

Supplementary information

Comparison of cadmium pathways in a high Cd accumulating cultivar versus a low Cd accumulating cultivar of *Theobroma cacao* L.

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1.1. Supplementary tables

Table S1: Soil properties in the top- and subsoil for the different cultivars.

		Soil properties					
		<i>High</i>		Low		Grafted	
Topsoil		Mean	SD	Mean	SD	Mean	SD
pH		4.13	<i>0.11</i>	4.20	0.09	4.27	0.04
OC	%	1.86	<i>0.36</i>	2.02	0.23	2.44	0.36
CEC	cmol _c /kg	12.2^b	<i>0.12</i>	11.7^b	0.16	14.3^a	0.47
Exchangeable Ca	cmol _c /kg	6.23^c	<i>0.58</i>	7.32^b	0.23	10.1^a	0.34
Exchangeable Mg	cmol _c /kg	2.72^a	<i>0.36</i>	2.05^b	0.06	2.28^{ab}	0.06
Exchangeable K	cmol _c /kg	0.22^b	<i>0.00</i>	0.21^b	0.00	0.31^a	0.02
Exchangeable Na	cmol _c /kg	0.14	<i>0.01</i>	0.11	0.01	0.14	0.02
Base saturation	%	76	<i>7</i>	83	2	90	3
Oxalate extractable P	g/kg	0.16^b	<i>0.03</i>	0.20^b	0.03	0.44^a	0.10
Oxalate extractable Al	g/kg	1.00^b	<i>0.05</i>	0.93^b	0.04	1.10^a	0.14
Oxalate extractable Fe	g/kg	4.86^b	<i>0.20</i>	5.50^b	0.61	9.07^a	0.98
Oxalate extractable Mn	g/kg	0.83	<i>0.10</i>	0.62	0.09	0.65	0.14
Ca(NO ₃) ₂ - Mn	mg/kg	248^a	<i>28</i>	197^{ab}	5.9	155^b	25
Ca(NO ₃) ₂ - Zn	mg/kg	3.64	<i>0.37</i>	4.15	0.95	6.10	2.98
Ca(NO ₃) ₂ - Cu	mg/kg	0.08	<i>0.01</i>	0.08	0.02	0.07	0.01
Ca(NO ₃) ₂ - Cd	mg/kg	0.08	<i>0.02</i>	0.07	0.01	0.10	0.01
Subsoil							
pH		3.74	<i>0.12</i>	3.84	0.06	3.78	0.06
CEC	cmol _c /kg	11.5^b	<i>0.39</i>	11.2^b	1.22	14.0^a	0.31
Exchangeable Ca	cmol _c /kg	4.07^b	<i>0.69</i>	4.82^b	0.96	7.27^a	0.18
Exchangeable Mg	cmol _c /kg	2.54	<i>0.18</i>	2.26	0.50	2.81	0.22
Exchangeable K	cmol _c /kg	0.23^b	<i>0.01</i>	0.25^b	0.04	0.39^a	0.01
Exchangeable Na	cmol _c /kg	0.19^b	<i>0.00</i>	0.17^b	0.02	0.33^a	0.03
Base saturation	%	61^b	<i>4</i>	67^{ab}	7	77^a	1
Oxalate extractable P	g/kg	0.09^b	<i>0.03</i>	0.11^{ab}	0.03	0.18^a	0.03
Oxalate extractable Al	g/kg	0.10	<i>0.02</i>	1.16	0.05	1.03	0.11
Oxalate extractable Fe	g/kg	3.20	<i>0.50</i>	4.14	0.09	5.05	0.89
Oxalate extractable Mn	g/kg	0.45	<i>0.13</i>	0.50	0.17	0.17	0.21
Ca(NO ₃) ₂ - Mn	mg/kg	109	<i>43.63</i>	130	40.97	68.0	3.52
Ca(NO ₃) ₂ - Zn	mg/kg	4.00^b	<i>0.49</i>	4.17^b	0.93	6.59^a	4.17
Ca(NO ₃) ₂ - Cu	mg/kg	0.13	<i>0.06</i>	0.10	0.03	0.15	0.01
Ca(NO ₃) ₂ - Cd	mg/kg	0.03	<i>0.01</i>	0.03	0.00	0.04	0.01

The data in italic was previously published in Blommaert et al. (2022).

