolomítica para modificar la concentración de cadmio en el cacao

Resumen: El cadmio (Cd) es un metal pesado tóxico presente de manera natural en los suelos y es fácilmente absorbido por las plantas comestibles, incluyendo el cacao, el principal ingrediente de los alimentos a base de chocolate. Los altos niveles de Cd en los granos de cacao representan un riesgo para la salud pública, lo que ha llevado a la implementación de estrictas regulaciones, especialmente en la Unión Europea, que afectan negativamente a los pequeños agricultores de cacao. Este estudio tuvo como objetivo evaluar los efectos del girasol (Helianthus annuus) y la cal dolomítica sobre los niveles de Cd en plantas de cacao de un mes (Theobroma cacao) y en los suelos donde crecieron. Se realizó un experimento en invernadero, donde se cultivaron girasol y cacao juntos bajo dos niveles de exposición al Cd en el suelo, con la adición de cal dolomítica en algunos tratamientos. Los resultados indicaron que la cal dolomítica redujo significativamente ($P < 0,05$) las concentraciones de Cd en el cacao, mientras que la presencia de girasol aumentó la acumulación de Cd en el cacao, posiblemente debido a las condiciones ácidas creadas por las raíces del girasol. Se observó una correlación negativa entre el pH del suelo y los niveles de Cd en el suelo. Estos hallazgos sugieren que la cal dolomítica puede ser una estrategia viable para mitigar la absorción de Cd en el cacao en suelos con niveles bajos a moderados de contaminación por Cd.

Palabras clave: Cadmio; Cal Dolomítica; Fitoextracción; Girasol

Experimento com girassol e cal dolomítica para modificar a concentração de cádmio no cacau

Resumo: O cádmio (Cd) é um metal pesado tóxico presente naturalmente nos solos e facilmente absorvido pelas plantas comestíveis, incluindo o cacau — principal ingrediente dos alimentos à base de chocolate. Os altos níveis de Cd nos grãos de cacau representam um risco à saúde pública, o que levou à implementação de regulamentos rigorosos, especialmente na União Europeia, afetando negativamente os pequenos produtores de cacau. Este estudo teve como objetivo avaliar os efeitos do girassol (*Helianthus annuus*) e da cal dolomítica sobre os níveis de Cd em plantas jovens de cacau de um mês (*Theobroma cacao*) e nos solos em que cresceram. Foi realizado um experimento em estufa, no qual o girassol e o cacau foram cultivados juntos sob dois níveis de exposição ao Cd no solo, com a adição de cal dolomítica em alguns tratamentos. Os resultados indicaram que a cal dolomítica reduziu significativamente ($P < 0,05$) as concentrações de Cd no cacau, enquanto a presença do girassol aumentou a acumulação de Cd no cacau, possivelmente devido às condições ácidas criadas pelas raízes do girassol. Observou-se uma correlação negativa entre o pH do solo e os níveis de Cd no solo. Esses achados sugerem que a cal dolomítica pode ser uma estratégia viável para mitigar a absorção de Cd pelo cacau em solos com níveis baixos a moderados de contaminação por cádmio.

Palavras-chave: cádmio; cal dolomítica; fitoextração; girassol

Introduction

Cadmium (Cd) is a non-essential metal for plants, with no biological function, and is primarily found in soils as a divalent cation complexed with other anions [1], [2]. Its high mobility within the soil–plant system facilitates its dissemination throughout the food chain, posing significant risks to ecosystems and human health [3–5]. Although Cd is not required for plant growth, many species can inadvertently absorb it [6]. In response to these concerns, the European Union implemented a regulation on January 1, 2019, limiting Cd levels in cocoa powder to 0.6 mg Cd kg^{-1}, creating challenges for cacao producers [7]. Exporting countries—particularly those in Central and Latin America, where high Cd concentrations have been reported in soils and cacao beans—are especially affected by these restrictions [8–16]. This situation is particularly concerning, as restricting the commercialization of cacao-based foods is likely to harm hundreds of thousands of farmers who depend on cacao production [17].

Cd accumulates in various parts of the cacao plant, including leaves, pod husks, and beans [18], [19]. Therefore, mitigating Cd uptake by cacao and its translocation to edible parts is a priority for managing soils moderately contaminated with this metal. Traditional remediation methods, such as vitrification, soil incineration, excavation, and soil flushing [20], are not only labor-intensive and costly but can also irreversibly alter soil biological and physicochemical properties [21], [22]. Consequently, alternative, more sustainable strategies for managing Cd in soils are needed.

