Phytoextraction of Cd by rice capable of accumulating Cd at high levels

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Abstract: Selecting a phytoextraction plant with high Cd-accumulating ability based on the plant's compatibility with mechanized cultivation techniques may yield more immediately practical results than selection based on high tolerance to Cd. Rice (cv. Nipponbare and Milyang 23), soybean (cv. Enrei and Suzuyutaka), and maize (cv. Gold Dent) were grown on three paddy soils with low to moderately Cd contamination in pots. Shoot Cd uptake was as follows: Gold Dent < Enrei and Nipponbare < Suzuyutaka and Milyang 23. Several soil Cd fractions after Milyang 23 harvesting decreased most. Milyang 23 accumulated 10–15% of the total soil Cd in its shoot.

Soybean is the major summer crops grown in upland fields (fields under aerobic soil conditions) that have been converted from paddies in Japan. To evaluate the effect of phytoextraction by rice on the seed Cd content of soybean cultivated subsequently, we grew Milyang 23 rice, and then grew soybeans in three paddy soils contaminated with moderate Cd concentrations in pots. The rice accumulated 7% to 14% of the total soil Cd in its shoots. The soybean seed Cd contents were 24% to 46% less than those grown on control soils.

In a Cd-polluted paddy field, phytoextraction using Indica-type rice cultivars capable of accumulating Cd at high levels was conducted. Phytoextraction with the Indica rice Chokoukoku grown for 2 years without irrigation after drainage reduced the total soil Cd content by 38%, and reduced the grain Cd content in subsequently grown Japonica food rice by 47% without decreasing yield. The results suggest that phytoextraction with Chokoukoku can remove Cd from paddy fields polluted with low to moderate levels of Cd and reduce the grain Cd concentration of Japonica food rice cultivars to below the Codex standard within a reasonable time frame.

Keywords: cadmium, phytoextraction, rice, soybean

1. Introduction

Cadmium (Cd) is more mobile and bioavailable than other metals, and it is toxic to humans at concentrations lower than those toxic to plants because its effects on humans are cumulative[1]. Soil pollution by Cd has been a public concern ever since daily ingestion of high-Cd rice (*Oryza sativa* L.) was found to be the main cause of *Itai-Itai* disease in the 1970s [2]. Since then, Japan has used engineering techniques in Cd-polluted paddy fields to combat this condition [3]. However, in accordance with a new international standard set by the Codex Alimentarius Commission for the Cd content of rice grain—0.4 mg kg^{-1} [4], which is stricter than the Japanese standard of 1 mg kg^{-1}—Japan must perform large-scale remediation of paddy fields that cover more than 40 000 ha and are polluted with Cd at low to moderate levels [5, 6]. The use of engineering techniques may satisfy this requirement, but such methods are extremely environmentally disruptive and expensive and are impractical for treating large areas [7]. Although phytoextraction by hyperaccumulating wild plants such as *Thlaspi caerulescens* has been proposed as a low-cost, environmentally friendly restoration technology for soils contaminated with toxic metals, the difficulties with sowing, weed and disease control, and harvesting of such hyperaccumulators suggest that they may be unsuitable for large areas [8-10]. The use of agricultural species adapted to these growing conditions may therefore be a better alternative. This paper consists of the 3 results of our phytoextraction researches.

Selecting a phytoextraction plant with high Cd-accumulating ability based on the plant's compatibility with mechanized cultivation techniques may yield more immediately practical results than selection based on high tolerance to Cd. Rice, soybean (*Glycine max* (L.) Merr.), and maize (*Zea mays* L.) are the major summer crops grown in paddy fields and in upland fields (fields under aerobic soil conditions) that have been converted from paddies in Japan. The cultivation systems for these crops are well established and highly mechanized. However, the study of phytoextraction using rice and soybean has not yet been examined. The purpose of our first research was to select a promising plant cultivar for the phytoextraction of paddy soils contaminated with relatively low concentration of Cd based on a comparison of CdU in shoots and an examination of which soil Cd fractions were decreased by plant growth, using five cultivars of three crop species (rice, soybean, and maize).

