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ARTICLE

Weed Species Associated with Cacao Cultivation Exhibiting Phytoremediation Potential for Cadmium and Lead: A Study Based on Bioconcentration and Translocation Factors

Lorena E. Romero-Chávez[1,*], Emilee Calero-Rios[2], Uriel Aldava-Pardave[3], Elvis Ottos-Díaz[1], Noelito Salgado-Veramendi[3], Eldhy S. Huerto-Pajuelo[4], Carolina Ibarra-Porras[4] and Richard A. Solórzano-Acosta[2]

[1]Estación Experimental Agraria Pichanaki, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria, Pichanaki District, Junin, Peru

[2]Centro Experimental La Molina, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria, La Molina, Lima, Peru

[3]Estación Experimental Agraria Pucallpa, Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria, Pucallpa, Peru

[4]Escuela Profesional de Ingeniería en Conservación de Suelos y Agua, Facultad de Recursos Naturales Renovables, Universidad Nacional Agraria de la Selva, Tingo Maria, Peru

*Corresponding Author: Lorena E. Romero-Chávez. Email: leromeroc@alumno.unsm.edu.pe

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ABSTRACT: The exploration and identification of spontaneous weed species in heavy metal–contaminated soils represent a relevant approach for understanding the role and their potential application in phytoremediation. In cacao cultivation, cadmium contamination poses a significant risk due to the restrictions established for soils and cacao-derived products, thereby threatening productive sustainability and export viability. The objective of this study was to identify weed species associated with cacao cultivation exhibiting accumulation patterns and phytoremediation potential for Cd and Pb, through the assessment of biomass production and the bioconcentration factor (BCF) and translocation factor (TF), in natural conditions. Soil samples were collected from seven cacao-growing zones, and the site with the highest Cd concentration (1.65 mg kg^{-1}) was selected, where ten dominant weed species were identified and evaluated. Significant interspecific differences were observed in biomass production and in Cd and Pb concentrations ($p < 0.05$). *Talinum paniculatum* exhibited the highest total biomass (6.15 g) and the highest TF for Cd, whereas *Cyperus aggregatus* showed the greatest total Cd accumulation (2.68 mg kg^{-1}). BCF and TF values enabled the identification of species with accumulation patterns consistent with Cd phytoextraction (*T. paniculatum*, *C. aggregatus*, *Pseudelephantopus spiralis*, *Bidens riparia*, *Euphorbia heterophylla*, and *Malvastrum coromandelianum*) and with phytostabilization (*Ocimum campechianum*). For Pb the observed patterns were consistent with potential phytoextractive behavior in *B. riparia*, *P. spiralis*, *E. heterophylla*, *Hilleria latifolia*, and *Acalypha stachyura.* Although these results do not constitute functional evidence of remediation, they describe heavy metal accumulation and translocation patterns in native weed species, providing a technical basis for the design of future experimental trials aimed at the sustainable remediation of contaminated soils.

KEYWORDS: Spontaneous weeds; cadmium; lead; plant biomass; bioconcentration factor; translocation factor; rhizosphere

1 Introduction

The contamination of agricultural soils with heavy metals, particularly cadmium (Cd) and lead (Pb), represents a significant risk to human health and food security [1]. Cadmium can be readily absorbed by plants and translocated from the soil to plant tissues depending on its bioavailability, which is influenced by a range of edaphic and environmental factors [2]. In contrast, Pb has no known biological function, and its entry into the food chain occurs primarily through irrigation practices, environmental contamination during field cultivation, and post-harvest handling and packaging of agricultural products [1]. Under natural conditions, soils generally contain low Cd concentrations; however, its bioavailability is strongly governed by soil pH, organic matter content, and texture [3], and can be substantially enhanced by agricultural practices such as phosphate fertilization and pesticide application [4,5].

This issue has been widely documented in cacao (*Theobroma cacao* L.) cultivation, where Cd availability in soils promotes its uptake and translocation to the beans [6,7], in some cases reaching concentrations higher than those detected in the soil itself [8]. In response to the increasing risks associated with heavy metal contamination and the growing demand for cacao-derived products, maximum permissible limits have been established for both soils and cacao beans. The Ministry of the Environment of Peru has established Environmental Quality Standards with maximum concentrations of 1.4 mg kg^{-1} for Cd and 70 mg kg^{-1} for Pb in agricultural soils [9]. Regarding cacao-derived products, regulatory limits include 0.6 mg kg^{-1} for ground cacao [10] and 0.7 mg kg^{-1} for chocolate with a cocoa solids content ≥30% and <50% [11].

According to projections by Thomas et al. [12], high concentrations of Cd in Peruvian cocoa beans and soils generally affect at least 20% of producing households from six regions, and 86% in Piura region. The regulations have raised concern among producers, as a substantial proportion of national cacao production is destined for international markets, with at least 75% [13–15]. Argüello et al. [14] reported that, to prevent cacao beans from exceeding Cd concentrations of 1 mg kg^{-1}, soil Cd levels should not surpass 0.4 mg kg^{-1} under acidic conditions (pH 5.0), whereas at neutral pH (7.0) the threshold may increase to as much as 1.0 mg kg^{-1}.

Against this backdrop, the development of technologies aimed at mitigating heavy metal contamination in agricultural soils is essential [16,17], in order to reduce carcinogenic risks and adverse effects on human health [18]. Within this context, phytoremediation has emerged as a low-cost and environmentally sustainable alternative [19], based on the ability of certain plant species to extract, immobilize, or transform soil contaminants [20], while also producing substantial biomass, adapting to adverse conditions, and exhibiting a high capacity for metal accumulation [21].

A wide range of plant species has been evaluated for the phytoremediation of contaminated soils, including woody species [22,23], agricultural crops [24,25], as well as grasses and other herbaceous plants [26]. However, the effectiveness of these species is often constrained by site-specific environmental and edaphoclimatic factors [27], highlighting the need to identify plant species adapted to each agroecosystem [28]. In this regard, weeds are characterized by aggressive growth, native origin, high adaptability, and tolerance to adverse conditions [29–31]. Their physiological and genetic traits confer remarkable ecological plasticity [32], positioning them as promising candidates for the phytoremediation of soils contaminated with Cd and Pb [33,34].

Despite these advances, a significant knowledge gap persists regarding the exploration and identification of weed species with phytoremediation potential associated with cacao cultivation, an agroecosystem that covers more than 250,000 ha in Peru. Within this context, the objective of this exploratory study was to identify and evaluate the phytoremediation potential of spontaneous weed species adapted to the edaphoclimatic conditions of cacao-growing systems in the central Peruvian rainforest. This prospective approach provides a technical foundation for the development of future controlled trials aimed at more precisely defining the role of these species in mitigating heavy metal contamination in agricultural soils.