Phytoremediation is widely recognized as an environmentally sustainable approach to remediating soils contaminated with toxic metals. Although international studies highlight its cost-effectiveness, its economic feasibility and contextual applicability remain insufficiently examined in Colombia [23–27]. Phytoremediation encompasses several strategies for managing soil contamination, including phytoextraction (plant uptake and removal of contaminants), Phyto stabilization (immobilization of pro contaminants in the rhizosphere), and phytovolatilization [28].

Phytoextraction relies on plants that transport and accumulate Cd in their roots, shoots, leaves, and vacuoles [29–31]. While some species can concentrate Cd to levels exceeding 100 μg g^{-1} [32], true Cd hyperaccumulators must also tolerate high levels of zinc (Zn) [33], [34]. In areas such as Zn mines or waste deposits, Zn concentrations are typically 100 times higher than Cd levels [35]. To reach 100 mg Cd kg^{-1} dry weight in shoots, plants must withstand around 10,000 mg Zn kg^{-1} in dry tissues. Consequently, many so-called "Cd hyperaccumulators" are not true hyperaccumulators, which is why this study adopts the term "Cd relative accumulator" for such plants [35], [36].

Advances in molecular biology and genetic engineering have enabled the manipulation of metal transporter genes and antioxidant defense pathways to enhance Cd tolerance and accumulation in hyperaccumulator species [24]. Despite these advances, phytoextraction is often slow, and achieving meaningful reductions in Cd requires plants that combine high biomass production with the capacity to tolerate and accumulate Cd over successive cropping cycles. Thus, the efficacy of phytoextraction depends on factors such as metal bioavailability, harvestable biomass, and plant growth rate [37].

Selecting an appropriate plant—or combination of species—to extract specific contaminants is challenging given the diversity of phytoextractors and their varying absorption mechanisms. Plant responses depend on factors such as soil type, climate, and the concentrations and interactions of bioavailable heavy metals. In most cases, soils are contaminated with multiple pollutants rather than a single one [38–41]. The bioconcentration factor (BCF), defined as the ratio of metal concentration in plant aerial tissue (PAT) to the total metal concentration in soil, is commonly used to evaluate metal transfer efficiency. A BCF greater than 1 indicates significant metal accumulation in plant shoots [42], [43].

Sunflower (Helianthus annuus L.) has been identified as a promising Cd relative phytoextractor [44–46]. A recent study conducted at the Slovak University of Agriculture in Nitra evaluated the Cd accumulation potential and physiological tolerance of four sunflower hybrids—NK Brio, NK Kondi, PR64A89, and LG 54.85—under controlled

Cd exposure [47]. The experiment employed artificially Cd-spiked soil with two treatments: a control (0 mg·kg^{-1}) and a Cd-contaminated treatment (6.5 mg·kg^{-1} Cd, applied as $CdCl_2 \cdot 2.5H_2O$). Plants were cultivated under greenhouse conditions in pots for 42 days. Results revealed significant genotypic variation in Cd uptake and translocation efficiency. The NK Kondi hybrid exhibited the highest BCF (0.67), indicating efficient Cd transfer from soil to shoots. It also showed the maximum total Cd accumulation per plant (71.4 ± 4.2 µg Cd·plant^{-1}), followed closely by NK Brio (69.1 ± 3.8 µg Cd·plant^{-1}) [47]. These findings suggest that sunflower hybrids possess enhanced Cd phytoextraction capabilities compared with the other tested varieties.

Phytoremediation can be further enhanced by combining it with in situ immobilization technologies, which reduce Cd levels in the plant–root zone [48–50]. This approach modifies soil characteristics with chemical amendments to immobilize contaminants [51]. Numerous studies identify soil pH as the primary factor influencing Cd bioavailability [52], [53], with an inverse relationship between soil pH and Cd mobility [15], [16], [54]. Among remediation techniques, liming is the most widely used method to reduce heavy metal bioavailability [11], [19], [37], [55]. By raising soil pH, lime enhances Cd binding to soil particles, as hydrogen ions primarily compete with Cd for sorption sites [56].

In this study, we combined sunflower (Sf) with one-month-old cacao plants in soils artificially contaminated with Cd, together with dolomitic lime ($CaMg(CO_3)_2$) (D), under greenhouse conditions. We evaluated soil pH, total Cd levels in soil and PAT, and the BCF. The primary objective was to assess the effects of Sf (as a Cd relative accumulator species) and D on Cd concentrations in the PAT of one-month-old cacao plants, as well as in the soil supporting their growth.