The Codex Alimentarius Commission set maximum levels for Cd in wheat, potato, many vegetables [11], and polished rice [4]. On the other hand, it discontinued work on developing a maximum level for Cd in soybeans, which it considered was not a major contributor to Cd intake [12]. However, soybean is a major summer crop in Japan and, via tofu, natto, and soy sauce, is the main source of dietary intake of Cd in Japan [13]. Thus, decreasing the Cd

content of soybean seeds is extremely important. The purpose of our second research was to evaluate the potential of Milyang 23 rice for removing Cd from three paddy soils moderately contaminated with Cd, and the effects on the seed Cd concentrations of soybeans subsequently grown on those soils.

In previous studies, we found several Indica-type rice cultivars that are capable of accumulating Cd at high levels [14, 15], and we reported the effects of phytoextraction by high-Cd-accumulating rice on the seed Cd content of subsequently grown soybean [16]. In our third research, we demonstrate that phytoextraction using these Indica-type rice cultivars was capable of accumulating Cd at high levels. We examined the reduction in soil Cd concentration in a paddy field with a moderate level of Cd contamination and the decrease in grain Cd concentration of a Japonica food cultivar after phytoextraction with these Indica-type rice cultivars.

2. Materials and Methods

1) Research 1

We performed a pot experiment using industrially contaminated paddy soils containing Cd under aerobic soil conditions in 2002. The chemical properties of these soils are given in Table 1. The soils used in this pot experiment were collected from the surface (top 15 cm) of three paddy fields with low concentration of Cd contamination (an Andosol and two Fluvisols; [17]) in Japan. The main sources of Cd appeared to be atmospheric deposition of soot from a zinc refinery (Fluvisol 1), the use of wastewater from an abandoned copper mine for irrigation (Andosol), and the use of wastewater from an abandoned zinc mine for irrigation (Fluvisol 2). Each soil that we collected was air-dried, crushed, passed through a 2-mm sieve, thoroughly mixed, separated into 550-mL portions, and placed in pots. The pH values of the two Fluvisols used for soybean cultivation were raised to 6 by adding lime ($CaCO_3$).

We selected the Milyang 23 (an Indica-Japonica hybrid) rice cultivar and the Suzuyutaka soybean cultivar as plants that would accumulate high amounts of Cd in their shoots, respectively ([13], [15]). We then selected Nipponbare, Enrei, and Gold Dent as the recommended commercial cultivars of Japonica rice, soybean, and maize, respectively, in Japan. We sowed 4 seeds of soybean or maize per pot, and then thinned the seedlings to 2 per pot 10 d after sowing. We sowed 10 rice seeds per pot, and then thinned the seedlings to 5 per pot 10 d after sowing. The plants were grown in a greenhouse under natural sunlight at ambient temperatures from May to July. The pot experiment followed a randomized-block design, with four replicates per soil-cultivar treatment. Watering was done daily to maintain the soil water content near at field capacity. At 60 d after sowing, the leaves of the soybean had begun to fall, so we harvested the shoots of all crops by cutting the stems approximately 1 cm above the soil. After harvesting, we carefully removed the roots from the soil, and then the soil of each pot was separately air-dried and passed through a 2-mm sieve. We used a 'no plant, fertilizer' control for all cultivars grown on all soils, except for the soybean cultivars grown on the two Fluvisols; for the latter plants, we used a 'no plant, fertilizer with lime' control.

