



Cadmium accumulation and allocation in different cacao cultivars

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HIGHLIGHTS

- Available soil Cd was closely related to Cd in vegetative parts but less to bean Cd.
- Soil factors could not fully explain the variation in bean Cd.
- Cd translocation into beans showed a significant influence of the cultivar.
- Translocation of Cd into cacao beans could be limited by selecting appropriate cultivars.

GRAPHICAL ABSTRACT

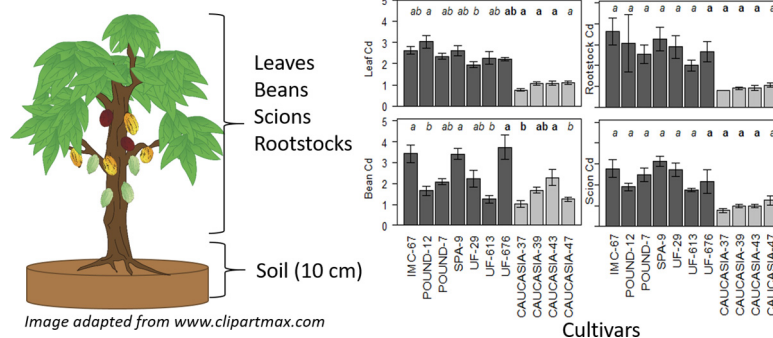


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ABSTRACT

Cadmium (Cd) is a biologically non-essential heavy metal that can cause toxic effects in plants, animals and humans already at low concentrations compared to other metals. After Cd concentrations in cacao beans of various provenances, particularly from Latin America, were found to exceed the new regulations enforced by the European Union in 2019, there is an urgent need to find measures to lower Cd accumulation in cacao beans to acceptable values. In this research, the long-term cacao cultivar trial CEDEC-JAS in northern Honduras was used to investigate differences between 11 cultivars in Cd uptake and translocation. Sampling of various plant parts, including rootstocks, scions, leaves and beans, from three replicate trees per cultivar and the soil around each tree was conducted at this site. Results indicate that concentrations of available soil Cd were more closely correlated with Cd concentrations of the rootstocks ($R^2 = 0.56$), scions ($R^2 = 0.59$) and leaves ($R^2 = 0.46$) than with bean Cd concentrations ($R^2 = 0.26$). In addition, Cd concentrations of rootstocks, scions and leaves showed close relationships to available soil Cd concentrations, with no significant differences between the cultivars. In contrast, bean Cd concentrations showed only weak correlations to available soil Cd and Cd concentrations in the vegetative plant parts, but significant variation among cultivars. Three cultivars, which were analysed in more detail, showed significant differences in Cd concentrations of mature beans, but not of immature beans. These results suggest that cultivar-related differences in bean Cd concentrations primarily result from differences in Cd loading during bean maturation, possibly due to cultivar-specific differences in the xylem-to-phloem transfer of Cd. The results show that selection of cultivars with low Cd transfer from vegetative parts into the beans has high potential to keep Cd accumulation in cacao beans at levels that are safe for consumption.

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1. Introduction

Cadmium (Cd) is a trace metal occurring naturally in soils due to weathering of Cd-containing parent material or volcanic input (Adriano, 2001). Other sources of Cd in soils are anthropogenic inputs, in particular with agricultural applications of phosphate fertilizers and bio-wastes, mining activities or aerial deposition (McLaughlin and Singh, 1999). Compared to other metals, Cd is quite mobile in soils and readily taken up by plants, although it does not have any essential metabolic functions (Benavides et al., 2005). On the contrary, accumulation of Cd can lead to toxic effects in plants, animals and humans already at low concentrations compared to other metals (Kabata-Pendias, 2001).

Since maximum values for Cd concentrations in cocoa-based products has been enforced in the European Union since 2019 (The European Commission, 2014), Cd uptake by cacao (*Theobroma cacao* L.) has received increasing attention in recent years, and a number of field studies have been carried out, in particular to identify the factors responsible for the high Cd concentrations found in cacao beans of many provenances from Latin America (Arevalo-Gardini et al., 2017; Barraza et al., 2017; Chavez et al., 2015; Ramtahal et al., 2015; Ramtahal et al., 2016; Gramlich et al., 2017; Lewis et al., 2018; Arguello et al., 2019; Zug et al., 2019). Whereas correlations between bean Cd and total soil Cd concentrations were generally weak, four studies found that between 50 and 72% of the variance in bean Cd concentrations was explained by available soil Cd concentrations, using various extraction methods (Mehlich 3, EDTA, DTPA, ammonium-bicarbonate-DTPA, HCl) or the diffusion-in-thin-films (DGT) method to determine available soil Cd (Chavez et al., 2015; Gramlich et al., 2018; Ramtahal et al., 2015; Arguello et al., 2019). While the relationship between Cd in leaves and beans showed large variation between different studies (ranging from $R^2 = 0.37$ (Lewis et al., 2018) to $R^2 = 0.9$ (Arguello et al., 2019)), available soil Cd was found to correlate more strongly with leaf than bean Cd concentrations. This indicates that a significant part of the variability in bean Cd was due to variation in physiological factors governing the translocation and allocation of Cd within the cacao plants (Gramlich et al., 2017; Gramlich et al., 2018; Barraza et al., 2017). Part of this variance may be related to the cultivation of different cacao cultivars and thus be due to variation in genetic factors (He et al., 2015). However, little is known about their contribution to the observed variability in leaf and bean Cd accumulation by cacao, as the role of cultivars was not or could not be studied in most of the previous studies.

Given that there is wide variation in the uptake and allocation of trace metals including Cd, not only between different plant species but often also among different genotypes or varieties of the same species (Broadley et al., 2001; Grant et al., 1997; Zhou et al., 2016), our hypothesis is that also cacao cultivars show variation in Cd accumulation and that this may offer an opportunity to reduce bean Cd concentrations in cacao production to safe levels through breeding and selection of appropriate cultivars. Variation in Cd uptake among cultivars has been studied primarily in annual crops (Arao et al., 2008; Grant et al., 1997), but much less in perennial crops. Especially for grafted trees, only little information on variation in Cd uptake among cultivars is available. In grafted crops, also the cultivar of the rootstock needs to be considered, as the root system plays a crucial role in metal uptake from the soil (Albacete et al., 2015; Savvas et al., 2010). Grafting *Malus*, *Prunus* and *Citrus* trees (which, like cacao, belong to the clade of Rosids) on rootstocks from different cultivars was found to have a strong influence on copper (Cu) concentrations in the scions (Francini and Sebastiani, 2010; Liu et al., 2011; Mozaffari et al., 1996). In a recent study on grafted apple trees, it was observed that the rootstocks of some cultivars accumulated less Cd than others, explaining lower Cd concentrations also in their aerial parts (Zhou et al., 2016). Only three studies regarding the role of cultivars in Cd uptake in cacao were found. The first one was a greenhouse study on cacao seedlings (Cryer and Hadley, 2012),

the second study, a field survey in Peru comparing bean and leaf Cd concentrations of cacao trees grafted on rootstocks of different cultivars (Arevalo-Gardini et al., 2017), and third a study using a cacao field trial at the International Cacao Genebank in Trinidad comparing leaf and bean Cd concentrations among 77 cultivars (Lewis et al., 2018). All three studies found significant variation in Cd uptake between different cultivars.

There is not only variation in Cd uptake from soil among plant species and cultivars, but also in the allocation of Cd in plant parts and tissues (Zhou et al., 2016; Clemens et al., 2013). While most plants accumulate more Cd in the roots than in the leaves and even less in their seeds (Clemens et al., 2013), root-to-shoot transfer shows large variability (Guo and Marschner, 2008). In trees, the Cd concentrations of leaves were mostly found to exceed those of the woody tissues of the trunks, but not always (Evangelou et al., 2013; Parraga-Aguado et al., 2014; Chen et al., 2015; Dai et al., 2013). Considerable variation in Cd concentrations can also be found within the same type of tissue in a plant. For example, Cd concentrations of old leaves were found to exceed those of young leaves in *Salix viminalis* L. (Cosio et al., 2006) and in *Populus tremula* L., indicating that Cd had been accumulated over time (Vollenweider et al., 2011). But also the opposite has been found; higher Cd concentrations in young than in old leaves, e.g. in *Brassica juncea*, *Thlaspi caerulescens* (Salt et al., 1995) and *Populus* (Gu et al., 2007). Very little is still known about Cd allocation in cacao trees. Some studies found that leaf Cd concentrations were higher than bean Cd (Arevalo-Gardini et al., 2017; Barraza et al., 2017; Fauziah et al., 2001; Gramlich et al., 2017; Gramlich et al., 2018; Ramtahal et al., 2016; Lewis et al., 2018; Arguello et al., 2019), while the opposite was reported in a study from Ecuador (Chavez et al., 2015). In particular, the study of Lewis et al. (2018) provides evidence that also genetic factors play a major role in the variation of Cd allocation patterns in cacao apart from site conditions.

A major problem in identifying the role of factors that control Cd accumulation in cacao beans is the amount of time, space and expenditures required to conduct controlled factorial trials that can be translated to field conditions, as trees do not only require much more space than herbs, but also several years before they produce fruits. In addition, the performance comparison of cultivars grown at different sites faces the problem of separating cultivar from site effects. One of the few field trials where a collection of cacao cultivars are grown side by side on the same experimental site is run by the Honduran Foundation for Agricultural Research (FHIA) in northern Honduras. At this site, 11 different common cacao cultivars were used in this study to compare Cd accumulation and allocation in trees. Cadmium concentrations in their rootstocks, scions, leaves and beans were determined and later associated through multiple linear regression to available soil Cd concentrations and other properties of the soil surrounding the stems. For the three selected cultivars, root samples, scion samples taken at different heights, wood and leaf samples of young and old twigs from different positions in the crown, as well as pod husk and bean samples of mature and immature fruits were analysed. A few samples of scion cores were further subjected to laser ablation inductively-coupled plasma mass spectrometry (LA-ICPMS) in order to analyse the radial distribution of Cd in scion bark and wood. We expected to find a decreasing strength in the relationships of plant Cd concentrations to available soil Cd in the sequence from rootstock, scion, leaf to bean samples and an increasing strength along the same sequence in the relationships between plant Cd concentrations and cultivar.