2 Materials and Methods

2.1 Study Area

The research was conducted in the district of San Martín de Pangoa, Satipo Province, Junín Region, Peru. The area corresponds to the tropical premontane pluvial forest (bp-PMT) life zone, according to the Holdridge World Life Zone Classification System [35]. The site is characterized by maximum and minimum temperatures of 31.6 and 19.3°C, respectively, a relative humidity of 81.5%, and a mean annual precipitation of 1898 mm $year^{-1}$ [36]. One of the main economic activities in the area is the cultivation of coffee and cacao for both domestic consumption and export markets.

2.2 Experimental Design

An exploratory observational study was conducted based on purposive field sampling. Seven cacao-growing areas were identified according to the following criteria: plots at the production stage, a minimum area of one-hectare, organic management, manual weed control, and the absence of weeding activities during the two months prior to sampling. To determine soil heavy metal concentrations and identify the area with the highest Cd levels, as well as to characterize its physicochemical properties, soil sampling was carried out following the approach proposed by Havlin et al. [37]. This area was selected to maximize the likelihood of identifying species with metal accumulation capacity, consistent with the exploratory nature of the study. Soil samples were collected at a depth of 30 cm from edaphically homogeneous areas, with three replicates obtained per study site.

Soil samples were analyzed within the Soil, Water, and Foliar Laboratory Network of the National Institute for Agrarian Innovation (LABSAF-INIA). Prior to physicochemical analyses and the determination of Cd and Pb concentrations, samples underwent a pre-treatment consisting of air-drying at temperatures below 40°C and sieving to obtain the <2 mm fraction, in accordance with the procedure established by the International Organization for Standardization [38].

The variables considered for soil characterization included the following parameters and reference methodologies: sand, silt, and clay contents determined by the Bouyoucos hydrometer method [39]. Soil pH measured in a 1:1 soil–water suspension [40]; electrical conductivity (EC) measured in a 1:5 soil–water extract [41]. Organic matter content determined by the Walkley and Black method [39]; total nitrogen measured by the micro-Kjeldahl method [42]. Available phosphorus in neutral and acidic soils determined using the Bray and Kurtz method [39]. Available potassium determined according to Bazán Tapia [43]; exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) extracted with ammonium acetate. Moreover, exchangeable acidity (H^{+}, Al^{3+}) extracted with potassium chloride [39]. Finally, effective cation exchange capacity (ECEC) was calculated as the sum of exchangeable bases and exchangeable acidity.

Total recoverable Cd concentrations were determined following the USEPA Method 3050B [44]. The resulting solutions were analyzed using flame atomic absorption spectrometry (FAAS). Standard quality assurance and quality control (QA/QC) procedures were applied throughout the analytical process. A calibration coefficient (R^2) of 0.9995 or higher was obtained for the standard calibration curve on the flame atomic absorption spectrophotometer prior to sample analysis. Following initial calibration, the calibration curve was verified using an initial calibration blank (ICB) and an initial calibration verification standard (ICV). The ICV was prepared from an independent certified reference material (second source) at or near the mid-range of the calibration curve, with acceptance criteria set at ±10% of the true value. Spike recovery of Cd from a certified reference material (90%–110%) and duplicate analyses performed every 10 samples were also included as part of the QA/QC protocol. The method detection limit for Cd using FAAS was 0.41 mg kg^{-1}.

Soil analysis results indicated that the area known as Ciudad de Dios exhibited the highest Cd concentration (1.65 mg kg^{-1}), exceeding the Peruvian Environmental Quality Standard (EQS) for agricultural soils established by the Ministry of the Environment of Peru [9]. Accordingly, this site was selected for the identification and analysis of spontaneous weed species associated with cacao cultivation.

2.3 Characterization of the Selected Study Area

The selected area comprises an 8-year-old cocoa plantation with a planting density of 3 m × 3 m, arranged in a triangular (staggered) pattern, and previously used for coffee cultivation. The plantation was classified as polyclonal, predominantly composed of CCN-51 (80%), followed by ICS-1 (5%), ICS-6 (5%), TSH-565 (3%), VRAE-15 (2%), VRAE-99 (2%), ICS-39 (1%), ICS-95 (1%), and other minor genotypes (1%). Agronomic management included pruning carried out as required and fertilization based on seabird guano and dolomite (Fertiphos Tropical), resulting in an average productivity of 1000 kg ha^{-1}. The presence of major diseases and pests was observed, including witches' broom (*Moniliophthora perniciosa*), frosty pod rot (*Moniliophthora roreri*), cocoa pod borer (*Carmenta foraseminis*), and brown spot disease (*Phytophthora* sp.).

Soil analysis indicated a clay loam texture, a high organic matter content (4.1%), and a moderately acidic pH of 6.10. Electrical conductivity was low (0.09 dS m^{-1}), while total N, available P, and exchangeable K contents were 0.21%, 6.48 mg kg^{-1} and 77.80 mg kg^{-1}, respectively. The cation exchange capacity (CEC) was 3.50 cmol (+) kg^{-1}, with exchangeable cation concentrations of Ca^{2+} (2.84), Mg^{2+} (0.56), K^{+} (0.08), and Na^{+} (0.02). In addition, to determine heavy metal (Cd and Pb) levels in cocoa plants, samples of leaves, pods, husks, and cotyledons were collected. Sampling followed a zigzag pattern, collecting plant material from ten trees, including five mature leaves with petioles and two mature pods per tree, free of visible disease or pest damage [45].

In the laboratory, leaves were washed with distilled water; cocoa pods were opened, cut into small fragments, and placed in beakers, while cocoa beans underwent a five-day fermentation process followed by shade drying. Leaves, pods, and cocoa beans were subsequently oven-dried at 70°C for 48 h until constant dry weight was achieved [46]. The husk was manually separated from the cotyledons, and each plant component was ground using porcelain mortars (Table 1).

Table 1: Cd and Pb concentrations in cocoa plant tissues.

Sample	Cd (mg kg^{-1})	Pb (mg kg^{-1})
Leaf	2.05	4.75
Cocoa pod husk	0.93	2.08
Shell	0.92	1.45
Cotyledon	0.89	1.81

2.4 Weed Sampling and Taxonomic Identification

For plant sampling, a systematic survey was conducted across the select plot in which dominant weed species were defined as those exhibiting the highest frequency of occurrence and visual cover within the evaluated plots. Based on this criterion, the selected species were collected, ensuring that sampled individuals per species were spatially distributed across different sectors of the field, thereby avoiding clustering in a single location. Each plant individual constituted an independent experimental unit, allowing the acquisition of biological replicates per species, and was processed individually for subsequent analyses.

