



Potential for phytoextraction of copper, lead, and zinc by rice (*Oryza sativa* L.), soybean (*Glycine max* [L.] Merr.), and maize (*Zea mays* L.)

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ABSTRACT

Phytoextraction by hyperaccumulators has been proposed for decreasing toxic-metal concentrations of contaminated soils. However, hyperaccumulators have several shortcomings to introduce these species into Asian Monsoon's agricultural fields contaminated with low to moderate toxic-metals. To evaluate the phytoextraction potential, maize (Gold Dent), soybean (Enrei and Suzuyutaka), and rice (Nipponbare and Milyang 23) were pot-grown under aerobic soil conditions for 60 d on the Andosol or Fluvisol with low to moderate copper (Cu), lead (Pb), and zinc (Zn) contamination. After 2 months cultivation, the Gold Dent maize and Milyang 23 rice shoots took up 20.2–29.5% and 18.5–20.2% of the 0.1 mol L⁻¹ HCl-extractable Cu, 10.0–37.3% and 8.5–34.3% of the DTPA-extractable Cu, and 2.4–6.5% and 2.1–5.9% of the total Cu, respectively, in the two soils. Suzuyutaka soybean shoot took up 23.0–29.4% of the 0.1 mol L⁻¹ HCl-extractable Zn, 35.1–52.6% of the DTPA-extractable Zn, and 3.8–5.3% of the total Zn in the two soils. Therefore, there is a great potential for Cu phytoextraction by the Gold Dent maize and the Milyang 23 rice and for Zn phytoextraction by the Suzuyutaka soybean from paddy soils with low to moderate contamination under aerobic soil conditions.

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1. Introduction

Agricultural soil pollution with toxic-metals is of increasing concern due to food safety issues and potential health risks [1]. Traditional methods of dealing with metal pollution are either the extremely costly process of removal and burial or simply isolation of the contaminated sites [2]. Thus, new methods based on environmentally friendly and low-cost technology are needed.

Phytoextraction by using hyperaccumulator plants has been proposed for decreasing the toxic-metal concentrations of contaminated soils [3,4]. However, hyperaccumulator plants are small and grow slowly, making them difficult to harvest mechanically [3]. The culture of hyperaccumulator species may be hampered by their susceptibility to certain diseases, the development of which is favored by prevailing humid and warm weather conditions [5]. Because the typical weather conditions of Asian Monsoon summer are humid and warm, it may be difficult to introduce these species into Asian Monsoon's agricultural fields. Thus, to maxi-

mize the efficiency of phytoextraction, it is important to select a plant with a strong metal-accumulating ability that is also compatible with mechanized cultivation techniques and local weather conditions. Selection of such plants may yield more immediately practical results than selection-based solely on high tolerance to toxic-metals [6].

Maize (*Zea mays* L.), soybean (*Glycine max* [L.] Merr.), and rice (*Oryza sativa* L.) are the major summer crops grown in paddy fields and/or in upland fields (fields under aerobic soil conditions) that have been converted from paddies in Japan. Systems for the cultivation of maize, soybean, and rice are well established and highly mechanized. In a previous work, we examined phytoextraction of cadmium (Cd) from industrially contaminated soils by rice, soybean, and maize. We found that the Milyang 23 rice cultivar was efficient at removing soil Cd [6].

Anthropogenic activities such as mining, smelting, and refining pollute soils with not just a single metal but multiple metals. Sphalerite is the major ore of zinc [7]. In mining, it is usually accompanied by galena (the primary ore of lead: PbS; [8]) and chalcopyrite (the major ore of copper: CuFeS₂; [9]). Thus, soil pollution by anthropogenic activities often contains several metals such as copper (Cu), lead (Pb), and zinc (Zn). Moreover, metal uptake by plants is subject to the antagonistic, additive, and synergetic effects that toxic-metals exert on each other [10]. For instance, the extent of Zn and Cu removal by plants was less in the presence of both metals than in treatments with a single heavy metal [11]. Walker et

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al. [12] reported that interactions among Cu, Pb, and Zn restricted their uptake by plants. In addition, anthropogenic metals generally transform very slowly to highly stable forms over time [13]. However, several phytoremediation studies have been conducted with artificially spiked soils that were allowed to equilibrate for only 15 d (e.g. [14,15]). The metal forms in artificially spiked soils equilibrating for short period did not correspond to those in industrially or naturally contaminated soils [16]. Therefore, phytoremediation studies should be performed on industrially or naturally contaminated soils with multiple metal contaminants.

In Japan, a single extraction with 0.1 mol L^{-1} HCl has been widely used to determine the soluble Cu and Zn concentrations in soils. Soil Cu concentration extracted with 0.1 mol L^{-1} HCl and DTPA were significantly correlated to the Cu concentration in rice plants, and that with DTPA was significantly correlated to the Cu uptake by rice plants [17]. DTPA-extractable Cu and Zn in soils were significantly correlated with Cu and Zn concentrations in maize [18]. In contrast, evaluation procedures for the toxic-metal fractions of soils usually rely on sequential extraction to provide several soil fractions that are useful indicators of the bioavailability of trace elements in soil [19]. Changes in the relative proportions of several metal pools affected by plant uptake may provide insights into the mechanisms underlying metal uptake by plants [20]. Thus, in phytoremediation trials, it is necessary to assess the potential of plants to remove metals from these various fractions.

The purpose of this study was to select a promising plant cultivar for the phytoextraction of soils contaminated with the low to moderate toxic-metals, especially in Cu, Pb, and Zn, based on a comparison of metal uptake by plant shoots and an examination of which soil metal fractions were changed by plant metal uptakes, using five cultivars of three crop species (maize, soybean, and rice).

2. Materials and methods

2.1. Experimental design

We conducted a pot experiment on paddy soils contaminated by several toxic-metals under aerobic soil conditions. These soils (Andosol and Fluvisol [21]) were collected from the top 15 cm of two Japanese paddy fields. The main sources of toxic-metals were the use of wastewater from an abandoned copper mine for irrigation (Andosol) and the atmospheric deposition of soot from a zinc refinery (Fluvisol, Table 1).

