

Phytoextraction by a High-Cd-accumulating Rice: Reduction of Cd Content of Soybean Seeds

SUPPORTING INFORMATION

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Number of tables: 2

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Soil and plant analyses

All soils were passed through a 2-mm sieve before analysis. We measured the soil pH in distilled water (1:2.5 w/v) with a pH meter (HM-50V, TOA DKK, Tokyo, Japan). Total soil C and N were determined with an NC analyzer (Sumigraph NC-900, Sumitomo, Osaka, Japan). To identify which soil metal fractions were decreased by plant uptake, we extracted the soils by using a single extraction with 0.01 or 0.1 mol L⁻¹ HCl (1:5 w/v, 1 h shaking side-to-side (1) and a sequential extraction method (2)). The sequential extraction method was used to determine five soil metal fractions: exchangeable metal, extracted with 0.05 mol L⁻¹ Ca(NO₃)₂ (1:10 w/v, 24 h shaking); inorganically bound metal, extracted with 2.5% acetic acid (1:10 w/v, 24 h shaking) from the residue of the 1st fraction; organically bound metal, extracted with 2.5% acetic acid (1:10 w/v, 24 h shaking) after organic matter was decomposed with 6% H₂O₂, from the residue of the 2nd fraction; oxide-occluded metal, extracted with a mixture of 0.1 mol L⁻¹ H₂C₂O₄ and 0.175 mol L⁻¹ (NH₄)₂C₂O₄ (1:30 w/v) in a boiling water bath for 1 h with occasional stirring, from the residue of the 3rd fraction; and residual metal, digested with 30% H₂O₂, 60% HClO₄, 48% HF, and concentrated HNO₃ from the residue of the 4th fraction. Total metal was the sum of these five fractions. These methods of soil analysis are explained in our previous report (3).

Harvested shoots and roots of rice and seeds of soybeans were dried at 65 °C for 48 h, then ground in a mill (Wonder Blender, Osaka Chemical, Osaka, Japan). Half a gram of each sample was then digested with 10 mL 60% HNO₃ in a heating digester (DK 20, VELP Scientifica, Milan, Italy). Plant and soil extracts were filtered through disposable 0.2-μm PTFE syringe filters (DISMIC-25HP, Advantec, Tokyo, Japan). We determined the metal concentration in these extracts by means of inductively coupled plasma–optical emission spectroscopy (ICP-OES; Vista-Pro, Varian, Mulgrave, Vic., Australia). This spectroscope is an axially viewed plasma system, and its detection limits of metals are 0.09 (Cd), 0.22 (Cu), 0.48 (Pb), and 0.13 (Zn) μg L⁻¹. Certified reference materials for plants (NIES CRM No. 1 ‘Pepper Bush’, National Institute for Environmental Studies, Tsukuba, Japan) and soil (NDG-7, Fujihira Industry Co., Ltd., Tokyo, Japan) were included in the analysis. The recoveries of metals were within the certified limits.