Materials and Methods

Soil sampling location and potting preparation

Soil was collected from the Soils and Water Management Experimental Farm at Universidad del Valle (coordinates: 3°22'22.57"N, 76°31'47.57"W) in Cali, Colombia. After collection, the soil was transported to a greenhouse, where it was stored and air-dried in preparation for Cd addition and incubation. A 1 kg subsample was taken for soil characterization, while the remaining soil was left to dry for five days. Once dried, the soil was sieved through a mesh with openings smaller than 5 mm to remove coarse woody debris, roots, and stones. Four kilograms of the processed soil were then placed into polyethylene pots, which were intentionally left without drainage holes to prevent leaching. The water content of the pots was maintained at 65–70% of the soil's maximum water-holding capacity, determined by weekly weighing and adjusted as needed to avoid anoxic conditions. Hoagland's solution was applied weekly to all experimental units to provide essential plant nutrients [57].

Each experimental unit consisted of one cacao plant and, in treatments including Sf, one sunflower (Sf) plant. Seedlings of similar size were selected, and each pot was considered a replicate. A completely randomized design was employed to minimize environmental heterogeneity: pots were randomly positioned within the greenhouse and rotated weekly. Soil moisture and nutrient status were maintained uniformly across treatments through regular weighing, watering, and weekly application of Hoagland's solution as described above. This design ensured that observed differences among treatments were attributable to the experimental factors rather than to uncontrolled spatial variability.

Soil analytical methods

The soil samples were sent to a specialized laboratory for analysis. Soil pH was measured by potentiometry using distilled water extracts at a 1:1 weight-to-volume ratio (Sartorius PT-10, Germany). Cation exchange capacity (CEC) was determined with 1 N ammonium acetate at pH 7, following the Colombian Technical Standard 5268. Soil organic matter content was assessed using the Walkley–Black method (Nelson and Sommers, 1982). Phosphorus (P) levels were determined using the modified Bray II method (Colombian Technical Standard 5350). Potassium (K), magnesium (Mg), and calcium (Ca) concentrations were measured with 1 N ammonium acetate at pH 7 (Colombian Technical Standard 5349). Zinc (Zn) and copper (Cu) levels were analyzed using the modified Mehlich I method (Colombian Technical Standard 5526). Soil particle size distribution was determined by the hydrometer method.

Total Cadmium detection

Digestion of Cd in soil and PAT samples followed US EPA Method 3051A [58] and US EPA Method 200.9 [59]. Briefly, Method 3051A is a microwave-assisted extraction procedure designed to replicate conventional heating extraction using HNO_3, or alternatively HNO_3 and HCl. Method 200.9 provides procedures for determining dissolved and total recoverable elements by graphite furnace atomic absorption in water (groundwater, industrial, and domestic), sediments, sludge, and soil. For total recoverable analysis of a solid or aqueous sample containing undissolved material, analytes are first solubilized by refluxing with HNO_3 and HCl. Cadmium quantification was performed using inductively coupled plasma atomic emission spectrometry (ICP-AES).

Cadmium relative accumulators and addition

The experimental units received one of the following Cd additions, applied as $CdCl_2$ salt (Thermo Fisher Scientific, Geel, Belgium) dissolved in distilled water: 0.0 mg Cd kg^{-1} soil (control; no Cd addition) or 5.0 mg Cd kg^{-1} soil. Prior to seed planting, the Cd solution was applied in a single dose, soils were thoroughly mixed, and the growth pots were incubated for 15 days. Sunflower (Sf; Helianthus annuus cv. Mammoth Gray Stripe) was selected because it has been described as a suitable Cd relative accumulator [42], [44–46], [60–62]. Sf seedlings (sown from seed for two weeks) were intercropped with one-month-old cacao plants (Theobroma cacao L. cv. IMC-67), following schemes described in recent studies [63].

The cacao seedlings were grown in a nursery, from which uniform and healthy plants were selected. Because cacao plants may naturally contain Cd, a representative subsample of nursery-grown seedlings (n = 10) was digested according to EPA Method 3051A, a standard microwave-assisted acid digestion protocol for total element determination in plant tissues. The resulting Cd concentrations were below the analytical detection limit, confirming that initial Cd levels were negligible relative to those measured after experimental treatments. Therefore, endogenous Cd content in the cacao seedlings was considered insignificant for interpretation.

The study employed a completely randomized factorial design with three factors: Sf presence (present vs. absent), Cd addition (0 vs. 5 mg Cd kg^{-1} soil), and dolomitic lime (D) addition (0 vs. 5 g $CaMg(CO_3)_2$ kg^{-1} soil). This $2 \times 2 \times 2$ design produced eight treatment combinations, each replicated three times. A total of 24 pots were randomly distributed on greenhouse benches and rearranged weekly to minimize positional effects.