OC: organic carbon (%), CEC: cation exchange capacity, ex: exchangeable bases, ox: ammonium oxalate extractable Fe, Mn and Al.

Ca(NO₃)₂ – extraction: 0.05 M Ca(NO₃)₂ (5g soil: 50 mL Ca(NO₃)₂).

Letters a,b, and c denote different statistical groups with the posthoc Tukey HSD test (p < 0.05).

Table S2: Nutrient concentrations measured in the organs of the cacao trees for the different cultivars.

Nutrient concentrations									
	High			Low			Grafted		
	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev
P (g kg⁻¹)									
Roots	3	0.34	0.28	3	0.62	0.17	3	1.08	0.85
Branch	3	0.85	0.29	3	1.26	0.27	3	1.23	0.15
Pod husk	5	1.21	0.12	6	0.91	0.27	1	0.76	
Testa	4	1.00	0.16	5	1.44	0.35	1	1.45	
Nib	5	4.45	0.61	6	5.67	0.45	1	5.49	
Leaves F2	0			2	5.59	0.38	1	4.73	
Leaves IF2	3	1.23	0.08	3	1.45	0.12	3	1.73	0.26
Leaves IF3	3	1.03	0.13	3	1.05	0.05	3	1.24	0.16
Cu (mg kg⁻¹)									
Roots	3	9.82	3.70	3	6.29	1.21	3	7.66	6.77
Branch	3	6.55	1.44	3	8.77	1.52	3	10.21	2.33
Pod husk	5	10.15	1.59	6	7.78	1.39	1	9.30	
Testa	4	11.52	1.54	5	11.46	4.60	1	15.54	
Nib	5	16.80	1.82	6	19.55	2.11	1	19.53	
Leaves F2	0			2	24.25	0.02	1	13.82	
Leaves IF2	3	4.58	0.25	3	5.28	0.47	3	6.29	1.32
Leaves IF3	3	3.81	0.26	3	3.98	0.49	3	5.41	1.28
S (g kg⁻¹)									
Roots	3	1.69	0.33	3	2.05	0.26	3	1.37	0.09
Branch	3	1.00	0.21	3	1.60	0.05	3	1.54	0.31
Pod husk	5	1.27	0.08	6	1.53	0.32	1	1.94	
Testa	4	1.17	0.07	5	1.00	0.11	1	1.21	
Nib	5	1.88	0.09	6	1.53	0.08	1	1.64	
Leaves F2	0			2	3.57	0.00	1	3.05	
Leaves IF2	3	2.32	0.09	3	2.11	0.19	3	2.32	0.35
Leaves IF3	3	1.93	0.08	3	2.42	0.52	3	2.16	0.54
K (g kg⁻¹)									
Roots	3	5.44	0.87	3	5.62	1.63	3	9.30	0.17
Branch	3	10.12	3.82	3	15.76	4.30	3	22.00	1.07
Pod husk	5	28.57	5.20	6	25.75	6.30	1	34.58	
Testa	4	10.46	0.37	5	15.55	1.43	1	30.00	
Nib	5	9.50	2.19	6	10.22	1.27	1	9.76	
Leaves F2	0			2	21.91	3.94	1	14.05	
Leaves IF2	3	11.58	2.01	3	15.95	2.82	3	20.28	1.78
Leaves IF3	3	8.35	2.09	3	9.88	0.97	3	15.37	1.90

