Mitigating the level of cadmium in cacao products: Reviewing the transfer of cadmium from soil to chocolate bar

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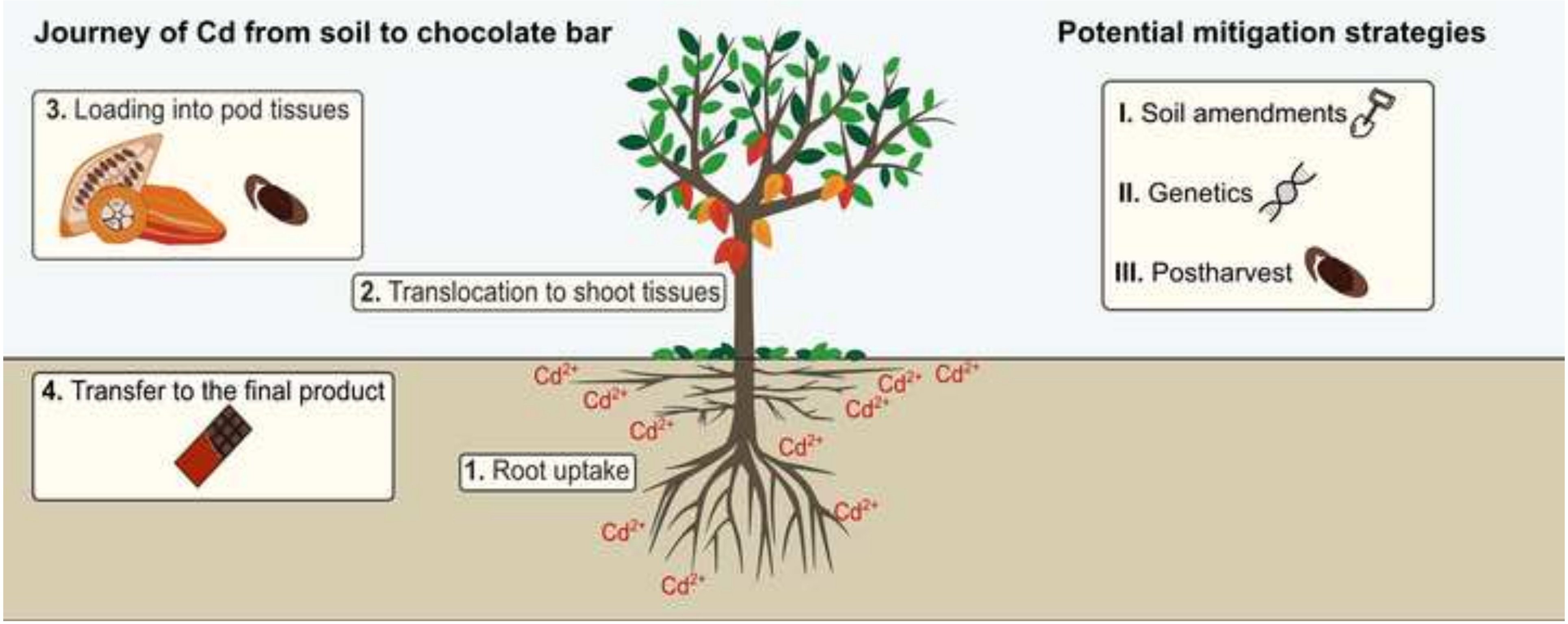
Graphical Abstract



Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

HIGHLIGHTS

- Cadmium concentrations are higher in Latin American cacao compared to other origins
- The source of Cd in cacao is mostly geogenic rather than anthropogenic
- The meta-analysis shows that bean Cd is explained by soil Cd, pH, and organic carbon
- Soil amendments reduce bean Cd but are limited by the rooting system of cacao
- Genetics based mitigation requires understanding of Cd uptake and translocation

Mitigating the level of cadmium in cacao products: reviewing the transfer of cadmium from soil to chocolate bar

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ABSTRACT

The new EU regulation on cadmium (Cd) in cacao-derived products affects the cacao market worldwide. Here, we reviewed the journey of Cd from soil to chocolate bar and collated current data on the topic, giving due attention to data quality. Cacao bean Cd concentrations are typically about a factor two larger compared to the soil on which the cacao tree grows, this is high but not unusual and, therefore, the cacao plant is not classified as a Cd hyperaccumulator. Mean Cd concentrations in cacao beans range 0.02–12 mg Cd kg^{-1} and are markedly higher in Latin America, where more than half of cacao bean samples exceed the commonly applied threshold for export to the EU (0.60 mg kg^{-1}). This regional enrichment is related to relatively high soil Cd concentrations in the young soils of Latin America. The source of Cd is, in general, likely geogenic rather than derived from phosphate fertilizers or contamination. A meta-analysis of 780 soil-plant paired data shows that soil Cd, soil pH and soil organic carbon largely explain cacao bean Cd concentrations. Detection of effects of cultivars, soil treatments, or agronomic practices are strongly hampered by the spatial variability in phytoavailable soil Cd concentrations. Application of lime or biochar has the potential to lower bean Cd in acid soils. In the long-term, breeding low Cd cultivars likely provides the highest potential for mitigation but genetics and breeding research is currently limited by the lack of understanding of how Cd is loaded into the developing cacao fruit of this cauliflorous tree. Postharvest practices such as fermentation can slightly lower Cd concentrations in the final product but also play a large role in product quality. In the short term, mixing of cacao from different origins may be the most feasible strategy to meet the EU limits.

KEYWORDS

Theobroma cacao L.

Cadmium

Soil-plant meta-analysis

Cadmium uptake

Cadmium translocation

Mitigation strategies

1. INTRODUCTION

Cadmium (Cd) is a potentially toxic trace metal that has no known biological function in humans. Cadmium accumulates in the human body and has a biological half-life of 10–35 years. Chronic Cd exposure has been related to several adverse health effects, including renal tubular dysfunction and osteomalacia (World Health Organization 2010). The human diet is the main source of body burden Cd in the non-smoking population and staple foods such as rice, wheat grain products or potatoes contribute largely to dietary Cd exposure because of their high consumption. Certain luxury foods such as chocolate, although ingested in smaller quantities, can also contribute to dietary Cd exposure due to their elevated Cd concentrations. To protect consumers, the European Commission approved a new regulation in 2014 (in force from 2019), which sets the maximum allowed Cd concentration in chocolates and cacao powders between 0.10–0.80 mg Cd kg^{-1}, depending on the cacao solids content of the product (European Commission 2014). Similar regulations have been, and are expected to be, implemented worldwide, e.g. in Australia and New Zealand (Australia New Zealand Food Standards Code 2017), Russia (Ministry of Health of the Russian Federation 2011) and the countries within the Southern Common Market (Mercosur 2011). In California (USA), products with elevated Cd concentration can be sold but it is mandatory to report this on the packaging (Meter et al. 2019). The Cd in cacao regulations have also been adopted by the Codex Alimentarius (Codex Alimentarius Commission 2018). Several products on the market have been reported to exceed these limits (Abt et al. 2018; Vanderschueren et al. 2019). As a result, the regulations have fueled research worldwide to monitor and mitigate Cd accumulation in cacao. The number of research papers on Cd in cacao was only 29 up to 2014 but rose almost exponentially to >100 in 2020.

Cadmium in cacao products originates from the cacao beans, rather than from contamination during processing. This is demonstrated by the strong correlations between the Cd concentrations in the final chocolate product and its cacao content, and between the Cd concentration in chocolate and the origin of the cacao (Villa et al. 2014; Yanus et al. 2014; Abt et al. 2018; Lo Dico et al. 2018; Vanderschueren et al. 2019). While all plants take up Cd to some extent, *Theobroma cacao* L. is rather effective in doing so. The oldest study reporting Cd concentrations in cacao beans and chocolate dates back to 1979 and that work already highlighted that elevated Cd concentrations in cacao are typically found in samples from Latin American origin, as well as in specialty origin chocolates (Knezevic 1979). The recently enforced EU limits and the Codex Alimentarius recommendations apply to the final product sold to consumers, not to the cacao beans. The cacao processing industry has translated the EU limits on Cd in cacao-derived products to requirements regarding the maximum Cd concentrations in the fermented cacao beans purchased from their suppliers, and requirements vary among companies. Those unofficial industry limits have been reported to range between 0.50 and 1.10 mg Cd kg^{-1} (Meter et al. 2019; CBI Ministry of Foreign Affairs n.d.), but some cacao farmers are confronted with purchaser thresholds as low as 0.10 mg Cd kg^{-1} (personal communication of first authors). To facilitate the discussion below, 0.60 mg Cd kg^{-1} was used as a threshold.

Mitigation of Cd accumulation has been intensively studied for several food crops including durum wheat, spinach, potatoes, and rice (McLaughlin et al. 2020), but for cacao this research is in its infancy. This review summarizes the existing knowledge on the various factors influencing Cd concentrations in cacao-derived products at all stages of the production process. This includes soil Cd phytoavailability, Cd uptake and translocation within the plant, postharvest processing, and the effect of dietary intake through chocolate consumption on the Cd body burden. A meta-analysis of the different Cd-cacao surveys is presented with due attention given to the quality of the analytical data, thereby only selecting data for which the quality of chemical analyses met quality criteria (more details below and in the Supplementary Information). Research gaps are identified and potential mitigation strategies at the different stages of the cacao value chain are discussed.

2. CADMIUM IN SOILS AND ITS RELATION TO CACAO PLANTS

2.1. Origin of cadmium in cacao beans: soil, air, fertilizer, and irrigation water

Generally, most of the Cd in plant tissues originates from soil through root uptake, while only a small fraction is derived from air through foliar uptake. This is likely also true for cacao, first because of the positive associations reported between soil Cd and cacao bean or leaf Cd concentrations (see below) and, secondly because of the large Cd concentrations found in cacao leaves and beans, which are well above what can reasonably be expected from foliar uptake. The contribution of airborne Cd to crop Cd concentrations can be measured using isotopes that indicate the Cd provenance, and can be quantified using air accumulation factors (AAFs, with units m^3 g^{-1}), calculated as the ratio of the Cd concentration in the plant to that in air. In the Amazon region of Ecuador, Barraza et al. (2017) did not find higher average Cd concentrations in soils and cacao beans from areas where Cd levels in aerosols could be higher due to oil activities, compared to pristine areas. For trace metals in general, the AAF can be estimated at 20 m^3 g^{-1} for plant leaves, with a range of 2–100 m^3 g^{-1} depending on the type of plant and trace metal (Mclaughlin et al. 2011). Air Cd concentrations in the rural areas where cacao is grown range 0.07–0.57 ng Cd m^{-3} [(Barraza et al. 2017), cacao production areas near oil activities]. Leaf Cd concentrations derived from air in these cacao growing areas will thus range 0.001–0.011 mg Cd kg^{-1} (considering an AAF of 20 m^3 g^{-1}), with a maximum of 0.06 mg Cd kg^{-1} (considering an AAF of 100 m^3 g^{-1}), which is only 6% of the typical leaf Cd concentrations (>1 mg Cd kg^{-1}) of cacao plants in Latin America (see below).

Cadmium occurs naturally in the environment, with background soil concentrations ranging from 0.1 to 1.0 mg Cd kg^{-1} and estimated global means of 0.1 to 0.3 mg Cd kg^{-1} (Smolders and Mertens 2013). In contrast, polluted soils (e.g. near smelting sites) can contain Cd concentrations up to three orders of magnitude higher than the background concentrations (He et al. 2015). The soil Cd concentrations in cacao producing areas differ worldwide and tend to be higher in the geologically young soils of Latin America with a range of means between 0.22 and 10.8 mg kg^{-1} (Chavez et al. 2015; Ramtahal et al. 2015; Arévalo-Gardini et al. 2017; Barraza et al. 2017; Gramlich et al. 2017; Barraza et al. 2018; Gramlich et al. 2018; Lewis et al. 2018; Argüello et al. 2019; Engbersen et al. 2019; Rodríguez Albarrcín et al. 2019; Scaccabarozzi et al. 2020), and 0.12 to 0.85 mg kg^{-1} in Asia (Fauziah et al. 2001; Zarcinas et al. 2004). Unfortunately, there is no reliable information to date about Cd concentrations in soils of cacao producing areas in Africa. The majority of global cacao is produced in western Africa where soils are old and weathered, i.e. they are expected to have low Cd concentrations. Indeed, studies in different crops of Ghana have reported total soil Cd concentrations of 0.026 mg kg^{-1} (Bortey-Sam et al. 2015). It is important to note that, while soil Cd concentrations are thus generally reported to be higher in Latin American soils compared to soils in other continents, soil Cd concentrations in Latin American cacao producing areas are mostly <1 mg kg^{-1} (see below), and thus generally not categorized as contaminated soils. Multiple researchers have indicated large geographical differences in cacao bean Cd, with elevated concentrations found in cacao from Latin American origin compared to cacao from other origins (e.g. Africa) (Bertoldi et al. 2016; Abt et al. 2018; Vanderschueren et al. 2019). The strong effect of geographical origin on bean Cd is outlined below (Section 2.2) and suggests that Cd in cacao generally does not originate from anthropogenic activity, as anthropogenic activities occur worldwide. Indeed, three independent studies by Gramlich et al. (2018), Argüello et al. (2019) and Scaccabarozzi et al. (2020) found that high soil Cd concentrations in cacao farms were associated with alluvial soils from sedimentary materials, which can be explained by the higher Cd concentrations usually found in sedimentary rocks compared to igneous rocks (Thornton 1981; Birke et al. 2017). The substrate or alluvium source is likely also of importance, as alluvial soils in Africa do not show similar Cd concentrations compared to their Latin American counterparts. Nevertheless, there are specific cacao producing areas in Latin America where soil Cd concentrations are exceptionally high (Rodríguez Albarrcín et al. 2019), likely due to historical enrichment related to mining activities.

The use of mineral P-fertilizer has been suggested to be at the origin of high Cd in cacao beans (Zug et al. 2019). However, this is highly unlikely on a large scale. Gramlich et al. (2017) measured the Cd concentration in P-fertilizers in a long-term system comparison trial on an experimental cacao farm in Bolivia and found a mean Cd concentration of 102 mg Cd kg^{-1} P_2O_5, which is high compared to fertilizers available in Europe [mean 28 mg Cd kg^{-1} P_2O_5, <10% of samples contained 60 mg Cd kg^{-1} P_2O_5 (Verbeeck et al. 2020)]. At a sustained annual dose of 20 kg P_2O_5 ha^{-1} year^{-1} with that local fertilizer, fertilization would add about 2 g Cd ha^{-1} year^{-1}, equivalent to a net addition to soil of 0.001 mg Cd kg^{-1} soil year^{-1} (at 15 cm incorporation depth) or an accumulation of only 0.1 mg Cd kg^{-1} soil after 100 years. This mineral fertilizer dose is unrealistically high, as most of the cacao production worldwide is in the hands of low-income smallholders who do not commonly use P-fertilizer (Snoeck et al. 2016; Vaast et al. 2016). This worst-case scenario calculation hence indicates that mineral P-fertilizers are not the main source of Cd in cacao plantations, at least not at a large scale.

