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

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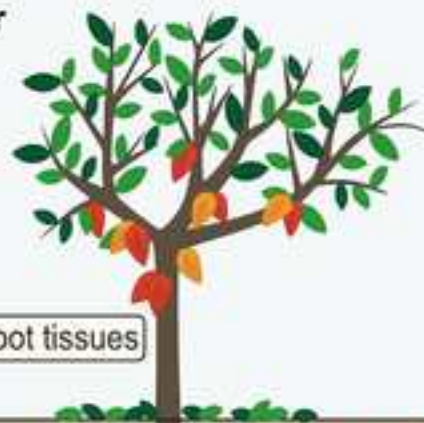
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Journey of Cd from soil to chocolate bar

3. Loading into pod tissues



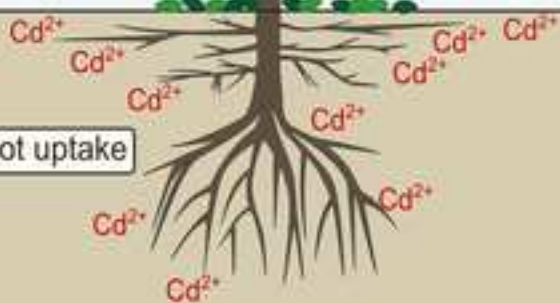
2. Translocation to shoot tissues




4. Transfer to the final product





1. Root uptake



Potential mitigation strategies

I. Soil amendments 

II. Genetics 

III. Postharvest 

1 HIGHLIGHTS

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

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

3

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25

26 ABSTRACT

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

46

47	KEYWORDS
48	<i>Theobroma cacao</i> L.
49	Cadmium
50	Soil-plant meta-analysis
51	Cadmium uptake
52	Cadmium translocation
53	Mitigation strategies
54	

55 1. INTRODUCTION

56 Cadmium (Cd) is a potentially toxic trace metal that has no known biological function in humans.
57 Cadmium accumulates in the human body and has a biological half-life of 10–35 years. Chronic
58 Cd exposure has been related to several adverse health effects, including renal tubular dysfunction
59 and osteomalacia (World Health Organization 2010). The human diet is the main source of body
60 burden Cd in the non-smoking population and staple foods such as rice, wheat grain products or
61 potatoes contribute largely to dietary Cd exposure because of their high consumption. Certain
62 luxury foods such as chocolate, although ingested in smaller quantities, can also contribute to
63 dietary Cd exposure due to their elevated Cd concentrations. To protect consumers, the European
64 Commission approved a new regulation in 2014 (in force from 2019), which sets the maximum
65 allowed Cd concentration in chocolates and cacao powders between 0.10–0.80 mg Cd kg⁻¹,
66 depending on the cacao solids content of the product (European Commission 2014). Similar
67 regulations have been, and are expected to be, implemented worldwide, e.g. in Australia and New
68 Zealand (Australia New Zealand Food Standards Code 2017), Russia (Ministry of Health of the
69 Russian Federation 2011) and the countries within the Southern Common Market (Mercosur
70 2011). In California (USA), products with elevated Cd concentration can be sold but it is
71 mandatory to report this on the packaging (Meter et al. 2019). The Cd in cacao regulations have
72 also been adopted by the Codex Alimentarius (Codex Alimentarius Commission 2018). Several
73 products on the market have been reported to exceed these limits (Abt et al. 2018; Vanderschueren
74 et al. 2019). As a result, the regulations have fueled research worldwide to monitor and mitigate
75 Cd accumulation in cacao. The number of research papers on Cd in cacao was only 29 up to 2014
76 but rose almost exponentially to >100 in 2020.

77 Cadmium in cacao products originates from the cacao beans, rather than from contamination
78 during processing. This is demonstrated by the strong correlations between the Cd concentrations
79 in the final chocolate product and its cacao content, and between the Cd concentration in chocolate
80 and the origin of the cacao (Villa et al. 2014; Yanus et al. 2014; Abt et al. 2018; Lo Dico et al.
81 2018; Vanderschueren et al. 2019). While all plants take up Cd to some extent, *Theobroma cacao*
82 L. is rather effective in doing so. The oldest study reporting Cd concentrations in cacao beans and
83 chocolate dates back to 1979 and that work already highlighted that elevated Cd concentrations in
84 cacao are typically found in samples from Latin American origin, as well as in specialty origin
85 chocolates (Knezevic 1979). The recently enforced EU limits and the Codex Alimentarius
86 recommendations apply to the final product sold to consumers, not to the cacao beans. The cacao
87 processing industry has translated the EU limits on Cd in cacao-derived products to requirements
88 regarding the maximum Cd concentrations in the fermented cacao beans purchased from their
89 suppliers, and requirements vary among companies. Those unofficial industry limits have been
90 reported to range between 0.50 and 1.10 mg Cd kg⁻¹ (Meter et al. 2019; CBI Ministry of Foreign
91 Affairs n.d.), but some cacao farmers are confronted with purchaser thresholds as low as 0.10 mg
92 Cd kg⁻¹ (personal communication of first authors). To facilitate the discussion below, 0.60 mg Cd
93 kg⁻¹ was used as a threshold.

94 Mitigation of Cd accumulation has been intensively studied for several food crops including durum
95 wheat, spinach, potatoes, and rice (McLaughlin et al. 2020), but for cacao this research is in its
96 infancy. This review summarizes the existing knowledge on the various factors influencing Cd
97 concentrations in cacao-derived products at all stages of the production process. This includes soil
98 Cd phytoavailability, Cd uptake and translocation within the plant, postharvest processing, and the
99 effect of dietary intake through chocolate consumption on the Cd body burden. A meta-analysis

100 of the different Cd-cacao surveys is presented with due attention given to the quality of the
101 analytical data, thereby only selecting data for which the quality of chemical analyses met quality
102 criteria (more details below and in the Supplementary Information). Research gaps are identified
103 and potential mitigation strategies at the different stages of the cacao value chain are discussed.

104 2. CADMIUM IN SOILS AND ITS RELATION TO CACAO PLANTS

105 **2.1. Origin of cadmium in cacao beans: soil, air, fertilizer, and irrigation water**

106 Generally, most of the Cd in plant tissues originates from soil through root uptake, while only a
107 small fraction is derived from air through foliar uptake. This is likely also true for cacao, first
108 because of the positive associations reported between soil Cd and cacao bean or leaf Cd
109 concentrations (see below) and, secondly because of the large Cd concentrations found in cacao
110 leaves and beans, which are well above what can reasonably be expected from foliar uptake. The
111 contribution of airborne Cd to crop Cd concentrations can be measured using isotopes that indicate
112 the Cd provenance, and can be quantified using air accumulation factors (AAFs, with units $\text{m}^3 \text{g}^{-1}$)
113 ¹), calculated as the ratio of the Cd concentration in the plant to that in air. In the Amazon region
114 of Ecuador, Barraza et al. (2017) did not find higher average Cd concentrations in soils and cacao
115 beans from areas where Cd levels in aerosols could be higher due to oil activities, compared to
116 pristine areas. For trace metals in general, the AAF can be estimated at $20 \text{ m}^3 \text{g}^{-1}$ for plant leaves,
117 with a range of $2\text{--}100 \text{ m}^3 \text{g}^{-1}$ depending on the type of plant and trace metal (Mclaughlin et al.
118 2011). Air Cd concentrations in the rural areas where cacao is grown range $0.07\text{--}0.57 \text{ ng Cd m}^{-3}$
119 [(Barraza et al. 2017), cacao production areas near oil activities]. Leaf Cd concentrations derived
120 from air in these cacao growing areas will thus range $0.001\text{--}0.011 \text{ mg Cd kg}^{-1}$ (considering an
121 AAF of $20 \text{ m}^3 \text{g}^{-1}$), with a maximum of $0.06 \text{ mg Cd kg}^{-1}$ (considering an AAF of $100 \text{ m}^3 \text{g}^{-1}$), which

122 is only 6% of the typical leaf Cd concentrations ($>1 \text{ mg Cd kg}^{-1}$) of cacao plants in Latin America
123 (see below).

