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'From soil to chocolate bar': identifying critical steps in the journey of cadmium in a Colombian cacao plantation

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ABSTRACT
Regulation of maximum levels of cadmium in chocolate is an issue for cacao exportation from many parts of Latin America, including Colombia. These limits are related to the final product, but buyers often request maximum levels of Cd in the beans. However, to date, there is neither a clear understanding of the relationship between the specified levels of Cd in chocolate and cocoa derivatives and levels in harvested beans or soil nor of the effect of post-harvest processes on the levels of Cd in the final product. To address this, the fate of Cd concentration from soil to chocolate bar was followed in a single farm in Santander district, Colombia. The concentration of Cd in soils was measured using ICP-OES and correlated with soil pH, soil organic matter (SOM), and the use of P-based fertilisers. Cd concentrations were also measured in unfermented seeds, fermented and dried beans, shell, nibs, and chocolate. SOM (2.93–3.78%), soil pH (4.7–4.9), soil P concentration (120–132 mg kg^{-1}) affect Cd availability. However, it is still unclear whether Cd concentration of P-based fertilisers (3–30 mg kg^{-1}) is important or not. While post-harvest treatments did not affect the Cd concentration of beans (4.17 ± 0.8 mg kg^{-1} on average), the removal of the shell (6.57 mg kg^{-1}) from the nibs (3.28 mg kg^{-1}), as well as the percentage of cocoa mass used contributes to a reduction in Cd concentration in the chocolate bar (1.60 mg kg^{-1}). This study provides clear indications on where research into mitigation measures should be focussed, as well as indicating the importance of carrying out analyses for Cd in the nib or cacao mass, rather than the whole bean, reducing Cd concentration by up to 40%.

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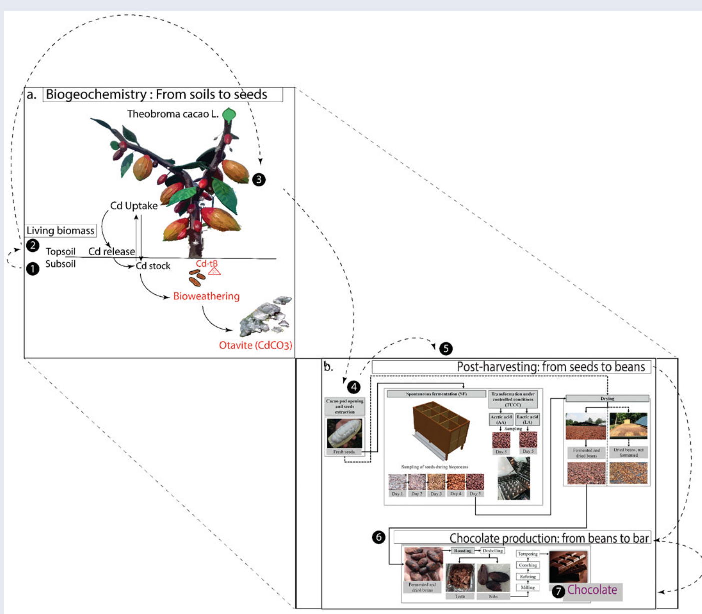
GRAPHICAL ABSTRACT



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Supplemental data for this article can be accessed here.

Introduction

To reduce the risks to human health from the consumption of high levels of cadmium (Cd) in the diet, many countries have imposed limits on the permitted level of Cd in food. The European Community has been a pioneer in legislating these limits in chocolate, cocoa powder, and derivatives, driven by the high consumption of chocolate of its population (European Commission 2014). The following limits of Cd have been in place since 2019: 0.10 mg kg^{-1} for milk chocolate with <30% total dry cocoa solids; 0.30 mg kg^{-1} for chocolate with <50% total dry cocoa solids and milk chocolate with ≥30% total dry cocoa solids; 0.8 mg kg^{-1} for chocolate with ≥50% total dry cocoa solids and 0.6 mg kg^{-1} for cocoa powder sold to the final consumer or as an ingredient in sweetened cocoa powder (European Commission 2014; FAO/WHO 2018; European-Union 2021). The high per capita consumption in Europe, including chocolate with a high percentage of cocoa solids, and single-origin cacao, means that these limits are important for the chocolate industry, with the potential to affect cacao producers. Limits on Cd levels in chocolate are also in place in Australia, Indonesia, New Zealand, Russia, and in the State of California in the USA. There is also active discussion regarding recommended limits for Cd to be included in the *Codex Alimentarius*.

The legislation is particularly relevant for Latin America where high Cd in cacao seeds (fresh material removed from the pods) from some areas of the subcontinent has been found (Vanderschueren et al. 2021). For Colombia (Bravo et al. 2021), cacao is the second most important crop with significant economic and social relevance for the country. While most of the cocoa is consumed within the country, export is important and is increasing. Last year, Colombia exported 9116 tons of cocoa beans, with a value of USD 23 million, of which 10% went to the European Union (FEDECACAO 2021).

These limits for Cd are related to the final product and buyers often request maximum levels of Cd in the beans. However, to date, there is neither a clear understanding of the relationship between the specified levels of Cd in chocolate and cocoa derivatives and levels in harvested beans or soil nor of the effect of post-harvest processes on the levels of Cd in the final product. A better understanding of these aspects could help predict levels in the final product, thus providing information for buyers as well as national legislative frameworks regarding maximum permissible levels of Cd in soil and cocoa beans, and could also identify stages in the production of chocolate where Cd appears to be reduced.

Our understanding of Cd uptake by cacao continues to improve. For example, the origin of Cd (Gil et al. 2021), Cd subsoil distribution (Bravo and Benavides-Erazo 2020), effects of soil pH, soil organic matter (SOM), phosphorus concentration (Bravo et al. 2021), Cd uptake, and translocation (Barraza et al. 2019; Moore et al. 2020) and post-harvest treatments on Cd concentrations (Kruszewski et al. 2018; Meter et al. 2019; Vanderschueren et al. 2020), as well as, Cd in P-based fertilisers (Bravo et al. 2018). A brief summary of the most relevant results from studies of Cd accumulation in cacao are shown in Supplementary Table S1.