2) Research 2

In 2003, we grew rice in 1/600-a pots containing industrially contaminated paddy soils under aerobic soil conditions. The soils were collected from the top 15 cm of three paddy fields with moderate concentrations of Cd ($2.5–4.3$ mg Cd kg^{-1}; an Andosol and two Fluvisols; [17]). Each soil was air-dried, crushed, and passed through an 8-mm sieve by a rotary crusher with a stainless steel sieve, and thoroughly mixed; 25 L was placed in each pot. We grew Milyang 23 rice as the phytoextractor [14]. We sowed three seeds at each of 16 spots at intervals of 10 cm in each pot. The plants were grown in a greenhouse from May to October. Pots were watered daily to maintain the soil water content at near field capacity. At 130 d after sowing, we harvested the shoots by cutting the stems approximately 5 cm above the soil. We then carefully removed the roots (with the residual stems) from the soil and air-dried all the soil and passed each soil through a 2-mm sieve.

In 2004, we grew soybeans in 1/5000-a Wagner pots containing the phytoextracted soils or control soils under aerobic soil conditions. The soils were passed through a stainless steel 8-mm sieve and thoroughly mixed, then 2.5 L was placed in each pot. Roots of one rice plant were added to each pot containing phytoextracted soil, since roots are left behind in the field. Before soybean culture, the pH values of the control and phytoextracted Fluvisols were raised to 6.0 by the addition of lime ($CaCO_3$). We grew Enrei and Suzuyutaka soybeans as cultivars that accumulate low and high amounts, respectively, of Cd in their seeds [13]. We sowed three seeds per pot and then thinned the seedlings to one per pot at 21 d after sowing. The plants were grown in a greenhouse from June to September. Pots were watered daily to maintain the soil water content at near field capacity. At maturity, 100 d after sowing, we harvested the seeds.

3) Research 3

In 2004, we cultivated three Indica-type rice cultivars (IR8, Milyang 23, and Moretsu) in the experimental paddy field(1.6 mg Cd kg^{-1}; Fluvisol; [17]). We also cultivated the Japonica cultivar Akitakomachi in order to compare shoot Cd uptakes by the Indica-type cultivars with that by the Japonica rice cultivar. In 2005, we also cultivated the Indica rice cultivar Chokoukoku which showed the ability to accumulate Cd at high levels (Ito et al., unpublished) as well as IR8, Milyang 23, and Moretsu. In 2006, the Indica-type IR8, Milyang 23, Moretsu, and Chokoukoku were cultivated. The treatments followed a randomized block design with two replicates. In late April of 2004, seeds of the

Indica-type IR8, Milyang 23, and Moretsu and the Japonica Akitakomachi were sown in seedling trays and grown for 1 month. The rice seedlings were then transplanted into the flooded subplots in late May by a transplanting machine. In 2005 and 2006, the Indica-type IR8, Milyang 23, Moretsu, and Chokoukoku were grown and transplanted in the same manner as the rice cultivars in the first year. We maintained the soils of all subplots under flooded conditions during tillering from first to third year in order to maximize the DW of the rice shoots. Once the floodwater was drained from the subplots, we maintained the soils under oxidizing conditions until harvesting in order to maximize Cd accumulation by the rice shoots. At harvesting, we harvested rice shoots of each cultivar to determine the DW, Cd concentration, and Cd uptake by cutting the stems 5 cm above ground level, because that is the lowest height of stem that can be harvested by the most common rice harvesters used in Japan.

To examine the effect of phytoextraction with Indica-type rice cultivars capable of accumulating Cd at high levels on the grain Cd concentrations of a food rice cultivar subsequently grown in the remediated soil, we used the traditional Japanese rice cultivation method to grow the Japonica food rice Yumesayaka in the control plot (in which no plants were grown) and in all the subplots that had undergone 3 years of phytoextraction by the Indica-type rice cultivars IR8, Moretsu, and Milyang 23 or 2 years of phytoextraction by the Indica rice cultivar Chokoukoku. The rice seedlings were then transplanted into the flooded subplots on late May by using transplanting machine. We performed Japanese typical water management "intermittent irrigation". In the early October of fourth year, we harvested the unhulled rice grains from six rice shoots per subplot. We sampled a single $16 \times 30 \times 15$-cm-deep block of soil from each subplot before plowing in each year. The stubble (the rice stem between ground level and 5 cm above the soil) and the root were combined and regarded as the root component of the biomass in our analysis, and this material was included in the soil sample. After sampling of the soil, the root component was carefully removed from each soil block. The soil was then air-dried and passed through a 2-mm stainless-steel sieve before soil analysis.