Table S2: continued

Zn (mg kg ⁻¹)									
Roots	3	46.80	10.24	3	22.27	5.25	3	33.10	2.40
Branch	3	139.22	25.15	3	108.12	31.96	3	155.90	72.34
Pod husk	5	71.41	6.92	6	51.42	12.06	1	64.16	
Testa	4	36.14	6.87	5	33.92	7.09	1	66.77	
Nib	5	46.70	4.90	6	38.88	4.17	1	45.77	
Leaves F2	0			2	85.21	11.76	1	66.85	
Leaves IF2	3	183.82	21.52	3	87.14	23.29	3	90.74	7.83
Leaves IF3	3	177.57	15.65	3	136.57	61.01	3	154.06	46.22
Mn (mg kg ⁻¹)									
Roots	3	101.15	54.52	3	19.97	4.32	3	30.68	10.42
Branch	3	465.08	114.25	3	160.87	71.42	3	391.08	164.57
Pod husk	5	308.42	134.74	6	98.44	66.24	1	128.18	
Testa	4	99.71	28.09	5	52.82	24.02	1	66.82	
Nib	5	93.58	24.77	6	49.09	16.60	1	62.95	
Leaves F2	0			2	184.44	28.13	1	77.11	
Leaves IF2	3	1776.86	287.47	3	239.08	69.06	3	406.20	108.08
Leaves IF3	3	1863.56	306.21	3	445.00	152.67	3	990.83	314.77
Ca (g kg ⁻¹)									
Roots	3	8.55	1.13	3	3.66	1.31	3	4.59	1.07
Branch	3	18.71	6.62	3	16.80	2.90	3	22.28	5.87
Pod husk	5	5.00	1.71	6	5.90	2.40	1	3.92	
Testa	4	4.45	1.67	5	6.61	2.11	1	4.87	
Nib	5	1.24	0.24	6	1.62	0.32	1	1.29	
Leaves F2	0			2	3.57	0.81	1	4.01	
Leaves IF2	3	15.28	2.53	3	7.97	1.75	3	7.54	0.97
Leaves IF3	3	16.21	1.52	3	14.51	0.91	3	13.92	0.36
Mg (g kg ⁻¹)									
Roots	3	6.32	1.04	3	3.24	0.84	3	4.80	0.87
Branch	3	5.10	1.92	3	5.46	1.09	3	4.34	1.63
Pod husk	5	3.12	0.67	6	3.37	1.18	1	3.12	
Testa	4	2.73	0.48	5	3.67	0.68	1	3.78	
Nib	5	3.44	0.26	6	3.61	0.18	1	3.64	
Leaves F2	0			2	4.62	0.28	1	4.03	
Leaves IF2	3	8.37	0.52	3	4.93	0.87	3	4.30	0.33
Leaves IF3	3	8.25	0.41	3	7.01	0.63	3	6.83	0.84

1 **Table S3:** Estimation of total uptake of Cd and nutrients for each cultivar (in g).

	Total uptake (in g)								
	High			Low			Grafted		
	Mean	SD	Group	Mean	SD		Mean	SD	
Cd	0.67	0.06	a	0.25	0.08	c	0.44	0.05	b
Mn	70.65	4.42	a	19.51	7.56	b	44.80	16.39	ab
K	1180	289	b	1708	426	ab	2425	107	a
S	170.4	26.2	b	235.2	13.4	a	208.2	30.9	ab
Ni	0.83	0.22		0.45	0.17		0.82	0.76	
P	100.2	21.0		144.7	27.5		161.0	36.9	
Ca	2092	612		1707	278		2191	461	
Cu	0.93	0.09		1.01	0.15		1.19	0.21	
Zn	16.02	2.47		11.56	3.49		16.10	6.55	

2 Total uptake was calculated based on biomass assumptions (see materials and methods). Letters a, b, and c
3 differences in Cd/nutrient uptake among cultivars (posthoc Tukey HSD test, $p < 0.05$).

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5 **Table S4:** Bulk concentration ratios ($\log[\text{nutrient}/\text{Cd}]$ ($\text{mmol kg}^{-1} \text{ DW}/\text{mmol kg}^{-1} \text{ DW}$)) for the different tree organs
6 for the different cultivar groups (low, high, and grafted).