Each soil sample was air-dried, crushed, passed through a 2-mm sieve, thoroughly mixed, separated into 550-ml portions (375 g for Andosol, 550 g for Fluvisol), and placed in 1/10,000 a pots. Liming is a prerequisite for increasing soybean yields on acidic soils [22]. Suitable soil pH values for optimizing soybean yields are 6.0–6.5 [23]. So before soybean cultivation, the pH values of the Fluvisol used for soybean cultivation was raised to 6.0 by the addition of lime (CaCO_3) according to the buffer curve method [24]. Basal fertilizer was supplied at a rate of 0.02 g of N, 0.15 g of P_2O_5 , 0.1 g of K_2O , and 0.5 g of $\text{Ca}(\text{Mg})\text{CO}_3$ per pot for soybeans and 0.1 g of N, 0.1 g of P_2O_5 , 0.1 g of K_2O , and 0.5 g of $\text{Ca}(\text{Mg})\text{CO}_3$ per pot for rice and maize. Nutrients were provided in the following forms: N as $(\text{NH}_4)_2\text{SO}_4$, P_2O_5 as a single superphosphate of lime, and K_2O as K_2SO_4 .

We selected the Milyang 23 (an Indica-Japonica hybrid) rice cultivar and the Suzuyutaka soybean cultivar as plants that would accumulate high amounts of toxic-metals in their shoots, respectively [25,26]. We then selected Nipponbare, Enrei, and Gold Dent as the recommended commercial cultivars of Japonica rice, soybean, and maize, respectively, in Japan. Four seeds of soybean or maize were sown per pot, and seedlings were thinned to two per pot

10 d after sowing. Ten rice seeds were sown per pot, and seedlings were thinned to five per pot 10 d after sowing. The plants were grown from May to July in a greenhouse under natural sunlight at ambient temperatures ($18\text{--}30^\circ\text{C}$). The pot experiment followed a randomized-block design, with four replicates per soil-cultivar treatment. Plants were watered daily to maintain the soil water content near the field capacity. At 60 d after sowing, the shoots of all plants were harvested by cutting the stems approximately 1 cm above the soil. After the shoots had been harvested, the roots were carefully removed from the soil, and then the soil from each pot was separately air-dried and passed through a 2-mm sieve. The shoots and roots were washed with tap water and rinsed with distilled water. We used a “no plant, fertilizer” control for all cultivars grown on all soils, except the soybean cultivars grown on the Fluvisol, for which we used a “no plant, fertilizer with lime” control because the pH value of the Fluvisol used for soybean cultivation was raised by adding lime (CaCO_3). We did not use chelating agents to enhance metal mobility in soil because of their environmental risk of leaching to ground water [27].

2.2. Soil and plant analysis

The soil pH in distilled water (1:2.5 w/v) was measured 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 changed by plant growth, a metal analysis of the soils was conducted by a single-extraction method with 0.01 or 0.1 mol L^{-1} HCl (1:5 w/v, 1 h shaking side-by-side; [28]) and with DTPA (0.005 mol L^{-1} DTPA, 0.1 mol L^{-1} TEA, 0.01 mol L^{-1} CaCl_2 , pH 7.3, 1:2 w/v, 2 h shaking side-by-side; [29]), and a sequential-extraction method [30]. The soil metal fractions determined by this sequential-extraction method were as follows: exchangeable fraction, extracted with 0.05 mol L^{-1} $\text{Ca}(\text{NO}_3)_2$ (1:10 w/v, 24 h shaking); inorganically bound fraction, extracted with 2.5% CH_3COOH (1:10 w/v, 24 h shaking) from the residue of the exchangeable fraction; organically bound fraction, extracted with 2.5% CH_3COOH (1:10 w/v, 24 h shaking) after decomposing organic matter with 6% H_2O_2 from the residue of the inorganically bound fraction; and oxide occluded fraction, extracted with a mixture of 0.1 mol L^{-1} $\text{H}_2\text{C}_2\text{O}_4$ and 0.175 mol L^{-1} $(\text{NH}_4)_2\text{C}_2\text{O}_4$ (1:30 w/v), and ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$, 1:1 w/w) in a boiling water bath for 1 h, with occasional stirring, from the residue of the organically bound fraction; and residual fraction (calculated as the difference between the sum of the four above-mentioned fractions and the total fraction). The total fraction of each soil metal was determined by digestion with 30% H_2O_2 , 60% HClO_4 , 48% HF, and 60% HNO_3 [28].

Harvested plant shoots and roots were dried at 65°C for 48 h and then ground with a mill (Wonder Blender, Osaka Chemical, Osaka, Japan). A total of 0.5 g of each sample was then digested with 10 ml of a mixture of 60% HNO_3 , 60% HClO_4 , and 97% H_2SO_4 (3:1:1 v/v) 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). The metal concentrations in these extracts were determined by means of inductively coupled plasma-optical-emission spectroscopy (Vista-Pro, Varian, Mulgrave, Australia). Certified reference materials for plant (NIES CRM No. 1 “Pepper Bush,” National Institute for Environmental Studies, Japan) and soil (NDG-7, Fujihira Industry Co. Ltd., Japan) were included in the analyses. The recovery of metals was within the certified limits.

Statistical analyses were performed with Excel Tokei software (Esumi, Tokyo, Japan). Treatments were compared by Bonferroni’s multiple-comparison test.

Table 1
Physicochemical properties and total metals of the two soils before sowing

Soil	Source of contamination	Classification ^a	Clay content (g kg ⁻¹)	Texture ^b	Bulk density (g cm ⁻³)	pH (H ₂ O)	Total C (g kg ⁻¹)	Total N	Total Cu (mg kg ⁻¹)	Total Pb	Total Zn
Andosol	Wastewater from abandoned copper mine	Umbic Andosol	164	CL	0.7	6.1	66.3	5.4	94.4	131.4	155.3
Fluvisol	Atmospheric deposition of soot from zinc refinery	Eutric Fluvisol	157	SCL	1.0	5.3	20.2	1.4	19.1	44.0	299.6

^a FAO et al. [21].

^b CL, clay loam; SCL, sandy clay loam.