Irrigation water contains Cd and moderate Cd contamination in the water may be a significant source of Cd in soil and plants. An annual irrigation application of 500 mm with irrigation water containing 1 µg L^{-1} is equivalent to 5 g Cd ha^{-1} $year^{-1}$ and leads to an accumulation of 0.25 mg Cd kg^{-1} soil, at a sustained irrigation after 100 years and 15 cm incorporation. Such accumulation is not unlikely in areas affected by mining activities. For example, in the Puyango-Tumbes river basin at the border between Ecuador and Peru, dissolved Cd concentrations range 1–10 µg L^{-1} due to upstream gold mining (Tarras-Wahlberg et al. 2001; Carling et al. 2013; Marshall et al. 2018). The elevated Cd concentrations in these rivers have been mentioned as a potential source of Cd to nearby cacao fields (Chavez et al. 2015).

Several soil profile studies have reported that soil Cd concentrations decline with increasing soil depth in cacao plantations. The Cd concentration in the top 0–15 cm of the soil is about a factor 1.5 larger than the Cd concentration at depths 15–60 cm (Chavez et al. 2015; Arévalo-Gardini et al. 2016; Barraza et al. 2017; Gramlich et al. 2018). Although this might be interpreted as evidence for anthropogenic sources, it is more plausible that this concentration profile reflects the cycling effect caused by decomposition of litter from the aerial biomass (Reimann et al. 2019). The dry leaf biomass production of cacao is about 3.9 Mg ha^{-1} y^{-1} (Heuveldop et al. 1988) with an average leaf Cd concentration of 2.6 mg kg^{-1} dry weight (dw) ($n = 762$, derived from the meta-analysis described below). Hence, the annual Cd flux returned to the soil by cacao leaf litter decomposition is about 10 g Cd ha^{-1} y^{-1}, which is much larger than the worst-case inputs calculated from application of contaminated P fertilizers or irrigation water as indicated above. Enriching the topsoil from a background soil concentration of 0.89 mg Cd kg^{-1} [estimated by the subsoil Cd concentrations reported by Gramlich et al. (2017)] to 1.09 mg Cd kg^{-1} [topsoil Cd concentrations reported by Gramlich et al. (2017)] would thus require approximately 50 years of leaf litter decomposition, which is not an unrealistic age for the many cacao orchards in the hands of small farmers. Indeed, an economic survey conducted in Ecuador (Vazquez et al., in preparation) reported that the age of cacao orchards ranged from 1 to 100 years, with an average of 13.8 years (n = 1534), and 11% of the orchards surpassed the age of 30. Barraza et al. (2019) determined the stable isotope ratio ($\delta^{114/110}$Cd) in cacao leaves and litter, and topsoil in cacao plantations. While the isotopic ratios in topsoil differed among different sites, there was a consistent fractionation between leaves and soil ($\Delta_{\text{leaf-soil}}$), indicating that the topsoils were likely enriched with heavier Cd isotopes originating from the leaf litter. Considering all the above, the main Cd source in cacao producing soils is likely geogenic rather than anthropogenic, and local high enrichments may be related to point sources such as mining activities, input of sedimentary materials or the use of contaminated irrigation water. The annual Cd cycling due to plant uptake and leaf litter decomposition can explain the larger Cd concentrations in surface soil compared to the subsoil.

2.2. Bean cadmium concentrations and their relationship with soil properties: a meta-analysis

Cacao bean Cd concentration data reported in different surveys have been collated here to identify general trends (Table 1). Studies included in this table were selected based on the quality criteria elaborated in the Supplementary Information, i.e. quality assurance of the chemical analysis combined with a minimal number ($n \geq 4$) of different samples to characterize the region, and information on sample treatment (drying, peeling and digestion procedure). Studies that used flame atomic absorption (AAS) or older inductively coupled plasma optical emission spectrometry (ICP-OES) instruments were excluded from this compilation because of their poor detection limits for Cd in plant samples (0.1–0.5 mg Cd kg^{-1} dry weight) and their propensity to overestimate the true Cd concentrations. Average or median bean Cd concentrations clearly vary by region, with cacao beans originating from Latin America showing Cd concentrations four to six times higher compared to cacao beans originating from Asia or Africa. These elevated cacao Cd concentrations are however only found in the Andean countries, not in Brazil. While most included studies have reported the data as bean Cd concentrations, sample treatments before analyses differ strongly among studies. Processing steps such as fermentation, peeling, or roasting influence the Cd concentration as explained in section 4.3. The spatial variability of bean Cd has been assessed systematically. The coefficient of variation of bean Cd concentrations within fruits ranges 2–27%, mean 12% (Vanderschueren et al., in preparation). No such data were found for the variability among fruits within a tree, but the coefficient of variation among trees within a single field ranges 0.62–110% depending on the field, mean 39% (Argüello et al. 2019). At larger distances, this variation logically increases. This spatial variation may be used to make a power analysis for the minimal replicates needed when testing remediation effects in cacao (see section 4.1).

Cadmium concentrations in crops depend on the availability of soil Cd, which increases with increasing total soil Cd, increasing soil acidity, decreasing soil organic matter, and in some cases, zinc deficiency and chloride salinity (Smolders and Mertens 2013). These relationships have also been corroborated for cacao (Gramlich et al. 2018; Argüello et al. 2019). To better illustrate these general trends, we collated the raw data of different soil-plant surveys. We found over 400 studies on soil-plant Cd relationships in cacao (including all plant tissues), however only seven (Table S 1) passed all quality criteria elaborated in the Supplementary Information. A total of 785 paired soil-plant data points were gathered from these seven studies, which were all based on surveys in Latin America [six published studies and one unpublished dataset (Table S 2)]. The selected studies reported total soil Cd, soil pH, soil organic carbon (SOC), and bean Cd, among other variables. In studies I and VII, bean Cd referred to the Cd concentration in the peeled bean or nib;

while in studies II, III, IV, V and VI, samples were not peeled and bean Cd thus referred to the total bean Cd concentration. The frequency distribution of these data (Figure 1) shows that bean Cd concentrations are 1.1 mg kg^{-1} (arithmetic mean) and 0.63 mg kg^{-1} (median; n = 779) with distinct differences among studies. A total of 403 samples (52% of these compiled data) showed bean Cd concentrations exceeding 0.60 mg kg^{-1}, the threshold commonly used for export to the EU (Figure 1). Per study, the number of observations exceeding this threshold ranged between 7 and 100%. This clearly emphasizes the magnitude of the impact of the new EU Cd regulations on cacao producers.

The average total topsoil Cd concentrations of each study (Table S 2), as well as the overall average (± standard deviation, stdev) of the compiled dataset (0.43 ± 0.41 mg Cd kg^{-1}, n = 776) are within the range for non-polluted soils. Only 50 soil samples across all studies exceeded 1.0 mg Cd kg^{-1}, such “hotspots” represent only 6% of these compiled data. Multivariate regression analysis was first applied to each individual dataset (details on methods can be found in the Supplementary Information). The results of this multivariate analysis (Table 2 and Supplementary Information Table S3) show a generally emerging trend: bean Cd increases with increasing total soil Cd, with decreasing pH (except in study II), and with decreasing soil organic carbon (except in study VI). The effect of organic carbon can be explained by the high affinity of Cd for sorption sites in organic matter (Christensen 1989). The effect of pH on Cd availability is due to the competition between Cd^{2+} and protons for soil sorption sites, as Cd^{2+} binds to oxygen atoms of carboxylic or phenolic groups present in organic matter and oxyhydroxides (Smolders and Mertens 2013). Total soil Cd alone might not be the best predictor for crop Cd concentrations as the majority of Cd is sorbed to different soil particles and is not directly available for plant uptake. Therefore, plant Cd concentrations are better predicted if soil pH is included in the prediction equation, as it is the key soil property controlling the availability of soil Cd. Available soil Cd has been proposed as a proxy to predict crop Cd concentrations. Available Cd is an operationally defined extraction that often refers to the soluble fraction in soil (McLaughlin et al. 2000). Indeed, study VII reported available Cd in the topsoil (measured either by simple extraction with ammonium-acetate-EDTA or using diffusive gradients in thin film, DGT), and available soil Cd was identified as a significant predictor for bean Cd for that study (Table 2 and Supplementary Information Table S3). Soil pH, which affects the soluble fraction of Cd in the soil, is logically not a significant variable when available soil Cd rather than total soil Cd is used. Multivariate regression was also applied to the compiled dataset including data from all seven studies, with correction for the disproportionate weight of their sample sizes (n = 334, methods are described in detail in the Supplementary Information). Total soil Cd, pH, and SOC explained >41% of the variance in bean Cd concentrations within the compiled dataset. The equation of the model reads as follows:

$$log_{10}[Bean\ Cd] = 1.34 + 0.86 * log_{10}[Total\ Soil\ Cd] - 0.18 * pH - 0.25 *\ log_{10}[SOC]$$

where bean Cd and total soil Cd are expressed in mg kg^{-1}, pH is standardized as pH$_{CaCl2}$ by the equation proposed by Kissel et al. (2009), and SOC is expressed as a percentage. This significant multivariate model suggests (i) that bean Cd increases almost proportionally to total soil Cd; (ii) that bean Cd increases by a factor of 1.5 per unit decrease in soil pH (Figure 2); and (iii) that doubling SOC reduces bean Cd by a factor of 1.8. The Cd in cacao issue is thus clearly not related to a single factor such as total soil Cd, but rather to an interaction of several factors, i.e. total soil Cd, soil pH and SOC which control the solubility of Cd and therefore also its availability to the cacao plant.

Because zinc (Zn) and Cd are considered analogues, low available soil Zn has been suggested to trigger plant Cd uptake (Oliver et al. 1994; Chaney et al. 2006; Chaney 2010). In a recent greenhouse study, Souza dos Santos et al. (2020) reported that increase of soil Zn inhibited uptake and translocation of Cd by CCN-51 cacao. However, this effect was only found in soils spiked to Cd concentrations well above environmentally relevant soil Cd concentrations. Argüello et al. (2020) found that Cd uptake in cacao from deeper soil layers was enhanced by surface liming and the authors related this to increased activity of deeper roots to cope with micronutrient deficiency in the topsoil (i.e. Zn) caused by surface liming. The multivariate regressions presented here did not identify total soil Zn as a significant factor to predict bean Cd concentrations (with exception of study II, Table 2), even when using leaf Zn concentrations as regressors (details not shown). Widespread Zn deficiency is unlikely . The overall average leaf Zn concentration in the meta-analysis is 97 mg kg^{-1} (n=700, data from studies I, II, IV and VII) which falls within the range reported as optimal (>20 mg Zn kg^{-1} dw) for plant development (Fageria et al. 2002). Only a small fraction of samples (<5%) presented suboptimal leaf Zn concentrations, which may explain why no significant effect of soil Zn was identified in the regression analysis. The lack of Zn deficiency within the included datasets may also indicate that Zn deficiency is not an issue in Latin American cacao production areas, where Cd accumulation is considerable. However, study II showed a significant statistical effect of total subsoil Zn and total topsoil Mn on bean Cd (regression II a, Table 2). Similarly, study VII showed a significant effect of available subsoil Zn and available topsoil Mn on bean Cd, when available soil Cd was used as a predictor instead of total soil Cd (regression VII b, Table 2). Additional research regarding the effect of available Zn and Mn is required to unravel their potential interaction with Cd and their effect on Cd accumulation in cacao.

Transfer factors (TF) indicate to what extent a crop accumulates Cd in its edible parts and are calculated as the ratio of the Cd concentration in either the plant leaf or the edible part, over the total Cd concentration of the soil. Average transfer factors for cacao (i.e. cacao bean Cd over soil Cd) in the different studies vary from 0.26 to 19 and are significantly affected by soil pH (Figure 3). Transfer factors for plants grown in acid soils are higher compared to TF for plants grown in more neutral or alkaline conditions, confirming the importance of soil pH for plant Cd availability. The TF expression shows that average bean Cd will remain below the 0.60 mg Cd kg^{-1} threshold if soil Cd is below 0.32 mg kg^{-1} (pH 7.0–8.0), below 0.29 mg kg^{-1} (pH 6.0–7.0), below 0.19 mg kg^{-1} (pH 5.0–6.0), or below 0.10 mg kg^{-1} for the most acid soils (pH <5.0). Transfer factors are also influenced by plant genetics and allow comparison of Cd accumulation among crops (see below in section 3.1).

2.3. Agronomic factors affecting Cd uptake in cacao

Some researchers have argued that Cd uptake is affected by cacao management, i.e. monoculture vs agroforestry and organic vs conventional mineral fertilization (Maddela et al. 2020). However, there is not enough information available to date to assess the effect of these agronomic practices on Cd in cacao, and the scarce existing information is not standardized among studies. Two studies investigated the effect of cropping systems (monoculture vs agroforestry) on Cd uptake in cacao. Argüello et al. (2019) found no significant effect of cropping systems on bean Cd concentration in their large national survey in Ecuador. Gramlich et al. (2017) also found no such effect in bean Cd concentrations in a field trial but found higher concentration of Cd in cacao leaves when cacao was grown as a monoculture compared to agroforestry. Gramlich et al. (2017) suggested that the higher plant density present in agroforestry systems may be the reason for a lower leaf Cd concentration in cacao plants in this cropping system. Higher plant density could be translated to higher competition for nutrients, water, and light, which may result in a lower growth rate for cacao. The authors proposed that plants with a lower growth rate take up less Cd, because lower growth rate infers lower nutrient uptake and Cd is usually taken up as a hitchhiker element along with essential nutrients such as Zn. The effect of fertilizers application, i.e. organic vs conventional fertilizers, on Cd uptake in cacao has not been widely studied.

Three studies presented the effect of fertilizer application on Cd uptake with inconsistent results. On one hand, Gramlich et al. (2017) found no significant differences in both soil and bean Cd concentrations between soils treated with either organic or conventional fertilization but this could be related to the low number of observations. On the other hand, Argüello et al. (2019) reported higher Cd concentrations in cacao beans from trees that received organic fertilizers compared to cacao beans from trees that received conventional fertilizers, whereas Zug et al. (2019) found higher Cd concentrations in cacao beans from trees that received conventional N fertilization but no effect of P-fertilizers. Even though the results are contradictory, both situations could be plausible. First, compost is the most common source of nutrients in organic farming. As compost is primarily made with vegetal residues, the quality of the compost product, i.e. amount of trace metals, would be directly related to the original composition of the raw materials. The quality of compost plus the high rates of application could result in higher Cd concentration in organically managed cacao as reported by Argüello et al. (2019). Second, increased N application has been mentioned to enhance Cd uptake and accumulation in plants (Yang et al. 2020). As N fertilization increases aboveground vegetative growth of plants, it stimulates active nutrient uptake and thus uptake of hitchhiker elements such as Cd will also increase. Additionally, N supplementation can lead to soil acidification and reduced pH may cause Cd mobilization (see section 2.2).