	High		Low		Grafted	
	Mean \pm SD		Mean \pm SD		Mean \pm SD	
$\log (\text{P}/\text{Cd})$						
Roots	5.53 \pm 1.27	c	7.61 \pm 0.19	d	7.45 \pm 0.78	c
Branch	6.18 \pm 0.35 ^x	bc	7.62 \pm 0.33 ^y	d	7.02 \pm 0.20 ^y	c
Pod husk	7.40 \pm 0.37	b	8.35 \pm 0.80	bcd	7.44	bc
Testa	7.33 \pm 0.15 ^x	b	8.92 \pm 0.31 ^y	bc	8.60 ^y	abc
Nib	8.97 \pm 0.17 ^x	a	10.66 \pm 0.47 ^y	a	9.55 ^{xy}	ab
F2 leaves			9.52 \pm 0.29	ab	10.45	a
IF2 leaves	7.18 \pm 0.30 ^x	b	8.59 \pm 0.15 ^z	bcd	7.92 \pm 0.23 ^y	bc
IF3 leaves	7.09 \pm 0.18 ^x	b	7.97 \pm 0.33 ^y	cd	7.11 \pm 0.06 ^x	c
$\log (\text{Cu}/\text{Cd})$						
Roots	1.66 \pm 0.62	bc	2.32 \pm 0.30	bcd	1.73 \pm 0.89	bc
Branch	0.62 \pm 0.14 ^x	d	1.94 \pm 0.43 ^y	cd	1.50 \pm 0.13 ^y	bc
Pod husk	1.87 \pm 0.42	b	2.89 \pm 0.64	bc	2.33	abc
Testa	2.16 \pm 0.24 ^x	ab	3.34 \pm 0.32 ^y	b	3.34	ab
Nib	2.68 \pm 0.15 ^x	a	4.27 \pm 0.49 ^y	a	3.19	ab
F2 leaves			3.36 \pm 0.22	ab	3.89	a
IF2 leaves	0.87 \pm 0.28 ^x	cd	2.26 \pm 0.14 ^z	bcd	1.57 \pm 0.28 ^y	bc
IF3 leaves	0.78 \pm 0.14 ^x	d	1.67 \pm 0.41 ^y	d	0.95 \pm 0.09 ^x	c
$\log (\text{S}/\text{Cd})$						
Roots	7.53 \pm 0.31 ^x	ab	8.80 \pm 0.45 ^y	ab	7.84 \pm 0.03 ^x	bc
Branch	6.33 \pm 0.17 ^x	c	7.85 \pm 0.33 ^z	b	7.21 \pm 0.10 ^y	d
Pod husk	7.41 \pm 0.52 ^x	b	8.86 \pm 0.72 ^y	ab	8.35 ^{xy}	b
Testa	7.47 \pm 0.20 ^x	ab	8.54 \pm 0.37 ^y	ab	8.38 ^{xy}	b
Nib	8.09 \pm 0.22 ^x	a	9.32 \pm 0.48 ^y	a	8.30 ^{xy}	b
F2 leaves			9.04 \pm 0.22	ab	9.98	a
IF2 leaves	7.78 \pm 0.27 ^x	ab	8.94 \pm 0.29 ^y	ab	8.18 \pm 0.22 ^x	b
IF3 leaves	7.69 \pm 0.13 ^x	ab	8.76 \pm 0.56 ^y	ab	7.62 \pm 0.06 ^x	c
$\log (\text{K}/\text{Cd})$						
Roots	8.50 \pm 0.42 ^x	cd	9.59 \pm 0.27 ^y	b	9.56 \pm 0.12 ^y	c
Branch	8.42 \pm 0.28 ^x	d	9.91 \pm 0.23 ^y	b	9.68 \pm 0.16 ^y	c
Pod husk	10.26 \pm 0.58 ^x	a	11.47 \pm 0.62 ^y	a	11.04 ^{xy}	a
Testa	9.47 \pm 0.18 ^x	abc	11.09 \pm 0.36 ^y	a	11.40 ^y	a
Nib	9.49 \pm 0.37 ^x	ab	11.01 \pm 0.51 ^y	a	9.89 ^{xy}	bc
F2 leaves			10.65 \pm 0.04	ab	11.30	a
IF2 leaves	9.18 \pm 0.40 ^x	bcd	10.75 \pm 0.11 ^y	ab	10.15 \pm 0.16 ^y	b
IF3 leaves	8.94 \pm 0.37 ^x	bcd	9.98 \pm 0.44 ^y	b	9.40 \pm 0.09 ^{xy}	c

7 Letters x, y, z denote different statistical groups within the same row for the different cultivars (horizontal). Letters
8 a, b, c, d denote different statistical groups within the same column for the different organs (vertical) (posthoc Tukey)

9 HSD test, $p < 0.05$). The bulk concentration ratios were log transformed to comply with the ANOVA assumptions
 10 (see Materials and Methods).