The variability of Cd concentration in crops, including cacao has been attributed to the 'total' soil Cd concentration and critical soil factors influencing Cd phytoavailability, such as soil pH, SOM, and to a lesser extent, clay content, texture, iron, or aluminium oxide levels (Alloway and Steinnes 1999; Grant et al. 1999; Chavez et al. 2016; Gramlich et al. 2017; Bravo et al. 2021). Research carried out in Ecuador (Argüello et al. 2019) and Colombia (Gil et al. 2021) show that it is possible to predict cocoa bean Cd from soil parameters. Both groups found that cocoa seed Cd concentration increased with increasing total soil Cd and with decreasing soil pH, oxalate-extractable manganese, and organic carbon. Available $Al^{3+}H^{+}$ and the altitude of cacao farms are also accurate predictors (R^2 = 0.72) of Cd concentration in cocoa seeds in Colombia (Gil et al. 2021). Studies from Peru indicate similar findings (https://cacaodiversity.org/).

The importance of soil pH on Cd accumulation is associated with its increased bioavailability with decreasing pH for crops in general (Grant et al. 1999; Welch and Norvell 1999), and cacao in particular (Bravo et al. 2018; Argüello et al. 2019; Ramtahal et al. 2019). However, pH is unlikely to be the only factor of importance and a range of physical and chemical parameters should be considered when studying Cd in soils and its potential to accumulate in cocoa seeds. For example, acidic soils with high levels of available Cd from the Santander district of Colombia were found to have high SOM content close to 5.2% (Bravo et al. 2018). Furthermore, when zinc concentration was high (average values of 11.6 ± 0.2 mg kg^{-1}), so was available Cd (Bravo et al. 2018).

In addition to soil factors, fertiliser application can influence Cd bioavailability if it is contaminated with this heavy metal (Wiggenhauser et al. 2019). Fertilisers can also influence Cd speciation and complexation, affecting both soil pH and ionic ligand interactions with the soil biota (Grant et al. 1999), and thus increase Cd mobility.

Processes that take place following the harvesting of cacao seeds to produce chocolate may also affect Cd levels. Key processes include the type of post-harvest transformation of cocoa from seeds (fresh material removed from the pods) to beans (fermented and dried cocoa), in which the fermentation and drying stages are highlighted, and the processing of beans into nibs (roasted cocoa beans without shells) in which the roasting and dehulling stages stand out. For example, it has been observed that the shells, while only comprising 10–17% of the mass of a bean, can contain between 2 and 4 times the concentration of Cd in the nib (Meter et al. 2019). However, there has been little research assessing how artisanal, post-harvest operations, such as fermentation roasting, refining, and conching, might influence the Cd concentration in beans and chocolate (Vanderschueren et al. 2019; 2020).

As summarised above, while there are isolated studies looking at the relationship of Cd in the field, or during specific stages of post-harvest, we are unaware of any study that has measured changes in Cd concentrations from soil to bar. The research presented here focuses on two main hypotheses regarding the journey of Cd from the soil to chocolate: (i) soil parameters, such as soil pH, SOM, soil P content and Cd concentration of P-based fertilisers have an impact on Cd concentration of soil and cocoa beans, and (ii) each postharvest operation, whereby the cocoa seeds are transformed into fermented, dried beans, and finally into chocolate, will affect Cd concentration. Assessing both soil and post-harvest Cd levels requires the use of several approaches to study physical, chemical, and microbiological properties of the system (Bravo et al. 2018). Additionally, as Cd in soils in cocoa fields tends to be highly heterogeneous, this study analysed the Cd concentration using cocoa from a single farm to reduce inherent variability. Considering the influence of soil parameters, fertiliser amendments, and post-harvest operating processes enables an understanding of the process as a whole and helps identify key phases where the reduction of Cd appears to be highest and could be the focus of future mitigation studies. This can improve the competitiveness of Colombian cocoa for export and also help protect public health within the country by establishing national quality control guidelines.

Materials and methods

Selecting the farm

A farm in the district of Santander, Colombia, was selected due to (i) the economic and social importance of cocoa in the district (representing 41% of national cacao production); (ii) the known issue of Cd in the cocoa-chocolate value chain in Santander, (iii) the diversity of the cacao varieties grown in Santander, comprising both universal (CCN51, EET8, IMC67, ICS1, ICS95, TSH565, and ICS60) and national cacao cultivars (TCS01, TCS06, FEC2, and FSV41), (iv) record of the highest soil Cd concentration found in the district (Bravo and Benavides-Erazo 2020; Bravo et al. 2021) and (v) the availability of appropriate level of technology in terms of methods and systems for post-harvest processing to obtain high-quality cocoa products (Escobar et al. 2020).

Cacao trees in the selected farm were organised at a spacing of 4 m, interspersed with two timber wood species (*Ceiba tolua* and *Cedrus brevifolia*). All varieties had an average age of eight years. Five kg of fresh cacao pods and 500 g of composite soil samples were collected one month after pruning.

Soil sampling

Soil samples were taken using Stanley stainless steel Shelby tubes of 20 by 4 cm (RA Ltda. Bogotá D.C., Colombia). Composite soil samples were obtained by mixing 500 g of soil collected within the Shelby tubes from each horizon. The samples were placed separately in plastic zip bags, according to the soil horizon, stored at 4 °C, and sent to the Laboratory of Soil Microbiology & Calorimetry of the Corporación Colombiana de Investigación Agropecuaria, AGROSAVIA, in Mosquera, Colombia.

Soil pH and SOM determination

Soil pH $_{[H2O]}$ was measured with a multi-parameter electrode (YSI 556 OH, USA) at each horizon of the rhizoplane pits. SOM and phosphorus content were determined by the modified Walkley-Black and the Bray II method, respectively, using a UV-Vis Thermo spectrophotometer (model Genesys 10S, ThermoFisher Scientific Inc., NY, USA) and UV-Vis Perkin Elmer spectrophotometer (model Lambda 25, USA) for SOM and P, respectively, according to a previous study (Bravo et al. 2018).