3. UPTAKE, TRANSLOCATION AND PARTITIONING OF CADMIUM WITHIN THE CACAO TREE

3.1. Soil-plant transfer of Cd in cacao compared to other plant species

Theobroma cacao L. is a perennial tree that displays cauliflorous flowering, i.e. flowers and pods develop on the trunk and thicker branches of the plant (Toxopeus 1985). The cacao fruit or pod is made up of a large woody outer pod husk, filled with 20–50 cacao beans (seeds) embedded in a white sugary mucilaginous pulp (Figure 4). The cacao beans are connected to the plant through a central tissue called the placenta, and each cacao bean comprises of two cotyledons (the nib), a germ or embryo (the radicle), and an outer shell (the testa) (Wood 1980). The nib is the only part of the cacao bean that is retained during chocolate processing.

Soil to plant transfer factors (TFs) allow identification of Cd accumulating characteristics, although they are influenced by genotype and external conditions such as soil Cd availability (see paragraph 2.2). Soil-plant TFs reported in cacao based on both leaves and beans, are higher compared to TFs reported for most other crops (Table 3). The soil-leaf TFs for Cd are close to those reported for other woody species like willow (Van Slycken et al. 2013) and poplar (Laureysens et al. 2004). The soil-seed TF for cacao range 2–6 depending on the study and are distinctly smaller than TF reported for wheat and rice, but of similar magnitude as TF reported for sunflower kernels (Table 3). The Cd accumulation potential of the cacao plant is thus high, but not unusual. Because of its high soil-plant TF, cacao has been described as a Cd accumulator although this term lacks a clear definition. Several criteria indicate whether a plant can be classified as a Cd hyperaccumulator: (i) in natural conditions the plant should be able to accumulate ≥100 mg Cd kg^{-1} ; (ii) both the transfer factor (TF) and the internal translocation factor ($ITF_{tissue\ 1-\ tissue\ 2} = Cd_{tissue\ 1} / Cd_{tissue\ 2}$) should be larger than one; and (iii) extreme metal tolerance should be achieved due to efficient biochemical detoxification (van der Ent et al. 2013). While cacao does not meet the first criterion, TF > 1 have been reported (Table 3). The mean Cd concentrations in the vegetative tissues of the cacao plant reported in different field studies are collated in Table 4, and these data suggest a relatively homogeneous Cd distribution between roots, scions, and leaves. Engbersen et al. (2019) reported a similar $ITF_{stem\text{-}root}$ (0.99) for cacao compared to $ITF_{stem\text{-}root}$ reported for known moderately Cd accumulating woody species [i.e. $ITF_{stem\text{-}root} \approx 1$ in poplar and cotton, (Vollenweider et al. 2011; Chen et al. 2015)]. To decide on the third criterion and to allow classification of cacao, additional research on the speciation of Cd in cacao tissues is crucial to elucidate the molecular detoxification mechanisms. Nonetheless, the Cd uptake potential of the cacao tree can be conceived as rather high compared to other agricultural crops, and more similar to woody species used for phytoextraction such as willow and poplar. Therefore, we suggest to term cacao a moderate Cd-accumulator.

It is unlikely that the accumulation of Cd in cacao is resulting in Cd toxicity in the field. Different critical Cd toxicity levels have been proposed for various crops in mature leaves [6–10 mg Cd kg^{-1} (Krämer 2010) and 5–30 mg Cd kg^{-1} (Kabata-Pendias 2010)]. Leaf Cd concentrations in cacao exceed the critical level of 10 mg Cd kg^{-1} in only 3% of these data included in the meta-analysis described above, suggesting that Cd toxicity to cacao plants is unlikely to occur at a larger scale. To the best of our knowledge, only one study is available to date discussing Cd toxicity in cacao seedlings (de Araújo et al. 2017). However, the soils were spiked to high Cd concentrations (50 and 100 mg Cd kg^{-1} soil) and the reported leaf Cd concentrations (230–390 mg kg^{-1}) were not in the range of leaf Cd concentrations found in most fields.

3.2. Uptake and translocation of Cd within the cacao plant: trends and mechanisms

Cadmium is known as a hitchhiker element, using transporters for essential elements such as Zn, Fe, Mn, and Ca at all steps of these nutrient pathways, from root uptake to grain or seed loading (Clemens and Ma 2016). Important transporter gene families for the uptake and translocation of Cd in plants are Zinc- Iron Permease (ZIP), Natural Resistance Associated Macrophage Proteins (NRAMPs), and heavy metal transporting ATPases (HMAs). These proteins transport divalent transition metals such as Fe(II), Zn, Mn, and Cd. The limited information available to date regarding the role of these transporter genes for Cd uptake in cacao is discussed below. For a more profound synthesis about the genetic and molecular mechanisms for Cd uptake in plants in general, the reader is referred to specialized reviews (Clemens and Ma 2016; Shahid et al. 2016). The identification of these key genes involved in Cd uptake and translocation in cacao is of great importance as it can be used to develop mitigation strategies by using these genes as targets for genetic engineering, or through marker-assisted breeding programs.

3.2.1. Uptake of Cd in the cacao root system

Ullah et al. (2018) identified five genes from the NRAMP family in cacao and demonstrated that TcNRAMP5 can encode for a protein which transports Cd, Mn(II) and Fe(II). Indeed, it has been reported that NRAMP5 plays a role in the entry of Cd in the root cells for rice (Sasaki et al. 2012; Ishikawa et al. 2012), barley (Wu et al. 2016), Polish wheat (Peng et al. 2018), and tobacco (Tang et al. 2017). More specifically, Ullah et al. (2018) showed that the TcNRAMP5 transcript was primarily expressed in the roots of cacao seedlings. Usually, the expression of NRAMP transporter genes is upregulated under divalent metal deficiency. In cacao seedlings, expression of the TcNRAMP5 transporter gene in the roots was downregulated in the presence of Cd, and upregulated under Fe deficiency, while Zn or Mn deficiency did not affect gene expression. To link the expression pattern to its function, Ullah et al. (2018) cloned five TcNRAMP genes and expressed them in yeast strains. The yeast cells expressing TcNRAMP5 accumulated up to three times more Cd compared to the empty vector control cells. Considering the above, TcNRAMP5 likely plays a major role in the regulation of Cd uptake in cacao plants (Figure 4) and may be targeted in a potential mitigation strategy through induction of loss-of-function mutations in TcNRAMP5. Yet, extrapolation of these findings to relevant cacao soil-plant systems is limited. First, activities used in the hydroponic experiment are about 100 times higher than natural soil Cd conditions. The expression pattern observed may thus be different than natural field conditions. Second, uptake in yeast may not be able to predict uptake in cacao plants.

The isotopic signature of Cd in plants is increasingly used to infer biogeochemical processes but only few studies have reported Cd isotope fractionation data in cacao. Several processes can generate stable isotope fractionation during the transfer of Cd from soil to plant tissues, such as membrane transport or chemical binding. Moore et al. (2020) compared the isotopic shift of TcNRAMP5 transporter expressed yeast relative to the empty vector control cells ($\Delta^{114/110}\mathrm{Cd}_{\mathrm{trans\text{-}ev}} \approx -0.8$ ‰), with the isotopic shift in the cacao seedlings relative to the hydroponic solution ($\Delta^{114/110}\mathrm{Cd}_{\mathrm{tot\text{-}sol}} = -0.22 \pm 0.08$ ‰). Because both systems showed a shift toward lighter isotopes and because the expressed TcNRAMP5 proteins were localized in the external plasma membrane of the yeast cells, the authors confirmed that TcNRAMP5 transporters can be a major pathway for Cd uptake in cacao. However, the observed similar fractionation does not exclude that other transporters can also show similar fractionation and be involved in Cd uptake. Compared to other plants, the total isotopic fractionation due to absorption of Cd from hydroponic solutions in cacao seedlings is less pronounced, but similar to the isotopic shift reported for Cd tolerant and accumulator plants (Wei et al. 2016). The reported fractionation towards lighter isotopes is similar to fractionation from the soil solution to plants in cereals and rice, although there is some variation in the rate of fractionation (Table 5). It is noteworthy that the cacao plants used in the hydroponic study of Moore et al. (2020) had leaf Cd concentration of about 200 mg Cd kg^{-1} which far exceeds the Cd toxicity threshold (10 mg Cd kg^{-1}, see above) and the relevant concentrations in the field (1–10 mg Cd kg^{-1}). This may have influenced the Cd uptake and translocation system due to the activation of internal systems to cope with Cd toxicity (de Araújo et al. 2017).

Experiments with the identification or induction of loss of function mutations in the TcNRAMP5 gene are indispensable to confirm the role of TcNRAMP5 for Cd uptake in cacao. Moreover, other proteins that have been previously reported as major Cd transporters [e.g. AtIRT1 in *A. Thaliana*, and OsIRT1 and OsIRT2 in rice (McLaughlin et al. 2021)] should be studied, since there is no information available regarding these transporters in cacao to date. Knowledge on such transporters is important to predict potential effects of Zn on Cd uptake. Recent work with cacao seedlings showed that cacao leaf Cd concentrations can be reduced by soil Zn addition (dos Santos et al. 2020), but these effects were found only in soils spiked to Cd concentrations well above the environmentally relevant range. Follow-up experiments with more realistic soil Cd concentrations are required.

3.2.2. Partitioning and translocation of Cd inside the cacao plant

From plant roots, Cd is generally transported radially across the root cells and loaded into the xylem, followed by long-distance transport via xylem and phloem and transfer into the plant organs (Clemens and Ma 2016). The rate of translocation of Cd from roots to above-ground tissues depends on vacuolar sequestration, xylem loading, and intervascular and xylem-to-phloem transfer (Figure 4). Cadmium can be sequestered in vacuoles during the radial transport through the root cells, as a protective measure to reduce the mobility of Cd for translocation to other tissues. A key transporter in this vacuolar sequestration is the Heavy Metal transporting ATPase 3 (HMA3) influx transporter, which is located at the membrane of vacuoles and which has been proven to be a major determinant in the sequestration of Cd in roots of rice (Wang et al. 2019), soybean (Wang et al. 2012), and Chinese cabbage and pak choi (L. Zhang et al. 2019). For a discussion of the further translocation of Cd loading in the xylem, root-to-shoot translocation, and intervascular transfer in plants in general, the reader is referred to more specialized reviews (Clemens and Ma 2016; McLaughlin et al. 2020). To the best of our knowledge, the role of the HMA family in Cd sequestration in cacao has not been determined to date. Moore et al. (2020) transformed yeast with HMA family transporters to compare the isotope fractionation pattern due to Cd sequestration in cacao seedlings. The data were however not conclusive on the specific role of HMA transporters in controlling Cd transport in cacao seedlings. In both rice and durum wheat, root-to-shoot Cd translocation via the xylem has been identified as a major determinant for shoot Cd accumulation. (Harris and Taylor 2004; Uraguchi et al. 2009). These observations highlight the need for studies focused on unravelling the mechanisms for Cd translocation from roots to aboveground tissues.

Cacao is a cauliflorous tree and mechanisms and pathways for Cd loading into the developing seeds (the cacao beans) thus may be different from other plants. The relative importance of either xylem or phloem in Cd loading in cacao beans has not been revealed to date. Most studies report leaf Cd concentrations to be higher than corresponding bean Cd concentrations (Table 4; Figure 5). The data from the meta-analysis described above show a markedly positive correlation between bean and leaf Cd concentrations, with average $\mathrm{ITF}_{\text{leaf-bean}}$ ranging between 1.1 to 4.2 among studies (Figure 5). This clear correlation may justify the use of leaf Cd concentration measurements as a proxy for bean Cd because leaf sampling is convenient as it does not depend on fruit availability. However, the use of leaf Cd measurements as a proxy for bean Cd may only be justified when considering a genetically homogeneous sample set. Indeed, both Engbersen et al. (2019) and Lewis et al. (2018) reported only moderate correlations between leaf and bean Cd concentrations in their genetically diverse studies.

Another approach to study the translocation of Cd within plants, is to quantify isotope discrimination between different tissues (Table 5). In the hydroponic system of Moore et al. (2020), cacao leaves were strongly enriched in heavy Cd isotopes compared to the roots ($\Delta^{114/110}$ $Cd_{leaf\text{-}root}$ = 0.26 ‰). Similar hydroponic studies with *Ricinus communis* and *Solanum nigrum* (Wei et al. 2016) showed a less systematic fractionation than reported in the cacao study of Moore et al. (2020). This indicates that cacao seedlings exert mechanisms to cope with Cd that differ from the Cd coping mechanisms in Cd-accumulator plants. A larger fractionation between roots and aboveground tissues may indicate that additional Cd retention mechanisms are invoked to inhibit the translocation of Cd to aerial plant part, as discussed before. In most plants, Cd becomes enriched in heavy isotopes in the order roots<stem<leaf<seed (Table 5). The only study available on Cd isotope discrimination in mature cacao trees suggests that the transfer of Cd from stem to leaves and seeds in cacao is different compared to isotope discrimination studies in other species available thus far (Table 5) (Barraza et al. 2019). This pilot field study in a soil-cacao system in Ecuador reported an average trend towards lighter isotopes in the beans compared to the leaves ($\Delta^{114/110}$ $Cd_{bean\text{-}leaf}$ = -0.27 ‰). This different isotopic signature may be related to the cauliflorous character of cacao and hints to the hypothesis that Cd is directly transferred from xylem to phloem in developing cacao beans, without first passing through the leaves.

3.2.3. Distribution of Cd within the cacao fruit

The Cd concentrations reported for the different cacao fruit tissues are summarized in Table 4. Vanderschueren et al. (2020) reported that weight-based Cd concentrations decreased with testa>nib~placenta~pod husk>mucilage in cacao fruits from four locations and reported testa Cd concentrations were 1.5 to 1.8 times larger compared to nib Cd concentrations. Lewis et al. (2018) also studied the partitioning of Cd between testa and nib, and found that testa Cd concentrations were, on average, twice as large as nib Cd concentrations. However, the authors reported that this concentration ratio (testa Cd over nib Cd) varied depending on the cacao genotype, ranging from 1.2 to >7. The testa accounts for only a small part of the total bean weight and is thus also responsible for only a small fraction of total bean Cd, despite its elevated Cd concentration. For example, Vanderschueren et al. (2020) reported that, in unfermented cacao beans, 91% of the total bean Cd mass was found in the nibs and only 9% originated from the testa.

The outer pod husk is known to be a storage organ providing nutrients to the developing cacao beans through the mucilage (Toxopeus 1985), but its role in Cd transport to the cacao beans is not known thus far. Several surveys have compared Cd concentrations between nibs and pod husks, and all reported similar Cd concentrations in both tissues, i.e. $\mathrm{ITF}_{\text{husk-nib}}$ close to one (Ramtahal et al. 2016; Barraza et al. 2017; Gramlich et al. 2018; Vanderschueren et al. 2020). It has been reported that different nutrient distribution pathways exist between cacao pod husks and beans (De Araujo et al. 2020). Engbersen et al. (2019) reported that Cd partitioning within the cacao fruit may change during maturation of the fruit on the tree. Their results showed decreasing Cd concentrations in the pod husk and increasing Cd in the bean (not peeled) with maturation, which indicates that there may be remobilization of Cd from the husk to the bean. However, additional research is required to corroborate this observation, as the observed differences in Cd concentration among fruits might have been related to the variation in Cd concentration among fruits on one tree, rather than to an effect of maturation.