2. Materials and methods

2.1. Site description

The sampling was carried out in October 2016 just before the beginning of the wet season at the research station “Centro Experimental y Demonstrativo de Cacao ‘Jesus Alfonso Sánchez’” (CEDEC-JAS) in

northern Honduras, which is located at an altitude of 18 m a.s.l. near the village La Masica in the department of Atlántida (15°38'43.5" N 87°05'59.5" W). The regional climate is classified as equatorial monsoon according to Köppen–Geiger (Kottek et al., 2006). The annual mean temperature varies between 20 and 35 °C and the monthly precipitation between 100 mm in spring and close to 500 mm in fall (Gramlich et al., 2018).

The experiment station, which was established in 1986, is operated by the FHIA. On several plantations, covering a total area of 43 ha, many different cacao varieties are grown in an agroforestry system in combination with 35 other tree species, mostly *Swietenia macrophylla*, *Cordia megalantha*, *Terminalia superba*, *Tabebuia rosea*, *Guarea grandifolia* and *Ilex tectonica*. The cacao trees were planted in parallel rows with one cultivar per row. All cacao trees were grafted onto rootstocks of unknown genetic identity. There was no irrigation system in place and no pesticides were used. Only mineral fertilizer was applied on a yearly basis. In 2016, a mixture of 136 kg N-P-K (15–15–15), 45.4 kg ammonium nitrate and 45.4 kg potassium chloride was applied. All plots were managed in the same way.

2.2. Sampling scheme

In October 2016, rootstocks, scions, leaves and beans were sampled from three replicate trees from each of 20 different cultivars from 7 different plots at the CEDEC-JAS trial. In addition, soil samples were taken around the stems of all sampled trees. The trees were selected based on the presence of mature fruits at the time of sampling. Based on the soil analysis, 11 cultivars grown on soils with similar available soil Cd concentrations were selected for the statistical analysis: 7 cultivars from the plot "Jardin Clonal" (JC) and 4 from the plot "Prueba Multilocal" (PM) (Fig. 1). The two plots are approximately 300 m apart. Three of the 11 cultivars, i.e. SPA-9, POUND-7 and IMC-67, were chosen for additional sampling to study Cd allocation patterns. The replicated trees of these three cultivars were located in three blocks, each containing one replicated tree of each cultivar in close proximity to each other (Fig. 1). The Cd and soil data for all sampled cacao trees are given in the Appendix in Tables A.1 and A.2.

2.2.1. Sampling procedure for cultivar screening

Around each tree, 8 soil cores were taken at a distance of 70 cm from the trunk at a depth of 0 to 10 cm using a 5 cm steel corer and combined

to one composite soil sample per tree. Two 5 cm deep wood cores were extracted on opposite sides from each rootstock (just below the soil surface) and 2 cores from the scion at 30 cm above soil surface with a 5 mm increment borer. In total, 10 leaves from 5 different branches from each tree were collected. Twigs that were branching off from the stem into different directions were chosen for sampling and the 9th and 10th leaf counted from the tips of these branches were collected following the procedure of (Gramlich et al., 2017; Gramlich et al., 2018). Finally, 2–4 mature fruits were collected from each tree. The pod husks were separated from the beans and discarded.

2.2.2. Sampling for additional analysis of three selected cultivars

From the trees of the cultivars SPA-9, POUND-7 and IMC-67, 4 root branches per tree at a distance of 70 cm from the trunk and a depth of 10 cm were collected. Additional wood cores at 120 and 170 cm above the soil surface, 3 branches with young and 3 with mature leaves, as well as 3 mature and 2 immature fruits were also gathered. Young and mature leaves were sampled from 3 positions each in the crown: one branch each from a high, sun-exposed position, one from a middle position, and one from a low position with no sun exposure. The root samples were pooled into one composite root sample per tree. The first six leaves of each branch, counting from the tip, were separated from the wooden axes of the branches and combined into one composite leaf sample of the respective branch. The wooden part of the branch from the tip to the 6th leaf was taken as branch wood sample. The fruits were separated into beans and husks. The immature fruits aged between 40 and 105 days.

2.3. Soil and plant analyses

Soil samples were oven-dried at 40 °C for at least 96 h and sieved to the ≤2 mm fraction. Soil pH was measured in suspension with deionized water (1:2 soil:liquid ratio). Organic matter content was determined using the Walkley-Black method (Nelson and Sommers, 1996). Soil texture was analysed using the Bouyoucos method (Bouyoucos, 1962). To determine 'total' soil metal concentrations, 1 g of soil was weighed into digestion tubes and 2 mL Nanopure water, 2 mL HNO₃ (65%) and 6 mL HCl (37%) were added. The tubes were heated on a DigiPREP digestion system with a hot plate for 90 min at 120 °C. Extracts were diluted up to 50 mL with Nanopure water and filtered with 20 µm filter paper (Whatman, No. 41).

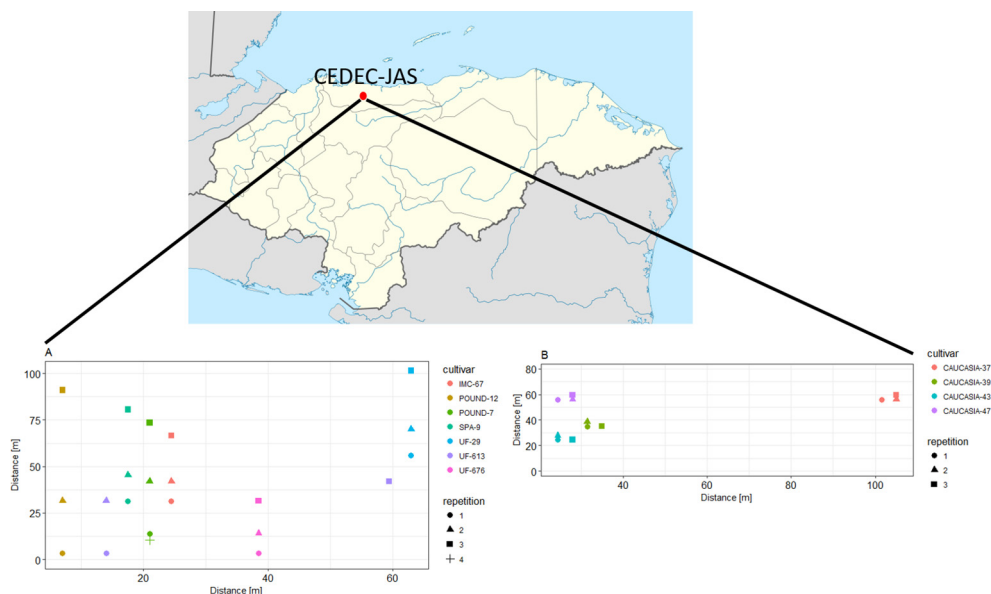


Fig. 1. Location of the CEDEC-JAS trial in Honduras (map from https://commons.wikimedia.org/wiki/File%3AHonduras_location_map.svg) and positions of the sampled cacao trees on the two experimental plots Jardin Clonal (A) and Prueba Multilocal (B). Symbols indicate cultivars as defined in the legend.

'Available' soil metal concentrations were determined using NaNO_3 (FAL RAC and FAW, 1996). Subsamples of 10 g each were suspended in 25 mL 0.1 M NaNO_3 , shaken for 2 h and passed through a 0.45 μm cellulose acetate filter. Nanopure water was added to each extract until its volume was 10 mL.

Root samples were washed with deionized water and air-dried. All other plant samples were oven-dried at 70 °C for at least 72 h. All plant samples were milled with a Retsch Mill (Vibratory Disc Mill RS 1). For Cd extraction, 50 mg (bean samples) or 100 mg (all other samples) aliquots of milled tissue were weighed into microwave Teflon tubes. After adding 2 mL H_2O_2 (30%) and 1 mL HNO_3 (65%), the samples were digested in a microwave (MLS-1200 MEGA ETHOS) for ~25 min at a maximum temperature of 220 °C. For chemical analysis, the digests were diluted to a sample volume of 10 mL with Nanopure™ water.

All plant and soil extracts were analysed for Cd, the soil extracts also for Zn and the macronutrient elements Ca, Mg and K, using ICP-MS (Agilent 7900, Agilent Technologies, USA). For quality control we used certified WEPAL (Wageningen Evaluating Programmes for Analytical Laboratories) reference materials: IPE-180 (oil palm leaf) and IPE-199 (wood pellets) for the plant samples and ISE-958 (sandy soil) and ISE-992 (sandy clay soil) for the soil samples. Respective recovery rates for Cd were $88 \pm 6\%$, $105 \pm 3\%$, $112 \pm 7\%$ and $135 \pm 12\%$.