Species-specific percentage cover was estimated across the study area, after which dominant weed species were randomly collected with ten replicates at the flowering stage [28]. These ten replicates were obtained to ensure a representative sampling, provide sufficient plant material for laboratory analyses, and support accurate taxonomic identification. From these, three individual plants per species were selected and analyzed independently for heavy metal determination. Samples consisted of both aboveground and root tissues [47]. In addition, targeted rhizospheric soil samples were collected for heavy metal analysis [28]. The ten most representative species throughout the study area were collected and deposited in herbarium presses to facilitate dehydration and prevent the growth of microorganisms, such as fungi [48]. Taxonomic identification was performed according to the APG IV system The Angiosperm Phylogeny Group [49], at the Herbarium of the Department of Biology, Universidad Nacional Agraria La Molina, Lima, Peru.

2.5 Parameter Evaluation

a. Weed biomass

Samples were washed with distilled water to remove adhering soil residues. Subsequently, the aboveground and root portions were separated. Each sample was oven-dried separately at 70°C until a constant weight was achieved [46]. The dried samples were ground using a stainless-steel mill and passed through a 100-mesh sieve [50]. Biomass was expressed on a dry weight basis, including aboveground biomass, root biomass, and total biomass, calculated from three replicates per weed species.

b. Bioconcentration factor (BCF) and translocation factor (TF)

The bioconcentration factor (BCF) indicates the ability of plants to remove chemical components from the soil [32]. BCF is defined as the ratio between the total concentration of a metallic element in plant tissues and its concentration in the soil [51]. BCF values < 1 indicate metal-excluding species, whereas BCF values > 1 indicate metal-accumulating species [52,53]. This factor was calculated as follow:

$$\mathrm{BCF} = \frac{\text{Concentration in the plant organ } \left(\text{mg kg}^{-1}\right)}{\text{Concentration in soil } (\text{mg kg}^{-1})}$$

The translocation factor (TF) determines the ability of a plant to transfer a compound from the roots to other plant organs [51]. A plant is considered to exhibit phytostabilization potential when metals are not translocated to other organs and remain immobilized in the root system (TF < 1) [54]. In contrast, TF values > 1 indicate the plant's capacity to translocate metals from roots to aboveground tissues, such as shoots, and are therefore indicative of bioaccumulation potential [55]. This factor was calculated using the following equation:

$$\mathrm{TF} = \frac{\text{Concentration in the aboveground part } \left(\text{mg kg}^{-1}\right)}{\text{Concentration in the root } (\text{mg kg}^{-1})}$$

2.6 Statistical Analysis

Statistical analyses were performed using R software version 4.5 through the RStudio interface (2024 edition). Cd and Pb concentrations, as well as biomass, were modeled using generalized linear models (GLMs) implemented with the *glmmTMB* package. Model selection was conducted using the *MuMIn* package based on the corrected Akaike Information Criterion (AICc). Model fit was evaluated using R^2 as implemented in the *performance* package. Assumption checking included residual normality, outlier detection, and homogeneity of variances, which were assessed using the *DHARMa* package. Multiple comparisons of heavy metal concentrations and biomass among weed species were performed using the *emmeans* package, considering a significance level of $\alpha = 0.05$ and applying false discovery rate (FDR) correction.

Additionally, the relationships between soil metal concentrations and biomass with metal contents in weed species were modeled using generalized linear models (GLMs) with spline functions (using different degrees of freedom and polynomial orders), implemented through the splines package. Predicted values were obtained using the ggeffects package, and results were visualized using the *ggplot2* package.

3 Results

3.1 Identified Weed Species

The weed species recorded in the study area were distributed across eight botanical families. The identified species included *Pseudelephantopus spiralis* (Less.) Cronquist, *Ocimum campechianum* Mill., *Bidens riparia* Kunth, *Tripogandra serrulata* (Vahl) Handlos, *Euphorbia heterophylla* L., *Talinum paniculatum* (Jacq.) Gaertn., *Hilleria latifolia* (Lam.) H. Walter (HIW), *Cyperus aggregatus* (Willd.) Endl., *Acalypha stachyura* Pax, and *Malvastrum coromandelianum* (L.) Garcke (Table 2). These species are locally known as achicoria, wild basil, chilco, siempre viva, leche-leche, verdolaga, cuerda de indio, pata de gallina, cola de zorro, and pichana, respectively.

Table 2: Identified weed species.

Species	Family
Pseudelephantopus spiralis (Less.) Cronquist	Asteraceae
Ocimum campechianum Mill.	Lamiaceae
Bidens riparia Kunth	Asteraceae
Tripogandra serrulata (Vahl) Handlos	Commelinaceae
Euphorbia heterophylla L.	Euphorbiaceae
Talinum paniculatum (Jacq.) Gaertn.	Talinaceae
Hilleria latifolia (Lam.) H. Walter	Petiveriaceae
Cyperus aggregatus (Willd.) Endl.	Cyperaceae
Acalypha stachyura Pax	Euphorbiaceae
Malvastrum coromandelianum (L.) Garcke	Malvaceae

3.2 Cadmium in Weed Species

The evaluated weed species showed significant differences in biomass production and Cd concentration ($p < 0.05$) (Table 3). *T. paniculatum* recorded the highest total biomass (6.15 g) and root biomass (3.72 g), and ranked second in shoot biomass (2.43 g). The rhizospheric soil associated with this species exhibited a Cd concentration of 0.91 mg kg^{-1}, ranking fourth among the evaluated species. In plant tissues, *T. paniculatum* showed a total Cd concentration of 0.78 mg kg^{-1}, with 0.59 mg kg^{-1} in shoots, and was notable for presenting the lowest Cd concentration in roots (0.19 mg kg^{-1}). In contrast, *M. coromandelianum* exhibited the highest shoot biomass (3.30 g); however, it was associated with the third-highest Cd concentration in soil (1.05 mg kg^{-1}) and ranked fourth in shoot Cd concentration (1.04 mg kg^{-1}).

In contrast, *T. serrulata* exhibited the lowest total biomass (0.57 g) and root biomass (0.10 g), and ranked among the species with the lowest shoot biomass (0.48 g). Nevertheless, this species grew in soils with the third-highest Cd concentration (1.05 mg kg^{-1}) and showed some of the lowest Cd concentrations in plant tissues, including total Cd (0.66 mg kg^{-1}), root Cd (0.42 mg kg^{-1}), and shoot Cd (0.24 mg kg^{-1}).

Conversely, *C. aggregatus* was recorded in soils with the highest Cd concentration in soil (1.49 mg kg^{-1}) and exhibited the highest values of total Cd accumulation in plant (2.68 mg kg^{-1}), as well as the highest root (1.04 mg kg^{-1}) and shoot Cd concentrations (1.64 mg kg^{-1}). However, this species ranked third in total biomass (3.20 g), second in root biomass (1.68 g), and fourth in shoot biomass (1.52 g).