Cd determination

In soils

Soil Cd concentration was quantified using an inductively coupled plasma spectrometer ICP-OES (Thermo ICAP-6500, ThermoFisher Scientific Inc., NY, USA) according to the methodology previously described (Bravo et al. 2018). Cd concentration was determined in each horizon (including soil litter) and spot-selected according to the soil profile characterization. The spectrometric determinations were carried out in replicate, and the standard deviations are shown graphically as vertical bars. To compare with the soil samples collected, the clay soil standards WEPAL ISE 961 (0.8 mg kg^{-1} Cd; Herveld, The Netherlands) and WEPAL ISE 970 (7.04 mg kg^{-1} Cd; River clay from Netherlands), were used to perform standard curves of calibration. The instrument limit of detection (LOD) and limit of quantification (LOQ) were 5 and 10 µg L^{-1} Cd, respectively, and the method detection limit (MDL) of blanks was 0.150 mg kg^{-1} Cd.

In fertilizers

Thirteen phosphate-based fertilisers were selected to be analysed for Cd concentration. The fertilisers were chosen according to the farmers' preferences in the municipality where the studied farm was located. The digestion method and measurements were performed according to the international EPA method 6010 C, as reported in previous studies (Rocco et al. 2018; Wiggenhauser et al. 2019).

In seeds, beans, nibs, shell, and chocolate

A microwave-assisted acid digestion protocol was applied, according to the protocol of a previous study (Jackson et al. 1986) with some modifications. Briefly, the modifications consisted of using 4 mL of concentrated Suprapur® nitric acid (HNO_3, 65% w/w, Merck, Darmstadt, Germany) and 1 mL of hydrogen peroxide (H_2O_2, 30% w/w, Merck, Darmstadt, Germany) together with 0.8 g of sample. The samples were heated in a microwave unit at 1500 W and 220 °C for 20 min with a pressure of 150 bar. The digested samples were diluted to 30 mL with nano-pure distilled water and filtered using a paper filter Whatman No. 1 (Sigma-Aldrich, USA) before the Cd counts.

The concentration of Cd in the samples was quantified by ICP-OES, using the same instrument used to perform the Cd measurements for soil (Thermo iCAP-6500, ThermoFisher Scientific Inc., NY, USA).

The LOQ for the ICP-OES analysis was 0.28 mg kg^{-1} dry matter. The concentration of the acid solution for digestion used was the same for both the samples and instrument blanks (nitric acid 65%). For all Cd analyses, triplicates were included in every 15 samples to evaluate reproducibility. The coefficient of variation (CV) for the triplicate digestions was in the range of 0.9–25% (average CV 7%). The certified reference material WEPAL-IPE-213 Milk Thistle Seed/ *Silybum marianum* (Cd 0.355 ± 0.016 mg kg^{-1})

was included in all digestions and treated the same way as the cocoa derived samples for quality assurance. The mean of recoveries of Cd with regard to the certified reference material was 93% ± 8.5 for cacao seeds and beans.

Post-harvest treatments

To analyse the effect of postharvest processes on the Cd concentration in cacao beans, we used a subsample of a single batch of fresh seeds in each of three postharvest transformation processes (Figure 1), using the following experimental conditions: two fermentation procedures based on previous studies (Santander et al. 2020, 2021), (i) a natural spontaneous fermentation process (SF) and (ii) fermentation under controlled conditions (TUCC) using acidic solutions. The latter treatment was used to evaluate the effect of pH on the Cd concentration of beans when they were subject to more acidic conditions. The third post-harvest treatment consisted of no fermentation, only drying, and processing of the beans into chocolate (roasting, winnowing, conching, and tempering). Table 1 shows the samples derived from these processes.

Spontaneous fermentation (SF)

SF was carried out using a mix of cocoa genotypes (Supplementary Figure S1). Fifty kg of

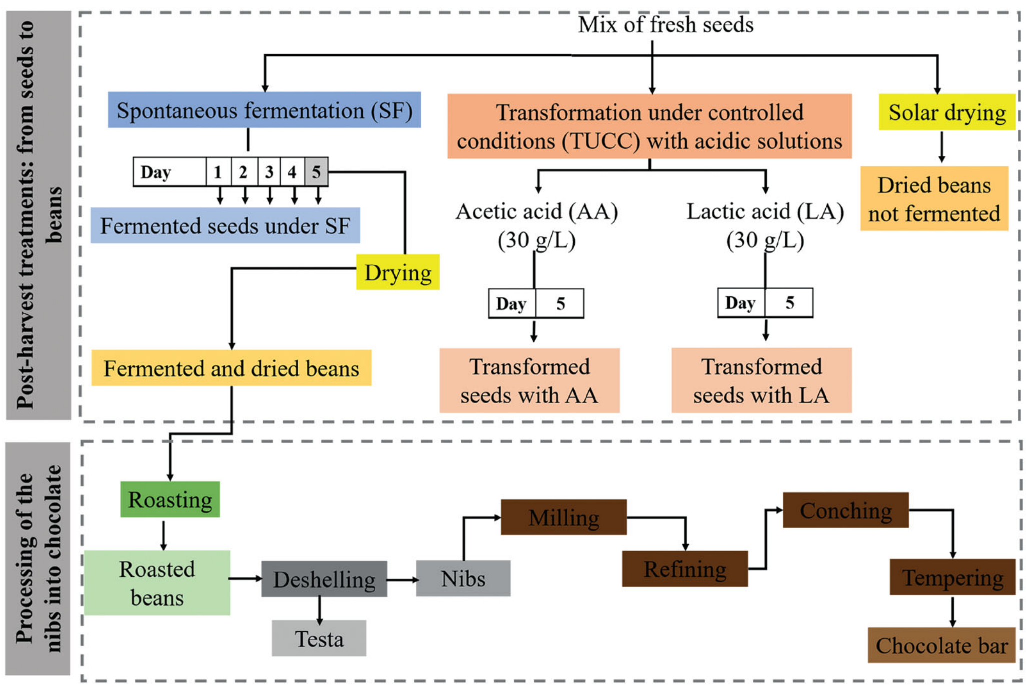

Figure 1. Representation of the treatments used for the transformation of cacao seeds to beans and chocolate. Dark colours are used for postharvest treatments, while opaque colours represents samples used for Cd analysis.