Information on the distribution and speciation of Cd within cacao beans and other cacao plant tissues remains limited. Thyssen et al. (2018) mapped the 2D distribution of Cd in sections of a fermented cacao bean using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Cadmium was enriched in the testa compared to the nib, and the authors reported a slight Cd enrichment in the meristematic part of the nib (radicle and hypocotyl). The Cd distribution was similar to the distribution of Zn, and to some extent Mg, K and P. Vanderschueren et al. (2020) also used LA-ICP-MS imaging to visualize the Cd distribution in unfermented cacao beans. They showed that the testa layer was visibly distinguishable from the cacao nib due to its elevated Cd content. In addition, they studied the Cd speciation in cacao using X-ray absorption near edge structure spectroscopy (XANES) and found that Cd in cacao nib and testa was bound to O/N-ligands. In contrast, Yan et al. (2020) showed that Cd concentrations in the crease of durum wheat grains was half-half associated with thiols (S ligands) and organic acids (O ligands). However, Cd speciation and localization are usually studied in plant systems exposed to high Cd concentrations. These studies do not allow comparison with the natural field conditions for cacao. More research on speciation and partitioning in crops grown in field conditions seems paramount to unravel the mechanisms used for Cd storage in cacao.

3.2.4. Cultivar-related differences in Cd uptake and partitioning

The cultivar effect on Cd uptake in cacao was first reported in 2018, with a factor 13 variation in bean Cd and factor 7 in leaf Cd concentrations among cultivars grown on the field (Lewis et al. 2018). The data suggest a differential partitioning of Cd between vegetative (leaf) and reproductive (bean) tissues, favoring the hypothesis that there are cultivar-specific differences in xylem-to-phloem transfer of Cd to the different tissues. Engbersen et al. (2019) reported that available soil Cd was closely related to Cd in vegetative parts ($R^2 \approx 0.50$) and to a minor extent to bean Cd (R^2

= 0.26), which was attributed to a cultivar effect in the loading of Cd into the beans. The authors used this as indirect evidence suggesting that cultivar-related differences in bean Cd concentrations are mainly due to cultivar-specific differences in xylem-to-phloem transfer. While Cd transfer to the leaves is probably mostly governed by the xylem, the contributions of either the xylem or phloem pathway for cacao bean Cd loading are yet to be revealed and may differ among cultivars. However, the cacao trees in the study of Engbersen et al. (2019) were grafted onto rootstocks of unknown genetic identity, which may have affected the uptake of metals and the further translocation from the root, obscuring the cultivar effect. The design of that study also did not allow to account for potential effects of local spatial variability, which may have influenced uptake and partitioning of Cd. Barraza et al. (2019) reported a genotype effect in the isotopic fractionation between cacao leaves and beans (Nacional: $\Delta^{114/110}Cd_{bean\text{-}leaf}$ = -0.34 to -0.40 ‰, CCN-51 hybrid: $\Delta^{114/110}Cd_{bean\text{-}leaf}$ = -0.08 ‰). A distinct isotope fractionation pattern has also been reported between shoots and roots of an excluder ($\Delta^{114/110}Cd_{shoot\text{-}root}$ = 0.19 ‰) and a non-excluder type of rice ($\Delta^{114/110}Cd_{shoot\text{-}root}$ = -0.02 ‰) (Wiggenhauser et al. 2020). The consistent isotope fractionation between cacao leaves and soil reported by Barraza et al. (2019) is intriguing and indicates clear differences in the soil-bean fractionation among cultivars. This confirms the previous hypothesis that the translocation and sequestration of Cd in different plant tissues varies among cultivars. This pilot study has some limitations due to the limited number of observations but confirms that the isotope approach may provide insights on Cd pathways in the plant and pave the road for further studies in the field.

4. MITIGATION STRATEGIES TO LOWER THE CADMIUM CONCENTRATION IN THE FINAL PRODUCT

The current Cd regulations in cacao products apply to the final food products, not the cacao beans. Therefore, mitigation strategies can be applied at all stages of the production process from tree to chocolate bar. Mitigation practices at different production steps will likely have to be combined in order to achieve a final product that complies with the new EU regulation. Mitigation practices can be based on soil amendments to reduce Cd uptake into the plant; plant-based strategies, i.e. selection of cultivars with reduced translocation of Cd to the cacao beans; or postharvest processing, i.e. fermentation and winnowing. Agronomic practices have also been suggested to influence Cd concentrations in cacao but additional research on their potential effects is required (see section 2.3).

4.1. Mitigating Cd uptake using soil amendments

While several soil amendment techniques exist, liming, biochar, gypsum, and Zn supplementation are the only soil amendments that will be discussed here as these are the only amendments that have been (or are being) investigated for the mitigation of Cd in cacao. A Reduction Factor (RF), defined as the ratio between the crop Cd concentration in the control (no treatment) and the Cd concentration in the treatment was calculated to quantify the effect of each soil amendment. A RF >1 indicates that crop Cd was reduced by the treatment.

4.1.1. Lime

The application of liming materials [i.e. $CaCO_3$, CaO, $Ca(OH)_2$, $CaMg(CO_3)_2$] has often been recommended as an agronomic practice to reduce the uptake and accumulation of Cd in edible parts of crops. Liming decreases the solubility of soil Cd (and thus decreases available soil Cd) by increasing soil pH and through competition between Ca^{2+} and Cd^{2+} at root uptake sites

(Christensen 1984; Bolan et al. 2003a). However, increasing the solution concentration of Ca^{2+} can also result in competitive desorption of surface bound Cd^{2+} (Christensen 1984). This latter is one of the plausible reasons why liming does not consistently decrease the concentration of Cd in plant tissue (Bolan et al. 2003b). Significant reductions in crop Cd by the application of lime have been reported in field experiments with paddy rice in China: the application of 7.5 Mg ha^{-1} $CaCO_3$ led to RF 3–4, and this was even sufficient to have an effect on rice grain Cd grown in the 2nd year crop (H. Chen et al. 2018); and application of 1.5 Mg ha^{-1} burned lime (75% CaO) resulted in RF of 1.5 (Zhu et al. 2016). However, it is unclear if such high RF can be reached with lime application in the case of cacao.

A recent study by Ramtahal et al. (2019) evaluated the effect of application of $Ca(OH)_2$ at different rates (0–6 Mg ha^{-1}) on Cd uptake in cacao plants grown in pots (6 month old cacao cuttings) and in the field (30 year old trees). Results from the pot trial showed effectively reduced leaf Cd concentrations 6 months after lime application, with a RF=3. However, in the field trial leaf Cd initially decreased by a factor 2 (measured 4 months after lime application), after which leaf Cd started to increase in both control and limed treatments so that for the last sampling (9 months after lime application), leaf Cd in the limed treatment was only reduced by a factor 1.1. Interestingly, soil pH remained at the target pH of 7.0 up until the 9th month after lime application. This contrast in lime effect between field trials and pot experiments is not uncommon. Earlier work conducted in potatoes (Maier et al. 1996; Sparrow and Salardini 1997), sunflower kernels (Li et al. 1996) and peanuts (McLaughlin et al. 1997) suggested that the difficulty in fully incorporating lime to deeper soil layers as an explanation for the lack of effect and/or limited effectiveness of liming in reducing crop Cd in the field. The lack of effect of liming in the cacao field trial may thus be related to Cd being taken up by deeper rooted soil horizons that were not affected by the lime treatment. In established plantations, lime incorporation to deeper soil horizons is not possible without damaging the root system, which may compromise the efficacy of the amendment. Indeed, Argüello et al. (2020) showed in a pot experiment with cacao seedlings that partially liming the rooted soil (lime in top compartment only as would be the case in field liming), enhanced Cd uptake from the non-limed bottom compartment.

4.1.2. Gypsum

The effectiveness of lime in reducing soil Cd availability to cacao is limited by the low solubility of liming materials and by the physical limitations to incorporate them into the soil. Amendments capable of reaching deeper soil layers, such as gypsum, may be able to overcome such limitations. The mechanism by which gypsum reduces soil Cd availability is yet to be revealed; it is not a liming material and thus does not increase soil pH. Nevertheless, studies have shown that adding gypsum as a soil amendment reduces Cd uptake in different crops: RF 1.9 with application of 3% w:w in pot trail with *Angelica gigas* (Kim et al. 2018); RF 2.5 with application of 0.15 g S kg^{-1} soil in pool experiment with rice (D. Zhang et al. 2019); and RF 2–3 with application of 0.8% w:w in a field trial with wheat and rice (Rehman et al. 2015). Based on the data available in other crops, gypsum might be a plausible amendment to ameliorate Cd accumulation in cacao beans. However, research is needed to reveal the potential of gypsum as a soil amendment to mitigate Cd in cacao.

4.1.3. Biochar

Biochar has received a lot of attention as an effective material for soil immobilization of metals such as Cd. The sorption properties of this carbon rich material are related to its high specific surface area, high cation exchange capacity (CEC), alkaline pH and the presence of surface functional groups (carboxylic, hydroxyl, phenolic) (Li et al. 2017). Because of the complex composition of biochar, the exact mechanism of metal sorption is not completely understood.

However, results from laboratory experiments indicate that, at least in the short term, metal immobilization is controlled by an increase in soil pH due to alkalinization processes and by intra-particle diffusion of the elements within the biochar pores (Rees et al. 2014). The effectiveness of biochar application also depends on soil properties, the type of crop and the type of biochar used. According to the meta-analysis of D. Chen et al. (2018), positive effects in reducing plant Cd are expected when biochar is applied in soils with acid pH, coarse texture and intermediate organic carbon content.

In cacao, the work by Ramtahal et al. (2019) evaluated the effect of a commercially available biochar (charcoal green®) on the phytoavailability of soil Cd in pot and field experiments. Biochar was applied at rates of 0, 326, 489, 652 kg ha^{-1} and the materials were incorporated at 20 cm depth. In the greenhouse study, biochar significantly increased soil pH by about one pH unit and reduced the concentration of Cd in the leaf of cacao seedlings, and this effect was dose dependent. Results from the field experiment with 30-year-old cacao trees showed that biochar did not increase soil pH in contrast to the pot trial. Biochar applied at the highest dose in the field trials reduced leaf Cd by factor 1.9 compared to the control, and the effect was more consistent over time than the effect observed for lime application (last sampling done 6 months after biochar application, see above). Results of studies with other crops are promising but the effectiveness of the treatment varies: RF 2 with application of 20–40 Mg ha^{-1} in a field study with rice grain and the effect of biochar application on rice grain could still be observed three years after application to the soil (Bian et al. 2013; Bian et al. 2014); and RF 1.2 determined one year after biochar application up to 40 Mg ha^{-1} in a field study with wheat on alkaline soil, but this effect was no longer present or highly variable in years 2 and 3 after application (Sui et al. 2018). Such high application rates of soil amendments would likely not be cost effective in cacao farming.

4.1.4. Fertilizer management: zinc supplementation

Although no studies in cacao to date have reported the effect of Zn fertilization in reducing Cd uptake; this topic is briefly described here because, based on information collected in other crops, the application of soil or foliar Zn can effectively reduce the concentration of Cd in crops. The effect of Zn fertilization on crop Cd depends on the status of Zn in the soil, with largest effects reported in Zn deficient soils: RF 2 with application of up to 5 kg Zn ha^{-1} in wheat grain (Oliver et al. 1994); RF 2 with application of 13 kg Zn ha^{-1} in brown rice (Fahad et al. 2015); and a linear decrease in Cd concentration in grain and shoots of yellow lupin with increasing Zn dose up to 6.4 kg Zn ha^{-1} (Brennan and Bolland 2014). It has been suggested that the effect of Zn application on plant Cd can be explained by competition between Zn and Cd at the root uptake sites, or that the application of Zn reduces the Zn deficiency of the plant, thereby preventing loss of integrity of the root cells which can facilitate non-selective Cd uptake through mass flow (Grant et al. 1999). In contrast, in studies conducted in soils where soil Zn levels were not deficient, Zn fertilization did not significantly affect the Cd concentration in wheat grain (Grant and Bailey 1998; Rojas-Cifuentes et al. 2012; Forster et al. 2018). An exception is the study on potatoes by McLaughlin et al. (1995), who showed that the addition of very large doses of Zn at planting (up to 100 kg Zn ha^{-1}) in non Zn-deficient soils could significantly decrease Cd in the tuber (RF 2) in one of the studied sites.

4.1.5. Factors complicating the use of soil amendments to remediate Cd in cacao

Theobroma cacao L. is a perennial tree and it is thus essential to test the efficacy of soil amendments in field conditions. The root system of the cacao tree is most dense in the near-surface soil (Nygren et al. 2013; Niether et al. 2019), which complicates effective incorporation of soil amendments. The poorly understood complexity of rhizosphere processes in the roots of cacao may also explain lack of effect for some soil amendments and additional research is needed to better understand how cacao roots shape the local biochemical condition in the rhizosphere. The high spatial variability is a major obstacle to detect effects of soil treatments on bean Cd concentrations. A power analysis was performed based on the variation in bean Cd within the same fields reported by Argüello et al. (2019) (Supplementary Information). Beans from only 5 trees per treatment are required to identify a statistically significant effect of treatment if a reduction factor 2 (RF) is expected and if the field has an average spatial variability; for fields with a realistic worst-case variability, that number increases to 15 trees per treatment. In contrast, an unfeasibly large number of 198 trees are required to identify significant effects if the treatments yield only RF 1.2 and if the variability is a realistic worst case. A RF 1.5 can have large consequences on the economy of the cacao sector (see below) but it is clear that detection of such moderate (but realistic) RFs is challenged by soil and bean Cd variability.

4.2. Genetics as a potential mitigation strategy for Cd in cacao

The research on the potential of genetic mitigation strategies (i.e. traditional genetic selection, plant breeding techniques, marker-assisted molecular breeding and/or genetic engineering) is still in an early phase as the genetic and molecular mechanisms for Cd uptake and partitioning in cacao are still being explored. There are indications that genetics-based techniques could offer effective and sustainable strategies for Cd mitigation in cacao. As discussed above, the work by Ullah et al. (2018) and Moore et al. (2020) identified TcNRAMP5 as a potentially important gene for Cd uptake in cacao, and thus as a potential target for genetic selection or modification. A recent greenhouse experiment with 53 wild and domesticated cacao genotypes proposed 11 cacao clones as low Cd accumulators which can be potentially exploited for future work (Arévalo-Hernández et al. 2020). However, soils in that study were spiked to extreme Cd concentrations (25 mg Cd kg^{-}

^{1}), a follow-up experiment with more environmentally relevant soil Cd concentrations is needed to truly confirm those cultivars as low Cd accumulators. Lewis et al. (2018) found significant differences in cacao bean and leaf Cd concentrations among cultivars grown in the field (up to factor 13) in conditions with comparable available soil Cd; while Engbersen et al. (2019) observed only a factor 2–3 difference in bean Cd among cacao cultivars. However, the differences in bean Cd concentrations among cultivars observed in these studies might also be related to other factors such as soil variability and other site conditions, specific growth dilution or differences in flushing and pod development cycles, rather than Cd uptake and translocation. Future studies of the cultivar effect on Cd in cacao should thus evaluate multiple sites and consider large genotype-environment interactions.

