Supplementary Information

To:

Stable isotope analyses reveal impact of Fe and Zn on Cd uptake and translocation by *Theobroma cacao*

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Table S1: Composition of the half-strength Hoagland solutions with additional $CdCl_2$ for the eight treatments

Component	Species	Concentration		Treatment
		mg L^{-1}	µmol L^{-1}	#
Major nutrients				
KH_2PO_4	K^+	19.55	500	All
	PO_4^{3-}	47.49	500	All
	H^+	2.002	1000	All
KNO_3	K^+	97.75	2500	All
	NO_3^-	155.0	2500	All
$Ca(NO_3)_2 \cdot 4H_2O$	Ca_2^+	100.2	2500	All
	NO_3^-	310.0	5000	All
$MgSO_4 \cdot 7H_2O$	Mg^{2+}	24.31	1000	All
	SO_4^{2-}	96.07	1000	All
Micronutrients				
H_3BO_3	H_3BO_3	1.430	23.1	All
Fe-EDTA[a,b]	Fe^{3+}	2.383	42.7	1, 2, 3, 6, 7
	$EDTA^{4-}$	12.30	42.7	1, 2, 3, 6, 7
$MnCl_2 \cdot 4H_2O$	Mn^{2+}	0.253	4.6	All
	Cl^-	0.326	9.2	All
$ZnSO_4 \cdot 7H_2O$[a]	Zn^{2+}	0.025	0.38	1, 2, 3, 4, 5
	SO_4^{2-}	0.037	0.38	1, 2, 3, 4, 5
$CuSO_4 \cdot 5H_2O$	Cu^{2+}	0.010	0.2	All
	SO_4^{2-}	0.015	0.2	All
$Na_2MoO_4 \cdot 2H_2O$	Na^+	0.002	0.1	All
	MoO_4^{2-}	0.003	0.04	All
$CdCl_2$[c]	Cd^{2+}	2.248	20.0	All
	Cl^-	2.836	40.0	All

[a] $C_{10}H_{12}FeN_2O_8$ [b] After an initial 28 days in the half-strength Hoagland solution without $CdCl_2$, the cacao seedlings were grown in eight different solutions for 14 days. These solutions included or excluded Fe-EDTA and $ZnSO_4$. [c] The $CdCl_2$ was present in all eight final solutions but excluded from the solutions used for the initial 28 days.

Table S2: Cadmium concentrations and isotope compositions (relative to NIST SRM 3108 Cd) obtained for reference and quality control materials and comparison with literature data

Material	**Type**	**n**	**[Cd] ± sd**	**$\delta^{114/110}$Cd ± 2sd**	**Reference**
			µg g^{-1}	‰	
BAM-I012	Cd Solution	4	**-**	**–1.32±0.06**	**This study**
			-	–1.33±0.04	[1]
			-	–1.32±0.06	[2]
			-	–1.33±0.06	[3]
NIST SRM 1570a	Spinach Leaf	2	**2.76±0.02**	**0.47±0.03**	**This study**
			2.87±0.06	-	Certificate
			2.71±0.01	0.44±0.06	[2]
			-	0.47±0.03	[3]
			2.87±0.02	0.45±0.05	[4]
BCR-679	White Cabbage	1	**1.69**	**0.22±0.04**	**This study**
			1.66±0.07	-	Certificate
			1.77±0.05	0.22±0.02	[5]

Table S3: Percentages of species present in the hydroponic solutions with or without Fe-EDTA and $ZnSO_4$, calculated using Visual MINTEQ 3.1

Species[a]	Fe-EDTA + $ZnSO_4$	Fe-EDTA	$ZnSO_4$	Neither
Cd^{2+}	87.7	87.1	88.3	88.3
$CdCl^{+}$	0.46	0.46	0.46	0.46
$CdSO_4$ (aq)	6.20	6.16	6.25	6.24
$Cd(SO_4)_2^{2-}$	0.07	0.06	0.07	0.07
$CdNO_3^{+}$	1.24	1.24	1.25	1.25
$CdHPO_4$ (aq)	3.62	3.59	3.64	3.64
$CdEDTA^{2-}$	0.66	1.38		
Cu^{2+}	32.5	19.4	83.6	83.6
$CuOH^{+}$	0.12	0.07	0.31	0.31
$CuSO_4$ (aq)	2.24	1.34	5.78	5.78
$CuNO_3^{+}$	0.47	0.28	1.21	1.21
$CuHPO_4$ (aq)	3.52	2.10	9.17	9.07
$CuEDTA^{2-}$	60.3	75.7		
$CuHEDTA^{-}$	0.89	1.12		
Fe-$EDTA^{-}$	99.1	99.4		
FeHEDTA (aq)	0.02	0.02		
$FeOHEDTA^{2-}$	0.35	0.35		
$Fe(OH)_2^{+}$	0.17	0.08		
$FeHPO_4^{+}$	0.36	0.17		
Zn^{2+}	90.8		91.3	
$ZnSO_4$ (aq)	5.97		6.01	
$Zn(SO_4)_2^{2-}$	0.04		0.04	
$ZnNO_3^{+}$	1.05		1.05	
$ZnHPO_4$ (aq)	1.53		1.53	
$ZnEDTA^{2-}$	0.56			
$ZnCl^{+}$	0.01		0.02	
$ZnOH^{+}$	0.01			