Table 1. Cocoa seed postharvest treatments and sampling stages to analyse the dynamics of the Cd concentration along of them.

Treatment	Stage	Sample
1	Harvest	Fresh seeds
2	Spontaneous fermentation	Seeds, day 1
3		Seeds, day 2
4		Seeds, day 3
5		Seeds, day 4
6		Seeds, day 5
7	Transformation under controlled conditions with acetic acid (TUCC-AA)	Seeds, day 5
8	Transformation under controlled conditions with lactic acid (TUCC- LA)	Seeds, day 5
9	Drying	Fermented and dried seeds
10		Only dried seeds
11	Roasting	Nibs
12		Shell
13		Chocolate

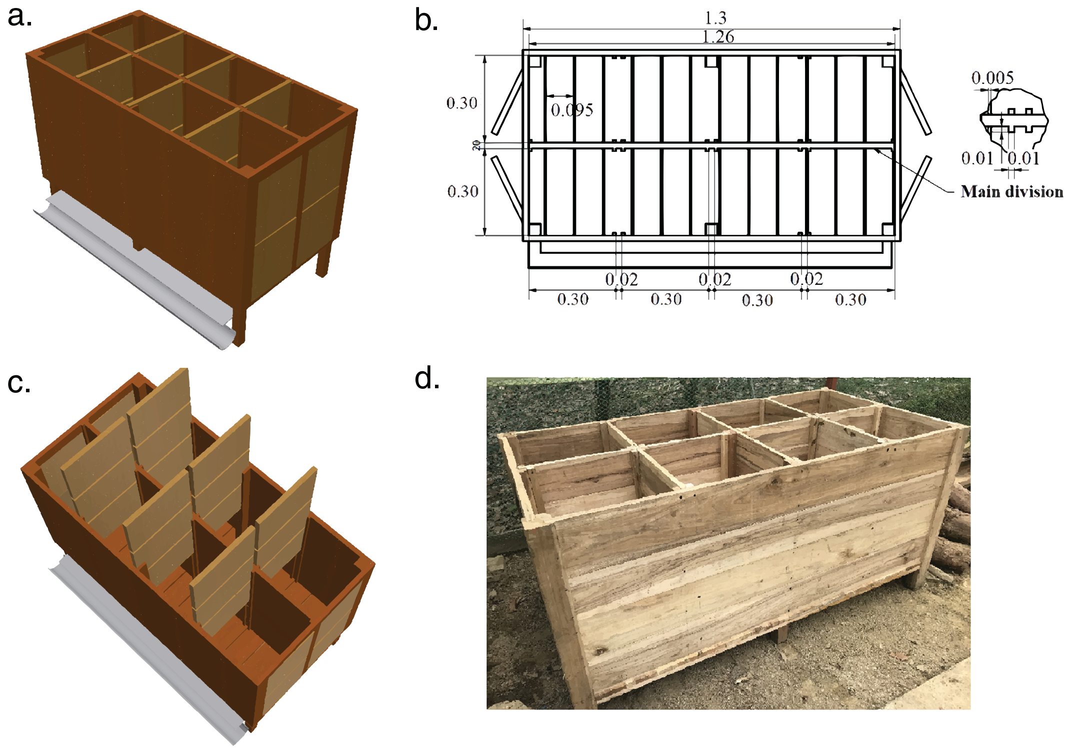

Figure 2. Seed fermentation system used for spontaneous fermentation (SF) and Cd assessments. (a) Front view. (b) Design and measurements. (c) Top view. (d) Side view.

fresh seeds from a mix of raw cocoa cultivars were placed in each of six compartments of the wooden fermenter shown in Figure 2. Each compartment was considered a repetition. Any contact with steel nails was avoided, ensuring no contamination of Cd. The SF lasted 120 h, and the anaerobic phase was maintained for 48 h. The aeration of the beans was performed manually every 12 h by moving them from one compartment to another. The cut test was carried out every 24 h to measure the fermentation degree to determine when to terminate the process. The internal temperature of the cocoa mass at the central point of each compartment of the fermenter system was monitored throughout with a thermocouple (Testo-735-1). The internal seed pH was determined by applying the protocol of John et al. (2019): the shell of the fermented seed was removed and the seed was ground with a blade grinder. Then 2.5 g of ground material was suspended in 22.5 mL MilliQ water in 50 mL centrifuge tubes and shaken head-over-head for 5 min. The suspension was centrifuged at 1000 rpm for 10 min, and the pellet was discarded. A pH meter (Mettler Toledo, Giessen, Germany) was used to measure the pH of the supernatant.

Transformation under controlled conditions (TUCC) using acidic solutions: acetic acid and lactic acid

The TUCC treatment was developed according to the protocol reported by Santander et al. (2021). This is a process by which the cocoa seeds, from the mix of cacao cultivars mentioned above, were transformed through simulation of the conditions of SF occurring on the field (Supplementary Figure S2). The TUCC process was developed under sterile conditions, for this, all equipment used was autoclaved or wiped with 70% ethanol. One kg of fresh seeds was depulped using sterilised plastic mesh pads inside a laminar flow work bench. Seeds were rinsed in 70% ethanol for 1 min to exclude any remaining microorganisms on the surfaces of the seeds. Two trials of TUCC were run using one of two fermentation by-products found in spontaneous fermentation: acetic acid (TUCC-AA; CH_3COOH, 100%, Merck, Darmstadt, Germany), and lactic acid (TUCC-LA; $C_3H_6O_3$, 98%, Merck, Darmstadt, Germany) that were used as incubation media to investigate the effect of pH on the Cd concentration in the seeds. Both TUCC-AA and TUCC-LA were carried out in triplicate. TUCC was carried out in a total of thirty-six Erlenmeyer flasks with 500 mL of work volume. Forty-five cacao seeds were placed in such glass flasks containing 280 mL of the incubation media. The incubation media contained acetic acid or lactic acid at 30 g L^{-1}. The glass flasks were placed in incubators under shaking at 200 rpm. The incubation temperature was adjusted to 30 °C on day one, 35 °C on day two, 45 °C until the end of the process. The pH was measured from each replica every 24 h. Ten grams of cacao seeds were collected from the six Erlenmeyers and stored at −20 °C until analysis.