3. Results and discussion

3.1. Soils before sowing

3.1.1. Physicochemical properties and total metals in soils before sowing

The physicochemical properties, total Cu, total Pb, and total Zn of the Andosol and Fluvisol are given in Table 1. Geometric means (range) for the background metal level of Japanese agricultural soils (mg kg⁻¹) are 19.0 (7.95–44.0) for Cu, 17.2 (9.25–41.8) for Pb, and 59.9 (16.0–105) for Zn [31]. In the studied soils, the concentrations of Cu (94.4) and Pb (131.4) in the Andosol and Zn in both soils (155.3 and 299.6 for the Andosol and Fluvisol, respectively) are regarded as low to moderate contamination levels in Japan. The total Cu, and total Pb in the Andosol were higher than those in the Fluvisol, whereas the total Zn in the Andosol were lower than those in the Fluvisol. The metal contamination source of the Andosol was wastewater from an abandoned copper mine. The main mineral ores in this copper mine were chalcopyrite and pyromorphite (Pb₅[PO₄]₃Cl). In contrast, the metal contamination source of the Fluvisol was atmospheric deposition of soot from a zinc refinery. In this refinery, Zn is made mainly from sphalerite. Thus, the total Cu and Pb concentrations in the Andosol would be higher than those in the Fluvisol.

3.1.2. Metals fractionated by sequential-extraction method in soils before sowing

The three metal concentrations in the eight fractions of the two soils before sowing were shown in Table 2. The dominant fractions among the five fractions assessed by sequential extraction were as follows: for Cu, the organically bound fraction in the Andosol and the organically bound and oxide occluded fractions in the Fluvisol; for Pb, the oxide occluded fraction in both soils; and for Zn, the oxide occluded and residual fractions in both soils. The ability of the organic soil constituents to bind Cu is well-recognized [32]. The higher selective adsorptions of Pb by oxide than by humus were reported [33,34]. In contrast, Zn appeared to occur in more readily soluble forms than Cu and Pb [32]. Therefore, the organically bound Cu and the oxide occluded Pb would thus be dominant fractions and the proportions of more mobile (exchangeable + inorganically bound) fraction to the total (12.1 and 14.1% for Andosol and Fluvisol, respectively) would be higher than those of Cu (1.2 and 3.6%) and Pb (1.2 and 1.1%) in both soils.

3.1.3. Metals fractionated by single extraction methods in soils before sowing

Among the three single extractions, the Cu, Pb, and Zn concentrations extracted by the 0.01 mol L⁻¹ HCl were the lowest in both soils (Table 2). In the Fluvisol, the Cu and Pb concentrations extracted by the 0.1 mol L⁻¹ HCl were higher than those by the DTPA. The Zn concentrations extracted by the 0.1 mol L⁻¹ HCl were higher than those by the DTPA in both soils. The concentrations

of Zn [35], and Cu, Pb, and Zn [36] in several soils extracted by the 0.1 mol L⁻¹ HCl were higher than those by the DTPA. The 0.1 mol L⁻¹ HCl solution dissolved various oxides of metals adsorbed on to soil colloids [35,37]. The oxide occluded Cu and Pb in the Fluvisol and Zn in both soils were the dominant fractions. The high ability of HCl solution to solubilize oxide occluded metals in the soils would thus explain why the Cu and Pb in the Fluvisol and the Zn in both soils extracted by the 0.1 mol L⁻¹ HCl were higher than those by the DTPA. In contrast, the Cu and Pb concentrations in the Andosol extracted by the DTPA were higher than those by the 0.1 mol L⁻¹ HCl. The Cu concentrations in the humus horizon (0–20 cm of soil depth) in an Andosol extracted by the DTPA was higher than that by the 0.1 mol L⁻¹ HCl [35]. The DTPA extracted effectively metal ions combined with soil organic materials by chelate formation [35]. The organically bound Cu in the Andosol was the dominant fractions. Moreover, the DTPA was able to dissolve some Pb in soils which was not solubilized by protons [38]. The higher ability of DTPA to solubilize organically bound Cu and some Pb in soils than protons would thus explain why the Cu and Pb in the Andosol extracted by the DTPA were higher than those by the 0.1 mol L⁻¹ HCl.

3.2. Plant shoots and roots

3.2.1. Dry weight of plant shoots and roots

The dry weights of shoots and roots of the five cultivars grown on the two soils at harvest are shown in Table 3. For both soils, the order of the shoot dry weights of the five cultivars was as follows: Gold Dent maize > Enrei soybean > Suzuyutaka soybean > Milyang 23 rice > Nipponbare rice. The root dry weight of Gold Dent maize was significantly greater than those of other cultivars in both soils ($p < 0.01$).

3.2.2. Metal concentrations in plant shoots and roots

The three metal concentrations in shoots and roots of the five cultivars at harvest are shown in Table 3. The shoot Cu concentration of Milyang 23 rice was the highest among five cultivars in both soils, whereas those of two soybean cultivars were the lowest in both soils. The shoot Pb concentrations of Suzuyutaka soybean and Nipponbare rice were higher than those of the other cultivars grown in the Andosol, whereas in the Fluvisol there was no significant difference among Pb concentrations in the soybean and rice cultivars. In contrast, no Pb was detected in the shoot of Gold Dent maize in both soils. The shoot Zn concentration of Milyang 23 rice was the highest among five cultivars in both soils, whereas that of Gold Dent maize was the lowest in both soils. The root Cu concentration of Milyang 23 rice was the highest among five cultivars in both soils. There were no significant differences in the root Pb concentrations among five cultivars grown in the Andosol, whereas the root Pb concentration of Enrei soybean was the highest in the Fluvisol. The root Zn concentrations of two rice cultivars were higher than those of the other cultivars in both soils, whereas that of Gold Dent maize was the lowest among five cultivars in both soils.