[a] The speciation of other elements was not significantly changed by the addition or removal of Fe-EDTA or $ZnSO_4$ and they are therefore omitted. Fe-EDTA and $ZnSO_4$ were both present in treatments 1, 2 and 3. Fe-EDTA, but not $ZnSO_4$, was present in treatments 6 and 7. $ZnSO_4$, but not Fe-EDTA, was present in treatments 4 and 5. Neither were present in treatment 8.

Table S4: Dry masses of plant tissue samples

T	Nutrient Solutions		Roots	Stems	Leaves	Total	Average Total	sd
#	Hydroponic	Foliar	g	g	g	g	g	g
C	Fe, Zn	-	0.10	0.17	0.38	0.65	0.69	0.27
			0.09	0.20	0.16	0.45		
			0.09	0.17	0.72	0.99		
1	Fe, Zn	-	0.11	0.16	0.36	0.63	0.78	0.21
			0.13	0.20	0.59	0.92		
2	Fe, Zn	Fe	0.15	0.17	0.52	0.84	0.91	0.11
			0.11	0.19	0.70	0.99		
3	Fe, Zn	Zn	0.10	0.17	0.69	0.96	0.86	0.14
			0.12	0.12	0.52	0.76		
4	Zn	-	0.09	0.11	0.36	0.56	0.67	0.15
			0.09	0.14	0.54	0.77		
5	Zn	Fe	0.11	0.15	0.52	0.79	0.69	0.14
			0.10	0.14	0.36	0.59		
6	Fe	-	0.08*	0.18	0.57	0.84	0.74	0.13
			0.11	0.18	0.51	0.79		
			0.10	0.13	0.36	0.59		
7	Fe	Zn	0.11	0.16	0.52	0.79	0.76	0.04
			0.10	0.13	0.50	0.73		
8	-	-	0.08	0.13	0.52	0.73	0.72	0.01
			0.09	0.19	0.44	0.72		
		Mean	**0.11**	**0.15**	**0.50**	**0.76**	**0.76**	0.12
		sd	0.02	0.03	0.11	0.13	0.09	0.06

T= treatment. C = control. Hydroponic solutions were half-strength Hoagland (pH 5.2) and T1-8 contained 2.25 mg L^{-1} Cd, administered as $CdCl_2$. Where present, the concentrations of Fe and Zn in the hydroponic solutions were 2.38 mg L^{-1} and 25 μg L^{-1}, respectively, and were administered as Fe-EDTA and $ZnSO_4$. The foliar treatments were sprays of 0.1% solutions of Fe- and Zn-EDTA. *Insufficient sample for Cd isotope composition analysis.

Table S5: Mass of Cd in roots, stems, leaves and total plants, translocation factors and fractions of total Cd in plant organs.

T	Nutrient Solutions		Cd (µg)								TF		Fraction of total Cd					
#	Hydroponic	Foliar	Roots	sd	Stems	sd	Leaves	sd	Total	sd	%	sd	Roots	sd	Stems	sd	Leaves	sd
1	Fe, Zn	-	38.8	2.4	51.3	8.7	52.1	3.1	142	9	268	47	0.27	0.04	0.36	0.04	0.37	0.00
2	Fe, Zn	Fe	44.6	10.1	49.5	9.5	92.8	17.9	187	38	324	11	0.24	0.01	0.27	0.00	0.50	0.00
3	Fe, Zn	Zn	38.4	1.1	36.5	10.8	66.0	14.6	141	24	268	74	0.28	0.06	0.26	0.03	0.47	0.02
4	Zn	-	44.4	0.7	20.0	6.1	17.3	7.6	82	14	84	30	0.55	0.09	0.24	0.03	0.21	0.06
5	Zn	Fe	51.4	4.8	29.8	1.6	19.4	2.7	101	0	96	17	0.51	0.05	0.30	0.02	0.19	0.03
6	Fe	-	39.3	6.2	42.9	29.5	36.2	32.2	118	68	191	127	0.38	0.16	0.35	0.05	0.27	0.12
7	Fe	Zn	32.9	5.4	43.8	5.9	45.7	15.6	122	27	270	21	0.27	0.02	0.36	0.03	0.37	0.05
8	-	-	31.6	5.3	13.6	5.7	13.9	15.0	59	15	94	81	0.57	0.24	0.22	0.04	0.21	0.20
		Mean	**40**	**5**	**36**	**10**	**43**	**14**	**118**	**22**	**199**	**51**	**0.38**	**0.08**	**0.29**	**0.03**	**0.32**	**0.06**
		sd	6	3	14	8	27	10	40	16	96	40	0.14	0.08	0.06	0.02	0.12	0.07
		Min	32	1	14	2	14	3	59	0	84	11	0.24	0.01	0.22	0.00	0.19	0.00
		Max	51	10	51	29	93	32	187	49	324	127	0.57	0.24	0.36	0.06	0.50	0.20