11
 12 **Table S4:** continued

	High		Low		Grafted	
	Mean \pm SD		Mean \pm SD		Mean \pm SD	
log (Zn/Cd)						
Roots	3.23 \pm 0.46	c	3.55 \pm 0.19	b	3.40 \pm 0.17	c
Branch	3.65 \pm 0.17 ^x	c	4.41 \pm 0.14 ^y	ab	4.14 \pm 0.35 ^{xy}	b
Pod husk	3.79 \pm 0.45	bc	4.74 \pm 0.58	a	4.23	abc
Testa	3.27 \pm 0.29 ^x	c	4.44 \pm 0.33 ^y	ab	4.77 ^y	ab
Nib	3.67 \pm 0.29 ^x	c	4.93 \pm 0.47 ^y	a	4.01 ^{xy}	bc
F2 leaves	.		4.59 \pm 0.08	ab	5.44	a
IF2 leaves	4.53 \pm 0.21 ^{xy}	ab	5.01 \pm 0.30 ^y	a	4.23 \pm 0.07 ^x	b
IF3 leaves	4.59 \pm 0.06 ^x	a	5.12 \pm 0.14 ^y	a	4.25 \pm 0.22 ^x	b
log (Mn/Cd)						
Roots	4.09 \pm 0.63	d	3.62 \pm 0.35	d	3.47 \pm 0.37	d
Branch	5.02 \pm 0.27	bc	4.93 \pm 0.42	c	5.23 \pm 0.36	bc
Pod husk	5.37 \pm 0.22	b	5.40 \pm 0.42	bc	5.09	abc
Testa	4.43 \pm 0.28 ^x	cd	4.99 \pm 0.28 ^y	c	4.94 ^{xy}	bc
Nib	4.52 \pm 0.30 ^x	cd	5.29 \pm 0.40 ^y	c	4.05 ^{xy}	cd
F2 leaves	.		5.53 \pm 0.06	abc	5.76	abc
IF2 leaves	6.97 \pm 0.20 ^y	a	6.19 \pm 0.44 ^x	ab	5.88 \pm 0.19 ^x	ab
IF3 leaves	7.11 \pm 0.07 ^y	a	6.50 \pm 0.25 ^x	a	6.29 \pm 0.20 ^x	a
log (Ca/Cd)						
Roots	8.82 \pm 0.31 ^x	bc	9.94 \pm 0.29 ^y	a	9.88 \pm ^y	a
Branch	9.11 \pm 0.39 ^x	abc	10.03 \pm 0.34 ^y	a	9.70 \pm ^{xy}	ab
Pod husk	8.47 \pm 0.10 ^x	c	9.88 \pm 0.29 ^y	a	8.80 ^x	cd
Testa	8.53 \pm 0.36 ^x	c	10.17 \pm 0.31 ^y	a	9.54 ^{xy}	abc
Nib	7.43 \pm 0.19 ^x	d	9.15 \pm 0.31 ^y	b	7.87 ^x	d
F2 leaves	.		8.88 \pm 0.45	b	10.11	a
IF2 leaves	9.49 \pm 0.28 ^{xy}	ab	10.12 \pm 0.41 ^y	a	9.19 \pm ^x	bc
IF3 leaves	9.66 \pm 0.12 ^x	a	10.55 \pm 0.40 ^y	a	9.34 \pm ^x	abc
log (Mg/Cd)						
Roots	8.46 \pm 0.71 ^x	c	10.00 \pm 0.21 ^y	ab	9.47 \pm 0.09 ^{xy}	b
Branch	8.29 \pm 0.42 ^x	c	9.38 \pm 0.23 ^y	b	8.51 \pm 0.32 ^x	c
Pod husk	8.56 \pm 0.27 ^x	bc	9.90 \pm 0.47 ^y	ab	9.09 ^{xy}	bc
Testa	8.64 \pm 0.15 ^x	abc	10.15 \pm 0.27 ^y	ab	9.82 ^y	ab
Nib	9.00 \pm 0.24 ^x	abc	10.50 \pm 0.48 ^y	a	9.41 ^{xy}	b
F2 leaves	.		9.62 \pm 0.30	ab	10.59	a
IF2 leaves	9.37 \pm 0.29 ^x	ab	10.11 \pm 0.39 ^y	ab	9.11 \pm 0.15 ^x	b
IF3 leaves	9.47 \pm 0.08 ^x	a	10.29 \pm 0.51 ^y	ab	9.09 \pm 0.06 ^x	bc

13 Letters x, y, z denote different statistical groups within the same row for the different cultivars, i.e. for one organ,
 14 cultivars falling in different groups x, y, z have significantly distinct X/Cd ratios. Letters a, b, c, d denote different
 15 statistical groups within the same column for the different organs, i.e. for one cultivar, organs falling in different
 16 group a, b, c, d have significantly different X/Cd ratios (posthoc Tukey HSD test, $p < 0.05$). The bulk concentration
 17 ratios were log transformed to comply with the ANOVA assumptions (see Materials and Methods).