Table 3: Cadmium concentration in soil, roots, and aerial parts, and biomass by weed species: ANOVA, model selection, and adjusted means.

Factors	Cd Soil (mg kg^{-1})	Cd Plant (mg kg^{-1})	Cd Root (mg kg^{-1})	Cd Shoot (mg kg^{-1})	Total Biomass (g)	Root Biomass (g)	Shoot Biomass (g)
Species	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Model selection							
Distribution	Gamma	lognormal	lognormal	Lognormal	Normal	lognormal	lognormal
AICc	−69.81	−46.77	−65.05	−61.36	−44.41	−66.29	−72.01
R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Estimated means by species							
BRK	1.22b	1.79c	0.59c	1.20c	0.69g	0.17e	0.52g
CAE	1.49a	2.68a	1.04a	1.64a	3.20c	1.68b	1.52d
EHL	1.50a	1.87b	0.9b	0.97e	0.99f	0.15e	0.85f
MCG	1.05c	1.92b	0.88b	1.04d	3.69b	0.39d	3.30a
OCM	0.30e	0.65e	0.42d	0.22g	2.18d	0.59c	1.58c
PSC	1.20b	1.93b	0.59c	1.35b	1.60e	0.58c	1.02e
TPG	0.91d	0.78d	0.19e	0.59f	6.15a	3.72a	2.43b
TSH	1.05c	0.66e	0.42d	0.24g	0.57h	0.10f	0.48g

Note: Different letters indicate significant differences among species ($p < 0.05$). PSC: *Pseudelephantopus spiralis* (Less.) Cronquist; OCM: *Ocimum campechianum* Mill; BRK: *Bidens riparia* Kunth; TSH: *Tripogandra serrulata* (Vahl) Handlos; EHL: *Euphorbia heterophylla* L.; TPG: *Talinum paniculatum* (Jacq.) Gaertn; CAE: *Cyperus aggregatus* (Willd.) Endl; MCG: *Malvastrum coromandelianum* (L.) Garcke.

The results indicate a clear relationship between soil Cd content and Cd accumulation in weed species, modeled using generalized linear models with spline functions based on different bases and polynomial degrees. Fig. 1A illustrates the functional relationship between total soil Cd and total Cd in weeds (R^2 = 0.67, df = 3; polynomial degree = 2). Soils with lower Cd concentrations were associated with *O. campechianum* and *T. paniculatum*, whereas *T. serrulata*; *M. coromandelianum* exhibited intermediate soil Cd levels. In contrast, the highest soil Cd concentrations were recorded in association with *E. heterophylla*, *C. aggregatus*, *B. riparia*, and *P. spiralis*, which also showed intermediate to high levels of Cd accumulation in their tissues. Regarding plant metal concentrations, *C. aggregatus* stood out by exhibiting the highest Cd concentration 2.68 mg kg^{-1}.

Fig. 1B depicts the relationship between total biomass and Cd accumulated in plant tissues (R^2 = 0.48, df = 4; polynomial degree = 2). Species with lower biomass, such as *T. serrulata* and *O. campechianum*, recorded lower Cd contents. *M. coromandelianum* showed intermediate biomass and Cd accumulation, whereas *C. aggregatus* displayed intermediate biomass but the highest Cd accumulation among the evaluated species. In contrast, *T. paniculatum*, despite producing the greatest biomass, exhibited one of the lowest Cd concentrations in plant tissues.

Fig. 1C relates shoot biomass to shoot Cd concentration (R^2 = 0.34, df = 4; polynomial degree = 2). The species with the lowest shoot biomass, *T. serrulata*, exhibited the lowest Cd concentrations in aerial tissues. Conversely, *E. heterophylla*, *P. spiralis*, despite also presenting low shoot biomass, showed intermediate levels of shoot Cd. *C. aggregatus*, stood out by exhibiting the highest shoot Cd concentration even under low shoot biomass conditions. Likewise, *T. paniculatum*, with intermediate shoot biomass, was among the species with the lowest Cd concentrations in aerial tissues; whereas *M. coromandelianum* displayed the highest shoot biomass accompanied by intermediate Cd storage.

Fig. 1D illustrates the functional relationship between root biomass and root Cd concentration ($R^2 = 0.86$, df = 5; polynomial degree = 2). Species with the lowest root biomass, such as *T. serrulata* and *O. campechianum*, exhibited the lowest levels of root Cd. In contrast, *P. spiralis*, *B. riparia*, *M. coromandelianum* and *E. heterophylla*, despite also presenting low root biomass, showed intermediate root Cd concentrations. *C. aggregatus* displayed intermediate root biomass but the highest root Cd accumulation among the evaluated species. Conversely, *T. paniculatum*, although exhibiting the greatest root biomass, was among the species with the lowest root Cd concentrations.