T = treatment. Hydroponic solutions were half-strength Hoagland (pH 5.2) and contained 2.25 mg L^{-1} Cd, administered as $CdCl_2$. Where present, the concentrations of Fe and Zn in the hydroponic solutions were 2.38 mg L^{-1} and 25 µg L^{-1} and were administered as Fe-EDTA and $ZnSO_4$. The foliar treatments were sprays of 0.1% solutions of Fe-EDTA and Zn-EDTA. Values are averages of two replicate plants per treatment. TF = translocation factor, which is calculated by dividing the total Cd in the shoots (leaves + stems) by the total Cd in the roots, multiplied by 100.

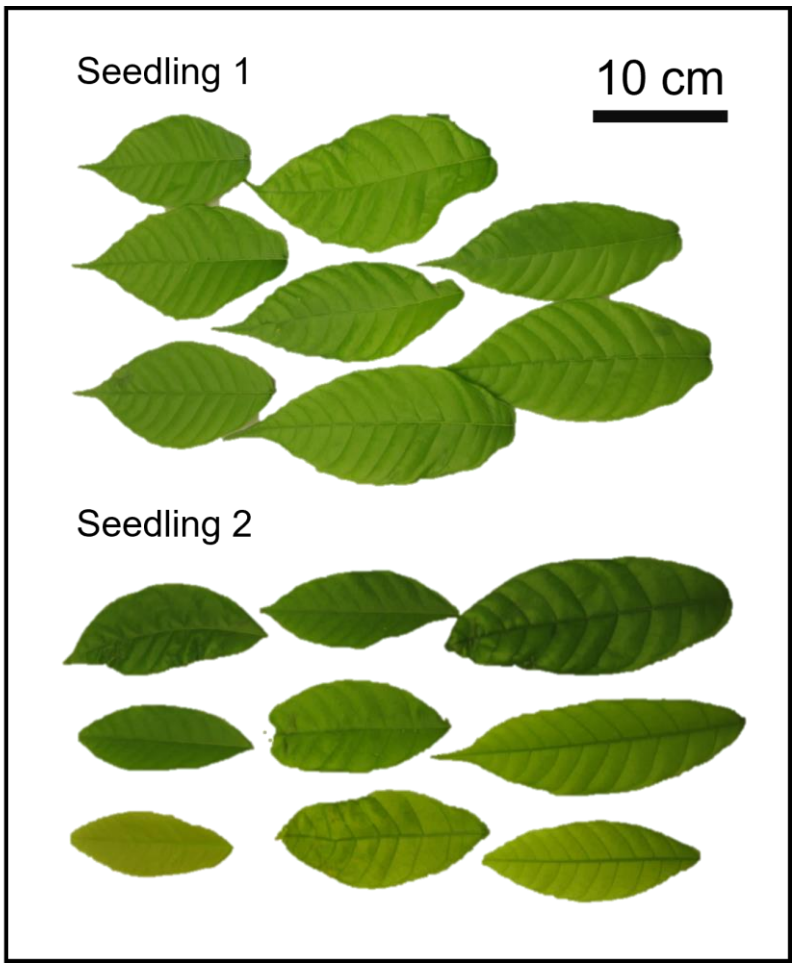

Figure S1: Post-harvest photograph of leaves from two cacao seedlings grown in hydroponics with the addition of 20 μM $CdCl_2$, at the Controlled Environment Laboratories (Reading, UK). No necrosis (death of leaf cells) or chlorosis (yellowing of the leaf from lack of chlorophyl) is evident.