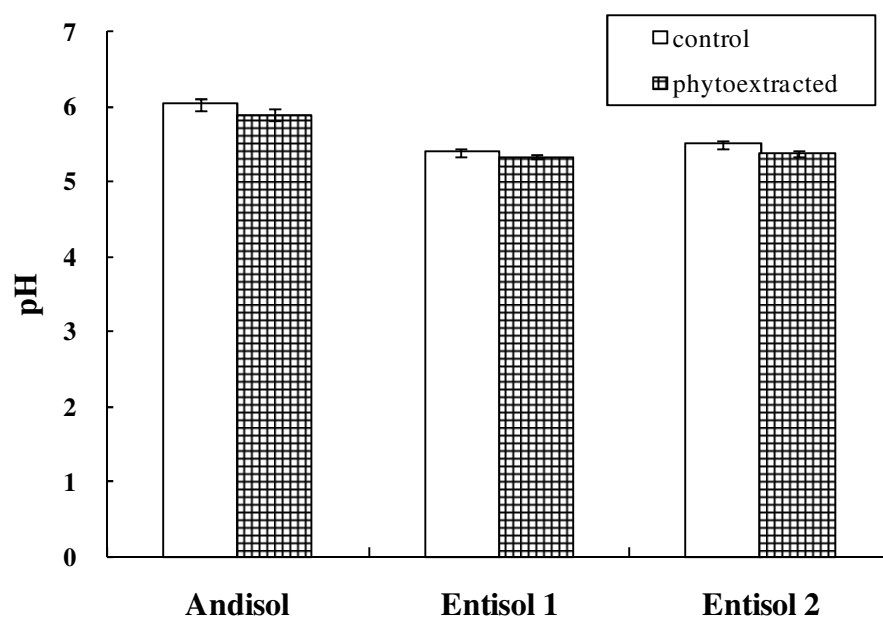


FIGURE S1. pHs of control and phytoextracted soils. Error bars show standard error ($n = 3$)