2.4. LA-ICPMS analyses of tree stem cores

LA-ICPMS analyses were carried out using an ArF excimer laser ablation system (Gunther et al., 1997) (193 nm, GeoLas C, Lambda Physik, Goettingen, Germany) coupled to an quadrupole ICP-MS instrument (Elan DRC Plus, Perkin Elmer, Norwalk, CT). The plasma was sustained with argon gas (99.996%, PanGas AG, Dagmersellen, Switzerland). LA and ICP operating conditions are given as supplementary information in Table S3. Laser ablation was carried out in a helium atmosphere (99.999%, PanGas AG, Dagmersellen, Switzerland) in a one-volume cell as described by Fricker et al. (2011). The instrument was tuned using the following procedure: NIST SRM 610, a standard reference material glass, was ablated using a 90 μm -diameter circular spot and a laser repetition rate of 10 Hz in line scanning mode at a scan speed of 20 $\mu\text{m s}^{-1}$. Gas flow rates and ICP-MS parameters were tuned to maximize sensitivity for ^{114}Cd and ^{111}Cd while maintaining high sensitivity, a low oxide ratio ($^{232}\text{Th}^{16}\text{O}^+ / ^{232}\text{Th}^+$) and similar $^{238}\text{U}^+$ and $^{232}\text{Th}^+$ sensitivities. For calibration, a powdered tree core sample (SPA-9b) was pressed into a pellet and ablated in the same manner as the reference samples.

In total, 1000 μm line scans of three core samples were recorded. Each sample was ablated two or more times from the outer xylem to the bark. The laser was focussed on the middle position of the lines. In addition, the xylem of each sample was ablated at six positions in radial direction further to the interior of the trunk, using the same parameters as for the line scans. Gas blank signal was recorded for at least 30 s before each ablation.

2.5. Data analysis

One-way ANOVA was applied to analyse differences between the 11 cultivars sampled on the two experimental plots JC and PM and between their soils. Tukey's HSD was used as post-hoc test. This analysis was performed separately for the two plots, as they differed substantially in some soil characteristics including available soil Cd concentration. Plots of residues vs fitted values and Q-Q normal plots were inspected visually to check for homogeneity of variance and for normal distribution of the residues. One replicate location of the cultivar UF-676 was considered as an outlier based on Q-Q plot inspection and therefore not included in the statistical analysis of the data set in order to meet the criterion of normal distribution (Fig. A.1).

Multiple linear regression with stepwise variable selection (forward procedure) was used to relate available soil Cd to other soil variables. The following variables were included as explanatory variables in the

start model: total soil Cd content, soil pH, organic matter content, clay, silt and sand content, total and available Zn contents, available K, Ca, Mg, cultivar and plot. The criteria for the selection of the best model were the significance of the variables and the improvement of the Bayesian Information Criterion (BIC). The same procedure was used to model plant Cd concentrations. Here, "available soil Cd" was included as explanatory variable in the start model in addition to the other explanatory variables mentioned before.

Additional statistical analysis was performed on the data set obtained for the three cultivars SPA-9, POUND-7 and IMC-67. For multiple regression analysis of these data, we used linear mixed-effect models with random effects to account for variability in soil and other site factors between the three replicate blocks. Two-way ANOVA was used to check for tissue and genotype effects on plant Cd concentrations. Multiple comparisons were performed by means of Tukey's post-hoc test.

All statistical analyses were carried out using the software package R (R Core Team, 2014). The lme function of the nlme package was used for linear mixed-effect models (Pinheiro et al., 2014), and the HSD.test function of the agricolae package to carry out post-hoc tests (Mendiburu, 2014). The significance level was set as $p \leq 0.05$, and, if not stated otherwise, means \pm standard errors (SE) are given. Data were \log_{10} (K, Ca, Mg, Cd_{tot} , $\text{Cd}_{\text{available}}$, Zn_{tot} , $\text{Zn}_{\text{available}}$, Cd in different plant tissues) or $\sqrt{\text{}}$ (organic matter, sand, silt, clay) transformed for statistical analysis in order to meet the criterion of normal distribution.

For the analysis of the LA-ICPMS data, background and ablation data signal traces were separated and the average background signal subtracted from the ablation signal. The elemental Cd mass fraction was determined according to the standard LA external-calibration approach developed by (Longerich et al., 1996). A pressed pellet of a branch wood sample from the cultivar SPA-9b with a Cd mass fraction of 2.68 $\mu\text{g g}^{-1}$ was used as external standard. ^{13}C was selected as internal standard, assuming a homogeneous carbon mass fraction of 40%. The Cd concentrations of the samples were calculated using the equation

$$w_{\text{Cd, sample}} = \left(\frac{S_{\text{Cd, sample}} w_{\text{Cd, reference}}}{S_{\text{Cd, reference}}} \right) \left(\frac{S_{^{13}\text{C, reference}} / w_{\text{C, reference}}}{S_{^{13}\text{C, sample}} / w_{\text{C, sample}}} \right) \quad (1)$$

where w denotes mass fraction in $\mu\text{g g}^{-1}$, S the LA signal in counts per second (cps), Cd the analyte (^{111}Cd or ^{114}Cd), ^{13}C the internal standard, and *reference* the external standard. It should be noted that Eq. 1 rests on the assumption of equal relative sensitivities of the applied LA method for both, the Cd analytes and ^{13}C , in the sample tissues and in the external standard. It should furthermore be recognized that the calculated Cd mass fraction ratios depend on the assumed carbon mass fraction ratio. Diagrams were generated using a fifth-order running average.

3. Results

3.1. Soil Cd and relations to other soil characteristics

The JC and PM plots differed significantly in several soil characteristics (Table 1). Our ANOVA analysis showed that total and available soil Cd concentrations were higher and the pH lower on JC than on PM. The difference between the two plots was particularly strong in available soil Cd. It ranged between 0.11 and 0.76 $\mu\text{g/ kg}$ with an average of $0.36 \pm 0.04 \mu\text{g/ kg}$ on JC, but only between 0.07 and 0.13 $\mu\text{g/ kg}$ with an average of $0.09 \pm 0.01 \mu\text{g/ kg}$ on PM. Total soil Cd ranged from 0.41 to 1.12 mg/ kg , averaging $0.63 \pm 0.04 \text{mg/ kg}$, on JC and from 0.26 to 0.54 mg/ kg , averaging $0.39 \pm 0.04 \text{mg/ kg}$, on PM. Also, soil texture was different on the two plots, with PM having a higher sand content and JC a higher clay content. Soil texture was mostly loamy on JC, varying from sandy-clay loam to clay-loam, while all sampled PM soils were sandy loams. Nonetheless, the soil was generally less heterogeneous on

JC than on PM, as no significant differences were observed in pH, organic matter, texture and total soil Cd concentration between sampled cultivar locations on JC, while significant differences in these characteristics were found on PM. Only magnesium (Mg) concentrations showed significant differences between cultivars on JC. Almost 90% ($R^2 = 0.89$) of the variance in available soil Cd (Cd_{av}) among the sampled locations of both plots together was explained by multiple regression on total soil Cd (Cd_{tot}), soil pH and clay content (clay), while the factors plot, organic matter and cultivar showed no significant effect:

$$\log_{10}(Cd_{av}) = 2.56 - 0.67 \text{ pH} + 0.86 \log_{10}(Cd_{tot}) + 1.94 \text{ asin}(\text{sqrt}(\text{clay})) \quad (2)$$

3.2. Plant Cd concentrations and their relationship to soil Cd

On both plots, bean Cd concentrations showed similar ranges of variation with some significant differences among the cultivars (Fig. 2). In contrast, the Cd concentrations of rootstocks, scions and leaves were all higher in the samples from the JC than from the PM plot, paralleling the difference in available soil Cd between the two plots. Also, they showed less variation than bean Cd among cultivars within each of the two plots.

In the best multiple linear regression models obtained after stepwise variable selection, the factor “plot” explained most of the variance in rootstock ($R^2 = 0.74$), scion Cd ($R^2 = 0.73$) and leaf Cd ($R^2 = 0.84$), whereas the “cultivar” was the dominant factor of similar strong influence ($R^2 = 0.78$) on bean Cd. In contrast, the factor “cultivar” showed no significant influence on the residual variation of Cd concentrations in the vegetative parts. Secondary factors of significant influence in the best models were soil silt content for rootstock Cd, soil Mg concentration for scion Cd, soil Ca concentration for leaf Cd (Table 2). Taking also clay content into account slightly improved the model for bean Cd. Similarly, accounting for organic matter slightly improved the model for rootstock Cd. As the influence of these variables however was not significant, they were not included in the final models presented in Table 2. It should be noted that the explanatory variables retained in the final models were not significantly correlated among each other.

The dominant influence of the factor “plot” on the Cd concentrations of rootstocks, scions and leaves in the regression models can be attributed to a large degree to the fact that it accounted for most of the

variation in ‘available’ soil Cd among the sampled locations. As Fig. 3 shows, the overall relationships between available soil Cd and plant Cd concentrations could be described reasonably well by common regression lines. Fig. 3 furthermore shows that the correlation between ‘available’ soil Cd and the Cd concentrations of rootstocks, scions, leaves and beans decreased in the order: scions ($R^2 = 0.59$) \approx rootstocks ($R^2 = 0.56$) > leaves ($R^2 = 0.46$) > beans ($R^2 = 0.26$).

3.3. Relationships between Cd concentrations in different plant parts

Paralleling their correlation with available soil Cd, the Cd concentrations of rootstocks, scions and leaves were closely correlated among each other, while their correlation with bean Cd was much weaker (Fig. 4). Bean Cd concentrations were more closely correlated with concentrations in scions and rootstocks ($R^2 = 0.39$ and $R^2 = 0.37$, respectively) than with those in the leaves ($R^2 = 0.27$). Comparing ratios between bean Cd and Cd in the other parts, there were some significant differences among cultivars. These ratios were significantly higher on PM for leaf and scion Cd in CAUCASIA-43 than in CAUCASIA-37 and CAUCASIA-47, and higher on JC for scion Cd in UF-676 than in UF-613. On JC, they were also higher for leaf Cd in UF-676 than in POUND-7, POUND-12 and UF-613 and higher in SPA-9 than in POUND-12 and UF-613 (Fig. A.2).