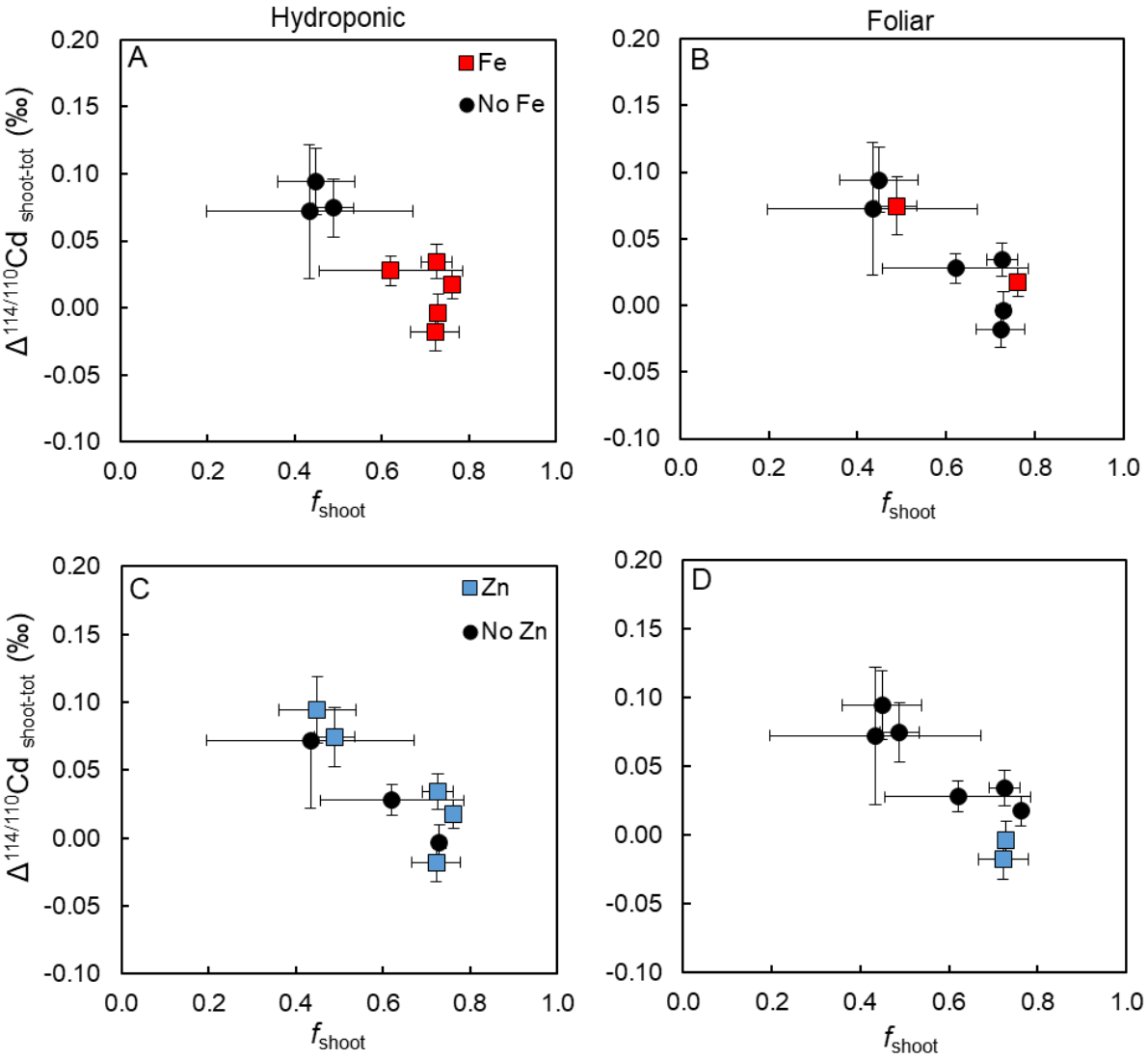

Figure S2: Apparent Cd isotope fractionation between shoots and total plant versus fraction (*f*) of total plant Cd in these shoots. Plots A and B highlight hydroponic and foliar Fe treatments, respectively. Plots C and D highlight hydroponic and foliar Zn treatments, respectively. Values are averages of two replicates for each treatment. Average isotope compositions were calculated using inverse-variance weighting.

References:

[1] Abouchami, W. et al. A Common Reference Material for Cadmium Isotope Studies - NIST SRM 3108. *Geostand. Geoanalytical Res.* 37, 5–17 (2013.

[2] Moore, R. E. T. et al. Cadmium isotope fractionation reveals genetic variation in Cd uptake and translocation by *Theobroma cacao* and role of natural resistance-associated macrophage protein 5 and heavy metal ATPase-family transporters. *Hortic. Res.* 7, 71 (2020).

[3] Lv, W., Yin, H., Liu, M., Huang, F., Yu, H. Effect of the Dry-Ashing Method on Cadmium Isotope Measurements in Soil and Plant Samples. *Geostand. Geoanalytical Res.* 45, 1–12 (2020).

[4] Borovička, J., Ackerman, L., Rejšek, J., Cadmium isotopic composition of biogenic certified reference materials determined by thermal ionization mass spectrometry with double spike correction. *Talanta*, 221, 121389 (2021).

[5] Wiggenhauser, M. et al. Cadmium transfer in contaminated soil-rice systems: Insights from solid-state speciation analysis and stable isotope fractionation. *Environ. Pollut.* 269, 115934 (2021)