Seed and bean sampling

Table 1 describes the treatments used to evaluate the Cd concentration of the seeds and beans during the post-harvest processes. In the case of SF, for all Cd analyses a sample was obtained from a mix of cocoa seeds which was taken from three parts of the fermentation chamber: upper, middle, and bottom zone. This sampling method was repeated for each of the six compartments of the fermenter (six replicas for each sample). For fresh seeds and fermented cocoa seeds, six samples of 100 g of seeds were sampled and stored in sterile plastic zip bags at −80 °C until further analysis. For the unfermented and dried cocoa beans, six samples of 100 g of fresh seeds, as replicates, were sampled from the upper, middle, and lower zone of the initial fermentation cocoa mass and placed in a drying system. The solar drying operation lasted for 120 h. The beans were spread to dry on a wooden platform with a sliding roof. Drying was considered complete when cocoa beans reached 7% w/w of moisture (Zahouli et al. 2010). For chocolate production, 300 g of fermented cocoa seeds from the fifth day were sampled from each of the six compartments of the wooden fermenter, dried at 50 °C for 3 days, and stored in bags at 25 °C. For the TUCC treatment, from each of the three replicas, 30 g of cocoa seeds from the fifth day of the process were sampled for analysis.

Chocolate production: from beans to bar

Chocolate production was completed in the Chocolate Factory Laboratory of the Institute of Food and Beverage Innovation of the University of Applied Sciences (Zürich, Switzerland) following a standard protocol (Chetschik et al. 2019). The chocolate samples were produced with 50% w/w of the nibs without shells.

Data analysis

A test for ANOVA was performed to compare differences in the means between both the soil Cd concentration and the Cd concentration of the fresh seeds and the fermented and dried beans. A Tukey test was performed to observe the meaningful differences ($p < 0.05$). A Pearson correlation was calculated for soil Cd concentration (mg kg^{-1}) to compare concentrations in the different soil profiles. The statistical analysis was performed using QtiPlot.

Data were processed using R v. 3.6.0 software and graphs (Cd values in fermented seeds and pH dynamics from different Colombian regions) were constructed using OriginLab 2015. Cd concentration means and standard errors of the mean were calculated for all samples mentioned in Table 1. To establish significant differences among samples derived from the different post-harvest treatments (to independently compare, on the one hand, the samples from 1 to 10 and, on the other hand, the samples 11–13 of Table 1), F tests were calculated in the ANOVA and a *post-hoc* Tukey's HSD (Honestly Significant Difference) test was carried out, to identify samples with significantly different Cd concentrations. Statistical significance was established at $p < 0.05$.

Results

Soil cd concentration and distribution

The farm was found to have a high pseudo-total soil Cd concentration (3.50 mg kg^{-1}) mostly present in the *A–B* horizon with average Cd values of 2.38 ± 0.3 and 1.37 ± 0.4 mg kg^{-1}, in the *AB* and *A* horizons, respectively. Farms from the neighbouring municipality of El Carmen de

Table 2. Soil Cd, pH, SOM, P concentration, and resistivity in the soil horizons assessed at the selected farm.

Horizon	Soil depth (cm)	Soil Cd (mg kg^{-1})	Resistivity (Ohm m^{-1})	SOM %	pH	P (mg kg^{-1})	Cd fertilizer (mg kg^{-1})
Ap	+3–0*	1.15 ± 0.20	950 ± 14	3.78	4.7	132.45	3–30
A	0–20	1.37 ± 0.40	477 ± 92	1.87	5.0	68.66	
B	21–72	2.38 ± 0.30	926 ± 70	2.93	4.9	120.62	
C	73–100	0.42 ± 0.70	370 ± 22	1.24	6.2	54.76	

*+3 = 3 cm above surface, mainly litter.

Chucurí, show similar patterns of subsoil Cd distribution in these horizons, with average Cd values of 3.24 ± 0.5 and 1.92 ± 1 mg kg^{-1}, respectively.

Moreover, in the *C* bottom horizon, the Cd concentration was related to a high concentration of rocky-type carbonate-like material (ranging from 75 to 100 cm in soil depth). These data were highly correlated with the reference material used in the calibration curve for electrical resistivity tomography analysis (see Supplementary Material S3) as observed in previous work (Bravo and Benavides-Erazo 2020).

A segregated patchy distribution of rocky material exhibited high SOM content with fragmented solid-state phase rocky types. A rock formation was observed from underground (*C* horizon) through to the surface (*A* horizon). The mineral composition of the rock in the *C* horizon was confirmed by performing an X-ray diffractogram (XRD), indicating the existence of otavite (see Supplementary Figure S3). Table 2 summarises the mean soil pseudo-total Cd values, soil pH, and SOM values at the three sampling sites of the cropped farm.

Cd in soils and phosphate-containing fertilisers

Soils in this farm were classified as *Typic Udorthents*, characterized to have high clay and SOM content, with 85 and 5.20%, on average, respectively (Bravo and Benavides-Erazo 2020). Interestingly in this farm, a high P content (above 900 mg kg^{-1} of assimilable P) indicates a large input of Cd related to contaminated P fertilizers, mainly at the interface of horizon *A-AB*.