On average there were no significant differences between the Cd concentrations of the different plant parts on JC (rootstocks: mean: 2.9; (range: 1.4–5.8) mg kg^{-1} DW, scions: 2.4 (1.6–3.6) mg kg^{-1} DW, leaves: 2.4 (1.7–3.5) mg kg^{-1} DW, beans: 2.5 (0.9–4.3) mg kg^{-1} DW), whereas Cd concentrations were on average higher in the beans (1.6 (0.9–2.0) mg kg^{-1} DW) than in the rootstocks (0.9 (0.8–1.2) mg kg^{-1} DW), scions (1.0 (0.8–1.7) mg kg^{-1} DW), and leaves (1.0 (0.6–1.3) mg kg^{-1} DW) on PM.

3.4. Cadmium allocation patterns in the three selected cultivars

The patterns of within-tree Cd allocation were similar in the three cultivars subjected to a more detailed analysis (Table 3). In all three, the highest Cd concentrations were found in branches and the lowest in immature beans. There was no significant difference between old and young branches, although there was a tendency towards increased Cd concentrations in the adult branches in SPA-9. Very similar Cd concentrations were also found in old and young leaves, and there was no

Table 1
General soil characteristics and soil Cd concentrations of the sampled locations, (average (\pm SE) of three replicates per cultivar) on the two experimental plots JC and PM, and ANOVA results relating to their variation within and between the two plots. Values marked with a common letter are not significantly different; “ns” indicates lack significant differences between the cultivars of the respective plot or between the averages of the two plots.

Cultivar	pH	OM	Sand	Silt	Clay	Exch. K	Exch. Ca	Exch. Mg	Cd total	Cd available
		[g/kg]	[g/kg]	[g/kg]	[g/kg]	[mg/kg]	[g/kg]	[g/kg]	[mg/kg]	[$\mu\text{g/kg}$]
JC										
IMC-67	5.3 \pm 0.2	0.46 \pm 0.05	4.16 \pm 0.41	3.63 \pm 0.3	2.21 \pm 0.18	91.2 \pm 13.4	2.31 \pm 0.1	0.48 \pm 0.0 ^{ab}	0.815 \pm 0.1	0.411 \pm 0.1
POUND-7	5.3 \pm 0.3	0.4 \pm 0.0	3.99 \pm 0.29	3.67 \pm 0.24	2.35 \pm 0.07	85.5 \pm 18.8	2.13 \pm 0.3	0.47 \pm 0.1 ^{ab}	0.73 \pm 0.1	0.327 \pm 0.1
POUND-12	5.3 \pm 0.2	0.35 \pm 0.01	4.28 \pm 0.35	3.37 \pm 0.29	2.35 \pm 0.07	184.4 \pm 44.3	2.24 \pm 0.4	0.35 \pm 0.0 ^{ab}	0.525 \pm 0.1	0.169 \pm 0.0
SPA-9	5.2 \pm 0.2	0.39 \pm 0.01	3.72 \pm 0.31	3.64 \pm 0.12	2.64 \pm 0.23	86.8 \pm 20.2	2.24 \pm 0.3	0.52 \pm 0.0 ^a	0.837 \pm 0.2	0.422 \pm 0.1
UF-29	5.2 \pm 0.2	0.38 \pm 0.02	3.81 \pm 0.13	3.77 \pm 0.18	2.41 \pm 0.07	171.1 \pm 1.3	2.02 \pm 0.0	0.43 \pm 0.0 ^{ab}	0.564 \pm 0.0	0.333 \pm 0.1
UF-613	4.8 \pm 0.2	0.37 \pm 0.05	4.39 \pm 0.71	3.16 \pm 0.32	2.45 \pm 0.43	127.0 \pm 11.4	1.65 \pm 0.2	0.33 \pm 0.00 ^b	0.453 \pm 0.0	0.528 \pm 0.2
UF-676	5.1 \pm 0.1	0.39 \pm 0.02	4.17 \pm 0.16	3.43 \pm 0.09	2.4 \pm 0.08	78.2 \pm 11	2.08 \pm 0.1	0.32 \pm 0.0 ^b	0.491 \pm 0.1	0.325 \pm 0.1
p-Value	ns	ns	ns	ns	ns	ns	ns	0.008	ns	ns
PM										
CAUCASIA-37	5.4 \pm 0.1 ^{ab}	0.22 \pm 0.02 ^c	5.08 \pm 0.50	34.9 \pm 4.4	1.43 \pm 0.07 ^b	60.9 \pm 10.6	1.81 \pm 0.2	0.14 \pm 0.0 ^b	0.296 \pm 0.0 ^{bc}	0.074 \pm 0.0
CAUCASIA-39	5.2 \pm 0.1 ^b	0.26 \pm 0.02 ^{bc}	4.52 \pm 0.53	33.3 \pm 3.3	2.15 \pm 0.24 ^a	133.6 \pm 14.4	2.17 \pm 0.2	0.31 \pm 0.0 ^a	0.257 \pm 0.0 ^c	0.134 \pm 0.0
CAUCASIA-43	5.9 \pm 0.1 ^a	0.35 \pm 0.03 ^{ab}	4.97 \pm 0.18	30.1 \pm 0.7	2.01 \pm 0.13 ^{ab}	122.7 \pm 20.6	2.78 \pm 0.3	0.34 \pm 0.0 ^a	0.538 \pm 0.1 ^a	0.081 \pm 0.0
CAUCASIA-47	5.5 \pm 0.1 ^{ab}	0.41 \pm 0.02 ^a	4.95 \pm 0.27	33.7 \pm 1.8	1.68 \pm 0.12 ^{ab}	134.6 \pm 21.8	2.64 \pm 0.2	0.37 \pm 0.0 ^a	0.480 \pm 0.0 ^{ab}	0.085 \pm 0.0
p-Value	0.014	0.002	ns	ns	0.039	ns	ns	0.002	0.006	ns
Variation between JC and PM										
p-value	0.01	0.002	0.002	ns	<0.05	ns	ns	0.001	0.001	<0.05
F-value	7.483	11.67	12.17	1.91	21.47	0.102	2.381	12.6	13.18	21.77

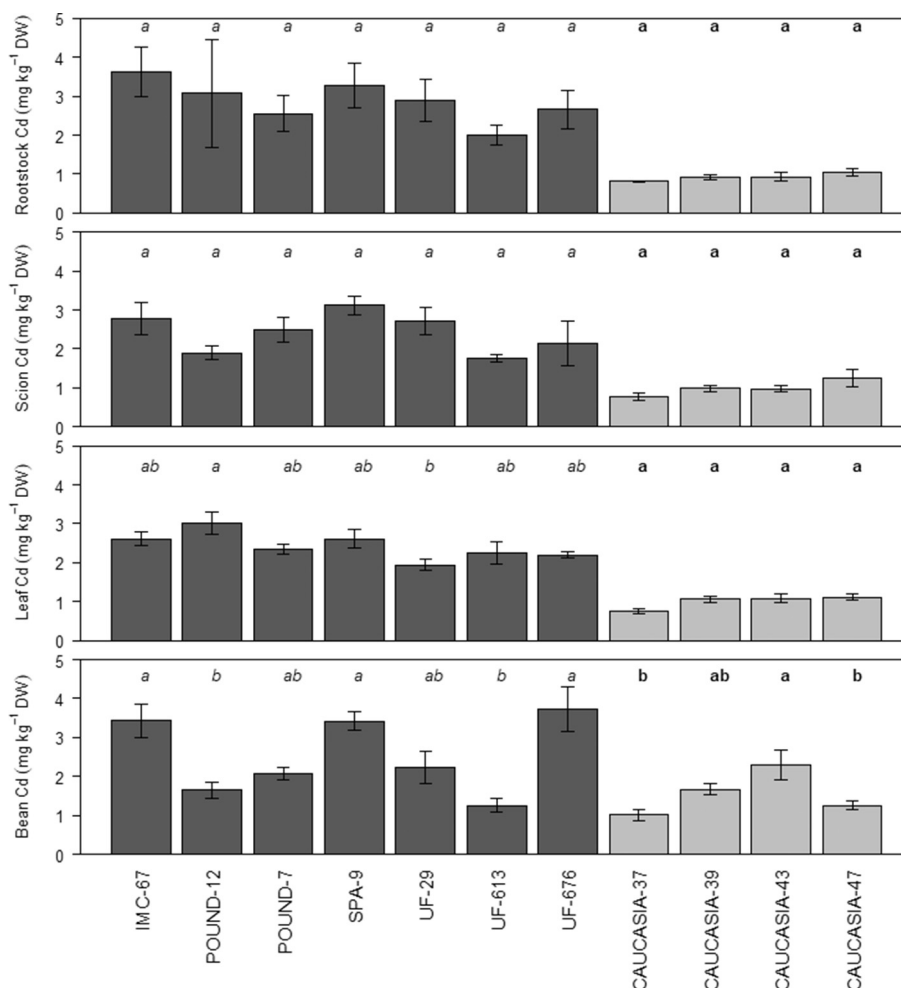


Fig. 2. Cadmium concentrations of different plants parts of cacao cultivars grown on the Jardin Clonal (dark grey) and the Prueba Multilocal (light grey) plot. Error bars represent standard errors. Different superscript letters indicate significant differences ($p < 0.05$) among cultivars within each plot (*italic*: Jardin Clonal; **bold**: Prueba Multilocal).

significant effect of the position in the crown on leaf and branch Cd concentrations (data not shown).