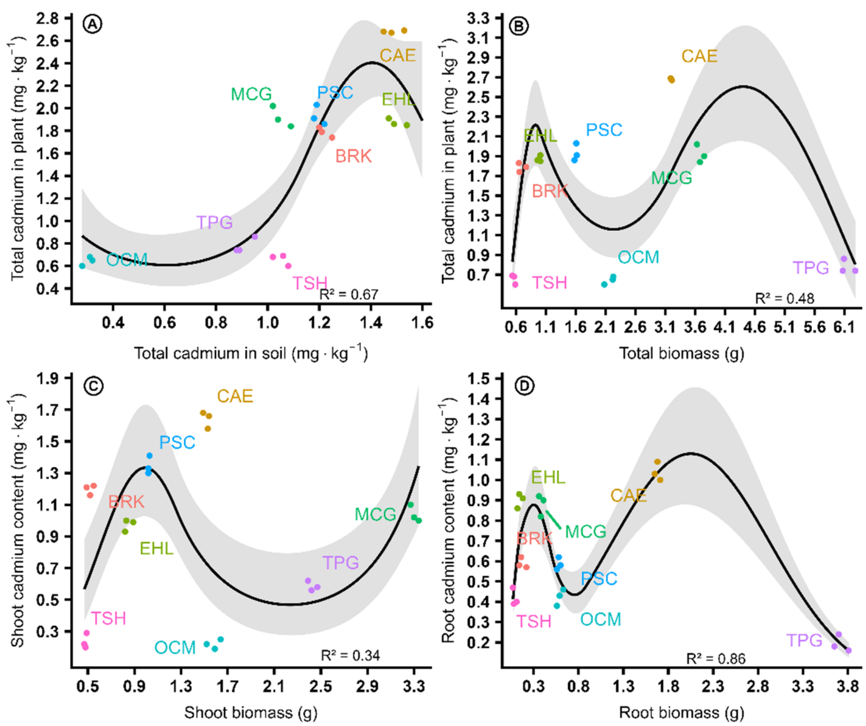

Figure 1: Functional relationships between: Ⓐ total Cd concentration in soil and total Cd concentration in the plant; Ⓑ total plant biomass and total Cd concentration in the plant; Ⓒ aboveground biomass and Cd concentration in the shoot; and Ⓓ root biomass and Cd concentration in the root. Colored points represent observations for each species. Solid black lines indicate fits from generalized linear models using spline functions, and the shaded areas denote the confidence intervals of the fitted models. The coefficient of determination (R^2) reflects the goodness of fit between the evaluated variables. Species abbreviations: PSC: *Pseudelephantopus spiralis* (Less.) Cronquist; OCM: *Ocimum campechianum* Mill; BRK: *Bidens riparia* Kunth; TSH: *Tripogandra serrulata* (Vahl) Handlos; EHL: *Euphorbia heterophylla* L.; TPG: *Talinum paniculatum* (Jacq.) Gaertn; CAE: *Cyperus aggregatus* (Willd.) Endl; MCG: *Malvastrum coromandelianum* (L.) Garcke.

3.3 Lead in Weed Species

The weed species exhibited significant differences in both biomass production and Pb concentration ($p < 0.05$) (Table 4). *T. paniculatum* recorded the highest total biomass (6.15 g) and root biomass (3.72 g), while ranking third in shoot biomass (2.43 g). This species was associated with one of the soils that ranked fourth in Pb concentration (12.08 mg kg^{-1}). In addition, *T. paniculatum* ranked fourth in total Pb accumulation in plant tissues (6.06 mg kg^{-1}), second in root Pb accumulation (3.98 mg kg^{-1}), and sixth in shoot Pb accumulation (2.08 mg kg^{-1}). In contrast, *A. stachyura* Pax exhibited the highest shoot biomass (4.03 g) and was associated with soils showing the highest Pb concentration (14.90 mg kg^{-1}), while also recording the highest Pb concentrations in aerial tissues (4.15 mg kg^{-1}).

T. serrulata exhibited the lowest total biomass (0.57 g), root biomass (0.10 g), and shoot biomass (0.48 g); however, its rhizospheric soil recorded the fourth highest Pb concentration (12.16 mg kg^{-1}). Regarding Pb levels in plant tissues, this species showed the highest total Pb concentration (11.19 mg kg^{-1}) and root Pb concentration (8.02 mg kg^{-1}), while ranking fourth in shoot Pb concentration (3.17 mg kg^{-1}). In contrast, the rhizospheric soil of *C. aggregatus* recorded a Pb concentration of 13.08 mg kg^{-1}, ranking second among the evaluated soils. Nevertheless, this species exhibited the lowest total Pb concentration in plant tissues (2.59 mg kg^{-1}) and the lowest shoot Pb concentration (0.56 mg kg^{-1}), while also ranking among the species with the lowest root Pb concentrations (2.03 mg kg^{-1}). Additionally, *C. aggregatus* ranked fourth in both total biomass (3.20 g) and shoot biomass (1.52 g), and third in root biomass (1.68 g).

Table 4: Lead concentrations in soil, roots, and shoots, and biomass across weed species: ANOVA, model selection, and estimated marginal means.

Factors	Pb Soil (mg kg^{-1})	Pb Total (mg kg^{-1})	Pb Root (mg kg^{-1})	Pb Shoot (mg kg^{-1})	Total Biomass (g)	Root Biomass (g)	Shoot Biomass (g)
Species	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model selection and fit							
Distribution	lognormal	lognormal	Gamma	Lognormal	lognormal	lognormal	Gamma
AICc	−18.18	−9.27	−31.06	−41.43	−39.88	−57.06	−62.90
R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Estimated marginal means by species							
ASP	14.90a	6.70c	2.55d	4.15a	5.75b	1.72c	4.03a
BRK	12.44c	5.36e	2.02f	3.33c	0.69g	0.18e	0.52g
CAE	13.08b	2.59g	2.03f	0.56g	3.20d	1.68c	1.52d
EHL	11.49e	4.55f	2.01f	2.53e	1.00f	0.15e	0.85f
HLW	11.25f	5.50e	2.25e	3.26cd	5.48c	2.51b	2.97b
PSC	11.18f	6.91b	2.96c	3.95b	1.61e	0.58d	1.02e
TPG	12.08d	6.06d	3.98b	2.08f	6.15a	3.72a	2.43c
TSH	12.16d	11.19a	8.02a	3.17d	0.57h	0.10f	0.48h

Note: Different letters indicate significant differences among species ($p < 0.05$). PSC: *Pseudelephantopus spiralis* (Less.) Cronquist; BRK: *Bidens riparia* Kunth; TSH: *Tripogandra serrulata* (Vahl) Handlos; EHL: *Euphorbia heterophylla* L.; TPG: *Talinum paniculatum* (Jacq.) Gaertn; HIW: *Hilleria latifolia* (Lam.) H. Walter; CAE: *Cyperus aggregatus* (Willd.) Endl; ASP: *Acalypha stachyura* Pax.

Fig. 2 illustrates the overall relationships between soil Pb content and weed biomass, modeled using generalized linear models with spline functions based on different bases and polynomial degrees. Fig. 2A shows the relationship between soil Pb concentration and Pb accumulation in weed species (R^2 = 0.67, df = 4, degree = 2). At lower soil Pb levels, *E. heterophylla*, *H. latifolia*, *B. riparia* and *T. paniculatum* were recorded and were associated with lower Pb concentrations; however, *T. serrulata*, even within this lower range, exhibited the highest Pb concentration among the species. Soils with intermediate Pb concentrations were represented by *C. aggregatus*, which showed the lowest Pb values, whereas soils with the highest Pb levels were associated with the presence of *A. stachyura*, which exhibited intermediate Pb concentrations.

Fig. 2B describes the relationship between total biomass and Pb concentration in weed species (R^2 = 0.28, df = 2, degree = 2). Species with lower biomass, such as *E. heterophylla*, *B. riparia* and *P. spiralis*, exhibited intermediate Pb concentrations; however, *T. serrulata*, despite belonging to this low-biomass group, recorded the highest Pb. Conversely, *C. aggregatus* showed intermediate biomass levels but the lowest Pb content, whereas *A. stachyura*, *T. paniculatum*, and *H. latifolia* were characterized by higher biomass and intermediate Pb concentrations. These patterns indicate that metal accumulation is not solely dependent on plant size, but rather on internal processes of uptake, transport, and partitioning associated with species-specific traits and edaphic metal availability, as widely documented in the phytoremediation literature [32].