124 Cadmium occurs naturally in the environment, with background soil concentrations ranging from
125 0.1 to $1.0 \text{ mg Cd kg}^{-1}$ and estimated global means of 0.1 to $0.3 \text{ mg Cd kg}^{-1}$ (Smolders and Mertens
126 2013). In contrast, polluted soils (e.g. near smelting sites) can contain Cd concentrations up to
127 three orders of magnitude higher than the background concentrations (He et al. 2015). The soil Cd
128 concentrations in cacao producing areas differ worldwide and tend to be higher in the geologically
129 young soils of Latin America with a range of means between 0.22 and 10.8 mg kg^{-1} (Chavez et al.
130 2015; Ramtahal et al. 2015; Arévalo-Gardini et al. 2017; Barraza et al. 2017; Gramlich et al. 2017;
131 Barraza et al. 2018; Gramlich et al. 2018; Lewis et al. 2018; Argüello et al. 2019; Engbersen et al.
132 2019; Rodríguez Albarrcín et al. 2019; Scaccabarozzi et al. 2020), and 0.12 to 0.85 mg kg^{-1} in Asia
133 (Fauziah et al. 2001; Zarcinas et al. 2004). Unfortunately, there is no reliable information to date
134 about Cd concentrations in soils of cacao producing areas in Africa. The majority of global cacao
135 is produced in western Africa where soils are old and weathered, i.e. they are expected to have low
136 Cd concentrations. Indeed, studies in different crops of Ghana have reported total soil Cd
137 concentrations of 0.026 mg kg^{-1} (Bortey-Sam et al. 2015). It is important to note that, while soil
138 Cd concentrations are thus generally reported to be higher in Latin American soils compared to
139 soils in other continents, soil Cd concentrations in Latin American cacao producing areas are
140 mostly $<1 \text{ mg kg}^{-1}$ (see below), and thus generally not categorized as contaminated soils. Multiple
141 researchers have indicated large geographical differences in cacao bean Cd, with elevated
142 concentrations found in cacao from Latin American origin compared to cacao from other origins
143 (e.g. Africa) (Bertoldi et al. 2016; Abt et al. 2018; Vanderschueren et al. 2019). The strong effect
144 of geographical origin on bean Cd is outlined below (Section 2.2) and suggests that Cd in cacao

145 generally does not originate from anthropogenic activity, as anthropogenic activities occur
146 worldwide. Indeed, three independent studies by Gramlich et al. (2018), Argüello et al. (2019) and
147 Scaccabarozzi et al. (2020) found that high soil Cd concentrations in cacao farms were associated
148 with alluvial soils from sedimentary materials, which can be explained by the higher Cd
149 concentrations usually found in sedimentary rocks compared to igneous rocks (Thornton 1981;
150 Birke et al. 2017). The substrate or alluvium source is likely also of importance, as alluvial soils
151 in Africa do not show similar Cd concentrations compared to their Latin American counterparts.
152 Nevertheless, there are specific cacao producing areas in Latin America where soil Cd
153 concentrations are exceptionally high (Rodríguez Albarrcín et al. 2019), likely due to historical
154 enrichment related to mining activities.

155 The use of mineral P-fertilizer has been suggested to be at the origin of high Cd in cacao beans
156 (Zug et al. 2019). However, this is highly unlikely on a large scale. Gramlich et al. (2017) measured
157 the Cd concentration in P-fertilizers in a long-term system comparison trial on an experimental
158 cacao farm in Bolivia and found a mean Cd concentration of 102 mg Cd kg⁻¹ P₂O₅, which is high
159 compared to fertilizers available in Europe [mean 28 mg Cd kg⁻¹ P₂O₅, <10% of samples contained
160 60 mg Cd kg⁻¹ P₂O₅ (Verbeeck et al. 2020)]. At a sustained annual dose of 20 kg P₂O₅ ha⁻¹ year⁻¹
161 with that local fertilizer, fertilization would add about 2 g Cd ha⁻¹ year⁻¹, equivalent to a net
162 addition to soil of 0.001 mg Cd kg⁻¹ soil year⁻¹ (at 15 cm incorporation depth) or an accumulation
163 of only 0.1 mg Cd kg⁻¹ soil after 100 years. This mineral fertilizer dose is unrealistically high, as
164 most of the cacao production worldwide is in the hands of low-income smallholders who do not
165 commonly use P-fertilizer (Snoeck et al. 2016; Vaast et al. 2016). This worst-case scenario
166 calculation hence indicates that mineral P-fertilizers are not the main source of Cd in cacao
167 plantations, at least not at a large scale.

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

177 Several soil profile studies have reported that soil Cd concentrations decline with increasing soil
178 depth in cacao plantations. The Cd concentration in the top 0–15 cm of the soil is about a factor
179 1.5 larger than the Cd concentration at depths 15–60 cm (Chavez et al. 2015; Arévalo-Gardini et
180 al. 2016; Barraza et al. 2017; Gramlich et al. 2018). Although this might be interpreted as evidence
181 for anthropogenic sources, it is more plausible that this concentration profile reflects the cycling
182 effect caused by decomposition of litter from the aerial biomass (Reimann et al. 2019). The dry
183 leaf biomass production of cacao is about $3.9 \text{ Mg ha}^{-1} \text{ y}^{-1}$ (Heuvel dop et al. 1988) with an average
184 leaf Cd concentration of 2.6 mg kg^{-1} dry weight (dw) ($n = 762$, derived from the meta-analysis
185 described below). Hence, the annual Cd flux returned to the soil by cacao leaf litter decomposition
186 is about $10 \text{ g Cd ha}^{-1} \text{ y}^{-1}$, which is much larger than the worst-case inputs calculated from
187 application of contaminated P fertilizers or irrigation water as indicated above. Enriching the
188 topsoil from a background soil concentration of $0.89 \text{ mg Cd kg}^{-1}$ [estimated by the subsoil Cd
189 concentrations reported by Gramlich et al. (2017)] to $1.09 \text{ mg Cd kg}^{-1}$ [topsoil Cd concentrations
190 reported by Gramlich et al. (2017)] would thus require approximately 50 years of leaf litter

191 decomposition, which is not an unrealistic age for the many cacao orchards in the hands of small
192 farmers. Indeed, an economic survey conducted in Ecuador (Vazquez et al., in preparation)
193 reported that the age of cacao orchards ranged from 1 to 100 years, with an average of 13.8 years
194 ($n = 1534$), and 11% of the orchards surpassed the age of 30. Barraza et al. (2019) determined the
195 stable isotope ratio ($\delta^{114/110}\text{Cd}$) in cacao leaves and litter, and topsoil in cacao plantations. While
196 the isotopic ratios in topsoil differed among different sites, there was a consistent fractionation
197 between leaves and soil ($\Delta_{\text{leaf-soil}}$), indicating that the topsoils were likely enriched with heavier Cd
198 isotopes originating from the leaf litter. Considering all the above, the main Cd source in cacao
199 producing soils is likely geogenic rather than anthropogenic, and local high enrichments may be
200 related to point sources such as mining activities, input of sedimentary materials or the use of
201 contaminated irrigation water. The annual Cd cycling due to plant uptake and leaf litter
202 decomposition can explain the larger Cd concentrations in surface soil compared to the subsoil.

203 **2.2. Bean cadmium concentrations and their relationship with soil properties: a meta-** 204 **analysis**

205 Cacao bean Cd concentration data reported in different surveys have been collated here to identify
206 general trends (Table 1). Studies included in this table were selected based on the quality criteria
207 elaborated in the Supplementary Information, i.e. quality assurance of the chemical analysis
208 combined with a minimal number ($n \geq 4$) of different samples to characterize the region, and
209 information on sample treatment (drying, peeling and digestion procedure). Studies that used flame
210 atomic absorption (AAS) or older inductively coupled plasma optical emission spectrometry (ICP-
211 OES) instruments were excluded from this compilation because of their poor detection limits for
212 Cd in plant samples ($0.1\text{--}0.5 \text{ mg Cd kg}^{-1}$ dry weight) and their propensity to overestimate the true
213 Cd concentrations. Average or median bean Cd concentrations clearly vary by region, with cacao

214 beans originating from Latin America showing Cd concentrations four to six times higher
215 compared to cacao beans originating from Asia or Africa. These elevated cacao Cd concentrations
216 are however only found in the Andean countries, not in Brazil. While most included studies have
217 reported the data as bean Cd concentrations, sample treatments before analyses differ strongly
218 among studies. Processing steps such as fermentation, peeling, or roasting influence the Cd
219 concentration as explained in section 4.3. The spatial variability of bean Cd has been assessed
220 systematically. The coefficient of variation of bean Cd concentrations within fruits ranges 2–27%,
221 mean 12% (Vanderschueren et al., in preparation). No such data were found for the variability
222 among fruits within a tree, but the coefficient of variation among trees within a single field ranges
223 0.62–110% depending on the field, mean 39% (Argüello et al. 2019). At larger distances, this
224 variation logically increases. This spatial variation may be used to make a power analysis for the
225 minimal replicates needed when testing remediation effects in cacao (see section 4.1).