Significant variation between cultivars was only observed in the Cd concentration of adult beans. Here, POUND-7 had ~40% lower Cd concentrations than SPA-9 and IMC-67 (Fig. 5). The Cd concentration of the immature beans was similar in the three cultivars and did not differ from that of the adult beans in POUND-7. While Cd concentrations tended to be higher in adult than in young beans of SPA-9 and IMC-67, the opposite trend was observed in the husks of all three cultivars (Fig. 5).

3.5. LA-ICPMS analyses

The LA-ICPMS analysis of the radial cores taken from the scions of two SPA-9 trees (Fig. 6 and Fig. A.3) and one POUND-7 tree (Fig. A.4), revealed substantial variation in Cd concentration along the axes of the cores with no consistent pattern. The range of variation was similar in

all three cores, and there was no indication of cultivar-specific patterns in these samples. Nonetheless, although the small sample size ($n = 3$) did not allow for a meaningful statistical analysis, the data show a clear tendency of higher Cd concentrations in the bark than in the wood (Table 4).

4. Discussion

Even though total soil Cd concentrations were within the range typically found on uncontaminated sites (0.6–1.1 mg/kg) according to Kabata-Pendias (2001), the bean Cd concentrations (mean values on JC: 2.48 ± 0.23 ; PM: 1.56 ± 0.17 mg/kg DW) exceeded the new EU limits of 0.6 mg/kg for cocoa powder that took effect since January 2019 (The European Commission, 2014). The propensity of cacao trees to accumulate comparatively high Cd concentrations in their beans and leaves has been observed also in previous studies performed in Ecuador, Malaysia and Honduras (Algreen et al., 2014; Chavez et al., 2015; Evangelou et al., 2013; Gramlich et al., 2018; Zarcinas et al., 2004; Lewis et al., 2018; Arguello et al., 2019).

4.1. Uptake and root-to-shoot transfer of Cd

The strong correlations between available soil Cd and Cd concentrations in rootstocks and scions, the solid influence of the factor “plot” and the lack of a significant “cultivar” effect on the accumulation of Cd in the vegetative plant parts suggest that Cd uptake was primarily influenced by soil Cd availability apart from other site factors we could not control

Table 2
Selected models using multiple linear regression with stepwise selection of variables optimizing the BIC-criterion and including only significant variables:

Predicted variable	Significant predictors	R ²
Bean Cd	Cultivar ($p < 0.01$)	0.78
Leaf Cd	Plot ($p < 0.001$) + Ca concentration ($p < 0.01$)	0.88
Scion Cd	Plot ($p < 0.001$) + Mg concentration ($p < 0.01$)	0.80
Rootstock Cd	Plot ($p < 0.001$) + silt content ($p < 0.01$)	0.74

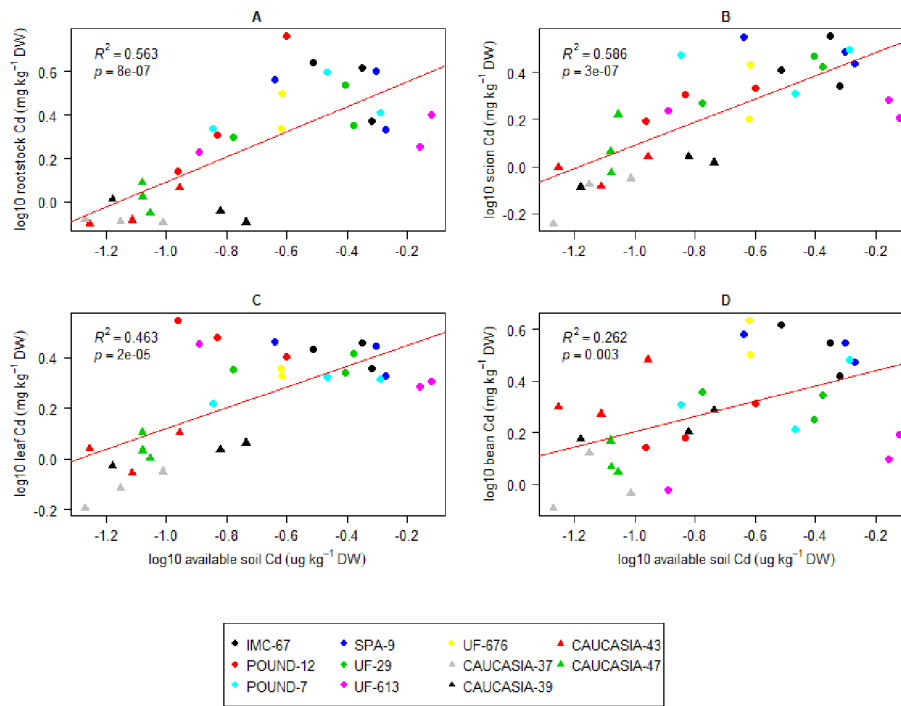


Fig. 3. Relationships between the log₁₀ transforms of available soil Cd and the Cd concentrations of the rootstocks (A), scions (B), leaves (C) and beans (D) for the 11 cacao cultivars sampled on the Jardin Clonal (circles) and the Prueba Multilocal plot (triangles). Red lines represent linear regression relationships across all sampling locations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

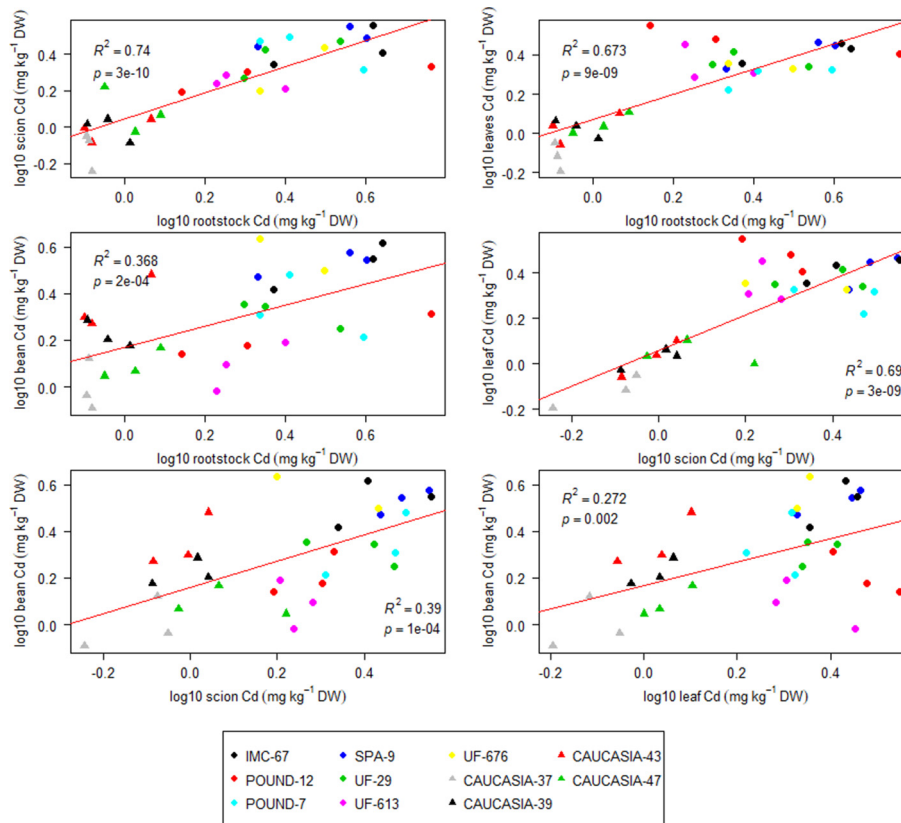


Fig. 4. Relationships between the log₁₀ transforms of the Cd concentrations of different plant parts. Red lines show linear regression. R² indicates squared Pearson correlation coefficient. Circles represent data from Jardin Clonal and triangles from Prueba Multilocal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Mean Cd concentrations \pm SE in mg kg^{-1} DW in tissues of the cacao tree. Significances were tested between tissues of one cultivar. Tissues with same letter indicate no significant difference.

	SPA-9	POUND-7	IMC-67
Adult beans	3.46 \pm 0.22 ^{ab}	2.10 \pm 0.10 ^c	3.44 \pm 0.26 ^{abc}
Immature beans	2.32 \pm 0.32 ^b	1.89 \pm 0.12 ^c	2.24 \pm 0.26 ^c
Adult husks	2.50 \pm 0.20 ^b	1.99 \pm 0.16 ^c	2.41 \pm 0.13 ^{bc}
Young husks	3.72 \pm 0.48 ^{ab}	2.71 \pm 0.19 ^{bc}	3.11 \pm 0.18 ^{bc}
Adult branches	6.34 \pm 1.30 ^a	4.40 \pm 0.41 ^{ab}	5.90 \pm 0.33 ^a
Young branches	3.56 \pm 0.65 ^{ab}	4.96 \pm 0.51 ^a	5.83 \pm 0.62 ^a
Adult leaves	2.65 \pm 0.39 ^b	2.24 \pm 0.18 ^c	2.62 \pm 0.18 ^{bc}
Young leaves	2.57 \pm 0.39 ^b	2.19 \pm 0.25 ^c	2.85 \pm 0.33 ^{bc}
Scions	3.01 \pm 0.33 ^{ab}	2.21 \pm 0.13 ^c	2.97 \pm 0.31 ^{bc}
Rootstocks	3.01 \pm 0.54 ^{ab}	3.00 \pm 0.47 ^{abc}	3.62 \pm 0.64 ^{abc}
Roots	3.34 \pm 0.66 ^{ab}	2.31 \pm 0.26 ^{bc}	4.27 \pm 1.09 ^{ab}

or account for by measurement in our study, such as variation in shade situation. It further indicates that plant-specific factors played no major role in the variation of Cd uptake among the various cultivars in this study. Unfortunately, the design of the trial did not allow for a clear separation of actual cultivar from location effects. Particularly, the differences in Cd accumulation between cultivars that was attributed by the model to the plot factor may have been due to some extent to cultivar effects. While our study did not reveal clear differences between cultivars in the accumulation of Cd in the vegetative parts of the trees that were not related to location effects, the results thus also do not exclude cultivar-specific differences. Variation in Cd accumulation has recently been observed in cacao cultivars by Lewis et al. (2018), and cultivar differences in Cd uptake and translocation have also been found in many other plants (Arevalo-Gardini et al., 2017; Francini and Sebastiani, 2010; Liu et al., 2011).