The selected Cd dose (5 mg Cd kg^{-1} soil) reflects moderate contamination levels commonly reported in cacao-growing soils across the Caribbean, Central and Latin America, including Colombia [11], [12], [16], [17], [38], [64–74]. A single Cd application prior to planting simulated a scenario of pre-existing soil contamination rather than continuous input. Dolomitic lime was applied once at 5 g kg^{-1} soil (~10 t ha^{-1}), a rate consistent with agronomic recommendations for raising soil pH and reducing Cd bioavailability in tropical soils. Control treatments (without Sf and without D) were also established.

After a nine-week growth period, the plant aerial tissue (PAT; leaves, stems, flowers) was harvested at the soil surface. Samples were rinsed with distilled water, cut into 5 cm pieces, and immediately weighed (Ohaus Ranger 300, USA) to obtain fresh weight. The PAT samples were then sent to a specialized laboratory for total Cd analysis.

All soil and plant tissue samples were subjected to strict quality assurance and quality control (QA/QC) procedures throughout chemical analysis. These included the use of certified reference materials and method blanks to monitor accuracy and detect potential contamination. Sample digestion and elemental quantification followed US EPA Methods 3051A and 200.9, ensuring consistent and reproducible Cd extraction and detection. Instrument calibration was conducted regularly with multi-point standard curves, and duplicate samples were analyzed to assess precision. Data validation involved checking detection limits, recovery rates, and repeatability to ensure reliability and accuracy of results.

Statistical analysis

When required, data were natural log-transformed to meet the assumption of Gaussian distribution and subsequently analyzed using parametric methods. A three-way ANOVA was performed to evaluate whether total Cd in cacao PAT, total Cd in soil, soil pH, and BCF differed as a function of sunflower (Sf) presence in the intercrop scheme, Cd addition (5.0 mg kg^{-1}), and D addition (5.0 g kg^{-1} soil). Correlation analyses were conducted between individual explanatory variables (e.g., total Cd in cacao PAT, total Cd in soil, and soil pH). Goodness of fit, expressed as the adjusted coefficient of determination (r^2), was tested using least-squares regression lines and comparisons. Unless otherwise specified, all reported differences were considered significant at $\alpha = 0.05$. Tukey's post hoc test was applied to identify significant differences between treatments. Statistical analyses were carried out using R software, version 4.5.1 [75].

The treatments used in this study are summarized in Table 1.

Table 1. Summary of the eight treatments in the 2 × 2 × 2 factorial design evaluating sunflower (Sf) presence, cadmium (Cd) addition, and dolomitic lime (D) application on cacao

Treatment code	Sf presence	Cd addition (mg Cd kg^{-1} soil)	D application (g kg^{-1} soil)	Description
T1	Absent	0	0	Cacao alone, control (no Cd, no D)
T2	Present	0	0	Cacao + Sf, no Cd, no D
T3	Absent	0	5	Cacao + D, no Cd
T4	Present	0	5	Cacao + Sf + D, no Cd
T5	Absent	5	0	Cacao alone, Cd
T6	Present	5	0	Cacao + Sf, Cd
T7	Absent	5	5	Cacao + D, Cd
T8	Present	5	5	Cacao + Sf + D, Cd

Source: Own elaboration.

Results

The soil used was classified as a Udertic Haplustoll, a clayey tropical soil formed under moderate rainfall with high organic matter content. Such soils, common in cacao-growing valleys, are moderately acidic, have low cation exchange capacity (CEC), and contain low native Cd. The Ca/Mg and Ca/K ratios were low, indicating potential Ca deficiencies, whereas the (Ca + Mg)/K ratio was within the optimal range. These properties influence Cd mobility and nutrient availability for plants (Table 2).

Table 2. Properties of soil

Properties	Units	Value
pH		5.5
Cd*	mg kg^{-1}	0.28 ± 0.06
K	mg kg^{-1}	194

Mg	mg kg^{-1}	805
Ca	mg kg^{-1}	510
CEC	meq/100 g	9.9
Zn	mg kg^{-1}	10.8
Cu	mg kg^{-1}	9.5
SOM	%	6.29
Sand	%	32
Silt	%	28
Clay	%	40
Soil textural class		Clay

Source: Own elaboration.

