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Cadmium Phytoextraction Efficiency of Arum (*Colocasia antiquorum*), Radish (*Raphanus sativus* L.) and Water Spinach (*Ipomoea aquatica*) Grown in Hydroponics

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Abstract Selection of a phytoextraction plant with high Cd accumulation potential based on compatibility with mechanized cultivation practice and local environmental conditions may provide more benefits than selection based mainly on high Cd tolerance plants. In this hydroponics study, the potential of Cd accumulation by three plant species; arum (*Colocasia antiquorum*), radish (*Raphanus sativus* L.) and water spinach (*Ipomoea aquatica*) were investigated. Arum (*Colocasia antiquorum* L.) plants were grown for 60 days in a nutrient solution with 0, 10 or 50 μM Cd, while radish and water spinach plants grew only 12 days in 0, 1.5, 2.5, 5 or 10 μM Cd. Growth of radish and water spinach plants decreased under all Cd treatments (1.5 to 10 μM), while arum growth decreased only at 50 μM Cd. At 10 μM Cd treatment, the growth of arum was similar to the control treatment indicating higher tolerance of arum for Cd than radish and water spinach. Cadmium concentrations in different plant parts of all plant species increased significantly with Cd application in the nutrient solution. Arum and water spinach retained greater proportions of Cd in their roots, while in radish, Cd concentration in leaves was higher than in other plant parts. Cadmium concentrations in arum increased from 158 to 1,060 in the dead leaves, 37 to 280 in the normal leaves, 108 to 715 in the stems, 42 to 290 in the bulbs and 1,195 to 3,840 mg kg^{-1} in the roots, when the Cd level in the solution was raised from 10 μM Cd to 50 μM Cd. Arum accumulated (dry weight×concentration) 25 mg plant^{-1} at 10 μM, while the corresponding values for radish and water spinach were 0.23 and 0.44 mg plant^{-1}, respectively. With no growth retardation at Cd concentrations as high as 166 mg kg^{-1} measured in entire plant (including root) of arum at 10 μM Cd in the nutrient solution, arum could be a potential Cd accumulator plant species and could be used for phytoremediation.

Keywords Metal accumulator · Nutrient solution · Phytoremediation · Translocation

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1 Introduction

Cadmium (Cd) is non-essential to biota, more mobile and bioavailable, potentially toxic to humans at lower concentrations than those toxic to plants (Singh and McLaughlin 1999). In Japan, mining wastes are the major source of rice field contamination with Cd. Cadmium concentrations in some soils of Japan were found to range from as low as 0.03 to as high as 19.7 mg kg^{-1} (Geochemmap 2004), the latter concentration was primarily caused by large input of Cd from industrial wastes. Management techniques such as isolation, cleansing, and 'inerting' are three general categories of conventional treatment for Cd contaminated soils. Isolation may involve removal of the top soil and then covering with concrete or non-contaminated soils. Cleansing involves the leaching of pollutants with acids. 'Inerting' is the addition of other chemicals to the soil that render the pollutants into a non-toxic form. Above-mentioned conventional procedures are expensive and, furthermore, these methods may leave the soil infertile, cause further pollution by leaching or only be a temporary solution (Robinson et al. 2000).

Chaney (1983) suggested that some heavy-metal contaminated soils may be cleaned up by growing crop plants which accumulate the metals, then harvesting the plants and disposing of them in a 'safe area'. This process was called phytoremediaton. Using hyperaccumulator plants for phytoremediaton has been proposed as an environmentally friendly, low cost technology for decreasing heavy metal contents of highly contaminated soils (McGrath et al. 2002; Ebbs et al. 1997). It has been reported that *Thlaspi caerulescens* (Chaney et al. 2004), *Arabidopsis halleri gemmifera* (Kashem et al. 2007; Kubota and Takenaka 2003) and *Sedum alfredii* hance (Yang et al. 2004) have substantial potential for phytoextraction at low cost for soil Cd remediation. Using such hyperaccumulators for phytoextraction of low Cd concentration in soils, competition from weeds may need to be controlled, although that is not the case in highly Cd contaminated soils (Robinson et al. 1998). Above-mentioned Cd accumulators may not always be suitable for large-scale remediation efforts because the plants are small, grow slowly and produce very low biomass. For phytoremediation, the ideal plant should possess multiple traits such as fast growing, having high biomass and deep roots, easily harvested, and should tolerate and accumulate a range of heavy metals in their aerial and harvestable parts (Clemens et al. 2002).