The use of low-Cd accumulating rootstocks for grafting (a common technique in cacao cultivation) may be promising to reduce Cd uptake in cacao. Grafting has been used to mitigate plant stress caused by adverse soil chemical conditions in the root environment (i.e. heavy metal uptake and accumulation) (Savvas et al. 2010). The role of the rootstock versus the scion on Cd accumulation in cacao beans is not yet known, and this is a key knowledge gap for breeding. Rootstock effects on above-ground Cd concentrations appear to be plant species dependent. For example, Arao et al. (2008) demonstrated that grafting eggplants onto *Solanum torvum* rootstock reduced Cd in the fruit by 63─74% compared to grafting onto *Solanum melongena* and *Solanum integrifolium*. In addition, the concentration of Cd measured in xylem sap was significantly lower in *S. torvum* than in *S. melongena*. This suggests that *S. torvum* can limit translocation of Cd from the root to the shoot. In contrast, studies conducted on potato cultivars showed that the rootstock is an important factor to regulate the total Cd uptake by the plant but that the scion regulates the distribution of Cd between the shoots and tubers (Mengist et al. 2018). The use of cacao rootstocks with low Cd uptake potential could be the basis to renovate old cultivars, and eventually reduce Cd concentrations in cacao beans and its by-products while maintaining the flavor of specific cacao cultivars which is likely under control of the scion.

4.3. Postharvest processing

There are four main approaches within the postharvest process that may affect the Cd concentration in the final cacao-derived product: fermentation, testa removal, choice of product recipe, and cacao bean mixing (Figure 6). First, fermentation has been suggested to cause mobilization of Cd within cacao beans due to acidification of the cacao nib (Vanderschueren et al. 2020). The potential impact of fermentation on Cd in cacao, and the need for research on this topic has been indicated (Meter et al. 2019), but published research remains scarce to date. Some authors have reported Cd concentrations measured in unfermented beans and intermediate products after fermentation. For example, Barraza et al. (2017) measured Cd in unfermented unpeeled cacao beans (1.02–1.37 mg kg^{-1}) and in cacao liquor (1.47–3.88 mg kg^{-1}), i.e. suggesting some Cd enrichment after fermentation and processing. Yanus et al. (2014) reported Cd concentrations in unfermented nibs (0.072 ± 0.001 mg kg^{-1}) and testa (0.085 ± 0.001 mg kg^{-1}), and in cacao powder (0.125 ± 0.011 mg kg^{-1}), again suggesting enrichment. However, it is unclear from these studies to what extent these trends are related to variability of samples or a true effect of processing steps. The systematic study of Vanderschueren et al. (2020) on the impact of fermentation on Cd in cacao indicated a potential migration of Cd from the nib to the testa during fermentation, resulting in a factor 1.3 decrease in nib Cd (RF 1.3). However, a significant decrease in nib Cd with fermentation was only observed when nib pH at the end of fermentation was <5 (which may be achieved by more extensive fermentation).

Second, optimal removal of the cacao testa likely reduces the Cd concentration in the final product, as testa Cd concentrations are generally larger (by approximately a factor 2) compared to nib Cd concentrations [Table 4 and (Lee and Low 1985; Yanus et al. 2014; Ramtahal et al. 2016; Lewis et al. 2018; Vanderschueren et al. 2020)]. The cacao testa is removed after roasting in a process called 'breaking and winnowing', after which the cacao nibs are ground to obtain cacao liquor. However, testa removal is not complete and the intermediate product cacao liquor is allowed to contain up to 5% (m/m) residual testa and/or germ (also referred to as radicle) on a fat-free dry weight basis or approximately 2.5% on a total dry weight basis. Considering that the testa comprises approximately 10% of the dry weight of a cacao bean, complete testa removal can result in a reduction of the Cd concentration by a factor 1.16 from intact cacao beans to cacao liquor. This also indicates that cacao beans may be unnecessarily rejected by the EU cacao processing industry because of elevated Cd concentrations if this decision is based on total bean Cd (nib and testa) instead of nib Cd.

Third, Cd is mostly partitioned in the non-fat cacao solids (Mounicou et al. 2003; Yanus et al. 2014; Kruszewski et al. 2018). Therefore, final consumer products made from the same cacao beans may have very different Cd concentrations depending on the intended product, i.e. Cd concentrations in white chocolate are negligibly small while fat-reduced cacao powder contains high Cd concentrations. Indeed, Cd concentrations are reported to be larger in cacao powders compared to cacao liquor and/or cacao beans (Mounicou et al. 2003; Kruszewski et al. 2018). Considering this, it can be relevant for cacao processing companies to take the intended final product into consideration when setting Cd requirements for their cacao suppliers (Figure 6). In addition, the new EU Cd limits differ depending on the cacao solids content of the final product. To produce a typical milk chocolate with 35% cacao solids (EU limit 0.30 mg Cd kg^{-1}), cacao beans can be used with nib Cd concentrations up to 0.86 mg Cd kg^{-1}, not considering effects of fermentation and/or testa removal on the Cd concentration of the cacao liquor. For dark chocolate with 70% cacao solids (EU limit 0.80 mg Cd kg^{-1}), cacao beans with nib Cd concentrations up to 1.14 mg Cd kg^{-1} could be used, which is much higher than the common industry requirement of 0.60 mg Cd kg^{-1}.

Fourth, it is common practice to mix cacao from different geographical sources during the production process. Considering the large geographical differences in Cd concentrations in cacao (Table 1), cacao from different origins can be mixed to ensure acceptable Cd concentrations in the final product (e.g. mixing cacao from West Africa which is generally reported to contain low Cd concentrations, with cacao from Central or South America where Cd concentrations are higher). This strategy can also offer viable solutions on a national scale, especially for countries specializing in fine flavor origin chocolate. For example, based on bean Cd concentrations measured by Argüello et al. (2019) and the cacao production per province reported by the Ecuadorian government between 2014 and 2019 (Instituto Nacional de Estadística y Censos - Gobierno de La República del Ecuador 2019), the average bean Cd concentration per province exceeds the recommended value of 0.60 mg Cd kg^{-1} in 10 of the 23 Ecuadorian provinces (corresponding to 32,000 metric tonnes, or 15.5% of the national production). While the extensive database of Argüello et al. (2019) indicates an average bean Cd concentration of 0.90 mg Cd kg^{-1} with 48% of fields exceeding the guideline of 0.60 mg Cd kg^{-1}, mixing all production at a national scale would result in an average bean Cd concentration of 0.59 mg kg^{-1} and the product would thus comply with the Cd requirements (Supplementary Information, Table S 6). Although mixing on a national scale is likely not practically feasible, this does indicate that there is potential for mixing strategies on smaller scales. The cacao market in many Latin American countries is already set up in a way that would allow structural mixing of cacao from different sources, as larger cooperatives often purchase the cacao from small scale farmers and then sell the (mixed) product to international clients.

5. FROM CHOCOLATE BAR TO BODY BURDEN

While different regulatory bodies are implementing limitations regarding the maximum allowed Cd concentration in chocolate and other cacao-derived products, controversy remains regarding tolerable intake levels, and regarding the relation between dietary intake and body burden. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a provisional tolerable monthly intake of 25 µg Cd kg^{-1} body weight (bw), which corresponds to a tolerable daily intake of 0.83 µg Cd kg^{-1} bw (FAO/WHO 2010). Conversely, the European Food Safety Authority (EFSA) set a stricter tolerable weekly intake of 2.5 µg Cd kg^{-1} bw, or a daily tolerable intake of 0.36 µg Cd kg^{-1} bw (EFSA 2011). Children and infants have been identified as a high-risk group within the population, due to differences in their diet compared to adults and because they consume more per unit body weight. However, as adverse effects due to dietary Cd intake are mostly related to accumulation in the human body over a lifetime of exposure, focusing on these young age groups may not be relevant. Although research has indicated that only a small part of dietary Cd is likely absorbed in the human body (European Chemicals Bureau 2007), regulations and health-based guidelines are based on dietary Cd intake rather than resulting Cd body burden. Most dietary Cd studies show that the Cd body burden increases less than proportional with increasing dietary Cd intake, in contrast to the assumption in the risk assessment models, and this is related to lower Cd bioavailability and/or changes in micronutrient status with diet when relying on high Cd diets (Vahter et al. 1996; Reeves et al. 2001). Only limited information is currently available regarding the gastro-intestinal absorption rate of Cd from cacao derived products, and studies thus far have focused only on *in vitro* experiments. Mounicou et al. (2002 and 2003) studied the *in vitro* bioaccessibility of Cd in cacao liquor and cacao powder and found accessible fractions ranging 10–50 %. Conversely, Barraza et al. (2017) determined the gastric bioaccessibility of Cd using the unified method from the Bioaccessibility Research Group of Europe and reported gastric bioaccessibility of >90% in cacao liquor from Ecuador. These studies have not highlighted if such fractions are lower than for other staple foods such as cereals. There is currently no chemical reason to expect lower Cd bioavailability and/or bioaccessibility in cacao compared to cereals, as cacao is unlikely to contain strong Cd chelating compounds (Vanderschueren et al. 2020). However, considering the strong influence of dietary status (e.g. dietary Zn, Fe, and Ca) on the gastro-intestinal absorption of Cd (Reeves and Chaney 2008), it remains to be demonstrated if long-term high chocolate consumption truly increases the body burden Cd to levels of concern. In-vivo duplicate diet studies with increased dietary Cd through chocolate consumption, would offer vital insights to assess the assumptions behind the existing (and future) food regulations.

6. CONCLUSION

Recent (and upcoming) regulations on the maximum allowed Cd concentration in cacao-derived products are threatening cacao producers worldwide and are especially causing concern in Latin America. Elevated soil Cd concentrations found in cacao production areas are likely related to geogenic origin rather than contamination through anthropogenic influences. Bean Cd concentrations are generally larger in cacao plants grown on acidic soils with low soil organic carbon, when considering equal total soil Cd. Several mitigation strategies are being explored to deal with elevated Cd concentrations in cacao. However, mitigation strategies are not a cure-all and their use comes at a cost, either due the financial cost of implementing the strategy or due to adverse effects on final product quality. First, soil amendments can reduce plant uptake of Cd by reducing the phytoavailable soil concentration (i.e. biochar, lime, or gypsum) or by saturating uptake mechanisms through which Cd can otherwise enter the plant (i.e. Zn fertilization). Application of lime and biochar in cacao field trials has been reported to result in reduction factors up to RF 1.9, but as both treatments are based on a liming effect (i.e. increasing soil pH), these amendments are mostly effective in acid soils. While soil amendments could offer an easily applicable medium-term solution, their incorporation is limited due to the rooting system of the perennial cacao tree. Second, root uptake and root-to-shoot translocation of Cd within the cacao plant may be targeted for selection of low Cd accumulating cultivars. While the research on uptake and translocation mechanisms of Cd in cacao is only in its early stages, genetics-based mitigation strategies can be highly valuable in the future. The available work on cacao indicates potential RF ranging from 2 up to 13. However, experiments on the same soil (i.e. same location, also termed common garden experiments) should be performed to identify low Cd accumulating cultivars (and their potential RF) and avoid current uncertainties related to the different soils under the different cultivars. The potential of using specific rootstocks in grafting should also be explored as this may offer the benefit of genetics-based mitigation (selection of a low Cd uptake rootstock) while maintaining the flavor profile of the original cacao cultivar in the scion. There is a need to better characterize the pathways of Cd loading into cacao beans to reveal the role of the xylem and phloem pathway. Third, postharvest mitigation strategies may offer the benefit of control for industrial scale cacao processers. Mitigation strategies interfering with processes such as fermentation have moderate potential (RF 1.3) but require additional research, and the effect of such strategies on the flavor quality of the final product should always be taken into consideration. On the short term, a realistic and easily implementable strategy is mixing of cacao from different origins (and thus with different bean Cd concentrations), both on larger and smaller scales. The intended final product should be kept in mind when setting Cd requirements for cacao suppliers, as current requirements may be overly strict depending on the cacao content of the final product. For example, to produce dark chocolate with 70% cacao solids, cacao beans containing up to 1.14 mg Cd kg^{-1} could be used, which is almost double the currently common requirement of 0.60 mg Cd kg^{-1}. Finally, considering the indications that cacao is a moderate Cd accumulator, farmers may be recommended to change to a lower Cd accumulating crop if mitigation for cacao is not feasible.

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FIGURES

Figure 1: Percentile distribution of cacao bean Cd concentrations of all the compiled data from Ecuador and Honduras (n = 780, overall mean 1.08 mg Cd kg^{-1}), with indication of the generally accepted threshold of 0.60 mg Cd kg^{-1} for export to the EU. Cacao beans were either peeled or not depending on the study (Table 1). A detailed description of the studies included in the meta-analysis can be found in the Supplementary Information.

Figure 2: Cacao bean Cd concentrations as influenced by total soil Cd and soil pH for the selected studies in the meta-analysis. Soil pH values for all studies are standardized to pH_{CaCl2} (Kissel et al. 2009). The dashed line indicates the generally accepted threshold of 0.60 mg Cd kg^{-1} for export of cacao beans to Europe. Details of the studies can be found in the Supplementary Information.

Figure 3: Average transfer factors (dry weight bean Cd concentrations divided by dry weight-based soil Cd concentrations, dimensionless) versus pH range per study (I-VII). Bars represent the standard error of the mean. For study III, no error bars are given in pH range 6–7 as only one observation was available. Details of the studies can be found in the Supplementary Information.

Figure 4: Uptake and translocation of Cd within the cacao plant-soil system. (1) Cadmium uptake in a simplified cacao root cell. TcNRAMP5 has been identified as an important transported for Cd uptake from soil solution, but it is likely not the only transporter. Potential further pathways include sequestration in vacuoles, and root-to-shoot translocation. (2) Translocation of Cd from root to cacao pod may occur through direct uptake from the xylem, or through remobilization of Cd from the leaves and transportation through phloem. (3) Loading of Cd in the different pod tissues. (4) Cycling of Cd from deeper soil layers to topsoil through leaf litter decomposition.

Figure 5: Cadmium concentrations in leaves and cacao beans are significantly correlated (Pearson correlation coefficient r = 0.82) and Cd concentrations are generally higher in leaves compared to beans. Studies III and V are not included as they did not include leaf Cd measurements. Details of the studies can be found in the Supplementary Information. ITF =internal transfer factor calculated as the ratio of leaf Cd over bean Cd concentrations.