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

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

245 The average total topsoil Cd concentrations of each study (Table S 2), as well as the overall average
246 (\pm standard deviation, stdev) of the compiled dataset (0.43 ± 0.41 mg Cd kg⁻¹, n = 776) are within
247 the range for non-polluted soils. Only 50 soil samples across all studies exceeded 1.0 mg Cd kg⁻¹,
248 such “hotspots” represent only 6% of these compiled data. Multivariate regression analysis was
249 first applied to each individual dataset (details on methods can be found in the Supplementary
250 Information). The results of this multivariate analysis (Table 2 and Supplementary Information
251 Table S3) show a generally emerging trend: bean Cd increases with increasing total soil Cd, with
252 decreasing pH (except in study II), and with decreasing soil organic carbon (except in study VI).
253 The effect of organic carbon can be explained by the high affinity of Cd for sorption sites in organic
254 matter (Christensen 1989). The effect of pH on Cd availability is due to the competition between
255 Cd²⁺ and protons for soil sorption sites, as Cd²⁺ binds to oxygen atoms of carboxylic or phenolic
256 groups present in organic matter and oxyhydroxides (Smolders and Mertens 2013). Total soil Cd
257 alone might not be the best predictor for crop Cd concentrations as the majority of Cd is sorbed to
258 different soil particles and is not directly available for plant uptake. Therefore, plant Cd
259 concentrations are better predicted if soil pH is included in the prediction equation, as it is the key

260 soil property controlling the availability of soil Cd. Available soil Cd has been proposed as a proxy
261 to predict crop Cd concentrations. Available Cd is an operationally defined extraction that often
262 refers to the soluble fraction in soil (McLaughlin et al. 2000). Indeed, study VII reported available
263 Cd in the topsoil (measured either by simple extraction with ammonium-acetate-EDTA or using
264 diffusive gradients in thin film, DGT), and available soil Cd was identified as a significant
265 predictor for bean Cd for that study (Table 2 and Supplementary Information Table S3). Soil pH,
266 which affects the soluble fraction of Cd in the soil, is logically not a significant variable when
267 available soil Cd rather than total soil Cd is used. Multivariate regression was also applied to the
268 compiled dataset including data from all seven studies, with correction for the disproportionate
269 weight of their sample sizes (n = 334, methods are described in detail in the Supplementary
270 Information). Total soil Cd, pH, and SOC explained >41% of the variance in bean Cd
271 concentrations within the compiled dataset. The equation of the model reads as follows:

$$272 \quad \log_{10}[\text{Bean Cd}] = 1.34 + 0.86 * \log_{10}[\text{Total Soil Cd}] - 0.18 * \text{pH} - 0.25 * \log_{10}[\text{SOC}]$$

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

281 Because zinc (Zn) and Cd are considered analogues, low available soil Zn has been suggested to
282 trigger plant Cd uptake (Oliver et al. 1994; Chaney et al. 2006; Chaney 2010). In a recent
283 greenhouse study, Souza dos Santos et al. (2020) reported that increase of soil Zn inhibited uptake
284 and translocation of Cd by CCN-51 cacao. However, this effect was only found in soils spiked to
285 Cd concentrations well above environmentally relevant soil Cd concentrations. Argüello et al.
286 (2020) found that Cd uptake in cacao from deeper soil layers was enhanced by surface liming and
287 the authors related this to increased activity of deeper roots to cope with micronutrient deficiency
288 in the topsoil (i.e. Zn) caused by surface liming. The multivariate regressions presented here did
289 not identify total soil Zn as a significant factor to predict bean Cd concentrations (with exception
290 of study II, Table 2), even when using leaf Zn concentrations as regressors (details not shown).
291 Widespread Zn deficiency is unlikely . The overall average leaf Zn concentration in the meta-
292 analysis is 97 mg kg^{-1} (n=700, data from studies I, II, IV and VII) which falls within the range
293 reported as optimal ($>20 \text{ mg Zn kg}^{-1} \text{ dw}$) for plant development (Fageria et al. 2002). Only a small
294 fraction of samples ($<5\%$) presented suboptimal leaf Zn concentrations, which may explain why
295 no significant effect of soil Zn was identified in the regression analysis. The lack of Zn deficiency
296 within the included datasets may also indicate that Zn deficiency is not an issue in Latin American
297 cacao production areas, where Cd accumulation is considerable. However, study II showed a
298 significant statistical effect of total subsoil Zn and total topsoil Mn on bean Cd (regression II a,
299 Table 2). Similarly, study VII showed a significant effect of available subsoil Zn and available
300 topsoil Mn on bean Cd, when available soil Cd was used as a predictor instead of total soil Cd
301 (regression VII b, Table 2). Additional research regarding the effect of available Zn and Mn is
302 required to unravel their potential interaction with Cd and their effect on Cd accumulation in cacao.

303 Transfer factors (TF) indicate to what extent a crop accumulates Cd in its edible parts and are
304 calculated as the ratio of the Cd concentration in either the plant leaf or the edible part, over the
305 total Cd concentration of the soil. Average transfer factors for cacao (i.e. cacao bean Cd over soil
306 Cd) in the different studies vary from 0.26 to 19 and are significantly affected by soil pH (Figure
307 3). Transfer factors for plants grown in acid soils are higher compared to TF for plants grown in
308 more neutral or alkaline conditions, confirming the importance of soil pH for plant Cd availability.
309 The TF expression shows that average bean Cd will remain below the 0.60 mg Cd kg⁻¹ threshold
310 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
311 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
312 also influenced by plant genetics and allow comparison of Cd accumulation among crops (see
313 below in section 3.1).

314 **2.3. Agronomic factors affecting Cd uptake in cacao**

315 Some researchers have argued that Cd uptake is affected by cacao management, i.e. monoculture
316 vs agroforestry and organic vs conventional mineral fertilization (Maddela et al. 2020). However,
317 there is not enough information available to date to assess the effect of these agronomic practices
318 on Cd in cacao, and the scarce existing information is not standardized among studies. Two studies
319 investigated the effect of cropping systems (monoculture vs agroforestry) on Cd uptake in cacao.
320 Argüello et al. (2019) found no significant effect of cropping systems on bean Cd concentration in
321 their large national survey in Ecuador. Gramlich et al. (2017) also found no such effect in bean Cd
322 concentrations in a field trial but found higher concentration of Cd in cacao leaves when cacao
323 was grown as a monoculture compared to agroforestry. Gramlich et al. (2017) suggested that the
324 higher plant density present in agroforestry systems may be the reason for a lower leaf Cd
325 concentration in cacao plants in this cropping system. Higher plant density could be translated to

326 higher competition for nutrients, water, and light, which may result in a lower growth rate for
327 cacao. The authors proposed that plants with a lower growth rate take up less Cd, because lower
328 growth rate infers lower nutrient uptake and Cd is usually taken up as a hitchhiker element along
329 with essential nutrients such as Zn. The effect of fertilizers application, i.e. organic vs conventional
330 fertilizers, on Cd uptake in cacao has not been widely studied.

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

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

350 **3.1. Soil-plant transfer of Cd in cacao compared to other plant species**

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

358 Soil to plant transfer factors (TFs) allow identification of Cd accumulating characteristics,
359 although they are influenced by genotype and external conditions such as soil Cd availability (see
360 paragraph 2.2). Soil-plant TFs reported in cacao based on both leaves and beans, are higher
361 compared to TFs reported for most other crops (Table 3). The soil-leaf TFs for Cd are close to
362 those reported for other woody species like willow (Van Slycken et al. 2013) and poplar
363 (Laureysens et al. 2004). The soil-seed TF for cacao range 2–6 depending on the study and are
364 distinctly smaller than TF reported for wheat and rice, but of similar magnitude as TF reported for
365 sunflower kernels (Table 3). The Cd accumulation potential of the cacao plant is thus high, but not
366 unusual. Because of its high soil-plant TF, cacao has been described as a Cd accumulator although
367 this term lacks a clear definition. Several criteria indicate whether a plant can be classified as a Cd
368 hyperaccumulator: (i) in natural conditions the plant should be able to accumulate $\geq 100 \text{ mg Cd kg}^{-1}$
369 ¹; (ii) both the transfer factor (TF) and the internal translocation factor ($\text{ITF}_{\text{tissue 1-tissue 2}} = \text{Cd}_{\text{tissue 1}}$
370 $/ \text{Cd}_{\text{tissue 2}}$) should be larger than one; and (iii) extreme metal tolerance should be achieved due to

371 efficient biochemical detoxification (van der Ent et al. 2013). While cacao does not meet the first
372 criterion, $TF > 1$ have been reported (Table 3). The mean Cd concentrations in the vegetative
373 tissues of the cacao plant reported in different field studies are collated in Table 4, and these data
374 suggest a relatively homogeneous Cd distribution between roots, scions, and leaves. Engbersen et
375 al. (2019) reported a similar $ITF_{\text{stem-root}}$ (0.99) for cacao compared to $ITF_{\text{stem-root}}$ reported for known
376 moderately Cd accumulating woody species [i.e. $ITF_{\text{stem-root}} \approx 1$ in poplar and cotton, (Vollenweider
377 et al. 2011; Chen et al. 2015)]. To decide on the third criterion and to allow classification of cacao,
378 additional research on the speciation of Cd in cacao tissues is crucial to elucidate the molecular
379 detoxification mechanisms. Nonetheless, the Cd uptake potential of the cacao tree can be
380 conceived as rather high compared to other agricultural crops, and more similar to woody species
381 used for phytoextraction such as willow and poplar. Therefore, we suggest to term cacao a
382 moderate Cd-accumulator.