Fig. 2C shows the relationship between shoot biomass and shoot Pb concentration (R^2 = 0.66, df = 4, degree = 2). Species with lower shoot biomass included *C. aggregatus*, which also recorded the lowest shoot Pb concentration, as well as *E. heterophylla*, *T. serrulata*, and *B. riparia*, which exhibited intermediate shoot Pb concentrations. Notably, *P. spiralis*, despite belonging to this low-shoot-biomass group, showed one of the highest Pb concentrations in aerial tissues. In contrast, *T. paniculatum* and *H. latifolia* displayed intermediate shoot biomass values accompanied by intermediate Pb concentrations, whereas *A. stachyura*, characterized by the highest shoot biomass, ranked among the species with the highest shoot Pb concentration.

Finally, Fig. 2D illustrates the relationship between root biomass and root Pb concentration (R^2 = 0.36, df = 2, degree = 2). Species with lower root biomass, such as *E. heterophylla*, *B. riparia*, and *P. spiralis*, exhibited the lowest root Pb concentrations. Likewise, species with intermediate root biomass values, including *C. aggregatus*, *A. stachyura*, and *H. latifolia*, as well as *T. paniculatum*, which exhibited the highest root biomass, were among the species with the lowest root Pb concentrations. However, *T. serrulata*, despite belonging to this group, showed the highest root Pb concentration.

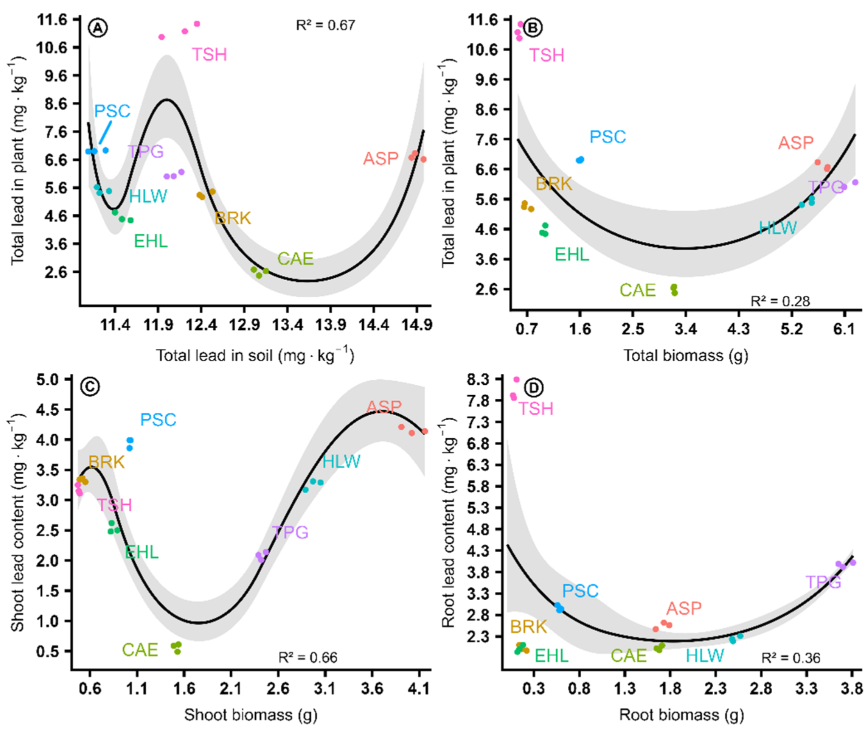

Figure 2: Functional relationships between: Ⓐ total Pb concentration in soil and total Pb concentration in the plant; Ⓑ total plant biomass and total Pb concentration in the plant; Ⓒ aboveground biomass and Pb concentration in the shoot; and Ⓓ root biomass and Pb concentration in the root. Colored points represent observations for each species. Solid black lines correspond to generalized linear model fits using spline functions, and the shaded areas represent the model confidence intervals. The coefficient of determination (R^2) indicates the goodness of fit between the evaluated variables. Species abbreviations: PSC: *Pseudelephantopus spiralis* (Less.) Cronquist; OCM: *Ocimum campechianum* Mill; BRK: *Bidens riparia* Kunth; TSH: *Tripogandra serrulata* (Vahl) Handlos; EHL: *Euphorbia heterophylla* L.; TPG: *Talinum paniculatum* (Jacq.) Gaertn; CAE: *Cyperus aggregatus* (Willd.) Endl.; MCG: *Malvastrum coromandelianum* (L.) Garcke.

3.4 Phytoremediation Potential Based on BCF and TF Factors

Fig. 3 shows the functional classification of the evaluated species according to their bioconcentration factor (BCF) and translocation factor (TF) for Cd and Pb, which allowed the identification of their potential roles as phytoextractors, bioaccumulators, or phytostabilizers.

For Cd (Fig. 3A), the evaluated species were distributed across three of the four quadrants defined by the BCF = 1 and TF = 1 thresholds. Species classified as phytoextractors (TF > 1 and BCF < 1) included *T. paniculatum* (TPG), *P. spiralis* (PSC), *C. aggregatus* (CAE), *B. riparia* (BRK), *E. heterophylla* (EHL), and *M. coromandelianum* (MCG). The phytostabilization quadrant (BCF > 1 and TF < 1) was represented by *O. campechianum* (OCM), indicating a predominant root retention of the metal and limited internal translocation. In contrast, no species were positioned within the bioaccumulator quadrant (BCF > 1 and TF > 1). Finally, *T. serrulata* (TSH) was located in the non-classified quadrant (TF < 1 and BCF < 1).

In the case of Pb (Fig. 3B), the distribution pattern of the species reflected a more pronounced differentiation of phytoremediation potential compared with Cd. *B. riparia* (BRK), *A. stachyura* (ASP), *H. latifolia* (HLW), *P. spiralis* (PSC), and *E. heterophylla* (EHL) were positioned within the phytoextraction quadrant (BCF < 1 and TF > 1). In contrast, no species were classified within the bioaccumulator (BCF > 1 and TF > 1) or phytostabilization (BCF > 1 and TF < 1) quadrants, suggesting a limited capacity of the evaluated species to combine high accumulation or effective root retention of Pb under the conditions of this study. Finally, *C. aggregatus* (CAE), *T. paniculatum* (TPG), and *T. serrulata* (TSH) were located in the non-classified quadrant (BCF < 1 and TF < 1).