3. Results and Discussion

1) Research 1

The shoot Cd uptake value was the second highest for the Milyang 23 rice, closely following the values for the Suzuyutaka soybean, and no significant difference was observed between the two cultivars (Table 1). However, soybean grown on Cd contaminated soil drops leaves that contain high concentration of Cd after the flowering stage, thus nearly half of the shoot Cd uptake of Suzuyutaka is lost as fallen leaves before harvesting [13]. This indicates that the Cd uptake value in the harvestable shoots of Suzuyutaka soybean would not be higher than that at the flowering stage even though cultivation continues beyond that stage. After 2 months of cultivation, the soybean had already begun flowering and dropping leaves, whereas the rice was still at its "maximum tiller number" stage and would have continued accumulating Cd for at least 2 months thereafter. Ito and Iimura [18] reported that the shoot Cd uptake of rice increased after heading in proportion to the biomass increase. After 2 months of cultivation, the soil Cd concentrations in the Nipponbare and Milyang 23 treatments were the lowest among the five cultivars, especially in the Andosol (data not shown), but Milyang 23 accumulated more soil Cd in its shoots than Nipponbare did (Table 1). The Milyang 23 rice extracted 12, 15 and 10% of the total Cd content in the Andosol, Fluvisol 1 and Fluvisol 2, respectively and accumulated in its shoot. These values are much higher than those reported for *B. juncea* (0.09%) and *T. caerulescens* (0.06%) grown on soil containing 40 mg kg^{-1} of total Cd for 6 weeks in pots [19]. The guide value of total soil Cd concentration by the Swiss ordinance is 0.8 mg kg^{-1} [20]. Assuming that the 0.8 mg kg^{-1} total soil Cd concentration is the maximum permissible value required to reduce Cd concentration of rice grain less than 0.4 mg kg^{-1}, and the shoot Cd uptake by Milyang 23 rice is constant, it would take 7, 5 and 1 croppings to remediate the Andosol, Fluvisol 1 and Fluvisol 2, respectively, indicating that it is economically feasible for phytoextraction.

See more detail: Murakami et al.,[14].

Table 1. Dry weights of, Cd concentrations in, and Cd uptakes by plant shoots grown in the three soils.

Cultivar	Andosol			Fluvisol 1			Fluvisol 2		
	DW (g pot^{-1})	CdC (µg g^{-1})	CdU (µg pot^{-1})	DW (g pot^{-1})	CdC (µg g^{-1})	CdU (µg pot^{-1})	DW (g pot^{-1})	CdC (µg g^{-1})	CdU (µg pot^{-1})
Gold dent maize	53.5 ± 2.5 a[1]	0.4 ± 0.0 d	19 ± 2 c	49.9 ± 2.0 a	0.4 ± 0.0 c	19 ± 2 c	28.3 ± 5.0 a	0.2 ± 0.0 c	6 ± 1 c
Enrei soybean	40.6 ± 0.9 b	2.4 ± 0.1 c	96 ± 4 b	32.2 ± 0.5 b	2.8 ± 0.4 c	90 ± 13 c	31.0 ± 0.8 a	1.0 ± 0.0 b	32 ± 1 b
Suzuyutaka soybean	38.7 ± 0.4 b	6.2 ± 0.5 b	240 ± 21 a	29.5 ± 0.5 b	9.8 ± 0.4 a	290 ± 14 a	30.9 ± 0.7 a	1.6 ± 0.3 b	45 ± 8 a
Nipponbare rice	15.3 ± 2.5 c	7.9 ± 0.6 b	116 ± 12 b	17.8 ± 1.1 c	7.5 ± 1.5 b	133 ± 29 b	20.3 ± 0.7 b	1.2 ± 0.0 b	24 ± 1 b
Milyang 23 rice	22.3 ± 2.0 c	8.5 ± 0.6 a	187 ± 14 a	18.6 ± 1.7 c	11.4 ± 2.4 a	225 ± 20 a	18.4 ± 1.2 b	2.5 ± 0.2 a	46 ± 2 a

[1]Mean±SE ($n = 4$), 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.