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

392 **3.2. Uptake and translocation of Cd within the cacao plant: trends and mechanisms**

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

405 3.2.1. Uptake of Cd in the cacao root system

406 Ullah et al. (2018) identified five genes from the NRAMP family in cacao and demonstrated that
407 TcNRAMP5 can encode for a protein which transports Cd, Mn(II) and Fe(II). Indeed, it has been
408 reported that NRAMP5 plays a role in the entry of Cd in the root cells for rice (Sasaki et al. 2012;
409 Ishikawa et al. 2012), barley (Wu et al. 2016), Polish wheat (Peng et al. 2018), and tobacco (Tang
410 et al. 2017). More specifically, Ullah et al. (2018) showed that the TcNRAMP5 transcript was
411 primarily expressed in the roots of cacao seedlings. Usually, the expression of NRAMP transporter
412 genes is upregulated under divalent metal deficiency. In cacao seedlings, expression of the
413 TcNRAMP5 transporter gene in the roots was downregulated in the presence of Cd, and
414 upregulated under Fe deficiency, while Zn or Mn deficiency did not affect gene expression. To

415 link the expression pattern to its function, Ullah et al. (2018) cloned five TcNRAMP genes and
416 expressed them in yeast strains. The yeast cells expressing TcNRAMP5 accumulated up to three
417 times more Cd compared to the empty vector control cells. Considering the above, TcNRAMP5
418 likely plays a major role in the regulation of Cd uptake in cacao plants (Figure 4) and may be
419 targeted in a potential mitigation strategy through induction of loss-of-function mutations in
420 TcNRAMP5. Yet, extrapolation of these findings to relevant cacao soil-plant systems is limited.
421 First, activities used in the hydroponic experiment are about 100 times higher than natural soil Cd
422 conditions. The expression pattern observed may thus be different than natural field conditions.
423 Second, uptake in yeast may not be able to predict uptake in cacao plants.

424 The isotopic signature of Cd in plants is increasingly used to infer biogeochemical processes but
425 only few studies have reported Cd isotope fractionation data in cacao. Several processes can
426 generate stable isotope fractionation during the transfer of Cd from soil to plant tissues, such as
427 membrane transport or chemical binding. Moore et al. (2020) compared the isotopic shift of
428 TcNRAMP5 transporter expressed yeast relative to the empty vector control cells ($\Delta^{114/110}\text{Cd}_{\text{trans-}}$
429 $_{\text{ev}} \approx -0.8 \text{ ‰}$), with the isotopic shift in the cacao seedlings relative to the hydroponic solution
430 ($\Delta^{114/110}\text{Cd}_{\text{tot-sol}} = -0.22 \pm 0.08 \text{ ‰}$). Because both systems showed a shift toward lighter isotopes
431 and because the expressed TcNRAMP5 proteins were localized in the external plasma membrane
432 of the yeast cells, the authors confirmed that TcNRAMP5 transporters can be a major pathway for
433 Cd uptake in cacao. However, the observed similar fractionation does not exclude that other
434 transporters can also show similar fractionation and be involved in Cd uptake. Compared to other
435 plants, the total isotopic fractionation due to absorption of Cd from hydroponic solutions in cacao
436 seedlings is less pronounced, but similar to the isotopic shift reported for Cd tolerant and
437 accumulator plants (Wei et al. 2016). The reported fractionation towards lighter isotopes is similar

438 to fractionation from the soil solution to plants in cereals and rice, although there is some variation
439 in the rate of fractionation (Table 5). It is noteworthy that the cacao plants used in the hydroponic
440 study of Moore et al. (2020) had leaf Cd concentration of about 200 mg Cd kg⁻¹ which far exceeds
441 the Cd toxicity threshold (10 mg Cd kg⁻¹, see above) and the relevant concentrations in the field
442 (1–10 mg Cd kg⁻¹). This may have influenced the Cd uptake and translocation system due to the
443 activation of internal systems to cope with Cd toxicity (de Araújo et al. 2017).

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

454 3.2.2. Partitioning and translocation of Cd inside the cacao plant

455 From plant roots, Cd is generally transported radially across the root cells and loaded into the
456 xylem, followed by long-distance transport via xylem and phloem and transfer into the plant organs
457 (Clemens and Ma 2016). The rate of translocation of Cd from roots to above-ground tissues
458 depends on vacuolar sequestration, xylem loading, and intervascular and xylem-to-phloem transfer
459 (Figure 4). Cadmium can be sequestered in vacuoles during the radial transport through the root
460 cells, as a protective measure to reduce the mobility of Cd for translocation to other tissues. A key

461 transporter in this vacuolar sequestration is the Heavy Metal transporting ATPase 3 (HMA3) influx
462 transporter, which is located at the membrane of vacuoles and which has been proven to be a major
463 determinant in the sequestration of Cd in roots of rice (Wang et al. 2019), soybean (Wang et al.
464 2012), and Chinese cabbage and pak choi (L. Zhang et al. 2019). For a discussion of the further
465 translocation of Cd loading in the xylem, root-to-shoot translocation, and intervascular transfer in
466 plants in general, the reader is referred to more specialized reviews (Clemens and Ma 2016;
467 McLaughlin et al. 2020). To the best of our knowledge, the role of the HMA family in Cd
468 sequestration in cacao has not been determined to date. Moore et al. (2020) transformed yeast with
469 HMA family transporters to compare the isotope fractionation pattern due to Cd sequestration in
470 cacao seedlings. The data were however not conclusive on the specific role of HMA transporters
471 in controlling Cd transport in cacao seedlings. In both rice and durum wheat, root-to-shoot Cd
472 translocation via the xylem has been identified as a major determinant for shoot Cd accumulation.
473 (Harris and Taylor 2004; Uraguchi et al. 2009). These observations highlight the need for studies
474 focused on unravelling the mechanisms for Cd translocation from roots to aboveground tissues.

475 Cacao is a cauliflorous tree and mechanisms and pathways for Cd loading into the developing
476 seeds (the cacao beans) thus may be different from other plants. The relative importance of either
477 xylem or phloem in Cd loading in cacao beans has not been revealed to date. Most studies report
478 leaf Cd concentrations to be higher than corresponding bean Cd concentrations (Table 4; Figure
479 5). The data from the meta-analysis described above show a markedly positive correlation between
480 bean and leaf Cd concentrations, with average $ITF_{\text{leaf-bean}}$ ranging between 1.1 to 4.2 among studies
481 (Figure 5). This clear correlation may justify the use of leaf Cd concentration measurements as a
482 proxy for bean Cd because leaf sampling is convenient as it does not depend on fruit availability.
483 However, the use of leaf Cd measurements as a proxy for bean Cd may only be justified when

484 considering a genetically homogeneous sample set. Indeed, both Engbersen et al. (2019) and Lewis
485 et al. (2018) reported only moderate correlations between leaf and bean Cd concentrations in their
486 genetically diverse studies.

487 Another approach to study the translocation of Cd within plants, is to quantify isotope
488 discrimination between different tissues (Table 5). In the hydroponic system of Moore et al.
489 (2020), cacao leaves were strongly enriched in heavy Cd isotopes compared to the roots ($\Delta^{114/110}$
490 $Cd_{\text{leaf-root}} = 0.26 \text{ ‰}$). Similar hydroponic studies with *Ricinus communis* and *Solanum nigrum* (Wei
491 et al. 2016) showed a less systematic fractionation than reported in the cacao study of Moore et al.
492 (2020). This indicates that cacao seedlings exert mechanisms to cope with Cd that differ from the
493 Cd coping mechanisms in Cd-accumulator plants. A larger fractionation between roots and
494 aboveground tissues may indicate that additional Cd retention mechanisms are invoked to inhibit
495 the translocation of Cd to aerial plant part, as discussed before. In most plants, Cd becomes
496 enriched in heavy isotopes in the order roots < stem < leaf < seed (Table 5). The only study available
497 on Cd isotope discrimination in mature cacao trees suggests that the transfer of Cd from stem to
498 leaves and seeds in cacao is different compared to isotope discrimination studies in other species
499 available thus far (Table 5) (Barraza et al. 2019). This pilot field study in a soil-cacao system in
500 Ecuador reported an average trend towards lighter isotopes in the beans compared to the leaves
501 ($\Delta^{114/110} Cd_{\text{bean-leaf}} = -0.27 \text{ ‰}$). This different isotopic signature may be related to the cauliflorous
502 character of cacao and hints to the hypothesis that Cd is directly transferred from xylem to phloem
503 in developing cacao beans, without first passing through the leaves.