Figure 6: Effect of postharvest processing steps on Cd concentrations in (intermediate) cacao-derived products, considering an initial total bean Cd concentration of 0.60 mg Cd kg^{-1}. Only the processing steps which can potentially reduce the Cd concentration in the final product are given: (1) adequate fermentation can cause migration of Cd from nib to testa, resulting in a reduction of nib Cd by up to a factor 1.3; (2) current regulations allow 2.5% (m/m) testa material to remain in cacao liquor, more efficient testa removal may result in lower Cd concentrations due to high testa Cd (i.e. in this case complete testa removal would result in cacao liquor with 0.42 mg Cd kg^{-1}); (3) Cd is contained in the non-fat cacao solids and thus Cd concentrations in the final product depend on product type.

TABLES

Table 1: Average bean Cd concentrations (mg kg^{-1}) in cacao from several surveys across the world clearly illustrate geographical differences, with the highest bean Cd concentrations reported for cacao grown in Central and South American countries (rows marked in grey), with exception of Brazil. Incomplete information on analytical data quality is indicated by superscripts with the sample origin. The variation is indicated either by the standard deviation or the range min-max; P = peeled; UP = unpeeled; F = fermented; UF = unfermented; R = roasted.

[a] No information was found on whether Certified Reference Materials (CRM) were included or not.

[b] CRM included with certified Cd concentrations above the reasonable range of cacao Cd concentrations.

[c] Reported sample Cd concentrations lower than the limit of detection of the equipment used for Cd analysis.

[d] Mean and variation values were calculated from the raw data which was kindly provided by the authors of these studies.

Table 2: Predictors for bean Cd concentrations for each of the included studies, determined by multivariate regression analysis. All variables were log-transformed before analysis except for pH. Sign in brackets corresponds to the sign of the coefficient of that predictor. Top = topsoil (0–20 cm), Sub = subsoil (20–40 cm), Av = available, SOC = soil organic carbon, DGT = diffusive gradient thin film.

[§] There are three regression models for study VII as the variables Total Cd Top, Av Cd Top and DGT Cd Top were highly correlated thus running only one regression with all variables included would have violated the assumption of no multicollinearity. For study II, two models are included because Total Cd Top was significantly correlated to Total Zn Sub and Total Mn Top.

[¥] The number of observations included in each regression can differ from the number of observations mentioned in the Supplementary Information (Table S 2) due to missing data for some of the predictors.

Table 3: Mean cadmium transfer factors (TF) (min-max) for cacao, i.e. Cd concentration ratios between cacao (leaf or bean = seed) and soil, and TF for other selected agricultural crops. The TF predicted from soil-plant regression models used median values of the soil properties from the surveys that yielded the corresponding models. Additional information regarding specific study conditions (i.e. study location and soil Cd) can be found in the Supplementary Information (Table S4).

[$]No range available, only mean is given

Table 4: Mean (min-max) dry weight-based Cd concentrations (mg Cd kg^{-1}) for different cacao plant tissues, including only unfermented cacao beans. Dashes indicate that the parameter was not reported for this specific study.

[$] Paired tissue Cd concentrations are displayed individually for each batch, as indicated by Vanderschueren et al. (2020).

[§] Analysis of scion wood cores.

Table 5: Average (min/max) or average ± stdev apparent Cd isotope fractionation ($\Delta^{114/110}Cd_{A-B} = \delta^{114/110}Cd_A - \delta^{114/110}Cd_B$) (‰) for different plants. A positive value indicates that tissue A is enriched in heavy isotopes compared to tissue B. If no average or min/max was reported in the article, it is not given in the table.

[$]Shoot=stem; [$$]Leaf = flag-leaves; [*]Rice accessions with functional OsHMA3 tonoplast transporter in roots; [**]Rice accessions without functional OsHMA3 tonoplast transporter; [a] Source: Hydroponic solution; [b] Source: $Ca(NO_3)_2$ soil extract; [c] Source: Soil solution

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TABLES

Table 1: Average bean Cd concentrations (mg kg^{-1}) in cacao from several surveys across the world clearly illustrate geographical differences, with the highest bean Cd concentrations reported for cacao grown in Central and South American countries (rows marked in grey), with exception of Brazil. Incomplete information on analytical data quality is indicated by superscripts with the sample origin. The variation is indicated either by the standard deviation or the range min-max; P = peeled; UP = unpeeled; F = fermented; UF = unfermented; R = roasted.

Sample origin	Bean Cd [mg kg^{-1}]			Cacao processing	Study
	Mean	Variation	N		
Ghana [a,c]	0.02	0.003	3	P, F	(Vītola and Ciproviča 2016)
Nigeria [a,c]	0.02	0.003	3	P, F	(Vītola and Ciproviča 2016)
Ghana [a,c]	0.05	0.045–0.058	30	UP, F	(Nnuro et al. 2020)
Cameroon [a,c]	0.05	0.01	3	P, F	(Vītola and Ciproviča 2016)
Ghana [a,c]	0.05	0.005–0.095	20	UP, F	(Amankwaah et al. 2015)
Ivory Coast [a,c]	0.05	0.04	9	P, F	(Yapo et al. 2014)
West Africa	0.09	0.04	21	UP, F	(Bertoldi et al. 2016)
Brazil [a]	0.10	/	1	P, F	(Knezevic 1979)
Ivory Coast [a]	0.12	0.09–0.14	3	P, F	(Knezevic 1979)
Sao Thomas and Principe [a]	0.12	0.09–0.15	4	P, F	(Knezevic 1979)
Dominican Republic	0.13	0.031	-	UP, F	(Kruszewski et al. 2018)
Tanzania [a]	0.13	/	1	P, F	(Knezevic 1979)
Ghana [a]	0.14	0.09–0.18	8	P, F	(Knezevic 1979)
Brazil (Bahía) [a]	0.19	0.09–0.29	8	P, F	(Knezevic 1979)
Ecuador [a,c]	0.20	0.04	3	UP, F	(Vītola and Ciproviča 2016)
Bolivia [a]	0.21	0.02	64	P, F	(Gramlich et al. 2017)
New Guinea [a]	0.22	0.14–0.29	8	P, F	(Knezevic 1979)
Samoa [a]	0.22	/	1	P, F	(Knezevic 1979)
Malaysia [a]	0.25	0.01–1.27	86	/	(Mohamed et al. 2020)
Sri Lanka [a]	0.26	0.24–0.27	2	P, F	(Knezevic 1979)
Ghana [a,c]	0.3	0.248–0.336	67	P, F	(Takrama et al. 2015)
Mexico [a]	0.28	/	1	P, F	(Knezevic 1979)
Asia	0.33	0.18	8	UP, F	(Bertoldi et al. 2016)
Ecuador	0.35	0.24	50	UP, UF	(Acosta and Pozo 2013)

Sample origin	Bean Cd [mg kg^{-1}]			Cacao processing	Study
	Mean	Variation	N		
East Africa	0.51	0.59	8	UP, F	(Bertoldi et al. 2016)
Indonesia (Sumatra) [a]	0.52	/	1	P, F	(Knezevic 1979)
Central America	0.54	0.30	10	UP, F	(Bertoldi et al. 2016)
Malaysia [a]	0.55	0.26	10	/	(Fauziah et al. 2001)
Brazil	0.55	0.10–1.50	36	P, F	(De Araujo et al. 2017)
Caribbean [a]	0.57	/	1	P, F	(Knezevic 1979)
Latin America	0.62	0.38	7	P, F, R	(Abt et al. 2018)
Ecuador	0.63	0.067	-	UP, F	(Kruszewski et al. 2018)
Malaysia [a]	0.67	0.20–1.68	5	/	(Zarcinas et al. 2004)
Trinidad & Tobago [a]	0.68	/	1	P, F	(Knezevic 1979)
Ecuador	0.75	0.27–1.72	81	UP, F	(Romero-Estévez et al. 2019)
Indonesia (Java) [a]	0.76	/	1	P, F	(Knezevic 1979)
Grenada [a]	0.77	/	1	P, F	(Knezevic 1979)
Ecuador	0.78	0.12–1.52	4	UP, UF	(Barraza et al. 2018)
Ecuador	0.90	0.09–3.51	31	UP, UF	(Barraza et al. 2017)
Ecuador	0.90	0.03–10.4	560	P, UF	(Argüello et al. 2019)
Malaysia (Sabah) [a]	0.94	0.59–1.29	2	P, F	(Knezevic 1979)
Ecuador [c]	0.94	0.02–3.00	19	P, UF	(Chavez et al. 2015)
Jamaica [a]	0.95	/	1	P, F	(Knezevic 1979)
Peru [a]	0.96	0.34	72	UP, UF	(Rosales-Huamani et al. 2020)
Trinidad & Tobago	0.98	0.50–2.34	45	P, F	(Ramtahal et al. 2015)
Trinidad & Tobago [b]	1.00	0.17–2.31	100	UP, UF	(Lewis et al. 2018)
Costa Rica [a]	1.02	/	1	P, F	(Knezevic 1979)
Honduras	1.10	0.10	110	P, UF	(Gramlich et al. 2018)
Malaysia [a]	1.14	0.45–1.83	9	P, F	(Knezevic 1979)
Peru [a, d]	1.13	0.11–6.30	70	UP, UF	(Arévalo-Gardini et al. 2017)
Peru [a]	1.31	1.26–1.36	3	P, F	(Knezevic 1979)
South America [a]	1.39	1.09	14	UP, F	(Bertoldi et al. 2016)
Venezuela [a]	1.96	1.75–2.17	3	P, F	(Knezevic 1979)
Costa Rica [a,c]	2.20	0.56–8.7	24	UP, UF	(Furcal-Beriguete and Torres-Morales 2020)
Trinidad & Tobago	2.27	1.78	402	P, UF	(Ramtahal et al. 2016)
Ecuador (Arriba) [a]	2.45	0.55–4.34	6	P, F	(Knezevic 1979)
Honduras	2.56	0.81–10.6	60	UP, UF	(Engbersen et al. 2019)

Sample origin	Bean Cd [mg kg^{-1}]			Cacao processing	Study
	Mean	Variation	N		
Ecuador [d]	2.68	1.26–3.92	5	UP, UF	(Barraza et al. 2019)
Colombia [a]	12.0	6.94	57	UP, F	(Rodríguez Albarrcín et al. 2019)

[a] No information was found on whether Certified Reference Materials (CRM) were included or not.

[b] CRM included with certified Cd concentrations above the reasonable range of cacao Cd concentrations.

[c] Reported sample Cd concentrations lower than the limit of detection of the equipment used for Cd analysis.

[d] Mean and variation values were calculated from the raw data which was kindly provided by the authors of these studies.

Table 2: Predictors for bean Cd concentrations for each of the included studies, determined by multivariate regression analysis. All variables were log-transformed before analysis except for pH. Sign in brackets corresponds to the sign of the coefficient of that predictor. Top = topsoil (0–20 cm), Sub = subsoil (20–40 cm), Av = available, SOC = soil organic carbon, DGT = diffusive gradient thin film.

Study	n ¥	Significant predictors	R^2
I	559	Total Cd Top (+); pH Top(-); SOC Top(-)	0.57
II a §	28	SOC Sub (-); Total Zn Sub (+); Total Mn Top (+)	0.64
II b §	28	Total Cd Top (+); SOC Sub(-)	0.49
VI	60	Total Cd Top (+); pH Top (-)	0.23
VII a §	107	Total Cd Top (+); pH Top(-); SOC Top(-)	0.36
VII b §	106	Av Cd Top (+); SOC Top (-); Av Zn Sub (+); Av Mn Top (-)	0.41
VII c §	105	DGT Cd Top (+); SOC Top (-)	0.43

§ There are three regression models for study VII as the variables Total Cd Top, Av Cd Top and DGT Cd Top were highly correlated thus running only one regression with all variables included would have violated the assumption of no multicollinearity. For study II, two models are included because Total Cd Top was significantly correlated to Total Zn Sub and Total Mn Top.

¥ The number of observations included in each regression can differ from the number of observations mentioned in the Supplementary Information (Table S 2) due to missing data for some of the predictors.

Table 3: Mean cadmium transfer factors (TF) (min-max) for cacao, i.e. Cd concentration ratios between cacao (leaf or bean = seed) and soil, and TF for other selected agricultural crops. The TF predicted from soil-plant regression models used median values of the soil properties from the surveys that yielded the corresponding models. Additional information regarding specific study conditions (i.e. study location and soil Cd) can be found in the Supplementary Information (Table S4).

TF calculated as concentration ratio

Plant	TF (leaf – soil)	TF (seed – soil)	n	Reference
Cacao	4.5 (0.34–42.8)	1.6 (0.13–12.5)	560	(Argüello et al. 2019)
	3.4 (0.56–20.1)	2.0 (0.26–7.8)	28	(Barraza et al. 2017)
	4.9 (1.9–29.2)	5.6 (1.4–29.8)	60	(Engbersen et al. 2019)
	7.1 (1.9–21.1)	2.7 (0.5–16.3)	108	(Gramlich et al. 2018)
Willow	(1.6–10.3)		8	(Van Slycken et al. 2013)
Oak	$0.26^{\$}$		2	(Sevel et al. 2009)
Poplar	(5.0–40.0)		13	(Laureysens et al. 2004)
Leafy vegetables	0.19 (0.001–4.5)		170	(Zhang et al. 2014)
Sunflower		2.2 (1.8–3.4)	200	(Li et al. 1995)
Cotton		$0.46^{\$}$	6	(Chen et al. 2015)
Wheat	0.27 (0.06–0.64)	0.15 (0.03–0.34)	40	(Puschenreiter and Horak 2000)
Rye	0.11 (0.03–0.29)	0.04 (0.01–0.16)	40	(Puschenreiter and Horak 2000)
Pistachio		0.03 (0.01–0.04)	220	(Shirani et al. 2018)

TF calculated from predicted crop Cd concentration model based on soil properties

Plant	TF (seed – soil)	n	Reference
Cacao	1.76	334	This review
Indica rice	0.76	1043	(Römkens et al. 2009)
Japonica rice	0.30	2155	(Römkens et al. 2009)
Wheat	0.27	246	(Adams et al. 2004)

$^{\$}$No range available, only mean is given

Table 4: Mean (min-max) dry weight-based Cd concentrations (mg Cd kg^{-1}) for different cacao plant tissues, including only unfermented cacao beans. Dashes indicate that the parameter was not reported for this specific study.