A three-way ANOVA was conducted on a sample of 24 plants to assess the effects of sunflower (Sf) presence, Cd addition, and dolomitic lime (D) application on four response variables: total Cd in PAT, total Cd in soil, soil pH, and BCF. The analysis revealed significant treatment interactions for total Cd in PAT ($F(7,16) = 3.815$, $P < 0.05$), total Cd in soil ($F(7,16) = 30.71$, $P < 0.001$), soil pH ($F(7,16) = 8.122$, $P < 0.005$), and BCF ($F(7,16) = 28.92$, $P < 0.001$). Notably, a significant difference was observed between the control and the highest Cd addition, with total Cd in soil increasing nearly sevenfold at 5.0 mg Cd kg^{-1} soil. The "cacao + Sf" treatment exhibited the highest total Cd in PAT, significantly exceeding the values recorded for the "cacao + D" and "cacao + Sf + D" treatments.

In treatments with dolomitic lime ("cacao + D" and "cacao + Sf + D"), soil pH increased significantly. Although the "cacao + Sf + D" treatment yielded the highest total Cd in soil, the difference was not statistically significant. BCF values above 1 were recorded across all treatments, indicating substantial Cd accumulation in plant shoots. Interestingly, the control treatment (no Cd addition) exhibited a significantly higher BCF compared with Cd-amended treatments. This elevated BCF is likely due to the relationship between total Cd in PAT and total Cd in soil, where the lower detected Cd in the 5.0 mg Cd kg^{-1} treatment skews the BCF calculation (Table 2). Future studies should consider the development of correction factors to address such discrepancies.

Table 2. Tukey post hoc analysis for the means of total Cd in PAT of cacao (mg kg^{-1}), total Cd in soil (mg kg^{-1}), soil pH, and BCF of cacao. Values are presented as mean ± SD; means followed by the same letter within columns are not significantly different ($P < 0.05$). Abbreviations: Sf = sunflower presence; D = dolomitic lime addition; PAT = plant aerial tissue; BCF = bioconcentration factor (total Cd in PAT/total Cd in soil)

Treatment	Total Cd in PAT of cacao (mg kg^{-1})	Total Cd in soil (mg kg^{-1})	Soil pH	BCF of cacao
Added Cd (mg kg^{-1})				
0.0	1.49 ± 0.33 a	0.28 ± 0.06 a	4.98 ± 0.54 a	5.58 ± 1.73 a
5.0	1.78 ± 0.89 a	7.28 ± 1.85 b	4.79 ± 0.45 a	0.27 ± 0.13 b
Treatments with Sf and D				
Cacao alone	1.55 ± 0.32 ab	2.92 ± 3.14 a	4.50 ± 0.11 b	3.15 ± 3.26 a
Cacao and Sf	2.35 ± 0.94 a	3.87 ± 4.00 a	4.50 ± 0.06 b	3.90 ± 4.06 a
Cacao and D	1.35 ± 0.40 b	3.82 ± 4.14 a	5.43 ± 0.45 a	2.45 ± 2.47 a
Cacao, Sf and D	1.30 ± 0.28 b	4.52 ± 4.69 a	5.12 ± 0.35 a	2.20 ± 2.29 a

Source: Own elaboration.

The modeled effects of the treatments on total Cd in PAT and total Cd in soil were significant; however, the model for total Cd in soil (with natural log transformation) was considerably stronger than that for total Cd in PAT (Table 3). This indicates a more robust association between the treatments and Cd levels in the soil compared to those in plant tissue.