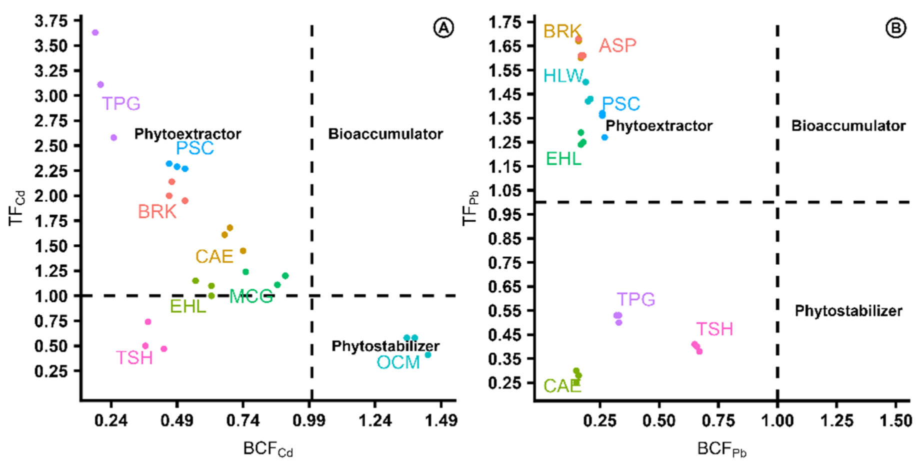

Figure 3: Functional classification of the evaluated weed species according to their phytoremediation potential, based on the Bioconcentration Factor (BCF) and the Translocation Factor (TF) for Cd and Pb. Ⓐ Distribution of species as a function of BCF and TF for Cd; Ⓑ corresponding distribution for Pb. Dashed lines at BCF = 1 and TF = 1 delineate the quadrants associated with phytoextraction, phytostabilization, bioaccumulation, and unclassified categories. Points represent the observed values for each species, and the abbreviations correspond to the evaluated species listed as follows: PSC: *Pseudelephantopus spiralis* (Less.) Cronquist; OCM: *Ocimum campechianum* Mill; BRK: *Bidens riparia* Kunth; TSH: *Tripogandra serrulata* (Vahl) Handlos; EHL: *Euphorbia heterophylla* L.; TPG: *Talinum paniculatum* (Jacq.) Gaertn; CAE: *Cyperus aggregatus* (Willd.) Endl; MCG: *Malvastrum coromandelianum* (L.) Garcke; HLW: *Hilleria latifolia* (Lam.) H. Walter; ASP: *Acalypha stachyura* Pax.

4 Discussion

Numerous studies have documented the bioremediation potential of weed species growing in soils contaminated with heavy metals [28,30,56]. In the present study, weed species associated with cacao cultivation were identified, which are naturally adapted to local edaphoclimatic conditions. This represents a clear advantage over introduced species commonly used in phytoremediation programs [32]. The recorded species were distributed across eight botanical families, with Asteraceae, Euphorbiaceae, Cyperaceae, and Malvaceae being the most representative. Species from these families have been widely reported in disturbed environments and in soils contaminated with heavy metals [57]. This floristic composition suggests a plant community exhibiting adaptive strategies linked to physiological resilience under environmental stress conditions.

Several of the identified species exhibit wide spatial and ecological distributions. *P. spiralis* and *B. riparia* show variation in their occurrence across zones, which has been associated with geographic and seasonal conditions [58]. *E. heterophylla* has been reported to grow in heavy metal–contaminated soils without significant impairment of its development, a response that may be linked to its rapid growth rate and invasive behavior [59,60]. *A. stachyura* has been documented across several regions of Peru, including Junín and Pasco, occurring between 100 and 1300 m a.s.l. in humid forest ecosystems [61]. Species of the genus *Ocimum* have been reported to possess the ability to block key ion transport channels involved in root-to-shoot translocation processes [62]. *T. serrulata* is a short-lived annual species widely distributed in warm and temperate regions, ranging from sea level up to 1600 m a.s.l [63,64]. *T. paniculatum*, broadly distributed throughout South America between 0 and 2000 m a.s.l., is characteristic of disturbed environments and can reach heights of up to 60 cm [65]. *H. latifolia* is a perennial herb commonly found in South America and tropical Africa, with stems that may reach up to 2 m in height [66]. *C. aggregatus* shows high adaptability to wet environments and has demonstrated the ability to establish in soils contaminated with hydrocarbons [67,68]. Finally, *Malvastrum coromandelianum* (L.) Garcke is frequently found in cultivated fields and disturbed areas and exhibits broad tolerance to varying light conditions [69].

Regarding biomass production, significant differences were observed between aboveground and belowground organs, with *T. paniculatum* standing out for its highest total biomass (6.15 g) (Table 3). *A. stachyura* and *M. coromandelianum* exhibited the greatest aboveground biomass (4.03 g and 3.30 g, respectively), with *A. stachyura* showing the highest Pb concentration in this compartment (4.15 mg kg^{-1}). Similarly, *T. paniculatum* and *H. latifolia* were characterized by high root biomass (3.72 g and 2.51 g, respectively), with *T. paniculatum* displaying one of the highest Pb concentrations in roots (3.98 mg kg^{-1}). *C. aggregatus* with an intermediate biomass weight, showed the highest Cd level in the whole plant (2.68 mg kg^{-1}). Biomass allocation among plant organs is governed by genotypic traits modulated by plant–environment interactions and depends on factors such as leaf area, climate, nutrient availability, and assimilate fluxes [70]. In species with phytoremediation potential, biomass production is as relevant as the ability to tolerate or accumulate heavy metals [71,72], since hyperaccumulator species such as *Thlaspi caerulescens* and *Arabidopsis halleri* present practical limitations due to their low biomass production [27]. In contrast, cacao-associated weed species such as *Cissus verticillata* have shown operational advantages owing to their higher biomass in soils with elevated Cd concentrations [73].

Phytoremediation potential was further assessed using the bioconcentration factor (BCF) and the translocation factor (TF), which are widely applied indicators for classifying the functional behavior of plants in response to heavy metal contamination [54,74]. According to Mellem et al. [51] and Hidayati et al. [32], BCF and TF values greater than 1 indicate a bioaccumulative behavior, whereas a BCF value exceeding 2 reflects high accumulation efficiency. In addition, a BCF > 1 combined with a TF < 1 is associated with phytostabilization, while a BCF < 1 and TF > 1 suggests phytoextraction potential.

In this study, significant differences in BCF and TF values for Cd and Pb were observed among the evaluated species (Fig. 3), revealing behaviors consistent with phytostabilization and phytoextraction strategies. *O. campechianum* exhibited a pattern compatible with Cd phytostabilization (BCF < 1, TF > 1), characterized by accumulation in roots and limited translocation to aerial tissues, a mechanism that contributes to metal immobilization in the rhizosphere [75]. Although no previous studies have directly evaluated this species for phytoremediation purposes, related evidence has been reported for *O. basilicum*, which has been described as a Cd bioaccumulator in roots while preserving essential oil quality [76,77]. For Pb, none of the evaluated species met the established criteria for phytostabilization. *C. aggregatus*, *T. serrulata*, and *T. paniculatum* did not fully conform to the classical functional categories. However, *T. serrulata* has been reported to exhibit enhanced root Pb accumulation, suggesting an exclusion behavior [78], which may be useful for metal stabilization and reducing trophic transfer. In the case of *C. aggregatus* although direct evidence is lacking, related species such as *C. elegans* have shown tolerance to Cd and enhanced root growth following inoculation with rhizobacteria [79].