2) Research 2

Milyang 23 accumulated 7% to 14% of the total soil Cd in its shoots, and decreased several Cd fractions by 8% to 42% and the total soil Cd by 10% to 19% (data not shown). Indian mustard (*Brassica juncea* (L.)) and the

hyperaccumulator *Thlaspi caerulescens* accumulated 0.09% and 0.06% of the total soil Cd (40 mg kg^{-1}) when grown for 6 weeks in pots [19]. *Nicotiana rustica* L. accumulated 6% and *N. tabacum* L. accumulated 20% of the total soil Cd (5.44 mg kg^{-1}) when grown for 8 weeks in containers [21]. Thus, Milyang 23 has the potential to phytoextract soil Cd with a similar efficiency as those *Nicotiana* species.   The Cd concentrations of the soybean seeds grown on the phytoextracted soils were 24% to 44% lower than that on the control soils (Fig. 1). These results suggest that phytoextraction by Milyang 23 has the potential to reduce the Cd concentrations of soybean seeds.
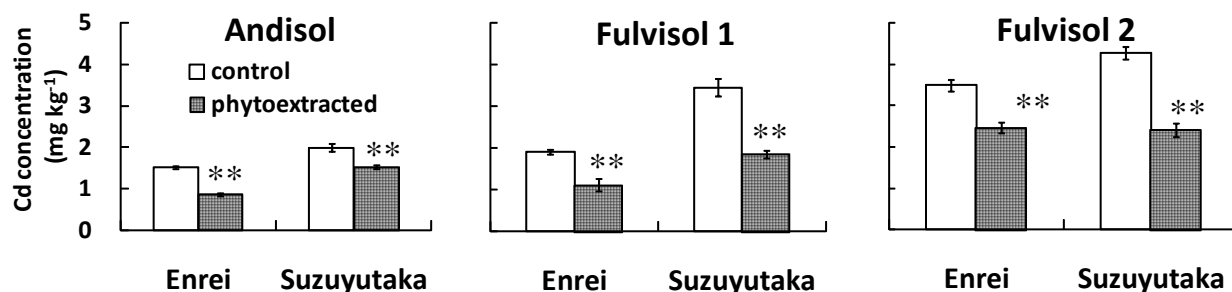
See more detail: Murakami et al., [16].

Fig. 1. Seed Cd concentrations of soybeans grown on control and phytoextracted soils. **P < 0.01 (*t*-test). Error bars show standard error (*n* = 3).