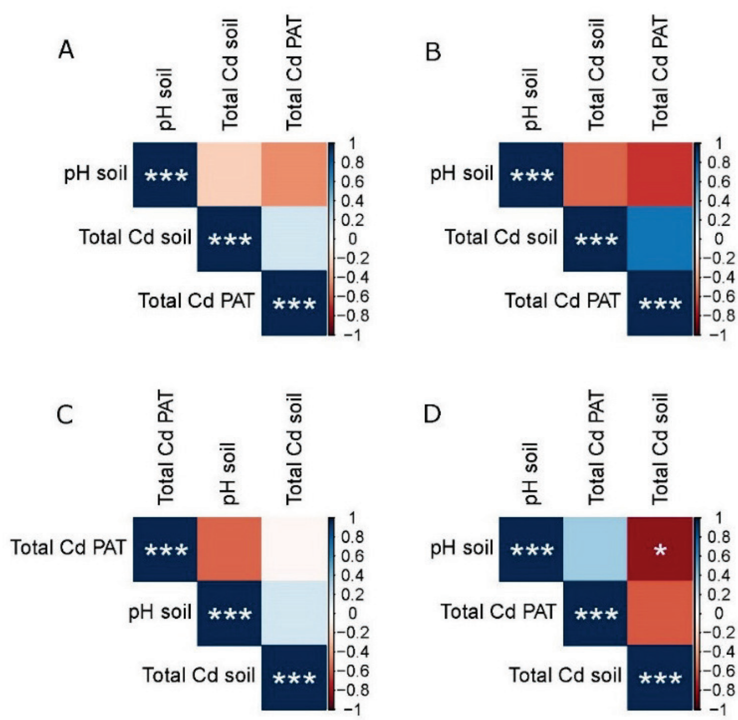
Table 3. Regression models

Linear regression models	Adjusted r^2	P-value
Total Cd in PAT = Sf × D × Added Cd	0.4614	< 0.05
Ln(Total Cd in soil) = Sf × D × Added Cd	0.9836	< 0.001

Source: Own elaboration.

Correlation analysis revealed a significant negative relationship ($P < 0.1$) between soil pH and total Cd in soil for the "cacao, Sf, and D" treatment. Although similar negative trends were observed in the "cacao alone" and "cacao and Sf" treatments, these relationships were not statistically significant. In contrast, the "cacao and D" treatment showed a positive but non-significant association between soil pH and total Cd in soil. Likewise, negative but non-significant correlations were identified between soil pH and total Cd in PAT across three of the four treatments: "cacao alone," "cacao and Sf," and "cacao and D" (Figure 1). Overall, these results suggest complex interactions between soil pH and Cd dynamics, underscoring the need for further research to clarify their ecological and agronomic implications.

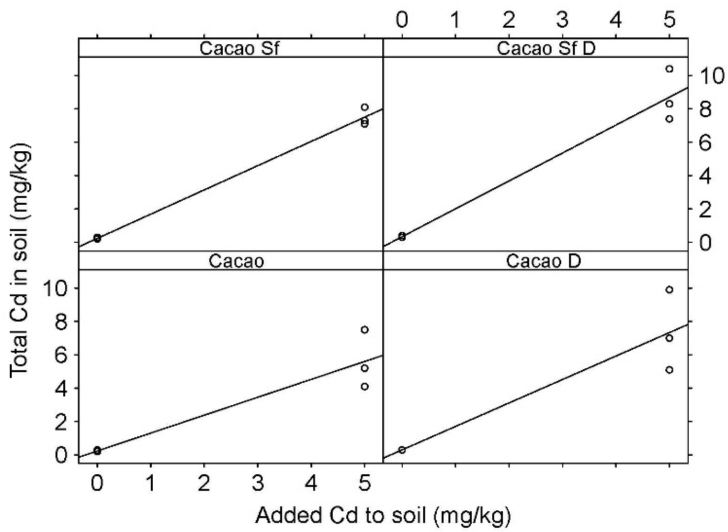
Figure 1. Correlations between predictor variables. A: Cacao alone; B: Cacao and sunflower; C: Cacao and dolomitic lime; D: Cacao, sunflower, and dolomitic lime. *P < 0.1, **P < 0.05, ***P < 0.001.

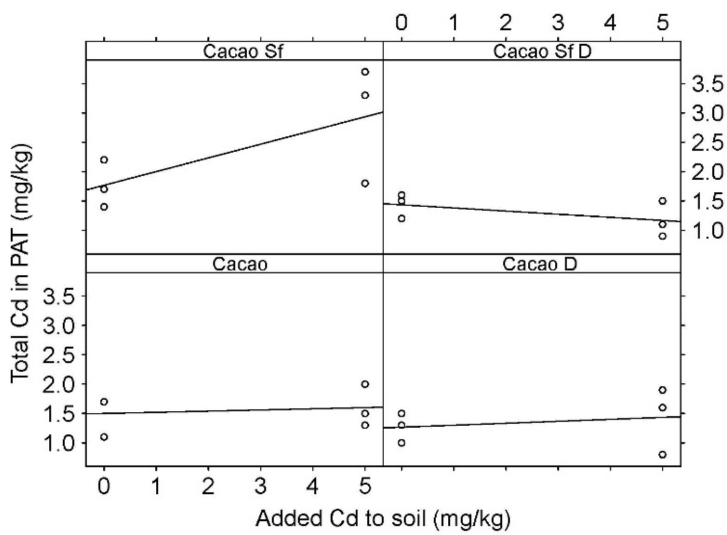


Source: Own elaboration.