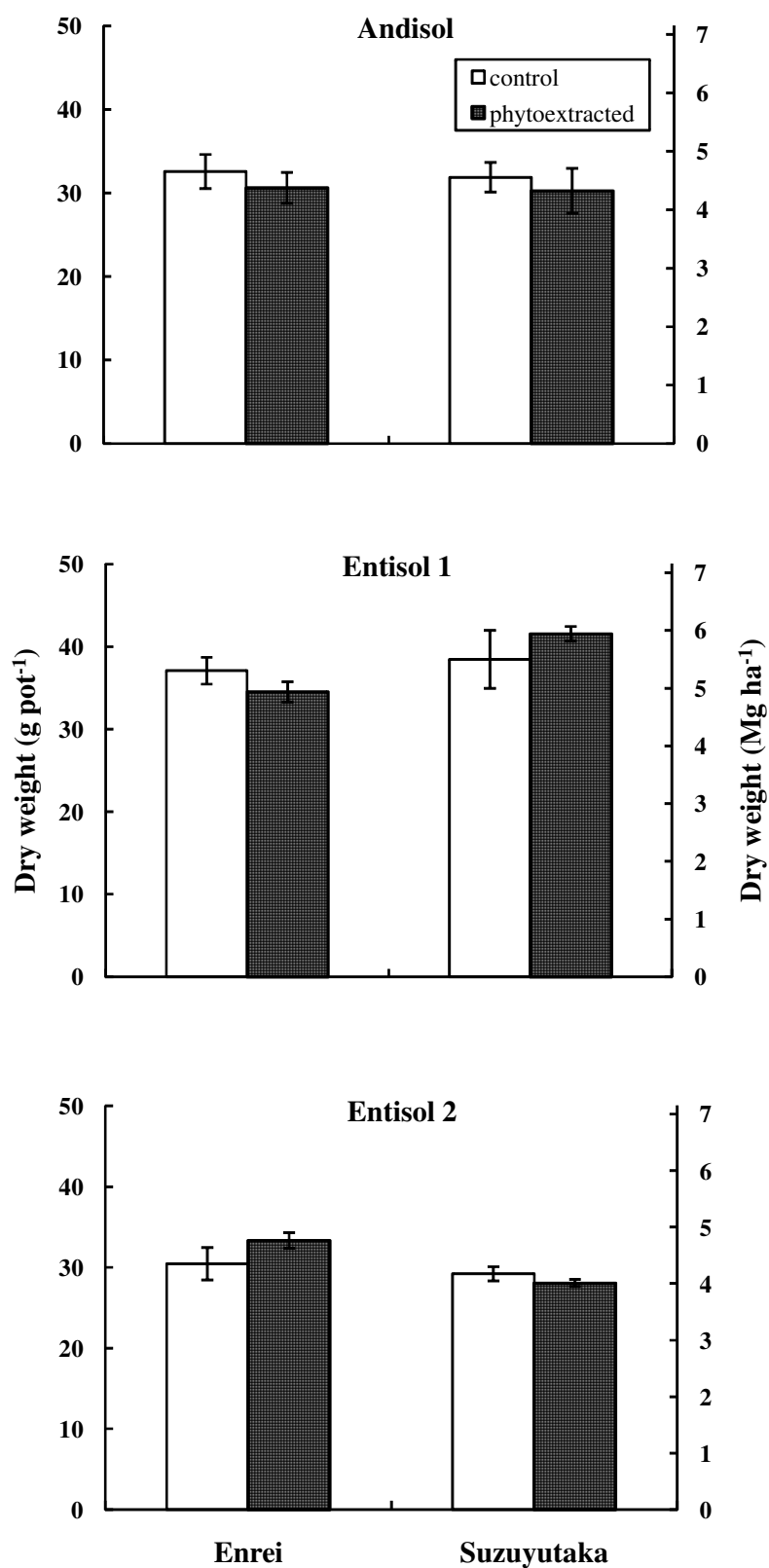


FIGURE S2. Seed dry weights of soybeans grown on control and phytoextracted soils. Error bars show standard error ($n = 3$). Values of the left Y-axes show pot-basis data, and those of the right Y-axes show a per-ha basis data ($143\,000\text{ plants ha}^{-1}$) converted from pot-basis data.