Differences between cultivars in Cd uptake from the soil may have been masked also by the variation in rootstock genotypes. A rootstock cultivar effect on Cd uptake by cacao was found in a recent study by Arevalo-Gardini et al. (2017). However, most rootstocks on the two experimental plots of our study had been planted in the 1980ies, and no attention had been paid at the time to their genetic identity. Genetic analysis revealed a very high genetic variability of the rootstocks sampled in our study even among replicates of the same tree cultivar grafted upon them (Table A.4), and the data set did not allow to account for the rootstock cultivar as a separate factor in the statistical analysis. Some influence of genetic differences among rootstocks on the variability of Cd uptake by cacao would be in line also with findings on grafted fruit trees.

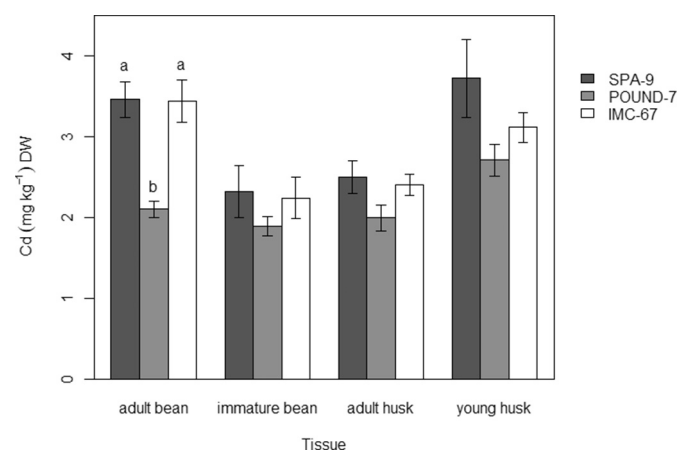


Fig. 5. Cadmium concentrations of beans and husks of the three cultivars SPA-9, POUND-7 and IMC-67. Columns give mean values and error bars represent standard deviations of bulked samples from 3 replicate trees of each cultivar. Different superscript letters indicate significant differences in adult beans between cultivars. Differences in the other tissues between cultivars were not significant.

Thus, soil-to-scion transfer of copper was found to be influenced by the genotype of the rootstock in trees of the genera *Prunus* and *Malus* (Francini and Sebastiani, 2010; Liu et al., 2011). In addition, a recent study found that low Cd accumulation in aerial tissues of grafted apple trees was related to weak expression of rootstock genes involved in Cd uptake and translocation in combination with strong expression of genes involved in Cd detoxification (Zhou et al., 2016). Furthermore, similar rootstock effects on metal accumulation were also reported in grafted non-woody plants. Savvas et al. (2010) observed that choosing appropriate rootstocks for grafting can help avoid metal toxicity effects in fruit vegetables. Moreover, these studies suggest that rootstocks cannot only affect the uptake of metals but also their root-to-shoot translocation. Also, Arao et al. (2008) found that Cd translocation to eggplant fruits (*Solanum melongena* L.) was reduced when plants were grafted onto tomato (*S. torvum*) rootstocks.

The results of this research indicate that the cultivar was the factor with the strongest influence on bean Cd concentration based on the regression model analysis. The strength of the correlation between available soil Cd and Cd in the various plant parts decreased from the scions to the leaves and largely disappeared with the transfer of Cd from the vegetative plant parts into the beans. Similar patterns were observed in trace metal uptake by other plants, e.g. in apple trees (Zhou et al., 2016); peanut (Zhang et al., 2007) and oilseed rape (Wu et al., 2015). It can be explained with the increasing number of transfer steps across tissue boundaries as a metal is transferred from the soil through the root cortex and endodermis into the xylem, translocated through the latter to the aboveground parts and here eventually transferred into the phloem and loaded into seeds (Salt et al., 1995). The notion that genetic control of Cd transfer within plants can be subject to substantial variation among cultivars is supported by several recent studies. For example, comparing Cd accumulation in two cultivars of *Brassica napus* (Wu et al., 2015) it was found that, due to a higher level of gene expression for Cd transport, one cultivar accumulated higher Cd concentrations in its shoots, however, it exhibited a lower ability for Cd uptake into the roots than the other cultivar. Similarly, Uruguchi et al. (2009) found that root-to-shoot translocation of Cd rather than root Cd uptake was responsible for different rates of shoot Cd accumulation in two different cultivars in *Oryza sativa*.

The finding that the relationship between scion and leaf Cd did not vary significantly between the cultivars indicates that the Cd transfer from the scions into the leaves only involved passive transport with the transpiration stream in the xylem, like the translocation from the roots into the scions, and no cultivar dependent control mechanisms. In contrast, the weak correlations between bean Cd concentrations and the Cd concentrations in the other plant parts indicate that xylem-to-phloem transfer is a necessary step in the loading of cacao beans with Cd, as it is the case in metal transfer from vegetative to generative parts in other plants (Waters and Sankaran, 2011). As phloem loading involves cellular trans-membrane transport, it is well suited as a control point for physiological regulation and thus also for genetic control of Cd translocation into fruits and seeds. In line with this notion, Stolt et al. (2003) observed large variation in the Cd concentrations of wheat grains among different cultivars, but not in shoot Cd concentrations. Also, a similar cultivar effect on Cd accumulation in wheat was also reported by others (Greger and Löfstedt, 2004; Ueno et al., 2008; Uruguchi et al., 2009; Wu et al., 2015). The large variation among the cultivars in the relationships between bean Cd and Cd in the vegetative parts, as compared to the much closer relationships between the various vegetative tissues, suggests that this may also be the case in cacao. In this context, it is interesting to note that Cd concentrations in scions and rootstocks were not only more closely correlated to available soil Cd but also to bean Cd than the concentration of Cd in the leaves (Fig. A.2). This observation suggests that a substantial amount of Cd is directly loaded from stems and branches into the beans, without prior passage through leaves. This could mean that substantial xylem-to-phloem transfer also occurs in the woody parts of cacao trees and that

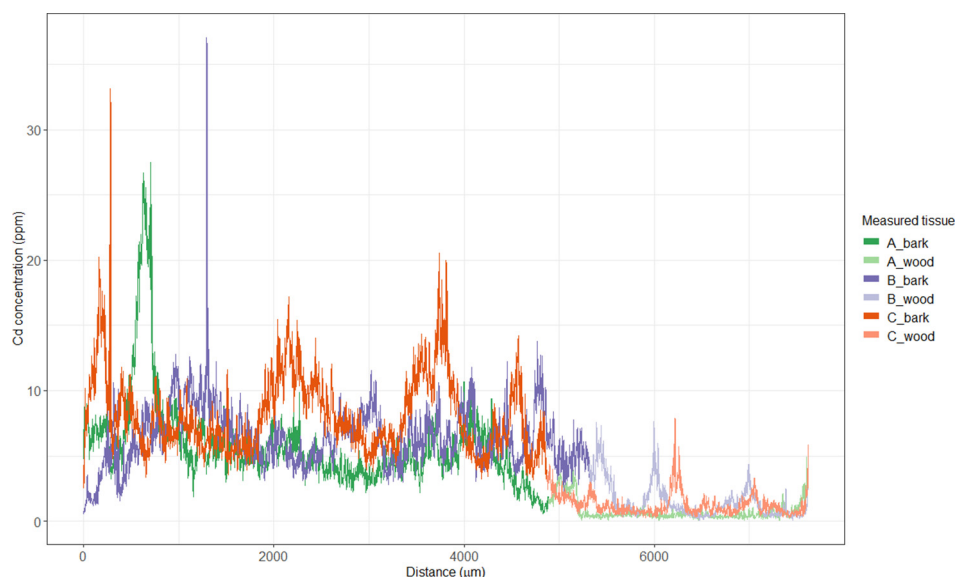


Fig. 6. LA-ICPMS analysis of Cd (sum of Cd-111 and Cd-114, in $\mu\text{g g}^{-1}$) in the bark (dark colours) and in the wood (light colours) of SPA-9b. Three different LA-ICPMS measurements were made along a radial core of a scion across the bark-wood border (A–C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cd remobilization from the leaves may not be the only relevant pathway for Cd transfer into the beans (Sankaran and Ebbs, 2008).

4.2. Cadmium allocation in different plant parts

In the cacao trees studied here, the Cd concentrations of the leaves were comparable to those of the beans and scions, but much lower than those of the branches. In other tree species (e.g. poplars, willows), Cd concentrations were found to be highest in the leaves, lower in the branches and lowest in the trunks (Evangelou et al., 2013; Parraga-Aguado et al., 2014; Chen et al., 2015). As the LA-ICPMS results of our study show, Cd was particularly enriched in the bark of the analysed scions as compared to the trunk wood. This is in line with previous studies generally finding higher metal concentrations in tree bark than in wood tissue (Pulford and Watson, 2003; Sander and Ericsson, 1998). Assuming that the distribution of Cd between bark and wood tissue was similar in branches and scions, it implies that the high Cd concentrations measured in the branches of the three intensively sampled cacao cultivars POUND-7, SPA-9 and IMC-67 were primarily due to high Cd concentrations in the bark of the branches. Furthermore, the higher fraction of bark biomass could then explain why the overall Cd concentrations of the branches were higher than those of the scions.