Six of the thirteen phosphate-based fertilisers tested (46%) had high levels of Cd (ranging from 3 to 30 mg kg^{-1}). For instance, one fertiliser of an international brand (called '12 M 15-8-12') was shown to have 3.68 mg kg^{-1} of Cd, whereas a national one ('Triple 15') was shown to have

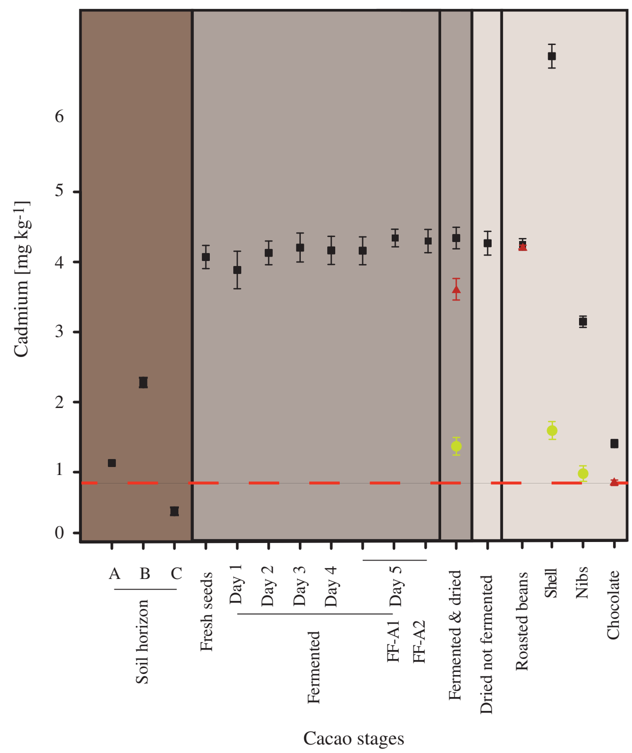

Figure 3. Path of Cd concentration from soils to chocolate according to the transformation stage. Vertical bars denote standard deviation (*SD*). Data are expressed as mean ± *SD* (*n* = 3). Black squares are data from the Colombian single farm in Santander district. Red triangles correspond to a single farm from Peru to compare the range of Cd between some of the stages assessed. Yellow circles correspond to a single farm from Trinidad and Tobago. The orange dotted line indicates the maximum Cd level allowed by EU regulation for chocolate with ≥50 to ≤70% cocoa solids.

29.9 mg kg^{-1} of this metal. Table 2 shows the distribution of soil P after fertilisation.

Cd concentration from seeds to chocolate bar

Figure 3 shows the Cd concentration from seed to bean and chocolate, at each stage of its transformation. The Cd concentration of unfermented seeds (to evaluate the direct effect of drying), and in samples of previously fermented cocoa seeds was quantified to determine the possible effect of endogenous bacterial populations that had participated in the process. There was no statistically

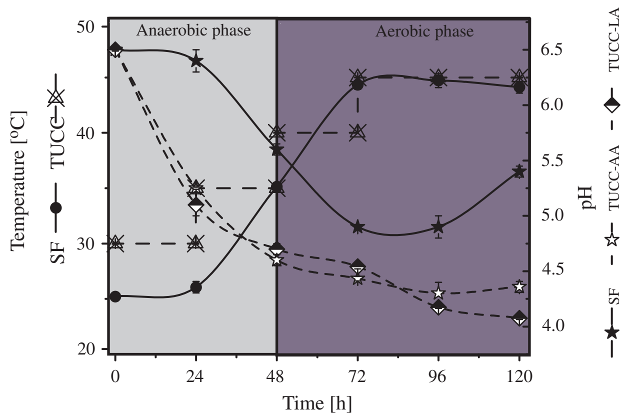

Figure 4. pH and temperature rates of cocoa seeds when using spontaneous (SF) and 'transformation under controlled conditions' (TUCC) treatments, at steady-state conditions. Vertical bars denote the standard deviation (*SD*). Data are expressed as mean ± *SD* ($n = 6$ for spontaneous fermentation and $n = 3$ for transformation under controlled conditions).

significant variation ($p > 0.05$) in the Cd concentration of these samples.

Furthermore, it is noteworthy that the fermentation process did not have a significant effect on Cd concentration from seeds to beans ($p > 0.05$).

Figure 4 shows the changes in pH and temperature during the post-harvest transformation of cocoa seeds according to the spontaneous fermentation (SF) and transformation under controlled conditions (TUCC). During SF, a drop of pH was observed between 0 and 72 h. Then it remained constant until 96 h, and, increased in the final hours to 5.5. The temperature reached 46 °C between 72 h and 96 h; however, after 96 h, the temperature was stable at 45 °C.

An evaluation of process variables was carried out to assess whether a stronger decrease in seed pH over time affects Cd concentration, using the TUCC treatment with two weak acids, acetic and lactic acid, using a temperature gradient along the process. It was observed that the pH decreased from 6.5 to 4.5 in both treatments between 0 and 72 h of fermentation; however, after 120 h, a second pH decrease was observed reaching 4.4 and 4.0 units in the TUCC-AA and TUCC-LA treatments, respectively (Figure 4). Thus, this postharvest process established more acidic pH conditions for seed transformation than that of spontaneous fermentation.

In both the SF and TUCC treatments, despite the observed differences in pH, there was no statistically significant variance ($p > 0.05$) in seed Cd concentration (Figure 4). The strongest and most sustained drop in pH over time was found when performing the cocoa transformation process with the assessed acids within the TUCC treatment, but it did not result in a lower concentration of Cd in the cocoa seeds at day 5 of fermentation even with pH values below 4.5.

During the cocoa transformation from beans to nibs, a significant difference was found in the Cd concentration of the nibs and shells (3.28 and 6.57 mg kg^{-1}, respectively $p < 0.05$).

Additionally, when the Cd concentration was assessed through the transformation of nibs into chocolate, made with 50% (w/w) of nibs, the resulting chocolate contained 1.60 mg $kg^{-1}$of Cd, which is, as expected, almost 50% of the reported Cd value in nibs.

Discussion

This study mapped the change of Cd concentration from soil, leaf litter, and fertilizers through seeds, post-harvesting processes, and finally to chocolate in a single farm's crop (Figure 5). Not only does the diagram indicate where the most important changes in Cd are, but it also illustrates that there is a need to reduce Cd accumulation by the trees as the chocolate was above the mandatory regulated level approved by the *Codex Alimentarius* (FAO/WHO 2018).