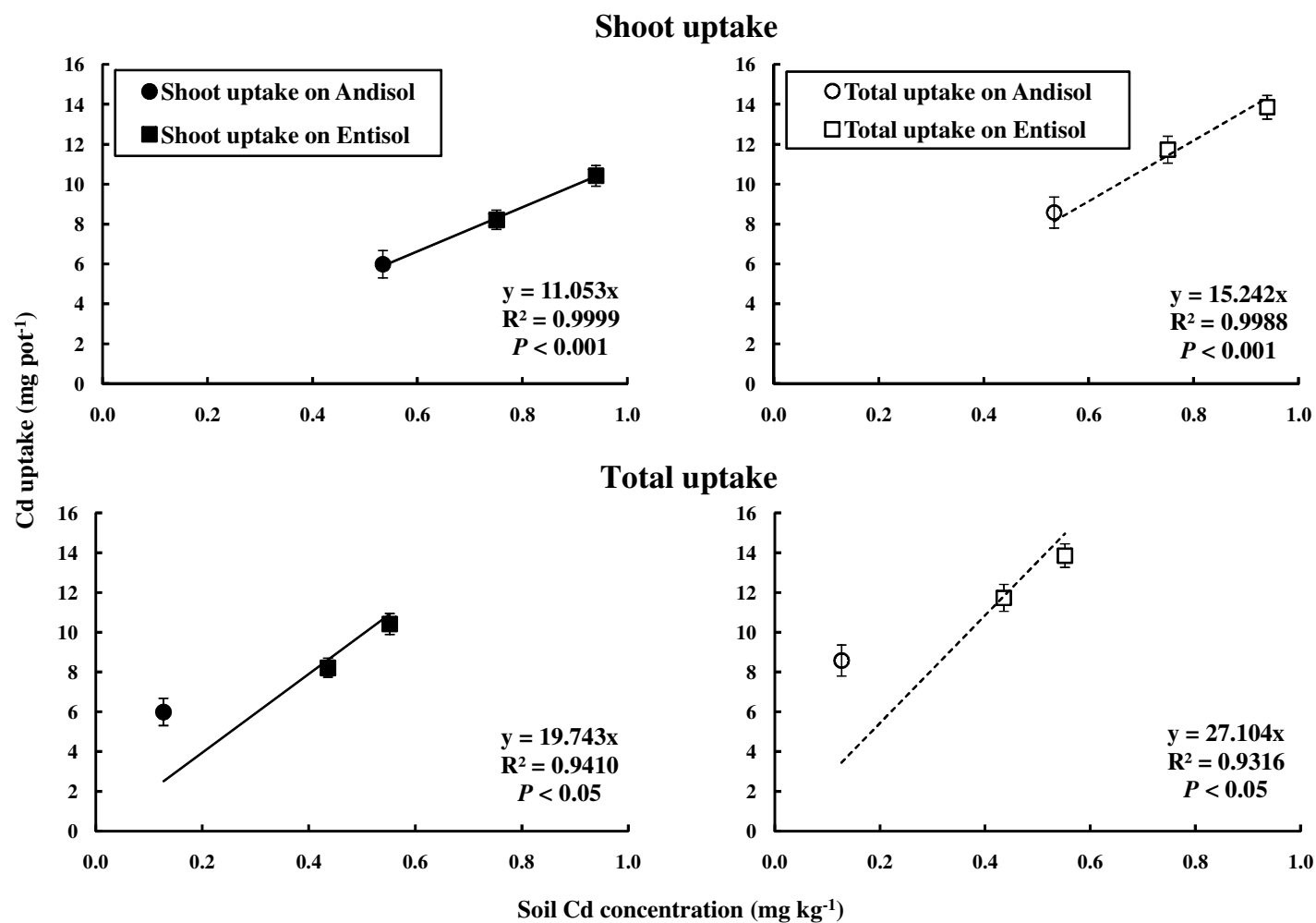


FIGURE S3. Relationships between Cd concentrations in exchangeable (upper) and 0.01 mol L⁻¹ HCl-extractable (lower) fractions of control soils and the Cd uptake in shoots (left) and shoots + roots (total; right) of Milyang 23 rice grown on the three soils. Error bars show standard error ($n = 3$). Exchangeable fraction: extracted with 0.05 mol L⁻¹ Ca(NO₃)₂.