504 3.2.3. Distribution of Cd within the cacao fruit

505 The Cd concentrations reported for the different cacao fruit tissues are summarized in Table 4.
506 Vanderschueren et al. (2020) reported that weight-based Cd concentrations decreased with

507 testa>nib~placenta~pod husk>mucilage in cacao fruits from four locations and reported testa Cd
508 concentrations were 1.5 to 1.8 times larger compared to nib Cd concentrations. Lewis et al. (2018)
509 also studied the partitioning of Cd between testa and nib, and found that testa Cd concentrations
510 were, on average, twice as large as nib Cd concentrations. However, the authors reported that this
511 concentration ratio (testa Cd over nib Cd) varied depending on the cacao genotype, ranging from
512 1.2 to >7. The testa accounts for only a small part of the total bean weight and is thus also
513 responsible for only a small fraction of total bean Cd, despite its elevated Cd concentration. For
514 example, Vanderschueren et al. (2020) reported that, in unfermented cacao beans, 91% of the total
515 bean Cd mass was found in the nibs and only 9% originated from the testa.

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

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

545 3.2.4. Cultivar-related differences in Cd uptake and partitioning

546 The cultivar effect on Cd uptake in cacao was first reported in 2018, with a factor 13 variation in
547 bean Cd and factor 7 in leaf Cd concentrations among cultivars grown on the field (Lewis et al.
548 2018). The data suggest a differential partitioning of Cd between vegetative (leaf) and reproductive
549 (bean) tissues, favoring the hypothesis that there are cultivar-specific differences in xylem-to-
550 phloem transfer of Cd to the different tissues. Engbersen et al. (2019) reported that available soil
551 Cd was closely related to Cd in vegetative parts ($R^2 \approx 0.50$) and to a minor extent to bean Cd (R^2

552 = 0.26), which was attributed to a cultivar effect in the loading of Cd into the beans. The authors
553 used this as indirect evidence suggesting that cultivar-related differences in bean Cd concentrations
554 are mainly due to cultivar-specific differences in xylem-to-phloem transfer. While Cd transfer to
555 the leaves is probably mostly governed by the xylem, the contributions of either the xylem or
556 phloem pathway for cacao bean Cd loading are yet to be revealed and may differ among cultivars.
557 However, the cacao trees in the study of Engbersen et al. (2019) were grafted onto rootstocks of
558 unknown genetic identity, which may have affected the uptake of metals and the further
559 translocation from the root, obscuring the cultivar effect. The design of that study also did not
560 allow to account for potential effects of local spatial variability, which may have influenced uptake
561 and partitioning of Cd. Barraza et al. (2019) reported a genotype effect in the isotopic fractionation
562 between cacao leaves and beans (Nacional: $\Delta^{114/110} \text{Cd}_{\text{bean-leaf}} = -0.34$ to -0.40 ‰, CCN-51 hybrid:
563 $\Delta^{114/110} \text{Cd}_{\text{bean-leaf}} = -0.08$ ‰). A distinct isotope fractionation pattern has also been reported
564 between shoots and roots of an excluder ($\Delta^{114/110} \text{Cd}_{\text{shoot-root}} = 0.19$ ‰) and a non-excluder type of
565 rice ($\Delta^{114/110} \text{Cd}_{\text{shoot-root}} = -0.02$ ‰) (Wiggenhauser et al. 2020). The consistent isotope fractionation
566 between cacao leaves and soil reported by Barraza et al. (2019) is intriguing and indicates clear
567 differences in the soil-bean fractionation among cultivars. This confirms the previous hypothesis
568 that the translocation and sequestration of Cd in different plant tissues varies among cultivars. This
569 pilot study has some limitations due to the limited number of observations but confirms that the
570 isotope approach may provide insights on Cd pathways in the plant and pave the road for further
571 studies in the field.

572 4. MITIGATION STRATEGIES TO LOWER THE CADMIUM CONCENTRATION IN THE 573 FINAL PRODUCT

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

583 **4.1. Mitigating Cd uptake using soil amendments**

584 While several soil amendment techniques exist, liming, biochar, gypsum, and Zn supplementation
585 are the only soil amendments that will be discussed here as these are the only amendments that
586 have been (or are being) investigated for the mitigation of Cd in cacao. A Reduction Factor (RF),
587 defined as the ratio between the crop Cd concentration in the control (no treatment) and the Cd
588 concentration in the treatment was calculated to quantify the effect of each soil amendment. A RF
589 >1 indicates that crop Cd was reduced by the treatment.

590 4.1.1. Lime

591 The application of liming materials [i.e. CaCO_3 , CaO , $\text{Ca}(\text{OH})_2$, $\text{CaMg}(\text{CO}_3)_2$] has often been
592 recommended as an agronomic practice to reduce the uptake and accumulation of Cd in edible
593 parts of crops. Liming decreases the solubility of soil Cd (and thus decreases available soil Cd) by
594 increasing soil pH and through competition between Ca^{2+} and Cd^{2+} at root uptake sites

595 (Christensen 1984; Bolan et al. 2003a). However, increasing the solution concentration of Ca^{2+}
596 can also result in competitive desorption of surface bound Cd^{2+} (Christensen 1984). This latter is
597 one of the plausible reasons why liming does not consistently decrease the concentration of Cd in
598 plant tissue (Bolan et al. 2003b). Significant reductions in crop Cd by the application of lime have
599 been reported in field experiments with paddy rice in China: the application of $7.5 \text{ Mg ha}^{-1} \text{ CaCO}_3$
600 led to RF 3–4, and this was even sufficient to have an effect on rice grain Cd grown in the 2nd year
601 crop (H. Chen et al. 2018); and application of 1.5 Mg ha^{-1} burned lime (75% CaO) resulted in RF
602 of 1.5 (Zhu et al. 2016). However, it is unclear if such high RF can be reached with lime application
603 in the case of cacao.

604 A recent study by Ramtahal et al. (2019) evaluated the effect of application of Ca(OH)_2 at different
605 rates ($0\text{--}6 \text{ Mg ha}^{-1}$) on Cd uptake in cacao plants grown in pots (6 month old cacao cuttings) and
606 in the field (30 year old trees). Results from the pot trial showed effectively reduced leaf Cd
607 concentrations 6 months after lime application, with a RF=3. However, in the field trial leaf Cd
608 initially decreased by a factor 2 (measured 4 months after lime application), after which leaf Cd
609 started to increase in both control and limed treatments so that for the last sampling (9 months after
610 lime application), leaf Cd in the limed treatment was only reduced by a factor 1.1. Interestingly,
611 soil pH remained at the target pH of 7.0 up until the 9th month after lime application. This contrast
612 in lime effect between field trials and pot experiments is not uncommon. Earlier work conducted
613 in potatoes (Maier et al. 1996; Sparrow and Salardini 1997), sunflower kernels (Li et al. 1996) and
614 peanuts (McLaughlin et al. 1997) suggested that the difficulty in fully incorporating lime to deeper
615 soil layers as an explanation for the lack of effect and/or limited effectiveness of liming in reducing
616 crop Cd in the field. The lack of effect of liming in the cacao field trial may thus be related to Cd
617 being taken up by deeper rooted soil horizons that were not affected by the lime treatment. In

618 established plantations, lime incorporation to deeper soil horizons is not possible without
619 damaging the root system, which may compromise the efficacy of the amendment. Indeed,
620 Argüello et al. (2020) showed in a pot experiment with cacao seedlings that partially liming the
621 rooted soil (lime in top compartment only as would be the case in field liming), enhanced Cd
622 uptake from the non-limed bottom compartment.

623 4.1.2. Gypsum

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

635 4.1.3. Biochar

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

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

648 In cacao, the work by Ramtahal et al. (2019) evaluated the effect of a commercially available
649 biochar (charcoal green[®]) on the phytoavailability of soil Cd in pot and field experiments. Biochar
650 was applied at rates of 0, 326, 489, 652 kg ha⁻¹ and the materials were incorporated at 20 cm depth.
651 In the greenhouse study, biochar significantly increased soil pH by about one pH unit and reduced
652 the concentration of Cd in the leaf of cacao seedlings, and this effect was dose dependent. Results
653 from the field experiment with 30-year-old cacao trees showed that biochar did not increase soil
654 pH in contrast to the pot trial. Biochar applied at the highest dose in the field trials reduced leaf Cd
655 by factor 1.9 compared to the control, and the effect was more consistent over time than the effect
656 observed for lime application (last sampling done 6 months after biochar application, see above).
657 Results of studies with other crops are promising but the effectiveness of the treatment varies: RF
658 2 with application of 20–40 Mg ha⁻¹ in a field study with rice grain and the effect of biochar
659 application on rice grain could still be observed three years after application to the soil (Bian et al.
660 2013; Bian et al. 2014); and RF 1.2 determined one year after biochar application up to 40 Mg ha⁻¹
661 in a field study with wheat on alkaline soil, but this effect was no longer present or highly variable
662 in years 2 and 3 after application (Sui et al. 2018). Such high application rates of soil amendments
663 would likely not be cost effective in cacao farming.