Reference	**Tissue Cd (mg kg^{-1})**						
	Root	**Scion**	**Leaf**	**Nib**	**Testa**	**Pod husk**	**Mucilage**
Argüello et al., 2019	/	/	2.62 (0.13–55.5) n = 560	0.90 (0.03–10.4) n = 560	/	/	/
Barraza et al., 2017	/	/	1.99 (0.19–7.9) n = 28	0.90 (0.09–3.5) n = 28	/	0.98 (0.08–4.4) n = 31	/
Engbersen et al., 2019	2.44 (0.68–9.70) n = 60	2.25 [§] (0.41–13.0) n = 60	2.31 (0.64–9.3) n = 60	2.56 (0.81–10.6) n = 60	/	/	/
Gramlich et al., 2018	/	/	2.64 (0.06–28.0) n = 110	1.06 (0.03–7.1) n = 109	/	1.12 (0.04–10.2) n = 108	/
Lewis et al., 2018	/	/	2.18 (0.48–5.2) n = 198	0.99 (0.17–2.3) n = 137	/	/	/
Ramtahal et al., 2016	/	/	3.56 (0.45–17.4) n = 482	2.27 (0.48–9.3) n = 402	2.55 (0.33–15.4) n = 441	2.55 (0.46–8.0) n = 241	/
Vanderschueren et al., 2020 – A [$]	/	/	/	0.52 (0.42–0.74) n = 3	0.94 (0.73–1.2) n = 3	0.59 (0.39–0.85) n = 3	0.08 (0.06–0.09) n = 3
Vanderschueren et al., 2020 – B [$]	/	/	/	0.39 (0.30–0.48) n = 3	0.66 (0.50–1.0) n = 3	0.28 (0.26–0.29) n = 3	0.09 (0.06–0.14) n = 3
Vanderschueren et al., 2020 – C [$]	/	/	/	2.4 (1.2–3.9) n = 6	3.7 (1.8–6.7) n = 6	/	0.48 (0.30–1.1) n = 6
Vanderschueren et al., 2020 – D [$]	/	/	/	9.6 (6.4–13) n = 6	16 (11–24) n = 6	/	/

[$] Paired tissue Cd concentrations are displayed individually for each batch, as indicated by Vanderschueren et al. (2020).

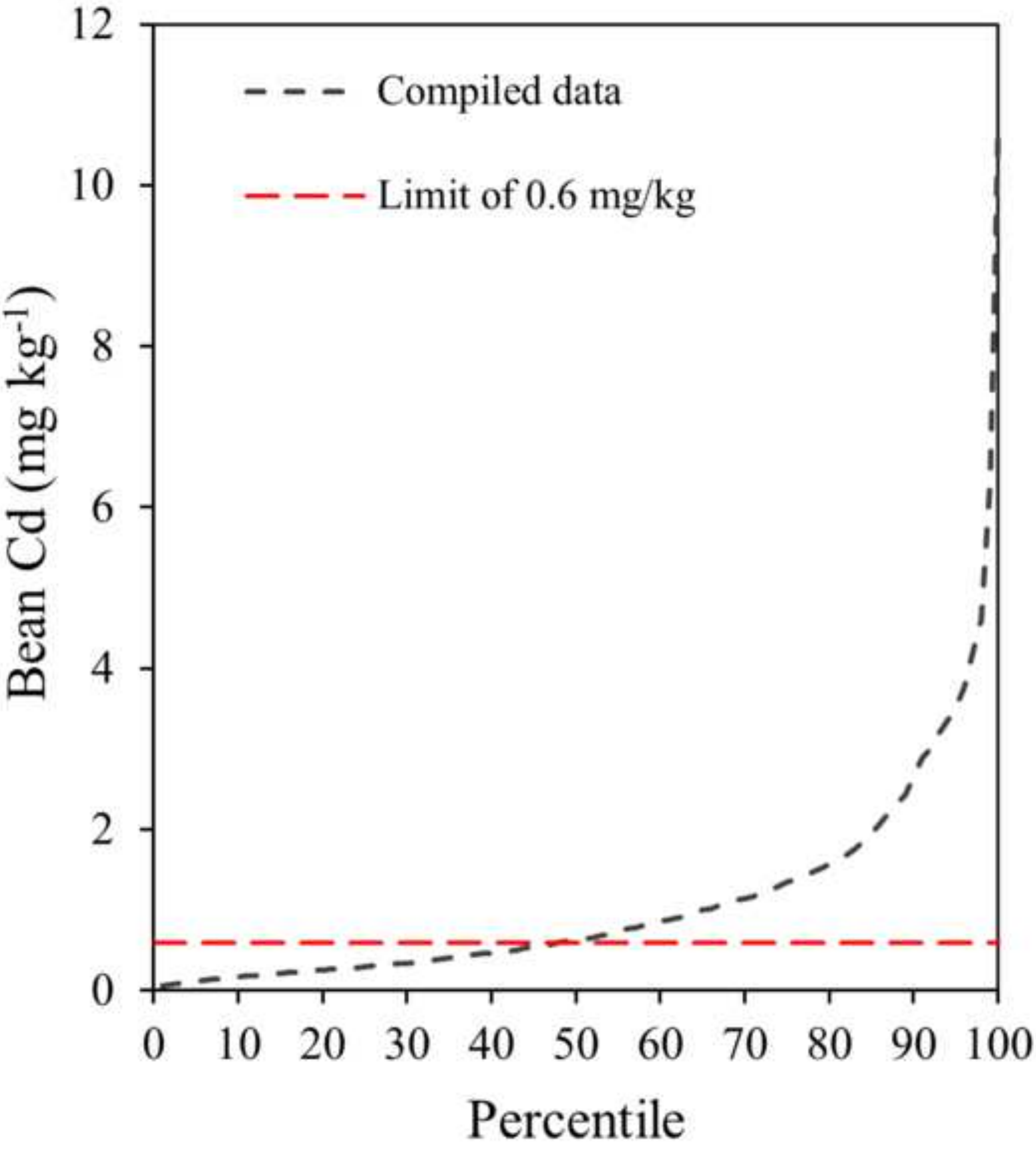
[§] Analysis of scion wood cores.

Table 5: Average (min/max) or average ± stdev apparent Cd isotope fractionation ($\Delta^{114/110}Cd_{A-B} = \delta^{114/110}Cd_A - \delta^{114/110}Cd_B$) (‰) for different plants. A positive value indicates that tissue A is enriched in heavy isotopes compared to tissue B. If no average or min/max was reported in the article, it is not given in the table.

Plant	$\Delta^{114/110}Cd_{A-B}$(‰)								
	Plant-substrate	**Plant-soil**	**Shoot-root**	**Leaf-stem**	**Leaf-root**	**Grain-stem**	**Grain-leaf**	**Remarks and conditions**	**Reference**
Hydroponics									
Ricinus communis	-0.38^{1} (-0.46/-0.32)		0.03 (-0.08/0.19) $^{\$}$	-0.11 (-0.18/-0.04)	-0.05 (-0.26/0.15)			2 mg Cd L^{-1}	(Wei et al. 2016)
Solanum nigrum	-0.41^{1}		0.02$^{\$}$	0.09	0.11			2 mg Cd L^{-1}	(Wei et al. 2016)
Cacao	-0.22 (-0.34/0.01) ^{a}				0.33 (0.13/0.9)			20 different clones 2.2 mg Cd L^{-1}	(Moore et al. 2020)
Pot experiments									
Wheat	-0.07 (-0.21/0.03) ^{b}	0.23 (0.13/0.39)	0.29 (0.21/0.41)			0.30 (0.1/0.5) $^{\$}$		Mean of 3 locations 0.19–0.51 mg Cd kg^{-1} soil	(Wiggenhauser et al. 2016)
Wheat	(-0.36/-0.20) ^{c}	(0.31/0.46)	0.26 (0.19/0.35)			0.32 (0.16/0.46) $^{\$}$		Mean of 3 locations 0.17–1.66 mg Cd kg^{-1} soil	(Imseng et al. 2019)
Barley	(-0.1/-0.06) ^{c}	(0.51/0.55)	0.34 (0.27/0.45)			0.59 (0.44/0.82) $^{\$}$		Mean of 3 locations 0.17–1.66 mg Cd kg^{-1} soil	(Imseng et al. 2019)
Rice	-0.30 ± 0.01^{c}		0.16 ± 0.03					Excluder*, flowering stage wet conditions 15 mg Cd kg^{-1} soil	(Wiggenhauser et al. 2020)
Rice	-0.43 ± 0.01^{c}		-0.02 ± 0.05					Non-excluder**, wet conditions 15 mg Cd kg^{-1} soil	(Wiggenhauser et al. 2020)
Rice			0.28	0.19$^{\$\$}$	0.44$^{\$\$}$		0.51	Excluder, maturity stage, wet conditions 15 mg Cd kg^{-1} soil	(Wiggenhauser et al. 2021)
Field experiments									

Cacao	0.34 (0.22/0.41)		-0.27 (-0.40/-0.08)	Mean of 4 sites ≤ 1.10 mg Cdkg soil	(Barraza et al. 2019)
Rice	0.45			Mean of 2 locations 2.25–8.29 mg Cd kg^{-1} soil	(Zhang et al. 2020)

$^{\$}$Shoot=stem; $^{\$\$}$Leaf = flag-leaves; *Rice accessions with functional OsHMA3 tonoplast transporter in roots; **Rice accessions without functional OsHMA3 tonoplast transporter; ^{a} Source: Hydroponic solution; ^{b} Source: $Ca(NO_3)_2$ soil extract; ^{c} Source: Soil solution.


Compiled data
Limit of 0.6 mg/kg
Bean Cd (mg kg-1)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
80
90
100
Percentile

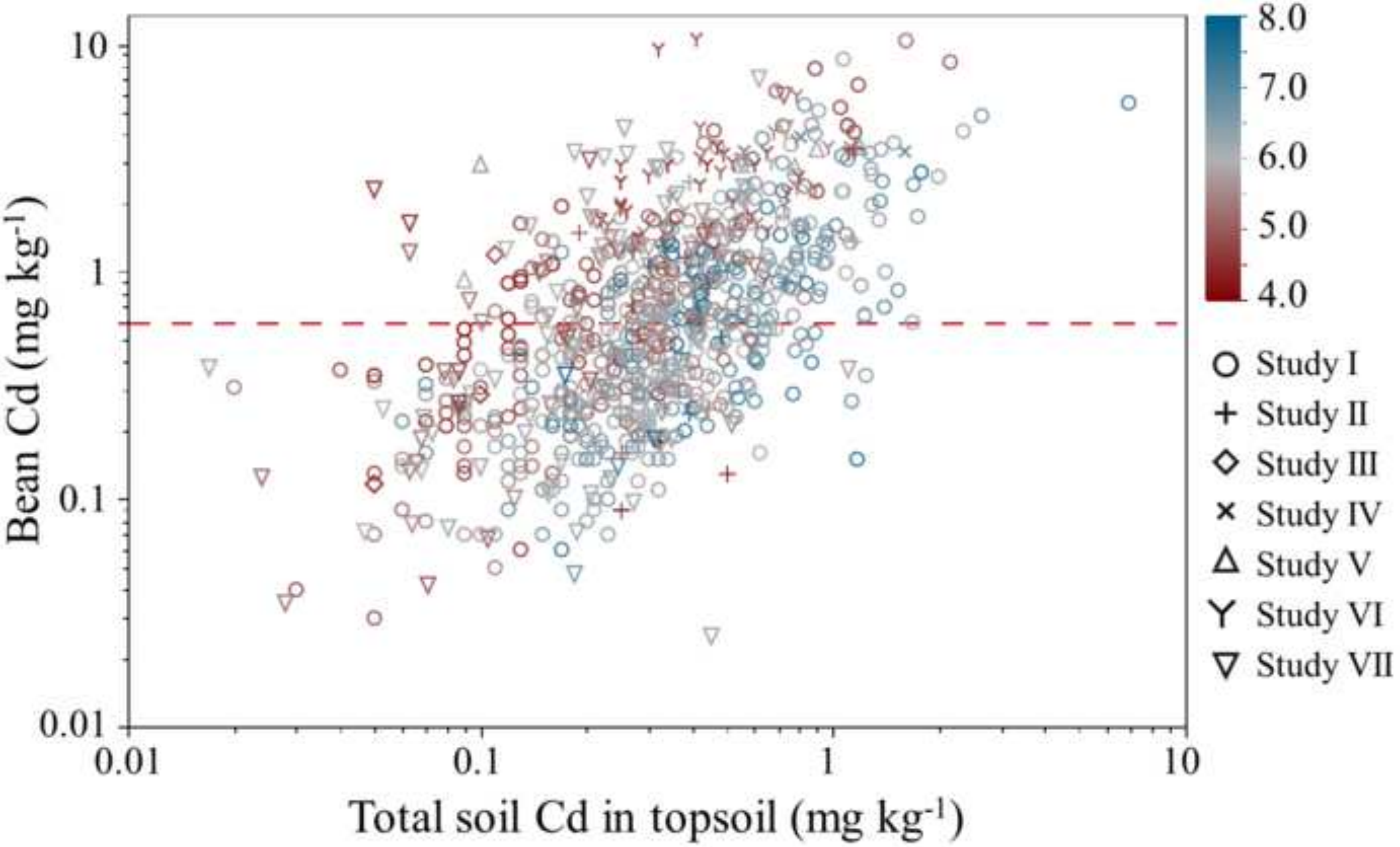
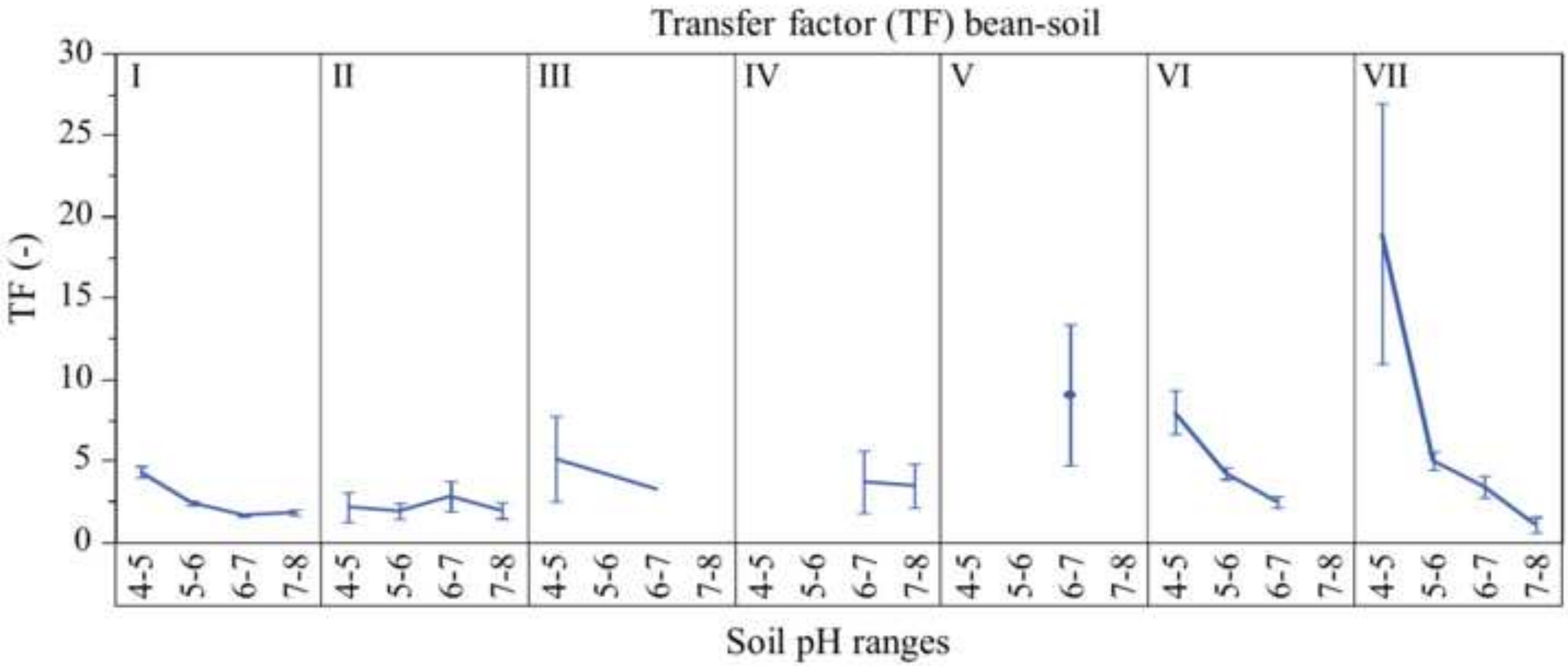