The finding that bean Cd concentrations were of similar magnitude as leaf Cd concentrations was not expected. Plants are generally found to accumulate significantly more Cd in leaves than in seeds (Clemens et al., 2013), and this was also found in most other studies investigating Cd accumulation in cacao beans and leaves (Arevalo-Gardini et al., 2017; Barraza et al., 2017; Fauziah et al., 2001; Gramlich et al., 2017; Gramlich et al., 2018; Ramtahal et al., 2016; Lewis et al., 2018). The only exception, to our knowledge, was the study of Chavez et al. (2015), who found even higher Cd concentrations in the beans than in

the leaves of cacao trees in Ecuador. The studies that found the opposite also include the survey performed by Gramlich et al. (2018) in Honduras. Although the >50 smallholder farmers participating in the latter survey did not know which cultivars were growing on their plantations, it is likely that they included cultivars studied here. Thus, we argue that the overall difference in the relationship between leaf and bean Cd concentrations between this and the latter study is not due to genetic differences between cultivars and that other factors must be responsible also for the divergent finding of Chavez et al. (2015).

Further noteworthy results of this study with respect to Cd allocation in cacao trees are the lack of significant differences in Cd concentrations between old and young leaves and the lack of an effect of leaf position in the crown on leaf Cd concentration. Differences in Cd concentrations have been found between leaves of different age in several other tree species (Vollenweider et al., 2011; Cosio et al., 2006), and leaf Cd concentrations were found to decrease with increasing height in poplars (Vollenweider et al., 2011).

The increase in bean Cd concentration in the two cultivars SPA-9 and IMC-67 was complementary to a tendency of decreasing Cd concentrations in the pod husks with ripening. This complementarity could suggest that Cd was remobilized in the husks and relocated into the developing beans when the latter started to develop. Similar relocation from temporary storage in other tissues to seeds has been observed for other micronutrient elements such as zinc (Waters and Sankaran, 2011). The pod husks are known as storage organs providing sugars and other nutrients required by the germinating seedlings via the mucilage coating to the ripening beans (Dand, 2011; Wood and Lass, 2008).

4.3. Implications for cultivar selection

Despite the rather large variability found not only between but also within the cultivars in bean Cd and its relationship to the Cd concentrations in the vegetative parts and available soil Cd, some cultivars were found to show bean Cd concentrations that were consistently above average, in particular the cultivars CAUCASIA-43, UF-676, and SPA-9. More importantly with respect to practical implications, the cultivar UF-613 was consistently below average in bean Cd and its relationships to Cd concentrations in other plant parts and to available soil Cd. Unfortunately, as IMC-67 was the only cultivar included in our study that was also studied by Lewis et al. (2018), the basis for a comparison of the performance of individual cultivars in the two studies is very limited. But it

Table 4

Mean Cd concentrations \pm SE in mg kg^{-1} DW in the wood and the bark of 2 or 3 replicate measurements for each core. Wood and bark Cd concentrations were significantly different from each other in all three cultivars.

Tissue	Core		
	SPA-9a	POUND-7	SPA-9b
Wood	1.41 \pm 0.03	1.1 \pm 0.02	1.16 \pm 0.01
Bark	4.95 \pm 0.05	4.93 \pm 0.13	6.75 \pm 0.03

may still be interesting to note that IMC-67 accumulated high bean Cd concentrations in our study, whereas it was found to accumulate only low bean Cd concentrations by Lewis et al. (2018). This discrepancy could mean that the performance of a given cacao cultivar in uptake and translocation of Cd may depend on site conditions and cultivation factors. Thus, while the results of our study agrees with the findings of Lewis et al. (2018) that there is high potential in breeding and selecting appropriate genotypes to mitigate the problem of Cd accumulation in cacao beans, it may be necessary to account for site conditions and cultivation schemes in pursuing this strategy.

5. Conclusions

The Cd concentrations of the cacao beans analysed in this study exceeded the threshold values that was imposed according to the new EU regulations for cacao products that will be brought into force in 2019 by factors of two or more. In contrast to the Cd concentrations measured in the vegetative parts, which were closely related to available soil Cd, the variation in bean Cd concentrations showed a significant influence of the cultivar and only a minor part of this variation was explained by soil factors including available soil Cd, suggesting that cultivar-specific genetic control of Cd uptake into the beans primarily occurred during Cd translocation from the vegetative parts into the fruits, probably in connection with xylem-to-phloem transfer. The finding that the Cd concentrations of the scions were more closely correlated to bean as well as available soil Cd concentrations than leaf Cd concentrations moreover suggests that this transfer step does not necessarily involve Cd passage through the leaves. In contrast to most previous studies, it was found in this study that bean Cd concentrations were of similar magnitude as and in no case much lower than leaf Cd concentrations. The fact that the relationship between leaf and bean Cd did not show cultivar-dependence suggests that this difference to other studies was not due to a specific selection of cultivars included in this study. The results of the current research indicate that there is potential to limit Cd accumulation in cacao not only by appropriate soil management but also by selection and development of genotypes with low propensity of Cd transfer from the vegetative parts into the beans.

CRedit authorship contribution statement

Nadine Engbersen: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Anja Gramlich:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Marlon Lopez:** Methodology, Investigation, Resources. **Gunnar Schwarz:** Investigation, Writing - original draft. **Bodo Hattendorf:** Validation, Resources, Writing - original draft, Project administration. **Osman Gutierrez:** Methodology, Resources. **Rainer Schulin:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.05.001>.