664 4.1.4. Fertilizer management: zinc supplementation

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

682 4.1.5. Factors complicating the use of soil amendments to remediate Cd in cacao

683 *Theobroma cacao* L. is a perennial tree and it is thus essential to test the efficacy of soil
684 amendments in field conditions. The root system of the cacao tree is most dense in the near-surface
685 soil (Nygren et al. 2013; Niether et al. 2019), which complicates effective incorporation of soil
686 amendments. The poorly understood complexity of rhizosphere processes in the roots of cacao

687 may also explain lack of effect for some soil amendments and additional research is needed to
688 better understand how cacao roots shape the local biochemical condition in the rhizosphere. The
689 high spatial variability is a major obstacle to detect effects of soil treatments on bean Cd
690 concentrations. A power analysis was performed based on the variation in bean Cd within the same
691 fields reported by Argüello et al. (2019) (Supplementary Information). Beans from only 5 trees
692 per treatment are required to identify a statistically significant effect of treatment if a reduction
693 factor 2 (RF) is expected and if the field has an average spatial variability; for fields with a realistic
694 worst-case variability, that number increases to 15 trees per treatment. In contrast, an unfeasibly
695 large number of 198 trees are required to identify significant effects if the treatments yield only
696 RF 1.2 and if the variability is a realistic worst case. A RF 1.5 can have large consequences on the
697 economy of the cacao sector (see below) but it is clear that detection of such moderate (but
698 realistic) RFs is challenged by soil and bean Cd variability.

699 **4.2. Genetics as a potential mitigation strategy for Cd in cacao**

700 The research on the potential of genetic mitigation strategies (i.e. traditional genetic selection,
701 plant breeding techniques, marker-assisted molecular breeding and/or genetic engineering) is still
702 in an early phase as the genetic and molecular mechanisms for Cd uptake and partitioning in cacao
703 are still being explored. There are indications that genetics-based techniques could offer effective
704 and sustainable strategies for Cd mitigation in cacao. As discussed above, the work by Ullah et al.
705 (2018) and Moore et al. (2020) identified TcNRAMP5 as a potentially important gene for Cd
706 uptake in cacao, and thus as a potential target for genetic selection or modification. A recent
707 greenhouse experiment with 53 wild and domesticated cacao genotypes proposed 11 cacao clones
708 as low Cd accumulators which can be potentially exploited for future work (Arévalo-Hernández
709 et al. 2020). However, soils in that study were spiked to extreme Cd concentrations (25 mg Cd kg⁻¹

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

720 The use of low-Cd accumulating rootstocks for grafting (a common technique in cacao cultivation)
721 may be promising to reduce Cd uptake in cacao. Grafting has been used to mitigate plant stress
722 caused by adverse soil chemical conditions in the root environment (i.e. heavy metal uptake and
723 accumulation) (Savvas et al. 2010). The role of the rootstock versus the scion on Cd accumulation
724 in cacao beans is not yet known, and this is a key knowledge gap for breeding. Rootstock effects
725 on above-ground Cd concentrations appear to be plant species dependent. For example, Arao et al.
726 (2008) demonstrated that grafting eggplants onto *Solanum torvum* rootstock reduced Cd in the fruit
727 by 63–74% compared to grafting onto *Solanum melongena* and *Solanum integrifolium*. In
728 addition, the concentration of Cd measured in xylem sap was significantly lower in *S. torvum* than
729 in *S. melongena*. This suggests that *S. torvum* can limit translocation of Cd from the root to the
730 shoot. In contrast, studies conducted on potato cultivars showed that the rootstock is an important
731 factor to regulate the total Cd uptake by the plant but that the scion regulates the distribution of Cd
732 between the shoots and tubers (Mengist et al. 2018). The use of cacao rootstocks with low Cd

733 uptake potential could be the basis to renovate old cultivars, and eventually reduce Cd
734 concentrations in cacao beans and its by-products while maintaining the flavor of specific cacao
735 cultivars which is likely under control of the scion.

736 **4.3. Postharvest processing**

737 There are four main approaches within the postharvest process that may affect the Cd concentration
738 in the final cacao-derived product: fermentation, testa removal, choice of product recipe, and cacao
739 bean mixing (Figure 6). First, fermentation has been suggested to cause mobilization of Cd within
740 cacao beans due to acidification of the cacao nib (Vanderschueren et al. 2020). The potential
741 impact of fermentation on Cd in cacao, and the need for research on this topic has been indicated
742 (Meter et al. 2019), but published research remains scarce to date. Some authors have reported Cd
743 concentrations measured in unfermented beans and intermediate products after fermentation. For
744 example, Barraza et al. (2017) measured Cd in unfermented unpeeled cacao beans (1.02–1.37 mg
745 kg⁻¹) and in cacao liquor (1.47–3.88 mg kg⁻¹), i.e. suggesting some Cd enrichment after
746 fermentation and processing. Yanus et al. (2014) reported Cd concentrations in unfermented nibs
747 (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
748 kg⁻¹), again suggesting enrichment. However, it is unclear from these studies to what extent these
749 trends are related to variability of samples or a true effect of processing steps. The systematic study
750 of Vanderschueren et al. (2020) on the impact of fermentation on Cd in cacao indicated a potential
751 migration of Cd from the nib to the testa during fermentation, resulting in a factor 1.3 decrease in
752 nib Cd (RF 1.3). However, a significant decrease in nib Cd with fermentation was only observed
753 when nib pH at the end of fermentation was <5 (which may be achieved by more extensive
754 fermentation).

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

768 Third, Cd is mostly partitioned in the non-fat cacao solids (Mounicou et al. 2003; Yanus et al.
769 2014; Kruszewski et al. 2018). Therefore, final consumer products made from the same cacao
770 beans may have very different Cd concentrations depending on the intended product, i.e. Cd
771 concentrations in white chocolate are negligibly small while fat-reduced cacao powder contains
772 high Cd concentrations. Indeed, Cd concentrations are reported to be larger in cacao powders
773 compared to cacao liquor and/or cacao beans (Mounicou et al. 2003; Kruszewski et al. 2018).
774 Considering this, it can be relevant for cacao processing companies to take the intended final
775 product into consideration when setting Cd requirements for their cacao suppliers (Figure 6). In
776 addition, the new EU Cd limits differ depending on the cacao solids content of the final product.
777 To produce a typical milk chocolate with 35% cacao solids (EU limit 0.30 mg Cd kg⁻¹), cacao

778 beans can be used with nib Cd concentrations up to 0.86 mg Cd kg⁻¹, not considering effects of
779 fermentation and/or testa removal on the Cd concentration of the cacao liquor. For dark chocolate
780 with 70% cacao solids (EU limit 0.80 mg Cd kg⁻¹), cacao beans with nib Cd concentrations up to
781 1.14 mg Cd kg⁻¹ could be used, which is much higher than the common industry requirement of
782 0.60 mg Cd kg⁻¹.

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

801 in a way that would allow structural mixing of cacao from different sources, as larger cooperatives
802 often purchase the cacao from small scale farmers and then sell the (mixed) product to international
803 clients.

804 5. FROM CHOCOLATE BAR TO BODY BURDEN

805 While different regulatory bodies are implementing limitations regarding the maximum allowed
806 Cd concentration in chocolate and other cacao-derived products, controversy remains regarding
807 tolerable intake levels, and regarding the relation between dietary intake and body burden. The
808 Joint FAO/WHO Expert Committee on Food Additives (JECFA) set a provisional tolerable
809 monthly intake of 25 $\mu\text{g Cd kg}^{-1}$ body weight (bw), which corresponds to a tolerable daily intake
810 of 0.83 $\mu\text{g Cd kg}^{-1}$ bw (FAO/WHO 2010). Conversely, the European Food Safety Authority
811 (EFSA) set a stricter tolerable weekly intake of 2.5 $\mu\text{g Cd kg}^{-1}$ bw, or a daily tolerable intake of
812 0.36 $\mu\text{g Cd kg}^{-1}$ bw (EFSA 2011). Children and infants have been identified as a high-risk group
813 within the population, due to differences in their diet compared to adults and because they consume
814 more per unit body weight. However, as adverse effects due to dietary Cd intake are mostly related
815 to accumulation in the human body over a lifetime of exposure, focusing on these young age
816 groups may not be relevant. Although research has indicated that only a small part of dietary Cd
817 is likely absorbed in the human body (European Chemicals Bureau 2007), regulations and health-
818 based guidelines are based on dietary Cd intake rather than resulting Cd body burden. Most dietary
819 Cd studies show that the Cd body burden increases less than proportional with increasing dietary
820 Cd intake, in contrast to the assumption in the risk assessment models, and this is related to lower
821 Cd bioavailability and/or changes in micronutrient status with diet when relying on high Cd diets
822 (Vahter et al. 1996; Reeves et al. 2001). Only limited information is currently available regarding
823 the gastro-intestinal absorption rate of Cd from cacao derived products, and studies thus far have

824 focused only on *in vitro* experiments. Mounicou et al. (2002 and 2003) studied the *in vitro*
825 bioaccessibility of Cd in cacao liquor and cacao powder and found accessible fractions ranging
826 10–50 %. Conversely, Barraza et al. (2017) determined the gastric bioaccessibility of Cd using the
827 unified method from the Bioaccessibility Research Group of Europe and reported gastric
828 bioaccessibility of >90% in cacao liquor from Ecuador. These studies have not highlighted if such
829 fractions are lower than for other staple foods such as cereals. There is currently no chemical
830 reason to expect lower Cd bioavailability and/or bioaccessibility in cacao compared to cereals, as
831 cacao is unlikely to contain strong Cd chelating compounds (Vanderschueren et al. 2020).
832 However, considering the strong influence of dietary status (e.g. dietary Zn, Fe, and Ca) on the
833 gastro-intestinal absorption of Cd (Reeves and Chaney 2008), it remains to be demonstrated if
834 long-term high chocolate consumption truly increases the body burden Cd to levels of concern. In-
835 vivo duplicate diet studies with increased dietary Cd through chocolate consumption, would offer
836 vital insights to assess the assumptions behind the existing (and future) food regulations.