3) Research 3

The average shoot DW of each Indica-type rice cultivar except IR8 at harvesting from 2004 to 2006 was nearly 8 Mg ha^{-1} cultivation^{-1} (data not shown); this was more than 10 times that of the hyperaccumulator *T. caerulescens* (0.7 Mg ha^{-1} cultivation^{-1}; [22]. The paddy field was maintained under flooded conditions for 2 months after transplanting to permit sufficient increase in the DW of the rice shoots [23]. Thereafter, the field was drained so that the soil remained under oxidizing conditions until harvesting, because the solubility of soil Cd is higher under these conditions than under reducing conditions [24]. The shoot Cd concentrations of the four Indica-type rice cultivars increased after drainage in late July (Table 2), indicating a successful increase in Cd availability under oxidizing conditions. The same result is reported [25]. In contrast, the shoot Cd concentration of the Japonica food rice cultivar Akitakomachi remained low (no more than 2.2 mg kg^{-1}) even after drainage in late July. In 2004, shoot Cd uptake by the Japonica Akitakomachi (23 g ha^{-1}, Fig. 2) was very low compared with that by the Indica Moretsu (358 g ha^{-1}). Shoot Cd uptakes by Japonica rice cultivars are lower than those by Indica-type rice cultivars [15], suggesting that Japonica rice cultivars are unsuitable for phytoextraction. In 2005 and 2006, shoot Cd uptake was highest in the Indica Chokoukoku (550 and 333 g ha^{-1}, respectively). The total shoot Cd uptake by the Indica Chokoukoku grown for 2 years (883 g ha^{-1}) was higher than those by the 3-year grown Indica-types Moretsu (869 g ha^{-1}), Milyang 23 (638 g ha^{-1}), and IR8 (532 g ha^{-1}) . This 2-year shoot Cd uptake by Chokoukoku from soil containing 1.63 mg kg^{-1} of total Cd was higher than the uptake by the hyperaccumulator *T. caerulescens* (540 g ha^{-1} after 3 years of cultivation in soil with a total Cd content of 2.8 mg kg^{-1}; [22], by willow *Salix viminalis* (170 g ha^{-1} after 5 years of cultivation in soil with a total Cd content of 2.5 mg kg^{-1}; [26], and by the poplar (*Populus*) clone Balsam Spire (57 g ha^{-1} after 2 years of cultivation in soil with a total Cd content of 0.75 mg kg^{-1}; [27]. In contrast, Cd uptake by the residual roots of the Indica Chokoukoku was lower than those of the other Indica-type rice cultivars (Fig. 2). Cadmium in the residual roots may be released gradually into the soil as the roots are decomposed by soil organisms. Because phytoextraction involves harvesting of plant shoots that have taken up toxic elements from the soil and removing harvestable material from contaminated fields, plants such as the Indica Chokoukoku, with high shoot Cd uptake and low root Cd uptake, are ideal for phytoextraction. Moreover, the rice plant can be cultivated continuously [28]. The shoot DWs of the four Indica-type rice cultivars did not decrease, even after two or three continuous cultivations without irrigation after drainage (data not shown), indicating that growth damage from continuous cultivation and the presence of toxic metals in the soil did not occur. This characteristic of rice is also useful for phytoextraction.

The exchangeable, inorganically bound, organically bound, and total soil Cd concentrations were lowest in the Indica Chokoukoku subplot, despite the fact that this cultivar was grown for only 2 years (Table 3). This suggests that this cultivar can take up Cd more efficiently than the other Indica-type rice cultivars from the more resistant (inorganically and organically bound) fractions, as well as from the more bioavailable (exchangeable) fraction. This uptake capability equaled that of the hyperaccumulator *T. caerulescens* when pot-grown [29]. The Cd uptake by the residual roots of the Indica Chokoukoku (29.5 g ha^{-1}, Fig. 2) corresponded to only 0.02 mg kg^{-1} of soil Cd. Even allowing for the return of this root Cd to the soil by microbial decomposition, the total soil Cd concentration in the Chokoukoku subplot was 38% less than the mean value in the subplots with no plants (a reduction from 1.63 to 1.01 mg kg^{-1}, Table 3).

The Japonica food rice cultivar Yumesayaka grown after phytoextraction by the four Indica-type rice cultivars and in the subplots without phytoextraction showed normal growth. The average of the grain yields of Yumesayaka grown in the four subplots after phytoextraction and in the no plant subplot (5.1 Mg ha^{-1}, Table 4) was similar to that of Japonica food rice cultivars in Japan in 2007 (5.2 Mg ha^{-1}; [6]). The grain Cd concentrations of Yumesayaka grown after 2 years of phytoextraction with the Indica Chokoukoku were reduced by 47% (to 0.54 mg kg^{-1}) of that of the same rice cultivar grown without phytoextraction (1.02 mg kg^{-1}; Table 4).