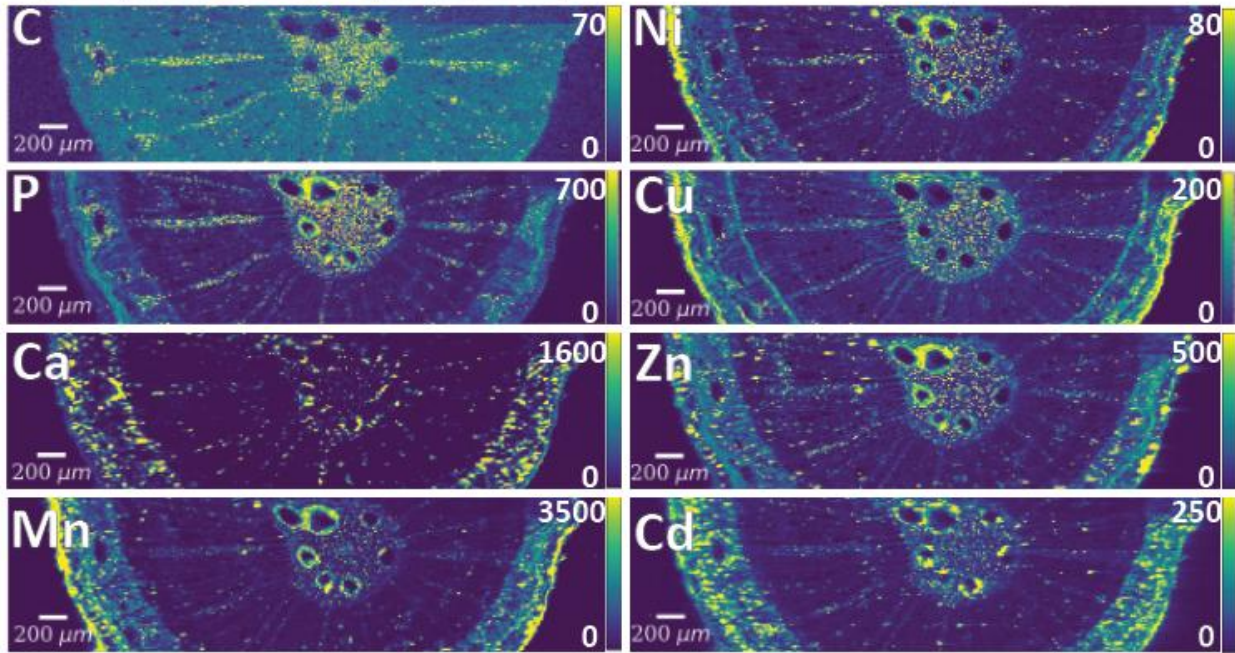
18 **Table S5:** Intensity of Cd signal detected by LA-ICP-MS in the different structures of the branch
 19 of the low Cd accumulating cultivar (replicates T15 and T3) and R^2 of regressions to intensities
 20 of other elements.

	Intensity ^s		R^2											
	Cd		Ca		Mn		Zn		Cu		Ni		P	
	T15	T3	T15	T3	T15	T3	T15	T3	T15	T3	T15	T3	T15	T3
1 Pith	8.74	1.73	0.42	0.21	0.44	0.28	0.47	0.32	0.17	0.27	0.28	0.28	0.27	0.19
2 Wood	1.00	1.00	0.28	0.29	0.36	0.27	0.37	0.16	0.25	0.16	0.16	0.12	0.29	0.18
3 Bark phloem	9.43	3.74	0.48	0.45	0.55	0.54	0.32	0.38	0.2	0.25	0.22	0.22	0.36	0.42
4 Phloem vessels	8.93	3.58	0.54	0.45	0.59	0.48	0.34	0.31	0.25	0.19	0.26	0.13	0.42	0.3
5 Phloem rays	10.50	6.38	0.45	0.42	0.55	0.46	0.34	0.31	0.21	0.12	0.21	0.09	0.32	0.25
6 Periderm	11.12	5.68	0.31	0.32	0.42	0.48	0.33	0.4	0.18	0.09	0.24	0.1	0.35	0.35

21 Elemental counts (cts) of each element were normalized to ^{13}C to account for differences in tissue hardness and
 22 water content within the sample. ^sThe Cd counts were normalized to the mean Cd counts in wood to compare the
 23 accumulation of Cd in the different structures of the branch. In the materials and methods of Blommaert et al.
 24 (2022), more details can be found regarding the analysis.