Table 2
Metal concentrations in the eight fractions of the two soils before sowing

Metal	Soil	Sequential extraction					Single extraction		
		Exchangeable	Inorganically bound	Organically bound	Oxide occluded (mg kg ⁻¹)	Residual (mg kg ⁻¹)	0.01 mol L ⁻¹ HCl	0.1 mol L ⁻¹ HCl	DTPA
Cu	Andosol	0.2 (0.2) ^a	0.9 (1.0)	65.2 (68.9)	13.0 (13.7)	15.3 (16.2)	0.2 (0.2)	7.8 (8.2)	23.0 (24.3)
	Fluvisol	0.1 (0.8)	0.5 (2.8)	7.7 (40.4)	8.1 (42.3)	2.6 (13.7)	0.1 (0.7)	6.1 (32.0)	3.3 (17.3)
Pb	Andosol	n.d. (0)	1.6 (1.2)	1.5 (1.2)	122.9 (93.5)	5.4 (4.1)	n.d. (0)	5.0 (3.8)	25.8 (19.6)
	Fluvisol	n.d. (0)	0.5 (1.1)	0.6 (1.4)	42.7 (96.6)	0.4 (0.9)	n.d. (0)	8.7 (19.7)	7.7 (17.4)
Zn	Andosol	3.7 (2.4)	15.0 (9.6)	26.7 (17.0)	51.1 (32.7)	60.0 (38.4)	1.2 (0.8)	28.3 (18.1)	15.8 (10.1)
	Fluvisol	23.2 (7.7)	19.4 (6.4)	21.7 (7.2)	159.8 (53.0)	77.6 (25.7)	13.8 (4.6)	50.3 (16.7)	33.0 (10.9)

^a Number in parentheses is the proportion of each chemical fraction to the total (%).

3.3. Metal uptakes by plants and soil metals after harvest

3.3.1. Maize

The three metal uptakes by shoots and roots of the five cultivars at harvest are shown in Table 3. The greatest shoot Cu uptake and the lowest shoot Pb and Zn uptakes were observed in Gold Dent maize in both soils. The Cu and Pb uptakes by Gold Dent maize root were the highest among five cultivars in the Andosol and in both soils, respectively. Fifty-six percent and 82% of total (shoot + root) Cu uptakes by the Gold Dent maize grown on the Andosol and Fluvisol, respectively, were able to be removed by harvesting only the aboveground parts. The Gold Dent maize shoot took up 29.5% and 20.2% of the 0.1 mol L⁻¹ HCl-extractable Cu, 10.0% and 37.3% of the DTPA-extractable Cu, and 2.4% and 6.5% of the total Cu in the Andosol and Fluvisol, respectively (Tables 1–3). The shoot Cu uptake by the Gold Dent maize was higher than that by hyperaccumulator *Alyssum murale* (0.05% of the total soil Cu [39]). These results suggest that the potential of Gold Dent maize for phytoex-

traction is higher for Cu but lower for Pb and Zn than that of the other cultivars that we tested here.

The Cu, Pb, and Zn concentrations in the eight fractions (exchangeable, inorganically bound, organically bound, oxide occluded, residual, 0.01 mol L⁻¹ HCl-extractable, 0.1 mol L⁻¹ HCl-extractable, and DTPA-extractable) and total in the Andosol and Fluvisol after harvest are shown in Tables 4–6. For Cu and Pb concentrations in the two soils of the Gold Dent maize treatment, the most marked post-harvest decreases compared with the control were found in the organically bound fraction for Cu ($p < 0.01$) in both soils and in the oxide occluded fraction for Pb ($p < 0.01$) in the Andosol (and in the Fluvisol, but not significantly so; Tables 4 and 5). The mucilage and high- and low-molecular-weight soluble exudates contained in maize root had a strong capacity to form complexes with Cu and Pb [40–42]. Soluble root exudates promoted the solubility of metals, possibly through the formation of soluble metal complexes [43]. Therefore, the enhancement of metal mobility by maize root exudates would cause the greatest

Table 3
Dry weights of, metal concentrations in, and metal uptakes by plant shoots and roots grown on the two soils