Recently, phytoextraction has been criticized by several researchers because of the long period required for restoration, the difficulty of producing a high-biomass crop of the desired species, and the lack of knowledge of agronomic practices and management [30, 31]. The results of our research should help to dispel these criticisms. The DW of, and Cd uptake by, the Indica rice Chokoukoku were higher than those in the hyperaccumulator *T. caerulescens* (Fig. 2; [22]). Paddy rice can be cultivated continuously [28], and its cultivation system is well-integrated and highly mechanized. The Indica rice Chokoukoku was managed by agricultural techniques familiar to farmers who grow Japonica food rice; it is therefore well suited to planting on a wide scale. The 2-year phytoextraction using Chokoukoku without irrigation after drainage reduced the total soil Cd concentration by 38%, and it reduced the Cd concentration in the grain of subsequently grown Japonica food rice by 47% without decreasing yield. However, the grain Cd concentration of the Japonica food rice was still above the Codex Alimentarius Commission's international standard for the Cd content of rice grain (0.4 mg kg^{-1}[4]). Although our study showed the shoot Cd uptake by the Indica IR8 was lower than that by the Indica Chokoukoku (Fig. 2), 3-year phytoextraction by Indica IR8 on a paddy field reduced the Cd concentration in soil Cd extracted with 0.1 mol L^{-1} HCl from 0.48 to 0.33 mg kg^{-1} and the Cd concentration in the grain of subsequently grown Japonica food rice to 0.11 mg kg^{-1} [32]. Even if the rate of reduction of soil Cd concentration by phytoextraction with Chokoukoku were to become half of that in the first 2 years, an additional 2 years of phytoextraction by Chokoukoku would reduce the grain Cd concentration of Japonica food rice Yumesayaka to below 0.4 mg kg^{-1}.

See more detail: Murakami et al., [33]

Table 2. Shoot Cd concentrations from after transplanting to harvesting in the Indica-type rice cultivars capable of accumulating Cd at high levels and in the Japonica food rice cultivar Akitakomachi, 2004 to 2006.

Cultivar	Shoot Cd concentration (mg kg^{-1})					
2004	23 June	7 July	12 Aug.	1 Sept.	15 Sept.	19 Oct.
IR8 (I)	0.2±0.1a*	0.1±0.0b	2.5±0.1b	6.1±0.8b	16.0±0.3a	23.2±0.1b
Moretsu (I)	0.1±0.0a	0.1±0.0b	3.1±0.1a	8.8±0.2a	16.4±1.8a	37.2±4.5a
Milyang 23 (IJ)	0.2±0.0a	0.2±0.0a	2.4±0.2b	7.2±0.3ab	10.9±0.7a	22.6±1.4b
Akitakomachi (J)	0.1±0.0a	0.1±0.0b	1.4±0.1c	2.2±0.2c	1.7±0.4b	1.8±0.3c†
2005	27 June	7 July	28 July	24 Aug.	15 Sept.	18 Oct.
IR8 (I)	0.3±0.0b	0.2±0.0ab	2.6±0.4b	4.6±0.0b	18.8±1.4b	22.1±2.5bc
Moretsu (I)	0.2±0.0b	0.1±0.1b	2.7±0.3b	6.4±0.3b	20.1±1.2ab	32.8±0.4b
Milyang 23 (IJ)	0.3±0.0b	0.2±0.0b	2.9±0.0b	5.2±1.7b	13.6±1.3b	16.6±3.0c
Chokoukoku (I)	0.5±0.0a	0.4±0.0a	4.8±0.4a	16.6±0.9a	26.6±0.4a	70.0±0.9a
2006	13 June	28 June	21 July	9 Aug.	13 Sept.	16 Oct.
IR8 (I)	0.1±0.0b	0.4±0.1a	0.7±0.0a	3.1±0.6a	13.7±2.8b	32.0±1.1a
Moretsu (I)	0.2±0.0b	0.5±0.2a	1.7±0.6a	3.3±1.0a	17.3±1.5b	28.8±0.0a
Milyang 23 (IJ)	0.1±0.0b	0.5±0.2a	1.1±0.0a	2.7±0.0a	16.0±0.4b	33.3±2.7a
Chokoukoku (I)	0.3±0.0a	0.5±0.2a	1.3±0.1a	5.7±0.6a	28.3±1.5a	33.9±4.5a

*Values represent means ± SE (*n* = 2). Means for a given year in the same column that are followed by the same letter are not significantly different (*P* < 0.05). †Date of harvesting of the Japonica food cultivar Akitakomachi was 29 September. I, Indica; IJ, Indica–Japonica; J, Japonica.