25 **1.2. Supplementary figures**

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27

28 **Figure S1:** Microscope image and LA-ICP-MS elemental maps of cross section of branch of the
29 low Cd accumulator (replicate T15). The color scale bar indicates the intensity expressed in total
30 counts (see Fig. 4 for explanation of the color scale) and is adjusted to the range of each element.
31 Cadmium was particularly enriched in the phloem rays and the periderm. The Cd in the pith
32 accumulated around the large secreting cavities in the pith, shown by white * .

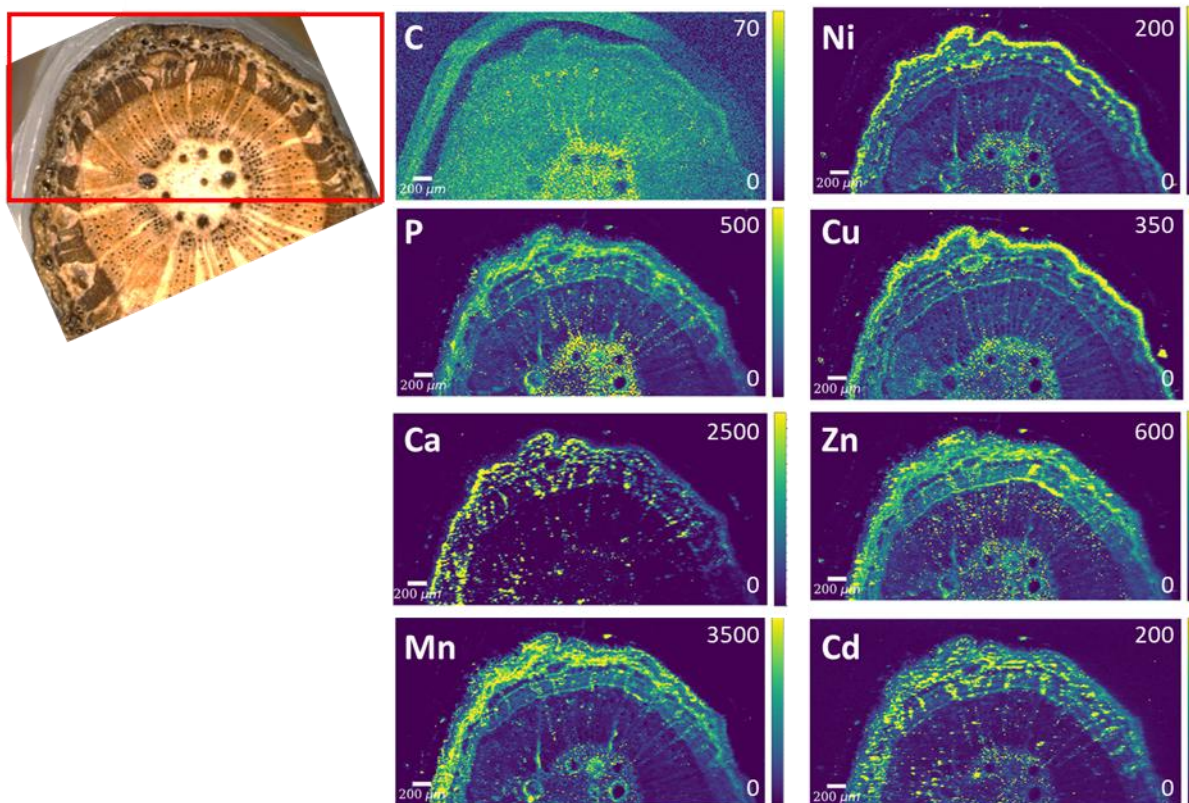


Figure S2: Microscope image and LA-ICP-MS elemental maps of a cross section of branch CL 10/3 (replicate T3). The intensity is expressed in total counts (cts). The color scale is adjusted to the range of each element.

1.3. References

Blommaert, H., Aucour, A., Wiggerhauser, M., Moens, C., Telouk, P., Landrot, G., *et al.* (2022) 'From soil to cacao bean : Unravelling the pathways of cadmium translocation in a high Cd accumulating cultivar of *Theobroma cacao* L . Front. Plant Sci. 13. <https://doi.org/10.3389/fpls.2022.1055912>.