Cultivar	Dry weight (g pot ⁻¹)	Metal concentration (mg kg ⁻¹)			Metal uptake (μg pot ⁻¹)		
		Cu	Pb	Zn	Cu	Pb	Zn
Andosol							
Shoot							
Gold Dent maize	53.5 ± 2.5 a ^a	16.5 ± 0.7 bc	n.d.	14.3 ± 1.3 c	886.8 ± 74.0 a	n.d.	762.2 ± 68.1 d
Enrei soybean	40.6 ± 0.9 b	9.8 ± 0.4 d	3.7 ± 0.5 bc	64.6 ± 1.5 b	398.7 ± 19.3 b	150.5 ± 18.2 b	2624.8 ± 87.9 b
Suzuyutaka soybean	38.7 ± 0.4 b	11.6 ± 0.6 cd	6.3 ± 0.3 a	82.7 ± 2.9 a	448.1 ± 21.6 b	243.8 ± 13.4 a	3198.2 ± 104.8 a
Nipponbare rice	15.3 ± 2.5 c	23.0 ± 2.7 bc	6.0 ± 0.8 ab	81.5 ± 6.7 ab	331.9 ± 23.8 b	87.9 ± 12.7 c	1196.8 ± 112.1 d
Milyang 23 rice	22.3 ± 2.0 c	34.0 ± 1.2 a	2.6 ± 0.3 c	89.8 ± 3.9 a	751.9 ± 48.3 a	58.2 ± 9.3 c	1997.1 ± 196.0 c
Root							
Gold Dent maize	19.2 ± 0.3 a	36.7 ± 1.4 c	69.2 ± 4.3 a	34.0 ± 1.6 d	704.1 ± 27.7 a	1325.8 ± 77.6 a	651.9 ± 30.5 c
Enrei soybean	6.5 ± 0.1 b	39.3 ± 2.2 c	65.7 ± 3.1 a	58.6 ± 5.1 c	255.5 ± 12.2 c	427.0 ± 16.5 bc	379.9 ± 27.8 d
Suzuyutaka soybean	6.0 ± 0.1 c	32.9 ± 0.7 c	62.3 ± 3.5 a	52.0 ± 2.8 c	196.9 ± 6.1 c	373.2 ± 26.5 bc	311.7 ± 19.9 d
Nipponbare rice	4.6 ± 0.1 d	83.9 ± 1.8 b	67.8 ± 0.8 a	232.3 ± 5.0 a	388.5 ± 7.4 b	314.2 ± 1.9 c	1077.1 ± 32.3 a
Milyang 23 rice	7.0 ± 0.1 b	96.9 ± 3.4 a	71.4 ± 2.7 a	122.6 ± 3.6 b	682.2 ± 17.3 a	502.8 ± 13.9 b	862.9 ± 17.3 b
Fluvisol							
Shoot							
Gold Dent maize	49.9 ± 2.0 a	13.7 ± 1.4 c	n.d.	42.4 ± 2.1 c	677.8 ± 41.1 a	n.d.	2113.7 ± 113.1 d
Enrei soybean	32.2 ± 0.5 b	8.4 ± 0.5 d	2.8 ± 0.2 a	195.8 ± 5.7 b	270.4 ± 14.8 c	90.3 ± 5.7 a	6295.8 ± 91.6 a
Suzuyutaka soybean	29.5 ± 0.5 b	8.4 ± 0.1 d	2.7 ± 0.1 a	216.4 ± 8.3 b	247.0 ± 5.9 c	79.4 ± 3.8 a	6374.2 ± 163.5 a
Nipponbare rice	17.8 ± 1.1 c	28.6 ± 0.7 b	2.5 ± 0.2 a	233.8 ± 14.6 ab	506.6 ± 20.3 b	45.2 ± 5.2 b	4106.6 ± 66.0 c
Milyang 23 rice	18.6 ± 1.7 c	34.0 ± 1.8 a	2.1 ± 0.2 a	264.1 ± 11.5 a	622.0 ± 27.7 ab	39.5 ± 5.2 b	4844.1 ± 249.4 b
Root							
Gold Dent maize	14.3 ± 0.3 a	10.1 ± 0.7 c	31.2 ± 1.4 b	80.1 ± 5.3 d	144.5 ± 8.0 c	445.8 ± 20.5 a	1143.3 ± 67.8 b
Enrei soybean	5.3 ± 0.3 c	14.9 ± 1.4 c	39.2 ± 2.4 a	200.1 ± 7.7 c	77.9 ± 6.5 d	206.4 ± 16.8 b	1050.1 ± 35.3 bc
Suzuyutaka soybean	4.4 ± 0.2 d	12.8 ± 0.7 c	21.8 ± 1.2 c	193.0 ± 9.7 c	56.0 ± 4.2 d	94.5 ± 1.2 c	845.1 ± 60.2 c
Nipponbare rice	6.3 ± 0.2 b	39.8 ± 1.3 b	21.0 ± 1.1 c	550.2 ± 14.9 b	250.2 ± 14.5 b	131.3 ± 5.1 c	3444.1 ± 13.9 a
Milyang 23 rice	6.1 ± 0.2 c	52.7 ± 1.3 a	23.9 ± 1.4 bc	602.7 ± 4.2 a	319.7 ± 2.7 a	144.8 ± 7.3 c	3659.9 ± 75.3 a

^a Mean ± S.E., means in the same column for each element followed by the same letter are not significantly different at $p < 0.05$ based on Bonferroni's multiple-comparison test.

Table 4
Copper concentrations in the eight fractions and the total of the two soils after harvest

Soil	Treatment	Sequential extraction					Single extraction			Total (HF–HClO ₄ –HNO ₃)
		Exchangeable	Inorganically bound	Organically bound	Oxide occluded	Residual (mg kg ⁻¹)	0.01 mol L ⁻¹ HCl	0.1 mol L ⁻¹ HCl	DTPA	
Andosol	No plant, no fertilizer	0.13 ± 0.01 ab ^a	0.97 ± 0.03 a	65.1 ± 0.2 b	12.8 ± 0.1 ab	15.9 ± 0.3 a	0.13 ± 0.01 bc	7.7 ± 0.2 a	22.6 ± 0.7 a	94.9 ± 0.3 a
	No plant, fertilizer	0.14 ± 0.01 ab	0.90 ± 0.04 a	67.2 ± 0.2 a	12.1 ± 0.2 ab	15.3 ± 0.4 a	0.18 ± 0.01 a	7.1 ± 0.2 b	23.0 ± 0.9 a	95.6 ± 0.1 a
	Gold Dent maize	0.08 ± 0.02 bc	0.86 ± 0.02 a	63.0 ± 0.2 c	12.1 ± 0.1 b	15.5 ± 0.2 a	0.15 ± 0.01 abc	5.3 ± 0.3 d	19.0 ± 0.2 b	91.5 ± 0.3 c
	Enrei soybean	0.17 ± 0.02 a	1.00 ± 0.05 a	65.6 ± 0.1 b	12.0 ± 0.1 b	15.1 ± 0.2 a	0.18 ± 0.01 a	6.9 ± 0.2 b	21.3 ± 0.2 ab	93.9 ± 0.2 b
	Suzuyutaka soybean	0.18 ± 0.01 a	1.04 ± 0.06 a	65.7 ± 0.1 b	12.1 ± 0.1 ab	14.8 ± 0.2 a	0.20 ± 0.01 a	6.5 ± 0.1 c	21.2 ± 0.4 ab	93.9 ± 0.2 b
	Nipponbare rice	0.04 ± 0.01 c	1.03 ± 0.03 a	65.5 ± 0.3 b	12.1 ± 0.2 ab	15.1 ± 0.5 a	0.10 ± 0.01 c	6.3 ± 0.3 c	21.2 ± 0.1 ab	93.8 ± 0.2 b
	Milyang 23 rice	0.08 ± 0.02 bc	0.98 ± 0.06 a	63.8 ± 0.2 c	12.0 ± 0.2 b	15.0 ± 0.5 a	0.19 ± 0.01 a	5.6 ± 0.5 d	19.3 ± 0.5 b	91.9 ± 0.1 c
Fluvisol	No plant, no fertilizer	0.10 ± 0.01 ab	0.57 ± 0.02 a	8.0 ± 0.1 a	8.4 ± 0.1 a	3.1 ± 0.4 a	0.21 ± 0.01 a	6.3 ± 0.1 a	3.3 ± 0.0 a	20.2 ± 0.2 a
	No plant, fertilizer with lime	0.07 ± 0.00 c	0.55 ± 0.04 a	7.8 ± 0.1 a	8.0 ± 0.2 a	2.7 ± 0.5 a	0.14 ± 0.01 ab	6.0 ± 0.1 ab	3.3 ± 0.2 a	19.2 ± 0.3 abc
	No plant, fertilizer	0.11 ± 0.00 a	0.59 ± 0.00 a	7.9 ± 0.1 a	8.2 ± 0.1 a	2.8 ± 0.3 a	0.20 ± 0.02 a	6.5 ± 0.1 a	3.1 ± 0.3 a	19.6 ± 0.2 ab
	Gold Dent maize	0.07 ± 0.01 c	0.54 ± 0.03 a	6.6 ± 0.1 c	8.0 ± 0.2 a	2.9 ± 0.3 a	0.08 ± 0.01 b	5.3 ± 0.1 c	2.1 ± 0.0 b	18.2 ± 0.1 cd
	Enrei soybean	0.06 ± 0.00 c	0.55 ± 0.02 a	7.3 ± 0.1 bc	8.0 ± 0.3 a	3.0 ± 0.2 a	0.10 ± 0.01 b	5.4 ± 0.2 bc	3.0 ± 0.2 ab	18.5 ± 0.2 bcd
	Suzuyutaka soybean	0.08 ± 0.01 bc	0.56 ± 0.02 a	7.3 ± 0.1 bc	8.1 ± 0.3 a	2.6 ± 0.4 a	0.11 ± 0.01 b	5.5 ± 0.2 bc	2.9 ± 0.2 ab	18.7 ± 0.1 bcd
	Nipponbare rice	0.02 ± 0.00 d	0.39 ± 0.03 b	6.8 ± 0.1 c	8.1 ± 0.2 a	2.9 ± 0.4 a	0.10 ± 0.02 b	5.3 ± 0.1 c	2.1 ± 0.1 b	18.2 ± 0.3 cd
	Milyang 23 rice	0.03 ± 0.00 d	0.47 ± 0.02 ab	6.6 ± 0.1 c	8.1 ± 0.3 a	2.8 ± 0.3 a	0.09 ± 0.02 b	5.3 ± 0.1 c	2.1 ± 0.2 b	18.0 ± 0.1 d