The variation in total Cd in PAT and total Cd in soil was explained with different levels of significance across treatments. Regression analyses for total Cd in PAT yielded weak fits for all treatments, with adjusted r^2 values of 0.3333 for "cacao and Sf" ($P > 0.05$), 0.2445 for "cacao, Sf and D" ($P > 0.05$), −0.2136 for "cacao alone" ($P > 0.05$), and −0.1861 for "cacao and D" ($P > 0.05$) (Figure 2, left). By contrast, regression analyses for total Cd in soil showed strong and significant fits ($P < 0.01$) across all treatments, with adjusted r^2 values of 0.9911 for "cacao and Sf," 0.948 for "cacao, Sf and D," 0.847 for "cacao alone," and 0.8299 for "cacao and D" (Figure 2, right Figure 2. Top: Total Cd in plant aerial tissue (PAT) by added Cd to soil; bottom: Total Cd in soil by added Cd to soil. Lines are the fitted regression lines.

Figure 2. Top: Total Cd in plant aerial tissue (PAT) by added Cd to soil; bottom: Total Cd in soil by added Cd to soil. Lines are the fitted regression lines





Source: Own elaboration.

As expected, across all experimental units, Total Cd in PAT increased with higher concentrations of Cd in the soil. However, a notable result was observed when Sf was planted alongside cacao: the Total Cd in cacao PAT increased significantly compared to the other treatments. This may be attributed to the rapid physiological development of Sf, which produces a dense root system. Research suggests that plants often release acid exudates as a defense mechanism [76], [77], [78]. In this case, both the dense roots of Sf and the less-developed roots of cacao likely produced these exudates, lowering the soil pH, as reflected in the data (Table 2).

The acidification of the rhizosphere by sunflower is driven by two complementary processes: (i) proton efflux associated with cation uptake and (ii) secretion of low-molecular-weight organic acids (LMWOAs). Plants typically release H^+ to maintain charge balance during uptake of basic cations and under Fe or P deficiency. In addition, sunflower roots exude LMWOAs such as citric, malic and oxalic acidify the rhizosphere and chelate metal cations. The resulting decrease in soil pH and complexation of Cd can markedly increase Cd solubility and availability for plant uptake [79].

Root exudates not only acidify the rhizosphere but also mobilize Cd through chelation. LMWOAs such as citric, glutaric, malic and oxalic acids—major components of sunflower root exudates—adjust redox coupling and cation–anion exchange processes in soils [80]. These organic acids can form stable complexes with Cd, decreasing the activity of free Cd^{2+} and enhancing its mobility and uptake [80]. Experimental evidence shows that exogenous malic or acetic acid applications reduce Cd toxicity while increasing Cd accumulation in sunflower roots [81]. Similarly, adding citric or glutaric acids to Cd-contaminated soils promotes sunflower biomass and increases Cd uptake [80]. Therefore, the dense root system of sunflowers likely mobilizes Cd by exuding LMWOAs, which chelate Cd and increased its solubility; this mobilized Cd can then be taken up by both sunflower and neighboring cacao plants. Thus, the dense root system of Sf likely acidified the soil around the roots, thereby explaining the observed decrease in soil pH in the intercropped treatments.

The interplay between dolomitic lime application and the presence of Sf significantly influenced Cd bioavailability and plant uptake through complex rhizosphere interactions. Liming elevated soil pH, promoting Cd precipitation and adsorption to soil particles, thereby reducing soluble Cd^{2+} available for plant uptake. Conversely, sunflower roots exuded various LMWOAs such as citric and malic acids, which acidified the rhizosphere microenvironment and mobilized Cd by chelation and disruption of soil Cd associations. This root-induced acidification could counteract the alkalinizing effect of dolomitic lime locally, increasing Cd solubility despite a globally higher soil pH.

Physiologically, cacao roots possess membrane transporters that facilitate Cd uptake, with expression potentially modulated by external Cd availability and rhizosphere chemistry [82], [83]. The acidified rhizosphere enhances Cd desorption and diffusion to root surfaces, increasing influx into cacao root cells. Moreover, the presence of Sf may alter soil microbial populations and root exudate composition, indirectly influencing Cd speciation and uptake kinetics [84], [85]. These variable interactions underscore the importance of considering both chemical amendments and intercropping species in managing Cd dynamics, as their combined effects determine net Cd accumulation in cacao tissues. Future research should investigate molecular transporter gene expression in cacao under such combined treatments to elucidate the underpinning physiological mechanisms.

Treatments involving D showed lower Total Cd in cacao PAT and higher soil pH, reinforcing the idea that raising soil pH significantly reduces Cd mobility in the soil. However, adding lime to soils was less effective at reducing Total Cd in plants when two crops were grown together. It is important to reiterate that this study used the IMC-67 cacao variety, classified as a low Cd accumulator [86], meaning that Cd accumulation in cacao depends significantly on the cultivar [87]. Therefore, not all cacao varieties function as effective phytoextractors, and their genetic variation influences their bioaccumulation and partitioning of Cd [88], [89]. Further research is necessary to explore how different cacao varieties respond to Cd contamination.