The relative importance of Cd from soil and fertilizers

High levels of soil Cd found in this case study are related to the low buffering capacity of acidic tropical soils, making Cd more active chemically and therefore available for uptake. In comparison with other elements, such as Pb, Cd is much more labile.

In the regional context, the average soil Cd concentration in Colombia of 1.43 mg kg^{-1} (Bravo et al. 2021) is higher than that reported in Ecuador of 0.44 mg kg^{-1} (Argüello et al. 2019), Bolivia, of 0.3 mg kg^{-1} (Gramlich et al. 2017), or Honduras, 0.25 mg kg^{-1} (Gramlich

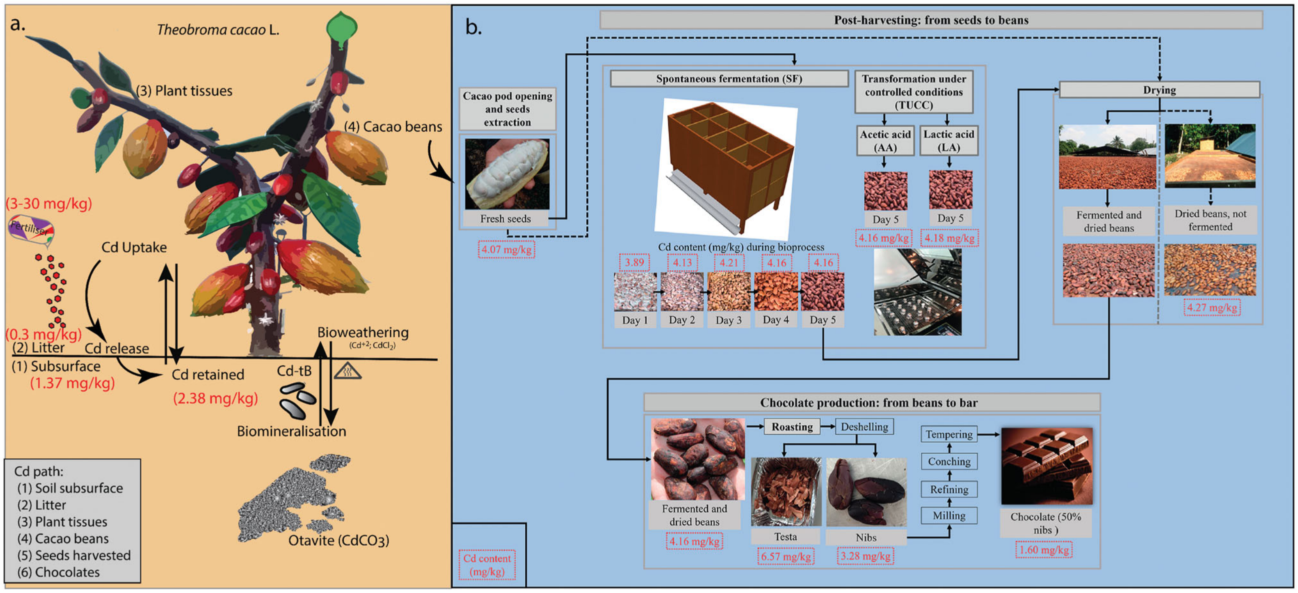

Figure 5. The Cd path from (a) cacao soils to (b) post-harvest and chocolate production, seen as a whole system.

et al. 2018), but lower than the values found in the north of Peru 2.46 mg kg^{-1} (Zug et al. 2019) and in Trinidad and Tobago 1.7 mg kg^{-1} (Ramtahal et al. 2016). A nationwide survey in Colombia (Bravo et al. 2021) found that even if Santander district showed high variability and had some farms with the highest Cd level in the country, farms with <1 mg kg^{-1} of soil Cd were also common and widespread. This heterogeneity is an important message especially when Cd assessment is a sensitive topic that can affect smallholder income and lead to social issues in rural areas.

Additionally, even in a single farm, the distribution of Cd in the subsoil is highly patchy and heterogenous. This could be caused by rock outcrops, mainly Batholite granitoids (Gil et al. 2021), and ligand dynamics due to climatic conditions (Liu et al. 2017; Bravo et al. 2018; Bravo and Braissant 2022).

Fertilizer application caused pH to decrease due to the formation of ligands between P and available protons in the soil solution, as has been also observed in other studies (McLaughlin and Singh 1999; Mourato et al. 2019). In addition to phosphates, the farm under study also applied animal manure and compost, both with levels of Cd similar to commercial P-based fertilizers (ranging from 7 to 13.3 mg kg^{-1}). While both national and international brands of these fertilizers have a wide range of Cd (3–30 mg kg^{-1}), international brands have on average half as much Cd as national brands (6.7 and 13.8 mg kg^{-1} Cd, respectively). It should be noted that the former is less commonly used due to much higher prices. At present in Colombia, there is no nationwide regulation for admissible Cd values in fertilizers for cacao. If we take the European regulation as a reference (Römkens et al. 2017), we find that all the samples of fertilizer had Cd levels within the regulatory limit of 60 mg kg^{-1} Cd in P_2O_5 fertilizers (European-Union 2020), suggesting that Colombian fertilizers may not represent a significant source of Cd to the plant.

One final Cd input into the system is through leaf litter, but this is very poorly studied. Our research (0.3 mg kg^{-1}), suggests that it could be an important source as it prevents Cd from leaving the system, constantly being recycled in a highly available form back into the soil in every dead leaf.

Effects of post-harvest on Cd concentration

Neither fermentation nor drying influenced Cd concentration in beans. Similar results to those reported here were found when spontaneous fermentation (SF) was carried out in other regions of Colombia (see Supplementary Material in

Table S2 and Figure S4). Thus, none of the physical or chemical processes inside the seed caused by the change of process variables, such as pH, oxygen concentration, and temperature during SF or TUCC, or drying leads to changes in Cd concentration of cocoa seeds. This is in contrast to previous work (Vanderschueren et al. 2020), where migration of Cd from the seed to the shell was observed when the seed pH dropped below 5, resulting in a reduced Cd concentration in hulled beans.