^a Mean ± S.E., means in the same column for each soil followed by the same letter are not significantly different at $p < 0.05$ based on Bonferroni's multiple-comparison test.

Table 5
Lead concentrations in the eight fractions and the total of the two soils after harvest

Soil	Treatment	Sequential extraction					Single extraction			Total (HF–HClO ₄ –HNO ₃)
		Exchangeable	Inorganically bound	Organically bound	Oxide occluded	Residual (mg kg ⁻¹)	0.01 mol L ⁻¹ HCl	0.1 mol L ⁻¹ HCl	DTPA	
Andosol	No plant, no fertilizer	n.d.	1.5 ± 0.0 a ^a	1.5 ± 0.1 a	122.0 ± 0.4 a	5.0 ± 0.4 a	n.d.	5.0 ± 0.0 a	26.0 ± 0.8 ab	130.0 ± 0.2 a
	No plant, fertilizer	n.d.	1.5 ± 0.0 a	1.5 ± 0.1 a	121.8 ± 0.4 a	5.6 ± 0.3 a	n.d.	5.0 ± 0.1 a	26.8 ± 0.3 a	130.4 ± 0.4 a
	Gold Dent maize	n.d.	1.4 ± 0.0 a	1.5 ± 0.0 a	118.6 ± 0.5 b	5.7 ± 0.5 a	n.d.	3.6 ± 0.1 c	24.2 ± 0.1 b	127.2 ± 0.4 b
	Enrei soybean	n.d.	1.5 ± 0.0 a	1.5 ± 0.0 a	120.8 ± 0.3 a	5.3 ± 0.4 a	n.d.	3.8 ± 0.1 bc	25.5 ± 0.4 ab	129.0 ± 0.4 a
	Suzuyutaka soybean	n.d.	1.5 ± 0.1 a	1.4 ± 0.0 a	120.2 ± 0.5 ab	5.7 ± 0.5 a	n.d.	3.6 ± 0.1 c	25.5 ± 0.6 ab	128.9 ± 0.3 ab
	Nipponbare rice	n.d.	1.5 ± 0.0 a	0.8 ± 0.1 b	121.4 ± 0.3 a	5.7 ± 0.4 a	n.d.	4.1 ± 0.1 b	25.4 ± 0.3 ab	129.5 ± 0.4 a
	Milyang 23 rice	n.d.	1.5 ± 0.0 a	0.7 ± 0.0 b	121.2 ± 0.4 a	5.7 ± 0.7 a	n.d.	4.1 ± 0.1 b	25.4 ± 0.3 ab	129.1 ± 0.3 a
Fluvisol	No plant, no fertilizer	n.d.	0.57 ± 0.02 a	0.60 ± 0.01 a	42.0 ± 0.5 a	0.9 ± 0.2 a	n.d.	8.8 ± 0.0 a	6.9 ± 0.3 a	44.1 ± 0.4 a
	No plant, fertilizer with lime	n.d.	0.47 ± 0.02 b	0.57 ± 0.01 a	41.9 ± 0.2 a	1.3 ± 0.2 a	n.d.	8.4 ± 0.1 ab	7.8 ± 0.4 a	44.3 ± 0.2 a
	No plant, fertilizer	n.d.	0.52 ± 0.02 ab	0.57 ± 0.01 a	42.0 ± 0.2 a	1.1 ± 0.5 a	n.d.	8.5 ± 0.1 ab	7.3 ± 0.2 a	44.2 ± 0.3 a
	Gold Dent maize	n.d.	0.55 ± 0.02 ab	0.56 ± 0.01 a	41.3 ± 0.8 a	1.1 ± 0.6 a	n.d.	8.3 ± 0.1 ab	7.3 ± 0.2 a	43.4 ± 0.1 a
	Enrei soybean	n.d.	0.55 ± 0.02 ab	0.54 ± 0.01 a	41.5 ± 0.5 a	1.3 ± 0.3 a	n.d.	8.0 ± 0.1 b	7.7 ± 0.3 a	43.9 ± 0.1 a
	Suzuyutaka soybean	n.d.	0.54 ± 0.00 ab	0.57 ± 0.02 a	41.7 ± 0.3 a	1.2 ± 0.2 a	n.d.	8.1 ± 0.1 b	7.7 ± 0.1 a	44.0 ± 0.3 a
	Nipponbare rice	n.d.	0.60 ± 0.01 a	0.55 ± 0.02 a	41.8 ± 0.3 a	1.0 ± 0.4 a	n.d.	8.3 ± 0.1 b	7.8 ± 0.1 a	44.0 ± 0.4 a
	Milyang 23 rice	n.d.	0.54 ± 0.02 ab	0.52 ± 0.01 a	41.8 ± 0.3 a	1.2 ± 0.5 a	n.d.	8.3 ± 0.1 abc	7.4 ± 0.2 a	44.0 ± 0.3 a