Moreover, Cd accumulation was not only evident in PAT but also in the residual Cd levels in the soil at the conclusion of the experiment. Total Cd in soil was lower in the "cacao alone" treatment and higher in the "cacao, Sf and D" treatment, although the difference was not statistically significant (Table 2). A negative correlation was observed between soil pH and Total Cd in soil in the "cacao, Sf and D" treatment (Figure 1). This supports the hypothesis that increased soil pH enhances Cd sorption, thereby reducing Cd in solution and lowering its phytoavailability [90]. Similarly, the rise in soil pH was associated with a reduction in Total Cd in cacao PAT, further indicating that pH plays a critical role in modulating the distribution of Cd between the solid and solution phases of the soil [91]. This finding aligns with previous studies that reported increased metal uptake with rising pH levels in soils [92].

Interestingly, the BCF value for the control treatment (with no added Cd) was significantly higher than for the treatment with added Cd. This may seem counterintuitive but is explained by the simple proportion used in the BCF calculation, where the denominator (Total Cd in soil) is lower than 1. As the BCF is not a fixed value and varies depending on soil chemical properties [43], it is essential to consider correction factors. The BCF of a plant can fluctuate due to biotic and abiotic conditions, especially in multi-crop systems, which can alter Cd bioavailability over time [93]. Further research should investigate these factors to ensure accurate BCF assessments.

Our factorial ANOVA revealed significant interactions among Sf presence, Cd addition and D application on Cd concentrations in cacao PAT and soil pH ($P < 0.05$). When Sf was intercropped with cacao under Cd addition, soil pH decreased and Cd accumulation in cacao increased. Physiologically, this can be explained by Sf's dense root system releasing protons and lowmolecularweight organic acids that acidify the rhizosphere and mobilize Cd [79], [80]. Liming counteracted this effect by raising soil pH and providing Ca^{2+} and Mg^{2+} ions that compete with Cd for sorption sites, thus reducing Cd solubility and uptake. Cd enters plant roots via cation transporters that normally transport essential elements; once inside, Cd can be sequestered in root vacuoles or translocated to shoots [79]. The observed decrease in Cd uptake in limed treatments indicates that raising soil pH and supplying Ca/Mg limited Cd transport to shoots. Therefore, the interactions among Sf presence, liming and Cd addition highlight the combined influence of rhizosphere chemistry and physiological Cd transport pathways on Cd accumulation in cacao.

Conclusions

Sunflower was identified as an effective relative accumulator of Cd. However, when cultivated alongside cacao, it appeared to promote rather than mitigate Cd accumulation in cacao plants. This effect may be attributed to the production of organic acids by sunflower's dense root system, which likely lowered soil pH and enhanced Cd mobility and uptake by cacao. The application of dolomitic lime significantly increased soil pH, thereby reducing Cd concentrations in the aerial tissues of cacao. Nonetheless, further research is warranted to examine the effects of both alkaline and acidic amendments.

A negative correlation was observed between soil pH and total Cd in the soil, underscoring the critical role of pH in determining Cd bioavailability. Although both sunflower and cacao exhibited BCF values greater than 1, these findings should be interpreted with caution, as BCF is not a constant and varies considerably depending on soil conditions.

This study is the first to assess Cd accumulation in an intercropping system involving sunflower and cacao under dolomitic lime application. However, additional research is required to better understand the dynamic interactions between Cd in agricultural soils and its accumulation in cacao. Future studies should explore novel Cd adsorbents and immobilization materials, particularly for sub-surface soil layers, and determine the time frame required for these amendments to effectively reduce Cd uptake. It is also recommended that similar research be extended to field conditions across diverse soil and climatic contexts, production systems (including agroforestry schemes), and cacao genotypes with varying Cd accumulation capacities.

Given cacao's economic importance and the growing concern over Cd contamination in tropical regions, integrated strategies combining amendment application and phytoremediation should be evaluated under local edaphoclimatic conditions. Our findings highlight that while liming remains a practical amendment to reduce Cd phytoavailability, intercropping decisions must carefully consider potential unintended facilitation of Cd mobilization. These insights provide valuable guidance for agronomic management and food safety policies in cacao production and reinforce the need for continued investigation into molecular and microbial interactions influencing heavy-metal dynamics in these systems.

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