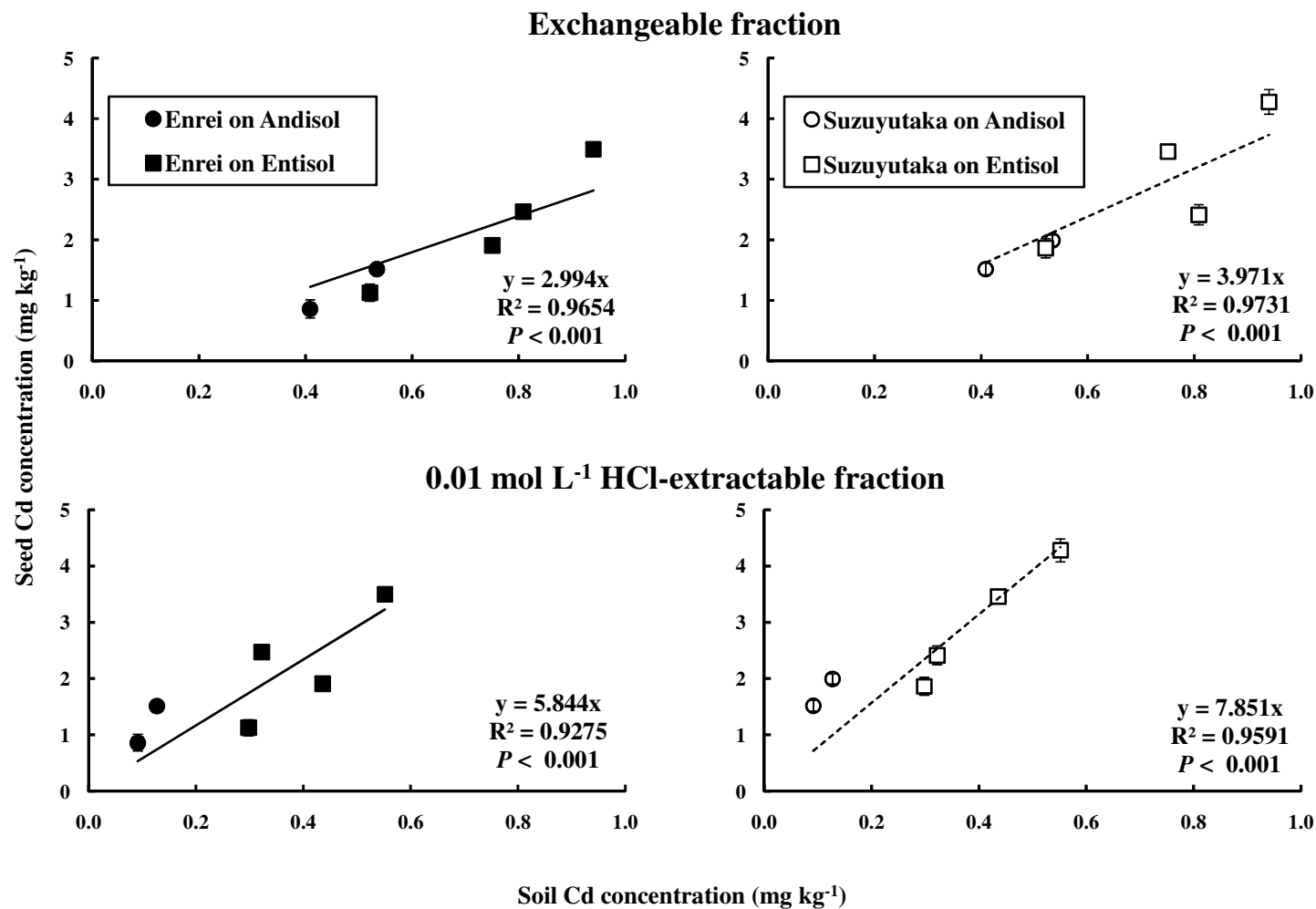


FIGURE S4. Relationships between Cd concentrations in exchangeable (upper) and 0.01 mol L⁻¹ HCl-extractable (lower) fractions of soils and seed Cd concentrations of Enrei (left) and Suzuyutaka (right) soybeans grown on the soils. Error bars show standard error ($n = 3$). Exchangeable fraction: extracted with 0.05 mol L⁻¹ Ca(NO₃)₂.

Table S1. Simple correlation matrix of the correlations between the shoot and total Cd uptakes by Milyang 23 rice and the Cd concentrations of seven fractions and total in control soils.

	Soil Cd							
	Exch [†]	Inorg [‡]	Org [§]	Ox [¶]	Res [#]	Total	0.01 mol L ⁻¹ HCl	0.1 mol L ⁻¹ HCl
Shoot Cd uptake (<i>n</i> = 3)	0.9994*	-0.7432	-0.8545	-0.9247	-0.7749	-0.9167	0.9681	-0.8113
Total Cd uptake (<i>n</i> = 3)	0.9974*	-0.8111	-0.9055	-0.8782	-0.7022	-0.9545	0.9895	-0.8697

**P* < 0.05. [†]Exchangeable fraction, [‡]Inorganically bound fraction, [§]Organically bound fraction, [¶]Oxide occluded fraction, [#]Residual fraction.

Table S2. Simple correlation matrix of the correlations between the seed Cd concentrations of Enrei and Suzuyutaka soybeans and the Cd concentrations of seven fractions and total in control and phytoextracted soils.

	Soil Cd							
	Exch [†]	Inorg [‡]	Org [§]	Ox [¶]	Res [#]	Total	0.01 mol L ⁻¹ HCl	0.1 mol L ⁻¹ HCl
Seed Cd concentration of Enrei (<i>n</i> = 6)	0.9628**	-0.3021	-0.5196	-0.8781*	-0.8174*	-0.4993	0.8195*	-0.3533
Seed Cd concentration of Suzuyutaka (<i>n</i> = 6)	0.8962*	-0.3220	-0.5481	-0.5990	-0.4602	-0.4364	0.9235**	-0.3706

P* < 0.05, *P* < 0.01. [†]Exchangeable fraction, [‡]Inorganically bound fraction, [§]Organically bound fraction, [¶]Oxide occluded fraction, [#]Residual fraction.

Literature Cited

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