837 6. CONCLUSION

838 Recent (and upcoming) regulations on the maximum allowed Cd concentration in cacao-derived
839 products are threatening cacao producers worldwide and are especially causing concern in Latin
840 America. Elevated soil Cd concentrations found in cacao production areas are likely related to
841 geogenic origin rather than contamination through anthropogenic influences. Bean Cd
842 concentrations are generally larger in cacao plants grown on acidic soils with low soil organic
843 carbon, when considering equal total soil Cd. Several mitigation strategies are being explored to
844 deal with elevated Cd concentrations in cacao. However, mitigation strategies are not a cure-all
845 and their use comes at a cost, either due the financial cost of implementing the strategy or due to
846 adverse effects on final product quality. First, soil amendments can reduce plant uptake of Cd by

847 reducing the phytoavailable soil concentration (i.e. biochar, lime, or gypsum) or by saturating
848 uptake mechanisms through which Cd can otherwise enter the plant (i.e. Zn fertilization).
849 Application of lime and biochar in cacao field trials has been reported to result in reduction factors
850 up to RF 1.9, but as both treatments are based on a liming effect (i.e. increasing soil pH), these
851 amendments are mostly effective in acid soils. While soil amendments could offer an easily
852 applicable medium-term solution, their incorporation is limited due to the rooting system of the
853 perennial cacao tree. Second, root uptake and root-to-shoot translocation of Cd within the cacao
854 plant may be targeted for selection of low Cd accumulating cultivars. While the research on uptake
855 and translocation mechanisms of Cd in cacao is only in its early stages, genetics-based mitigation
856 strategies can be highly valuable in the future. The available work on cacao indicates potential RF
857 ranging from 2 up to 13. However, experiments on the same soil (i.e. same location, also termed
858 common garden experiments) should be performed to identify low Cd accumulating cultivars (and
859 their potential RF) and avoid current uncertainties related to the different soils under the different
860 cultivars. The potential of using specific rootstocks in grafting should also be explored as this may
861 offer the benefit of genetics-based mitigation (selection of a low Cd uptake rootstock) while
862 maintaining the flavor profile of the original cacao cultivar in the scion. There is a need to better
863 characterize the pathways of Cd loading into cacao beans to reveal the role of the xylem and
864 phloem pathway. Third, postharvest mitigation strategies may offer the benefit of control for
865 industrial scale cacao processors. Mitigation strategies interfering with processes such as
866 fermentation have moderate potential (RF 1.3) but require additional research, and the effect of
867 such strategies on the flavor quality of the final product should always be taken into consideration.
868 On the short term, a realistic and easily implementable strategy is mixing of cacao from different
869 origins (and thus with different bean Cd concentrations), both on larger and smaller scales. The

870 intended final product should be kept in mind when setting Cd requirements for cacao suppliers,
871 as current requirements may be overly strict depending on the cacao content of the final product.
872 For example, to produce dark chocolate with 70% cacao solids, cacao beans containing up to 1.14
873 mg Cd kg⁻¹ could be used, which is almost double the currently common requirement of 0.60 mg
874 Cd kg⁻¹. Finally, considering the indications that cacao is a moderate Cd accumulator, farmers may
875 be recommended to change to a lower Cd accumulating crop if mitigation for cacao is not feasible.

876

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892 FIGURES

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

898

899 Figure 2: Cacao bean Cd concentrations as influenced by total soil Cd and soil pH for the selected
900 studies in the meta-analysis. Soil pH values for all studies are standardized to $\text{pH}_{\text{CaCl}_2}$ (Kissel et al.
901 2009). The dashed line indicates the generally accepted threshold of $0.60 \text{ mg Cd kg}^{-1}$ for export of
902 cacao beans to Europe. Details of the studies can be found in the Supplementary Information.

903

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

908

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

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

921

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

931

932 TABLES

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

939

940 ^a No information was found on whether Certified Reference Materials (CRM) were included or
941 not.

942 ^b CRM included with certified Cd concentrations above the reasonable range of cacao Cd
943 concentrations.

944 ^c Reported sample Cd concentrations lower than the limit of detection of the equipment used for
945 Cd analysis.

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

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

953

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

958 ¥ The number of observations included in each regression can differ from the number of
959 observations mentioned in the Supplementary Information (Table S 2) due to missing data for
960 some of the predictors.

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

967

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

969

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

972

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

974 § Analysis of scion wood cores.

975 Table 5: Average (min/max) or average \pm stdev apparent Cd isotope fractionation ($\Delta^{114/110}\text{Cd}_{A-B} = \delta^{114/110}\text{Cd}_A - \delta^{114/110}\text{Cd}_B$) (‰)
976 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
977 was reported in the article, it is not given in the table.

978

979 \$Shoot=stem; \$\$Leaf = flag-leaves; *Rice accessions with functional OsHMA3 tonoplast transporter in roots; **Rice accessions without
980 functional OsHMA3 tonoplast transporter; ^a Source: Hydroponic solution; ^b Source: Ca(NO₃)₂ soil extract; ^c Source: Soil solution

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1 TABLES

2 Table 1: Average bean Cd concentrations (mg kg^{-1}) in cacao from several surveys across the world
 3 clearly illustrate geographical differences, with the highest bean Cd concentrations reported for
 4 cacao grown in Central and South American countries (rows marked in grey), with exception of
 5 Brazil. Incomplete information on analytical data quality is indicated by superscripts with the
 6 sample origin. The variation is indicated either by the standard deviation or the range min-max; P
 7 = 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 Albarracín et al. 2019)

8 ^a No information was found on whether Certified Reference Materials (CRM) were included or
9 not.

10 ^b CRM included with certified Cd concentrations above the reasonable range of cacao Cd
11 concentrations.

12 ^c Reported sample Cd concentrations lower than the limit of detection of the equipment used for
13 Cd analysis.

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

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

21
 22 [§] There are three regression models for study VII as the variables Total Cd Top, Av Cd Top and
 23 DGT Cd Top were highly correlated thus running only one regression with all variables included
 24 would have violated the assumption of no multicollinearity. For study II, two models are included
 25 because Total Cd Top was significantly correlated to Total Zn Sub and Total Mn Top.

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

29 Table 3: Mean cadmium transfer factors (TF) (min-max) for cacao, i.e. Cd concentration ratios
 30 between cacao (leaf or bean = seed) and soil, and TF for other selected agricultural crops. The TF
 31 predicted from soil-plant regression models used median values of the soil properties from the
 32 surveys that yielded the corresponding models. Additional information regarding specific study
 33 conditions (i.e. study location and soil Cd) can be found in the Supplementary Information (Table
 34 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 ^s		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 ^s	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	(Römken et al. 2009)
Japonica rice		0.30	2155	(Römken et al. 2009)
Wheat		0.27	246	(Adams et al. 2004)

35 ^sNo range available, only mean is given

36 Table 4: Mean (min-max) dry weight-based Cd concentrations (mg Cd kg⁻¹) for different cacao plant tissues, including only unfermented
 37 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
Argiuello 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	/	/