Table 3. Soil Cd concentrations in the fractions obtained by means of sequential extraction, and their totals for each subplot, sampled before plowing in 2007.

Plot	Exchangeable (mg kg^{-1})	Inorganically bound	Organically bound	Oxide occluded	Residual	Total
No plants	0.62±0.02a*	0.44±0.03a	0.39±0.00a	0.11±0.01a	0.07±0.01a	1.63±0.01a
IR8	0.52± 0.04ab	0.32±0.02b	0.27±0.04b	0.08±0.01a	0.07±0.01a	1.26±0.04b
Moretsu	0.43±0.03bc	0.30±0.01b	0.24±0.01b	0.09±0.00a	0.05±0.00a	1.11±0.03cd
Milyang 23	0.50±0.00abc	0.32±0.00b	0.27±0.02b	0.10±0.01a	0.06±0.01a	1.24±0.02bc
Chokoukoku	0.37±0.01c	0.25±0.01b	0.23±0.00b	0.08±0.01a	0.05±0.00a	0.99±0.02d

*Values represent means ± SE (*n* = 2). Means in the same column that are followed by the same letter are not significantly different (*P* < 0.05).

Table 4. Grain yields and grain Cd concentrations of Yumesayaka, a Japonica food cultivar grown after phytoremediation using Indica-type rice cultivars capable of accumulating Cd at high levels.

Plot	Grain yield of Yumesayaka ($Mg\ ha^{-1}$)	Grain Cd concentration of Yumesayaka ($mg\ kg^{-1}$)
No plants	4.9±0.3a*	1.02±0.07a
IR8	5.4±0.1a	0.89±0.00ab
Moretsu	4.9±0.2a	0.74±0.01bc
Milyang 23	4.9±0.1a	0.86±0.05ab
Chokoukoku	5.4±0.4a	0.54±0.05c

*Values represent means ± SE ($n = 2$), Means in the same column that are followed by the same letter are not significantly different ($P < 0.05$).

Fig. 2. Shoot and root Cd uptakes by Indica-type rice cultivars capable of accumulating Cd at high levels, and shoot Cd uptake by the Japonica food rice cultivar Akitakomachi. Means in the same year (shoot or root) labeled with the same letter do not differ significantly ($P < 0.05$). J, Japonica; I, Indica; IJ, Indica–Japonica. Shoots were harvested in mid-October from 2004 to 2006. Residual roots were sampled in early May 2007.

3. Conclusions

These results suggest that phytoextraction with the Indica rice cultivar Chokoukoku can remove Cd from paddy fields polluted with Cd at low to moderate levels and can reduce the grain Cd concentration of the Japonica food rice cultivar Yumesayaka to below the Codex standard within a reasonable time frame. However, a potential hazard is inadvertent use of the phytoextractor grain as a food for humans and domestic animals. Large numbers of people were poisoned in Iraq in the early 1970s when mercury-treated grain meant for seed was eaten in homemade bread; poisoning also occurred in the USA in 1969 when treated grain was fed to hogs, whose meat was subsequently eaten [34, 35]. Phytoextraction by Indica rice cultivars capable of accumulating Cd at high levels will be applicable to the remediation of paddy fields in Monsoon Asia that have low to moderate levels of Cd contamination, provided that careful attention is paid to disposal of the high-Cd rice. Use of the phytoextraction techniques described here will help reduce the risk of Cd contamination of rice from paddy fields.

Acknowledgement

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