10
1
0.1
0.01
Bean Cd (mg kg^{-1})
0.01
0.1
1
10
Total soil Cd in topsoil (mg kg^{-1})
8.0
7.0
6.0
5.0
4.0
○ Study I
+ Study II
◇ Study III
× Study IV
△ Study V
Y Study VI
▽ Study VII

Figure 3


Transfer factor (TF) bean-soil
TF (-)
0
5
10
15
20
25
30
I
II
III
IV
V
VI
VII
4-5
5-6
6-7
7-8
Soil pH ranges

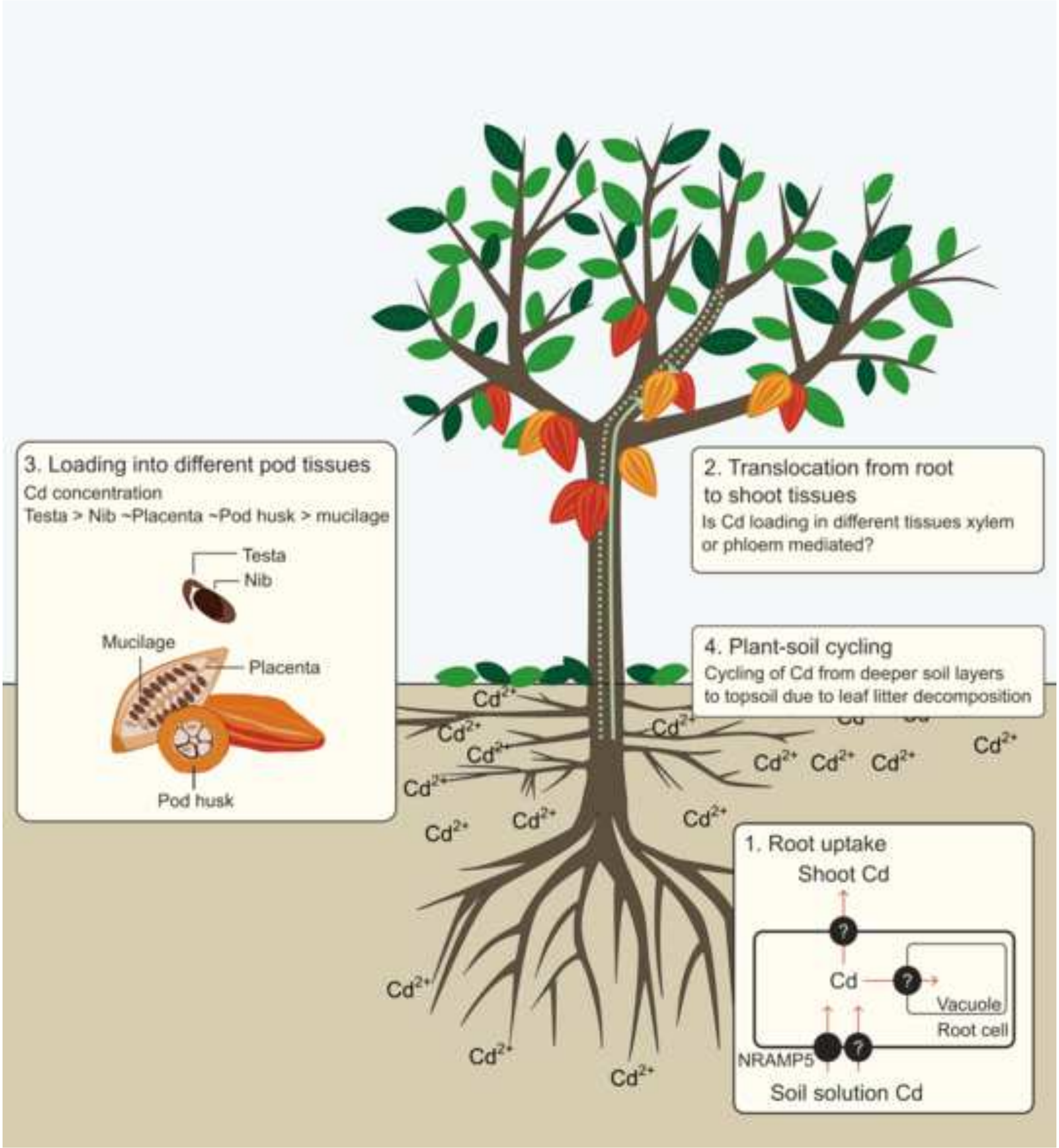
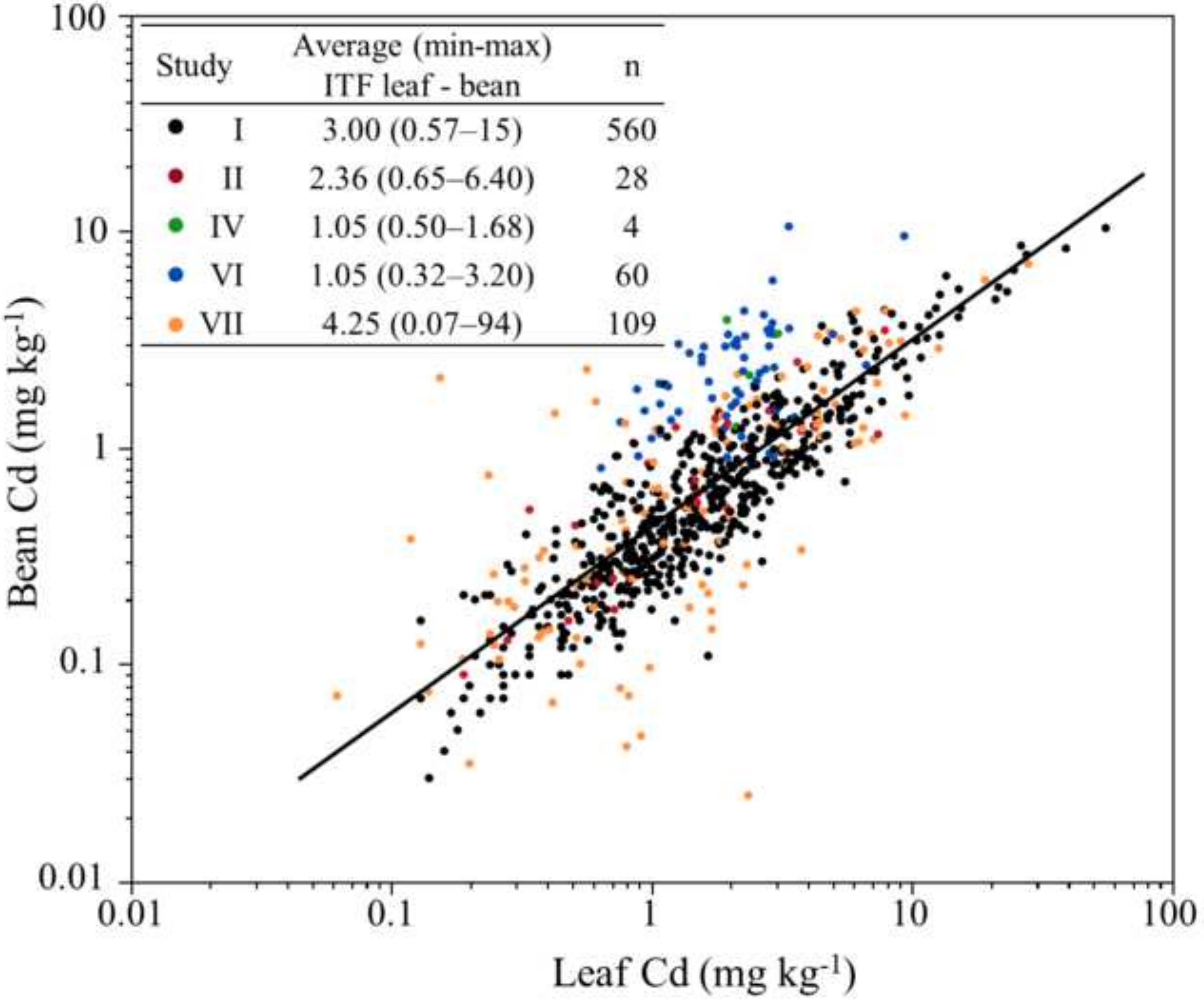
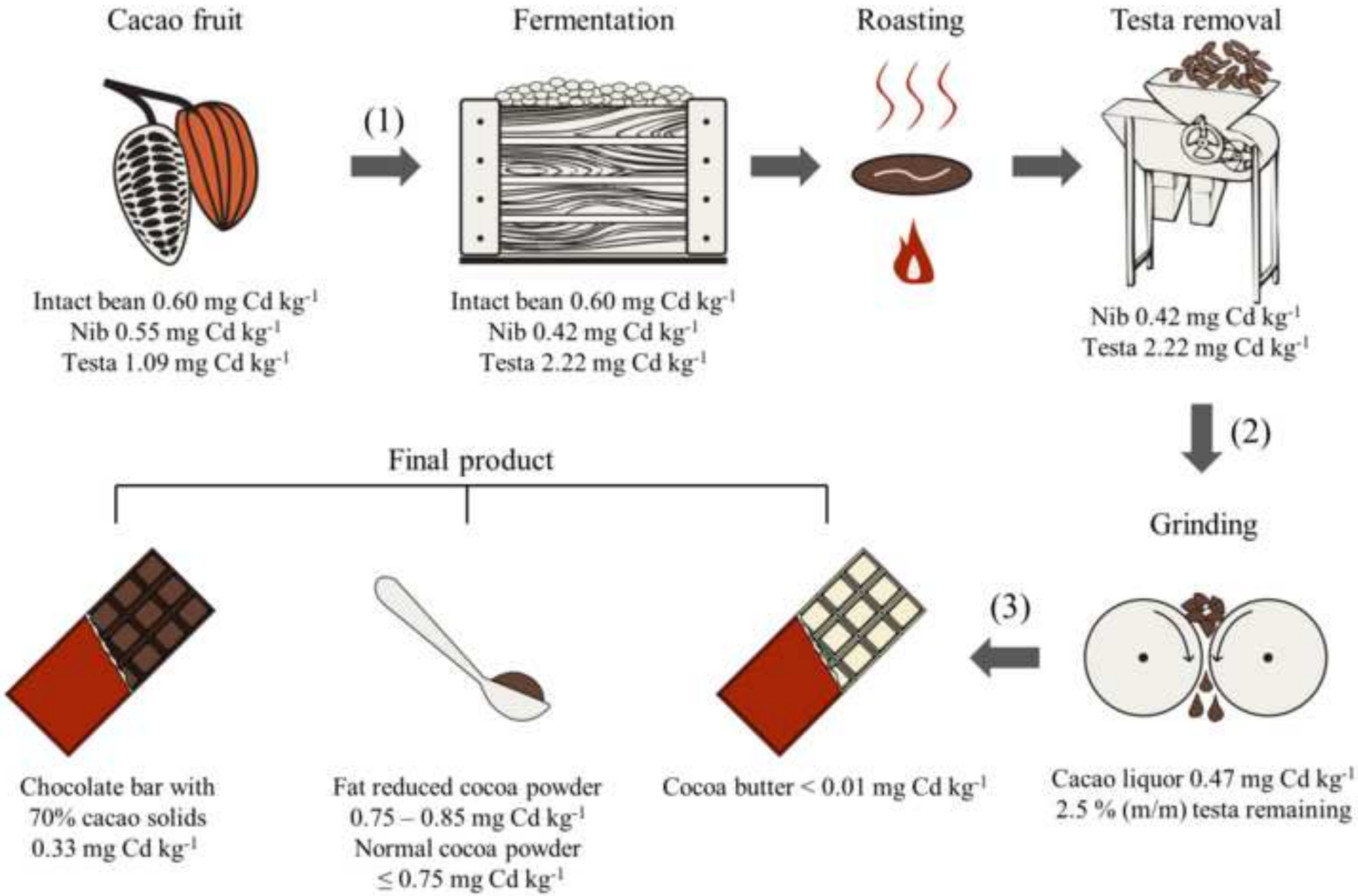

3. Loading into different pod tissues
Cd concentration
Testa > Nib ~Placenta ~Pod husk > mucilage
Testa
Nib
Mucilage
Placenta
Pod husk
2. Translocation from root to shoot tissues
Is Cd loading in different tissues xylem or phloem mediated?
4. Plant-soil cycling
Cycling of Cd from deeper soil layers to topsoil due to leaf litter decomposition
Cd2+
1. Root uptake
Shoot Cd
?
Cd
?
Vacuole
Root cell
NRAMP5
?
Soil solution Cd

Figure 5


Study
Average (min-max) ITF leaf - bean
n
● I 3.00 (0.57–15) 560
● II 2.36 (0.65–6.40) 28
● IV 1.05 (0.50–1.68) 4
● VI 1.05 (0.32–3.20) 60
● VII 4.25 (0.07–94) 109
100
10
1
0.1
0.01
Bean Cd (mg kg-1)
0.01
0.1
1
10
100
Leaf Cd (mg kg-1)


Cacao fruit
Fermentation
Roasting
Testa removal
(1)
Intact bean 0.60 mg Cd kg^{-1}
Nib 0.55 mg Cd kg^{-1}
Testa 1.09 mg Cd kg^{-1}
Intact bean 0.60 mg Cd kg^{-1}
Nib 0.42 mg Cd kg^{-1}
Testa 2.22 mg Cd kg^{-1}
Nib 0.42 mg Cd kg^{-1}
Testa 2.22 mg Cd kg^{-1}
(2)
Final product
Grinding
(3)
Chocolate bar with
70% cacao solids
0.33 mg Cd kg^{-1}
Fat reduced cocoa powder
0.75 – 0.85 mg Cd kg^{-1}
Normal cocoa powder
≤ 0.75 mg Cd kg^{-1}
Cocoa butter < 0.01 mg Cd kg^{-1}
Cacao liquor 0.47 mg Cd kg^{-1}
2.5 % (m/m) testa remaining