^a Mean ± S.E., means in the same column for each soil followed by the same letter are not significantly different at $p < 0.05$ based on Bonferroni's multiple-comparison test.

Table 6
pH and zinc concentrations in the eight fractions and the total of the two soils after harvest

Soil	Treatment	pH (H ₂ O)	Sequential extraction			Oxide occluded	Residual (mg kg ⁻¹)	Single extraction			Total (HF-HClO ₄ -HNO ₃)
			Exchangeable	Inorganically bound	Organically bound			0.01 mol L ⁻¹ HCl	0.1 mol L ⁻¹ HCl	DTPA	
Andosol	No plant, no fertilizer	5.5 ± 0.0 b ^a	3.4 ± 0.1 a	15.1 ± 0.2 ab	26.2 ± 0.6 a	51.4 ± 0.4 a	61.3 ± 1.1 a	0.8 ± 0.1 b	28.2 ± 0.3 a	14.0 ± 0.5 a	157.4 ± 0.9 a
	No plant, fertilizer	5.1 ± 0.1 c	3.7 ± 0.2 a	15.6 ± 0.2 a	25.7 ± 0.3 a	49.7 ± 0.5 a	61.2 ± 0.7 a	1.4 ± 0.2 a	28.0 ± 0.4 a	14.1 ± 0.2 a	156.0 ± 0.4 a
	Gold Dent maize	6.0 ± 0.0 a	1.4 ± 0.1 d	14.2 ± 0.2 bc	25.7 ± 0.2 a	49.7 ± 0.5 a	61.5 ± 0.7 a	0.2 ± 0.0 cd	24.6 ± 0.3 b	12.7 ± 0.1 ab	152.5 ± 0.3 b
	Enrei soybean	5.6 ± 0.1 b	2.4 ± 0.1 b	12.9 ± 0.3 e	22.1 ± 0.5 bc	49.6 ± 0.4 a	61.4 ± 1.3 a	0.7 ± 0.0 b	22.7 ± 0.2 cd	12.4 ± 0.5 abc	148.3 ± 0.9 c
	Suzuyutaka soybean	5.5 ± 0.0 b	2.2 ± 0.1 bc	12.5 ± 0.2 e	21.4 ± 0.2 c	49.2 ± 0.7 a	61.8 ± 0.5 a	0.7 ± 0.0 bc	22.0 ± 0.3 d	11.4 ± 0.2 bcd	147.0 ± 0.6 c
	Nipponbare rice	5.9 ± 0.0 a	1.8 ± 0.1 cd	13.8 ± 0.1 cd	23.6 ± 0.2 b	49.3 ± 0.8 a	61.7 ± 1.4 a	0.4 ± 0.1 bcd	24.0 ± 0.3 bc	10.6 ± 0.4 cd	150.2 ± 0.8 bc
Milyang 23 rice		6.0 ± 0.0 a	1.5 ± 0.1 d	13.2 ± 0.2 de	22.9 ± 0.4 bc	49.3 ± 0.7 a	61.7 ± 0.9 a	0.2 ± 0.0 d	23.1 ± 0.3 bcd	9.7 ± 0.6 d	148.7 ± 0.5 c
Fluvisol	No plant, no fertilizer	4.7 ± 0.1 d	23.7 ± 0.3 a	19.2 ± 0.5 bc	21.0 ± 0.3 b	159.6 ± 1.8 a	77.7 ± 1.8 ab	19.5 ± 0.6 a	50.3 ± 0.5 a	25.4 ± 0.6 ab	301.3 ± 0.3 a
	No plant, fertilizer with lime	5.1 ± 0.0 c	14.5 ± 0.2 b	25.7 ± 0.3 a	24.6 ± 0.7 a	161.7 ± 1.5 a	72.1 ± 2.1 b	12.5 ± 0.4 b	50.3 ± 0.4 a	28.9 ± 1.2 a	298.7 ± 0.9 a
	No plant, fertilizer	4.4 ± 0.1 e	23.8 ± 0.4 a	17.9 ± 0.3 c	20.9 ± 0.2 b	154.3 ± 1.1 a	83.1 ± 1.2 a	18.2 ± 0.4 a	48.8 ± 0.5 a	26.9 ± 0.7 a	300.1 ± 0.7 a
	Gold Dent maize	5.7 ± 0.0 a	5.8 ± 0.4 e	26.0 ± 0.3 a	24.9 ± 0.4 a	154.6 ± 0.8 a	82.7 ± 1.3 a	3.8 ± 0.1 d	43.3 ± 0.4 b	21.4 ± 0.6 c	294.0 ± 0.4 b
	Enrei soybean	5.4 ± 0.0 b	10.5 ± 0.4 c	20.3 ± 0.4 b	21.5 ± 0.4 b	160.5 ± 2.2 a	72.5 ± 2.7 b	9.2 ± 0.3 c	38.2 ± 0.8 c	22.1 ± 0.6 b	285.2 ± 0.6 c
	Suzuyutaka soybean	5.3 ± 0.1 bc	10.3 ± 0.3 c	20.0 ± 0.5 b	21.4 ± 0.4 b	160.7 ± 1.5 a	72.8 ± 1.5 b	8.9 ± 0.2 c	38.2 ± 0.4 c	22.3 ± 0.7 b	285.3 ± 0.6 c
Nipponbare rice	5.2 ± 0.1 bc	10.5 ± 0.3 c	18.0 ± 0.4 c	20.9 ± 0.7 b	154.3 ± 1.8 a	82.5 ± 2.5 a	8.0 ± 0.2 c	35.7 ± 0.3 d	14.2 ± 0.5 d	286.1 ± 0.4 c	
Milyang 23 rice		5.2 ± 0.1 bc	8.2 ± 0.6 d	17.3 ± 0.4 c	20.4 ± 0.4 b	153.9 ± 1.5 a	84.7 ± 1.2 a	5.2 ± 0.6 d	33.5 ± 0.4 d	13.4 ± 1.1 d	284.5 ± 0.3 c