The observed change in pH values and temperature in spontaneous fermentation can be explained by the activity of both the lactic acid bacteria (LAB) and acetic acid bacteria (AAB) populations. The pH increase noted at the end of the fermentation is explained by a decrease in substrate availability for bacteria and, consequently, the acidic metabolites that should migrate inside the seeds were not generated, as described previously (De Vuyst and Weckx 2016). Additionally, when the SF treatment time was prolonged, the pH increased again (see also the Supplementary Figure S4). This also contrasts with the above-mentioned study (Vanderschueren et al. 2020) which observed a decrease in pH with prolonged fermentation. In the processing of bean to chocolate, no change in Cd concentration was found due to roasting.

In this study, by separating the shell from the nib, ∼18% of the Cd concentration was removed. In Figure 4, data from two other studies have been added, one from Peru and the other from Trinidad and Tobago. Both studies measured Cd in whole bean, nibs, and shell. Both support the results from Colombia of a significant decrease in Cd comparing nibs with whole beans (a reduction of an average of 19% in the former and 30% in the later). Further details are given in Supplementary Tables S3, S4. A similar decrease has also been observed elsewhere (Lewis et al. 2018; Vanderschueren et al. 2020), where higher average Cd concentrations were found in the shell (5.33 of Cd mg kg^{-1} and 1.83 mg kg^{-1}, respectively) than in the nibs (3.22 of Cd mg kg^{-1} and 0.88 mg kg^{-1}, respectively). It is assumed that a complete separation of the shell from the nibs occurred at the dehulling stage and thus it can be stated that the lower Cd concentrations in the final product are the result of removal of the shells by winnowing (Okiyama et al. 2017). The lower Cd level in the nibs compared to the shells has been associated with the fact that cocoa nibs consist of ∼50% fat (Ramos-Escudero et al. 2021), which is known not to contain Cd (Mounicou et al. 2003; Abt and Robin 2020). In contrast, cocoa shells are predominantly cellulose (Meunier et al. 2003; Panak Balentić et al. 2018), which has the potential to chelate a significant amount of Cd (Mounicou et al. 2003).

The reduction of Cd when nibs were processed to chocolate is due to the percentage of nibs used in the recipe. These results are similar to those of recent studies (Abt et al. 2018; Vanderschueren et al. 2019), where Cd values in chocolate were found to be closely related to the percent of cocoa solids and cacao origin, rather than from other ingredients or contamination related to the processing stages of chocolate.

Manipulating the journey of Cd: where next?

Cd found in the chocolate made from beans from the studied farm was above the mandatory regulated level approved by the *Codex Alimentarius* (FAO/WHO 2018) and the European Union. It is likely that a mixture of farm-level interventions will be the most effective for the reduction of Cd accumulation in terms of cost while maintaining cacao quality (Vanderschueren et al. 2021). The exact solution will be a nuanced and site-specific manipulation of soil conditions, genotype selection, plantation age, and management, and will depend on the origin and distribution of Cd in the soil (Maddela et al. 2020). However, while the general principles of mitigating cadmium accumulation are known, knowledge of how to interpret these in a cacao plantation is still in its infancy with fundamental gaps, such as those related to cacao physiology; and the translocation and bioaccumulation rates of different cultivars still remaining. Another understudied area of research is the use of soil Cd-tolerant bacterial (CdtB) and cocoa bean endophytic bacterial populations (ECdtB), to remove Cd from the system (Bravo and Braissant 2022). Additionally, in the longer term, there may be scope to manipulate Cd in post-harvest processes using bioremediation and nanoparticles, i.e., Ag-NPs, hydroxides, and carbonates (Bravo and Braissant 2022) to chelate Cd in the final stages of chocolate production to thus avoid loss of quality.

Conclusions

The results from this study carried out on a single farm, suggest that there are two critical points in the journey of Cd from soil to bar. One is soil management and the second is the successful removal of the shell during processing.

Reducing the soil available Cd in chemical species, such as Cd hydroxides, Cd carbonates, or even Cd sulphates by increasing soil pH values is a first step to suggest for this farm and appears to be more important than the input of Cd from P-based fertilisers.

No significant changes in the Cd values were observed following a variety of post-harvest steps and processing from seed to the fermented and dried bean. However, removing the shell during winnowing decreases the Cd of nibs and thus of the resulting chocolate bar.

At present, buyers of cacao request Cd concentration of the beans before purchase. While beans are normally sent for analysis, it is unclear whether laboratories remove the shell or not before running their tests. We believe it is unlikely to occur as dehulling beans before toasting is very difficult. The results presented here show that measuring Cd in nibs can give values 40–50% lower than in whole beans. This difference can make or break a sale, and we suggest that it is important for both sellers and buyers to measure Cd in cocoa nibs, cocoa mass, or liquor and not whole beans.

Additionally, while there are many trials underway to determine effective solutions to reduce Cd uptake from the soil, one option that has not been well explored is the use of cadmium-tolerant bacteria (CdtB) and bacterial endophytes (ECdtB), which may result in a change in the labile fraction of Cd in both the soil and cocoa beans.

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Author contributions

DB conceived and designed the experiments; performed the experiments; analysed and interpreted the data; contributed reagents, materials, analysis tools, or data; drawn figures; wrote the paper and performed the final edition. MS performed the experiments; analysed and interpreted the data; drawn figures; wrote the paper and assisted the final edition. JR conceived and designed the experiments; performed the experiments; analysed and interpreted the data; analysis tools or data; wrote the paper and collaborate in the final edition. SE conceived and designed the experiments; performed the experiments; analysed and interpreted the data; contributed reagents, materials, analysis tools, or data; drawn figures; wrote the paper and assisted in the final edition. RA analysed and interpreted data from Peru to compare with the available data from Colombia and assisted in the final edition. GR analysed and interpreted data from Trinidad and Tobago to compare with data from Colombia. Both RA and GR also contributed to the final discussion and conclusions of the study.

Disclosure statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Data availability statement

The datasets generated during and/or analysed during the current study are not publicly available. However, related data at the municipality may be available upon request to the corresponding author.

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