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

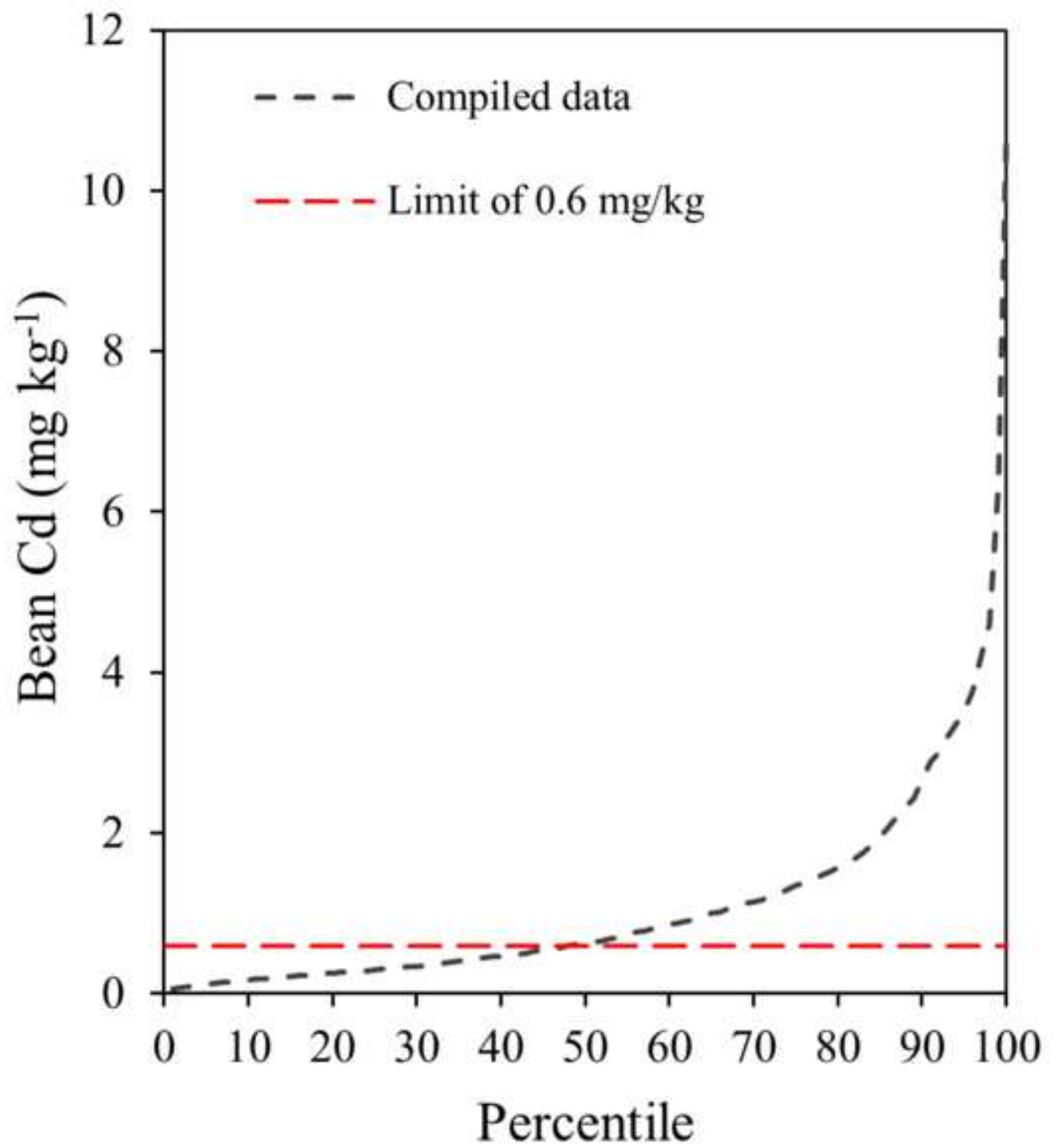
39 [§] Analysis of scion wood cores.

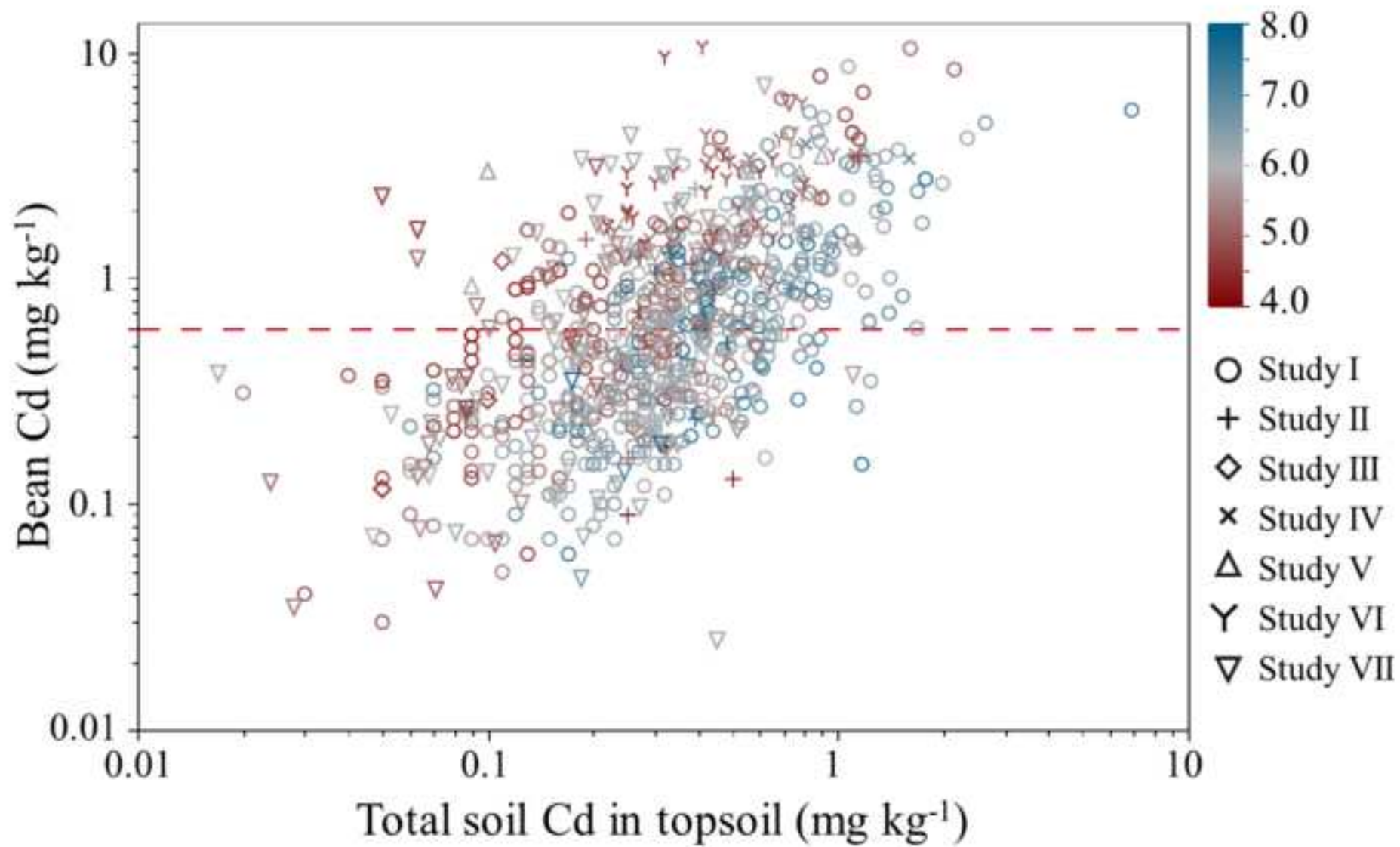
40 Table 5: Average (min/max) or average \pm stdev apparent Cd isotope fractionation ($\Delta^{114/110}\text{Cd}_{A-B} = \delta^{114/110}\text{Cd}_A - \delta^{114/110}\text{Cd}_B$) (‰)
 41 for different plants. A positive value indicates that tissue A is enriched in heavy isotopes compared to tissue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

Plant	$\Delta^{114/110}\text{Cd}_{A-B}$ (‰)						Remarks and conditions	Reference
	Plant-substrate	Plant-soil	Shoot-root	Leaf-stem	Leaf-root	Grain-stem		
Hydroponics								
<i>Ricinus communis</i>	-0.38 ¹ (-0.46/-0.32)		0.03 (-0.08/0.19) ^s	-0.11 (-0.18/-0.04)	-0.05 (-0.26/0.15)		2 mg Cd L ⁻¹	(Wei et al. 2016)
<i>Solanum nigrum</i>	-0.41 ¹		0.02 ^s	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) ^s	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) ^s	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) ^s	Mean of 3 locations 0.17–1.66 mg Cd kg ⁻¹ soil	(Imseng et al. 2019)
Rice	-0.30 \pm 0.01 ^c		0.16 \pm 0.03				Excluder*, flowering stage wet conditions 15 mg Cd kg ⁻¹ soil	(Wiggenhauser et al. 2020)
Rice	-0.43 \pm 0.01 ^c		-0.02 \pm 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)

43 ^{\$}Shoot=stem; ^{\$\$}Leaf = flag-leaves; ^{*}Rice accessions with functional OsHMA3 tonoplast transporter in roots; ^{**}Rice accessions without
44 functional OsHMA3 tonoplast transporter; ^a Source: Hydroponic solution; ^b Source: Ca(NO₃)₂ soil extract; ^c Source: Soil solution.





Transfer factor (TF) bean-soil