References

- Adriano, D.C., 2001. Chapter 8: cadmium. Trace elements in terrestrial ecosystems. Springer, New York.
- Albacete, A., Martinez-Andujar, C., Martinez-Perez, A., Thompson, A.J., Dodd, I.C., Perez-Alfoceta, F., 2015. Unravelling rootstock x scion interactions to improve food security. *J. Exp. Bot.* 66 (8), 2211–2226.
- Algreen, M., Trapp, S., Rein, A., 2014. Phytoscreening and phytoextraction of heavy metals at Danish polluted sites using willow and poplar trees. *Environ. Sci. Pollut. Res.* 21, 8992–9001.
- Arao, T., Takeda, H., Nishihara, E., 2008. Reduction of cadmium translocation from roots to shoots in eggplant (*Solanum melongena*) by grafting onto *Solanum torvum* rootstock. *Soil Science and Plant Nutrition* 54 (4), 555–559.
- Arevalo-Gardini, E., Arevalo-Hernandez, C.O., Baligar, V.C., He, Z.L., 2017. Heavy metal accumulation in leaves and beans of cacao (*Theobroma cacao* L.) in major cacao growing regions in Peru. *Sci. Total Environ.* 605–606, 792–800.
- Arguello, D., Chavez, E., Laurysen, F., Vanderschueren, R., Smolders, E., Montalvo, D., 2019. Soil properties and agronomic factors affecting cadmium concentrations in cacao beans: a nationwide survey in Ecuador. *Sci. Total Environ.* 649, 120–127.
- Barraza, F., Schreck, E., Leveque, T., Uzu, G., Lopez, F., Ruales, J., Prunier, J., Marquet, A., Maurice, L., 2017. Cadmium bioaccumulation and gastric bioaccessibility in cacao: a field study in areas impacted by oil activities in Ecuador. *Environ. Pollut.* 229, 950–963.
- Benavides, M.P., Gallego, S.M., Tomaro, M.L., 2005. Cadmium toxicity in plants. *Braz. J. Plant Physiol.* 17 (1), 21–34.
- Bouyoucos, G.J., 1962. Hydrometer method improved for Makin particle size analysis of soils. *Agron. J.* 54, 464–465.
- Broadley, M.R., Willey, N.J., Wilkins, J.C., Baker, A.J.M., Mead, A., Philip, J.W., 2001. Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytol.* 152, 9–27.
- Chavez, E., He, Z.L., Stoffella, P.J., Mylavarapu, R.S., Li, Y.C., Moyano, B., Baligar, V.C., 2015. Concentration of cadmium in cacao beans and its relationship with soil cadmium in southern Ecuador. *Sci. Total Environ.* 533, 205–214.
- Chen, Z., Zhao, Y., Fan, L., Xing, L., Yang, Y., 2015. Cadmium (cd) localization in tissues of cotton (*Gossypium hirsutum* L.), and its phytoremediation potential for cd-contaminated soils. *Bull. Environ. Contam. Toxicol.* 95 (6), 784–789.
- Clemens, S., Aarts, M.G., Thomine, S., Verbruggen, N., 2013. Plant science: the key to preventing slow cadmium poisoning. *Trends Plant Sci.* 18 (2), 92–99.
- Cosio, C., Vollenweider, P., Keller, C., 2006. Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.). *Environ. Exp. Bot.* 58 (1–3), 64–74.
- Cryer, N.C., Hadley, P., 2012. Cadmium uptake and partitioning within the cocoa plant. International Workshop on Cadmium in Cocoa and Chocolate Products/London.
- Dai, H.-P., Shan, C.-J., Jia, G.-L., Yang, T.-X., Wei, A.-Z., Zhao, H., Wu, S.-Q., Huo, K.-K., Chen, W.-Q., Cao, X.-Y., 2013. Responses to cadmium tolerance, accumulation and translocation in *Populus × canescens*. *Water Air Soil Pollution* 224, 1–9.
- Dand, R., 2011. The International Cocoa Trade. Woodhead Publishing Limited.
- Evangelou, M.W., Robinson, B.H., Gunthard-Goerg, M.S., Schulin, R., 2013. Metal uptake and allocation in trees grown on contaminated land: implications for biomass production. *Int J Phytoremediation* 15 (1), 77–90.
- FAL RAC & FAW, F. A. R.-T. (1996). Methode NaNO₃-Ex "Extraktion von Schwermetallen mit Natriumnitrat (1:2.5)". In Schweizerische Referenzmethoden der Eigenössischen landwirtschaftlichen Forschungsanstalten (Ed F. Agroscope).
- Fauziah, C.I., Rozita, O., Zauyah, S., Anuar, A.R., Shamsuddin, J., 2001. Heavy metals content in soils of peninsular Malaysia grown with cocoa and in cocoa tissues. *Malaysian Journal of Soil Science* 5, 47–58.
- Francini, A., Sebastiani, L., 2010. Copper effects on *Prunus persica* in two different grafting combinations (*P. Persica* × *P. Amygdalus* and *P. Cerasifera*). *J. Plant Nutr.* 33 (9), 1338–1352.
- Fricker, M.B., Kutscher, D., Aeschlimann, B., Frommer, J., Dietiker, R., Bettmer, J., Günther, D., 2011. High spatial resolution trace element analysis by LA-ICP-MS using a novel ablation cell for multiple or large samples. *Int. J. Mass Spectrom.* 307 (1–3), 39–45.
- Gramlich, A., Tandy, S., Andres, C., Chincheros Paniagua, J., Armengot, L., Schneider, M., Schulin, R., 2017. Cadmium uptake by cocoa in agroforestry and monoculture systems under conventional and organic management. *Sci. Total Environ.* 580, 677–686.
- Gramlich, A., Tandy, S., Gauggel, C., Lopez, M., Perla, D., Gonzalez, V., Schulin, R., 2018. Soil cadmium uptake by cocoa in Honduras. *Sci. Total Environ.* 612, 370–378.
- Grant, C.A., Buckley, W.T., Bailey, L.D., Selles, F., 1997. Cadmium accumulation in crops. *Can. J. Plant Sci.* 78, 1–17.
- Greger, M., Löfstedt, M., 2004. Comparison of uptake and distribution of cadmium in different cultivars of bread and durum wheat. *Crop Sci.* 44, 501–507.
- Gu, J., Qi, L., Jiang, W., Liu, D., 2007. Cadmium accumulation and its effects on growth and gas exchange in four *Populus* cultivars. *Acta Biologica Cracoviensis* 49 (2), 7–14.
- Gunther, D., Frischknecht, R., Heinrich, C.A., Kahlert, H.J., 1997. Capabilities of an argon fluoride 193 nm excimer laser for laser ablation inductively coupled plasma mass spectrometry microanalysis of geological materials. *J. Anal. At. Spectrom.* 12 (9), 939–944.
- Guo, Y., Marschner, H., 2008. Uptake, distribution, and binding of cadmium and nickel in different plant species. *J. Plant Nutr.* 18 (12), 2691–2706.
- He, S., He, Z., Yang, X., Stoffella, P.J., Baligar, V.C., 2015. Chapter four - soil biogeochemistry, plant physiology, and phytoremediation of cadmium-contaminated soils. In: Donald, L.S. (Ed.), *Advances in Agronomy*. vol.134. Academic Press, pp. 135–225.
- Kabata-Pendias, A., 2001. Trace Elements in Soils and Plants. CRC Press, Boca Raton.
- Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World map of the Köppen-Geiger climate classification updated. *Meteorol. Z.* 15 (3), 259–263.
- Lewis, C., Lennon, A.M., Eudoxie, G., Umaharan, P., 2018. Genetic variation in bioaccumulation and partitioning of cadmium in *Theobroma cacao* L. *Sci. Total Environ.* 640–641, 696–703.

- Liu, C.-S., Sun, B.-Y., Kan, S.-H., Zhang, Y.-Z., Deng, S.-H., Yang, G., 2011. Copper toxicity and accumulation in potted seedlings of three apple rootstock species: implications for safe fruit production on copper-polluted soils. *J. Plant Nutr.* 34 (9), 1268–1277.
- Longerich, H.P., Jackson, S.E., Günther, D., 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. *J. Anal. At. Spectrom.* 11, 899–904.
- McLaughlin, M.J., Singh, B.R., 1999. Cadmium in soils and plants. *Developments in Plant and Soil Sciences*. vol. 85. Springer Science + Business, Dordrecht.
- Mendiburu, F., 2014. *Agricolae: Statistical Procedures for Agricultural Research*. Vol. R Package Version 1.2–1.
- Mozaffari, M., Alva, A.K., Chen, E.Q., 1996. Relation of copper extractable from soil and pH to copper content and growth of two citrus rootstocks. *Soil Sci.* 161 (11), 786–792.
- Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matters. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis. Part 3. Chemical Methods*. SSSA Book Series, Madison, WI, pp. 961–1010 Vol. 5.
- Parraga-Aguado, I., Querejeta, J.I., Gonzalez-Alcaraz, M.N., Conesa, H.M., 2014. Metal(loid) allocation and nutrient retranslocation in *Pinus halepensis* trees growing on semiarid mine tailings. *Sci. Total Environ.* 485–486, 406–414.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2014. *Nlme: Linear and nonlinear mixed effects models*. Vol. R Package Version 3.1–117.
- Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees - a review. *Environ. Int.* 29 (4), 529–540.
- R Core Team, 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ramtahal, G., Chang Yen, I., Ahmad, N., Bekele, I., Bekele, F., Maharaj, K., Wilson, L., Harrynanan, L., 2015. Prediction of soil cadmium bioavailability to cacao (*Theobroma cacao* L.) using single-step extraction procedures. *Commun. Soil Sci. Plant Anal.* 46 (20), 2585–2594.
- Ramtahal, G., Yen, I.C., Bekele, I., Bekele, F., Wilson, L., Maharaj, K., Harrynanan, L., 2016. Relationships between cadmium in tissues of cacao trees and soils in plantations of Trinidad and Tobago. *Food Nutr. Sci.* 07 (01), 37–43.
- Salt, D.E., Prince, R.C., Pickering, I.J., Raskin, I., 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol.* 109, 1427–1433.
- Sander, M.L., Ericsson, T., 1998. Vertical distributions of plant nutrients and heavy metals in *Salix viminalis* stems and their implications for sampling. *Biomass Bioenergy* 14, 57–66.
- Sankaran, R.P., Ebbs, S.D., 2008. Transport of Cd and Zn to seeds of Indian mustard (*Brassica juncea*) during specific stages of plant growth and development. *Physiol. Plant.* 132, 69–78.
- Savvas, D., Colla, G., Roupael, Y., Schwarz, D., 2010. Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. *Sci. Hortic.* 127 (2), 156–161.
- Stolt, J.P., Sneller, F.E.C., Bryngelsson, T., Lundborg, T., Schat, H., 2003. Phytochelatin and cadmium accumulation in wheat. *Environ. Exp. Bot.* 49, 21–28.
- The European Commission, 2014. Commission Regulation (EU) no 488/2014 of 12 May 2014 amending regulation (EC) no 1881/2006 as regards maximum levels of cadmium in foodstuffs. *Off. J. Eur. Union* 488, 75–79.
- Ueno, D., Iwashita, T., Zhao, F.J., Ma, J.F., 2008. Characterization of Cd translocation and identification of the Cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri*. *Plant Cell Physiol.* 49 (4), 540–548.
- Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arai, T., Ishikawa, S., 2009. Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J. Exp. Bot.* 60, 2677–2688.
- Vollenweider, P., Menard, T., Gunthardt-Goerg, M.S., 2011. Compartmentation of metals in foliage of *Populus tremula* grown on soils with mixed contamination. I. from the tree crown to leaf cell level. *Environ. Pollut.* 159 (1), 324–336.
- Waters, B.M., Sankaran, R.P., 2011. Moving micronutrients from the soil to the seeds: genes and physiological processes from a biofortification perspective. *Plant Sci.* 180 (4), 562–574.
- Wood, G.A.R., Lass, R.A., 2008. *Cocoa*. Wiley, Oxford.
- Wu, Z.C., Zhao, X.H., Sun, X.C., Tan, Q.L., Tang, Y.F., Nie, Z.J., Hu, C.X., 2015. Xylem transport and gene expression play decisive roles in cadmium accumulation in shoots of two oilseed rape cultivars (*Brassica napus*). *Chemosphere* 119, 1217–1223.
- Zarcinas, B.A., Ishak, C.F., McLaughlin, M.J., Cozens, G., 2004. Heavy metals in soils and crops in southeast Asia. 1. Peninsular Malaysia. *Environ. Geochem. Health* 26, 343–357.
- Zhang, W.-H., Zhou, Y., Dibley, K.E., Tyerman, S.D., Furbank, R.T., Patrick, J.W., 2007. Review: nutrient loading of developing seeds. *Funct. Plant Biol.* 34, 314–331.
- Zhou, J., Wan, H., Qin, S., He, J., Lyu, D., Li, H., 2016. Net cadmium flux and gene expression in relation to differences in cadmium accumulation and translocation in four apple rootstocks. *Environ. Exp. Bot.* 130, 95–105.
- Zug, K.L.M., Huamaní Yupanqui, H.A., Meyberg, F., Cierjacks, J.S., Cierjacks, A., 2019. Cadmium accumulation in Peruvian cacao (*Theobroma cacao* L.) and opportunities for mitigation. *Water Air Soil Pollut.* 230 (3).