^a Mean ± S.E., means in the same column for each soil followed by the same letter are not significantly different at $p < 0.05$ based on Bonferroni's multiple-comparison test.

decrease in the more resistant fractions (i.e., the organically bound fraction for Cu and the oxide occluded fraction for Pb) in both soils.

For Zn concentration in the two soils of the Gold Dent maize treatments, the most marked post-harvest decrease compared with the control was found in the exchangeable fraction in the Fluvisol (Table 6). In contrast, significant post-harvest increases of Zn were found in the inorganically bound and organically bound fractions in the Fluvisol. The decreased soil Zn concentration in the exchangeable fraction in the Fluvisol was higher than those found in the 0.1 mol L⁻¹ HCl-extractable, DTPA-extractable fractions and total. Several studies have reported that shifts in Zn from the more bioavailable to the more resistant soil fractions occur in response to the pH increase that results from liming [44–46] and the high-pH of maize root exudates [47]. Ammonium-based fertilizers, such as the (NH₄)₂SO₄ used here, may yield nitrates by nitrification [48]. In NO₃⁻-fed maize, the external pH increased over time [49]. Among the five cultivars, the greatest soil pH increase compared with the control was found in Gold Dent maize, especially in the Fluvisol (Table 6). Therefore, the high-pH root exudates of maize and the alkalization of the maize rhizosphere by nitrates uptake would cause the Zn shift from the more bioavailable (exchangeable) fraction to the more resistant (inorganically bound and organically bound) fractions in the Fluvisol. This explains why the post-harvest decrease in Zn concentration in the exchangeable fraction in the Fluvisol was greater than those in the 0.1 mol L⁻¹ HCl-extractable, DTPA-extractable fractions and total. This type of metal shift in Fluvisol under maize cultivation was also reported for Cd [6].

3.3.2. Soybeans

The shoot Pb and Zn uptakes by the Enrei and Suzuyutaka soybeans were significantly higher than those by the other cultivars in both soils ($p < 0.05$ and $p < 0.01$ for the Andosol and Fluvisol, respectively; Table 3). The Suzuyutaka soybean took up less soil Pb and Zn in its root than the other cultivars in both soils. Forty percent and 46% of the total Pb, and 91% and 88% of the total Zn uptakes by the Suzuyutaka soybean grown on the Andosol and Fluvisol, respectively, were able to be removed by harvesting only the aboveground parts. The Suzuyutaka soybean shoot took up 12.7% and 1.7% of the 0.1 mol L⁻¹ HCl-extractable Pb, 2.5% and 1.9% of the DTPA-extractable Pb, and 0.5% and 0.3% of the total Pb, and 29.4% and 23.0% of the 0.1 mol L⁻¹ HCl-extractable Zn, 52.6% and 35.1% of the DTPA-extractable Zn, and 5.3% and 3.8% of the total Zn in the Andosol and Fluvisol, respectively (Tables 1–3). The shoot Pb and Zn uptakes by the Suzuyutaka soybean were higher than that by hyperaccumulator *Thlaspi caerulescens* (0.03% and 2.9% of the total soil Pb [27] and Zn [50], respectively). For Zn concentrations in the two soils of the Suzuyutaka soybean treatments, the significant post-harvest decreases compared with the control were found in six fractions other than the oxide occluded and residual fractions, and total in both soils ($p < 0.01$; Table 6). However, for Pb, the significant post-harvest decreases compared with the control were found only in the 0.1 mol L⁻¹ HCl-extractable fraction in the Andosol ($p < 0.01$, Table 5). These results suggest that the Suzuyutaka soybean has a greater potential for Zn phytoextraction than the other cultivars tested.

3.3.3. Rice

The shoot Cu uptake by Milyang 23 rice was the second highest, closely following that by the Gold Dent maize, no significant difference was found between these two cultivars (Table 3). However, the Milyang 23 rice took up less soil Pb and Zn in its shoot than the two soybean cultivars did in both soils. Fifty-two percent and 62% of the total Cu uptakes by the Milyang 23 rice grown on the

Andosol and Fluvisol, respectively, were able to be removed by harvesting only the aboveground parts. The Milyang 23 rice shoot took up 25.0% and 18.5% of the 0.1 mol L⁻¹ HCl-extractable Cu, 8.5% and 34.3% of the DTPA-extractable Cu, and 2.1% and 5.9% of the total Cu in the Andosol and Fluvisol, respectively. The shoot Cu uptake by the Milyang 23 rice was also higher than that by hyperaccumulator *Alyssum murale* (0.05% of the total soil Cu [39]). These results suggest that both the Milyang 23 rice and the Gold Dent maize have a great potential for Cu phytoextraction.

4. Conclusion

After 2 months of cultivation, the Gold Dent maize and the Milyang 23 rice shoots took up more soil Cu, and the Suzuyutaka soybean shoot took up more soil Zn of the Andosol and Fluvisol than the other cultivars did. The bioavailable (the 0.1 mol L⁻¹ HCl-extractable and the DTPA-extractable) fractions and the total of the two soils were decreased most for Cu by the Gold Dent maize and the Milyang 23 rice and for Zn by the Suzuyutaka soybean. Therefore, there is a great potential for Cu phytoextraction by the Gold Dent maize and the Milyang 23 rice and for Zn phytoextraction by the Suzuyutaka soybean from paddy soils with low to moderately contamination levels under aerobic soil conditions.

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