P. spiralis, *B. riparia*, *E. heterophylla*, *M. coromandelianum*, *C. aggregatus*, and *Talinum paniculatum* exhibited patterns compatible with phytoextraction potential for Cd. Whereas for Pb, *P. spiralis*, *B. riparia*, *E. heterophylla*, *H. latifolia*, and *A. stachyura* were the most prominent species. Nevertheless, phytoremediation effectiveness also depends on additional attributes such as vigorous growth, biomass production, root system architecture, metal tolerance, and agronomic management [80,81], as well as metal bioavailability in the rhizosphere and its translocation to aboveground biomass [82]. In this study, the soil exhibited a pH of 6.3, a slightly acidic range that favors Cd availability and plant uptake [83], which may partly explain the observed responses. Likewise, the organic matter content (4.1%) plays a key role in metal bioavailability, as it modulates complexation and adsorption processes that can reduce the fraction available for plant uptake [84]. Therefore, the BCF and TF values obtained should be interpreted within the specific edaphic conditions of the study site, as they may vary considerable across cocoa-growing with different physicochemical characteristics, particularly as a function of soil pH and organic matter content.

The behavior of *P. spiralis* and *B. riparia* (Asteraceae) is particularly noteworthy, as both species are widely documented for their phytoextraction capacity [85–88]. The genus *Bidens* has demonstrated accumulation potential for both Cd and Pb [89–93], in addition to contributing to improvements in soil structure and fertility. *P. spiralis* has been classified as a Cd hyperaccumulator in soils with pH values between 6 and 7, possibly due to the release of root exudates that acidify the rhizosphere and enhance metal bioavailability [73]. *E. heterophylla* exhibits differential affinity for metals in the order Cd > Hg > Pb > As [59], whereas *A. stachyura* has been identified as a Pb accumulator, with antioxidant mechanisms that mitigate metal toxicity [94,95]. In contrast, *M. coromandelianum* has been reported as a phytostabilizer in industrial soils contaminated with Pb and Cu [56], a behavior that differs from the patterns observed in the present study. Finally, *T. paniculatum* stood out by exhibiting the highest TF value for Cd, consistent with previous reports highlighting its rhizofiltration potential, tolerance to edaphic stress, and capacity for the degradation of organic contaminants [96,97].

Similarly, it is important to emphasize that the practical implementation of these species in association with cocoa cultivation should be evaluated through controlled experimental trials that integrate both remediation efficiency and ecological and economic impact. The development of specific management protocols will be essential to ensure that environmental benefits outweigh potential risks and that the strategy remains viable in terms of productive sustainability. Although these weed species may pose certain challenges, previous studies have reported favorable outcomes. For instance, interspecific competition (i.e., the coexistence between weeds and cocoa under regulated management) has been shown to improve soil structure and enhance moisture retention [28]. Additionally, the propagation capacity of weeds can be harnessed as ground cover to reduce soil erosion [30], while metal-enriched biomass may be repurposed for biochar production or composting [20].

Overall, this study enabled the identification of weed species associated with cacao cultivation that exhibit traits compatible with potential phytoextraction or phytostabilization performance. Although the present results do not constitute direct functional evidence of remediation, they expand current knowledge of spontaneous vegetation in cacao-based systems and provide a technical foundation for the design of controlled experiments aimed at quantifying their actual contribution to the mitigation of heavy metals in agricultural soils. In this context, the findings strengthen the prospect of biologically based solutions applicable to one of the major challenges facing the cacao sector.

5 Limitations

Although these results allow the identification of promising patterns of Cd and Pb accumulation and translocation in weed species associated with cacao cultivation, their interpretation should consider an inherent limitation of the study design. While the research initially involved the assessment of seven cacao-producing sites, the identification and detailed analysis of species were ultimately focused on the site exhibiting the highest Cd concentration. This approach is consistent with the explanatory nature of the study, which aimed to maximize the detection of weed weed species with potential accumulation rate. Targeted sampling strategies in highly contaminated sites have been employed in previous phytoremediation prospecting studies [28,30,56], as they enable the observation of functional responses under real stress conditions. The findings are not intended to be generalizable across all edaphoclimatic conditions of the region; rather, they provide a technical basis for the development of future studies incorporating experimental designs that include multiple sites and management. Therefore, the observed patterns should be interpreted with caution and validated in subsequent multisite and controlled studies to assess their consistency and applicability across different cacao production systems. BCF and TF values obtained should be interpreted within the specific edaphic conditions of the study site, as these indicators may vary considerably in cacao soils with different physicochemical properties, particularly as a function of soil pH and organic matter content. This underscores the importance of subsequent controlled trials to demonstrate the removal of heavy metals under those conditions. In this context, it is essential to develop experimental studies that quantify the removal of Cd and Pb from soils over time and, across different plant growth stages, as well as to evaluate extraction or stabilization efficiency as a function of metal bioavailability and edaphic conditions This approach will enable validation of the patterns inferred from bioconcentration and translocation factors and support their application in sustainable strategies for the management of contaminated soils.

6 Conclusions

The present study enabled the identification of spontaneous weed species associated with cacao cultivation that exhibit contrasting patterns of biomass production, bioconcentration, and translocation of Cd and Pb, revealing behaviors consistent with potential phytoextraction and phytostabilization strategies. The integrated assessment of plant biomass production together with bioconcentration factor (BCF) and translocation factor (TF) proved effective for the functional discrimination of the evaluated species under field conditions. Among the analyzed weeds, *P. spiralis*, *B. riparia*, *E. heterophylla*, *M. coromandelianum*, *C. aggregatus* and *T. paniculatum* exhibited patterns compatible with potential Cd phytoextraction, whereas *P. spiralis*, *B. riparia*, *E. heterophylla*, *H. latifolia* and *A. stachyura* showed similar responses with respect to Pb. In contrast, *O. campechianum* displayed a behavior consistent with Cd phytostabilization, characterized by metal retention in the root system and limited translocation to aerial tissues. Biomass production emerged as a key component for interpreting phytoremediation potential, complementing the information provided by BCF and TF indices. In this context, species that combine tolerance to heavy metals with higher biomass production appear viable candidates, for future experimental evaluations. Although the results obtained do not constitute functional evidence of soil remediation, they provide relevant insights into the behavior of native weed species under the edaphoclimatic conditions of cacao agroecosystems. These findings expand current knowledge on spontaneous vegetation in cacao-based systems and establish a technical basis for the design of controlled trials aimed at evaluating the actual efficiency of these species in heavy-metal management and mitigation strategies in agricultural soils.

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