Considering soil contamination with Cd and need for its remediation, researchers have tried to find some plant species which can accumulate Cd (Chaney et al. 2004; Kashem et al. 2007; Yang et al. 2004). We assumed that root crops may be appropriate for phytoremediation if root-bulbs can accumulate significant amounts of Cd, and that root-bulbs are easy to harvest for removal of Cd in soil. In this investigation, we selected three common and locally popular plant species (arum, radish and water spinach). Water spinach is the leaf crop used for comparison with the root crops. These crops are commonly grown in agricultural fields in Asia. These plants can grow in both dry and marshy conditions. Under such conditions it must be determined whether these plants display a high ability for Cd phytoextraction. In addition, there are very few studies on the selection of plant species that could be used for phytoremediation of soils with relatively low levels of Cd, such as those found in Japanese paddy fields (Kurihara et al. 2005). Hydroponics provides potential to examine metal tolerance and magnitude of metal accumulation in plant species with greater precision than soil studies. Grispen et al. (2006) suggested that metal accumulators should be selected under standard and repeatable conditions using both hydroponics and soil cultures.

The aim of this study was to investigate the phytoextractive potential of high biomass producing, commonly found plant species that can grow in Cd contaminated dry land or marshy conditions. The distribution of Cd in different parts of arum, radish and water spinach was also investigated.

2 Materials and Methods

2.1 Plant Culture

The experiment was carried out in the greenhouse of the Laboratory of Plant Nutrition and Physiology at Iwate University, Morioka, Japan under natural light conditions. Three plant species, arum (*Colocasia antiquorum*), radish (*Raphanus sativus* L.) and water spinach (*Ipomoea aquatica*), were used in three different experiments. Healthy and uniform size arum bulbs were sown in perlite in a plastic tray. After 1 month, buds with seedlings were transplanted to 15-l ceramic pots containing 12 l half-strength nutrient solution, as described below. Two weeks after transplanting, the three treatments (0, 10 and 50 μM Cd) of Cd (as $CdSO_4$) were imposed in the half-strength nutrient solution. There were three replicates of each treatment, and each replicate consisted of one bud. Arum plants were grown for 60 days (d) after Cd treatment.

Seeds of radish (*Raphanus sativus* L.) and water spinach (*Ipomoea aquatica*) were sown in perlite in a plastic tray. After 12 days, seedlings were selected for uniformity and transplanted to 10-l pots containing 9 l half-strength nutrient solution and allowed to grow for 7 days. The composition of half-strength modified Hoagland-Arnon nutrient solution (standard solution) was: 3.0 mM KNO_3; 2.0 mM $Ca(NO_3)_2$; 0.5 mM $NH_4H_2PO_4$; 1.0 mM $MgSO_4$; 10 μM Fe–EDTA; 1.5 μM H_3BO_3; 0.25 μM $MnSO_4$; 0.1 μM $CuSO_4$; 0.2 μM $ZnSO_4$; and 0.025 μM H_2MoO_4. After 7 days of radish and water spinach growth in nutrient solution, Cd ($CdSO_4$) was added to the nutrient solution at the rate of 0 (control), 1.5, 2.5, 5.0 and 10.0 μM Cd with three replications. The solution was aerated continuously for radish and water spinach, but not for arum. The level of the solution was maintained by adding deionized water and renewing once every 7 days. The pH level of the solutions was adjusted to 5.5 daily either with 1 M NaOH or 1 M HCl using a digital pH meter. The temperature of the greenhouse was about 22°C during the day and 15°C at night during the whole growth period.

2.2 Harvest and Analysis of Plant Material

Arum was harvested after 60 days, and radish and water spinach after 12 days of Cd treatment, washed with tap water and then deionized water. Arum plants were then separated into dead leaves, normal leaves, stems, buds and roots; radish into shoots and roots; and water spinach into leaves, stems and roots. All plant parts were oven dried (65°C) to a constant weight. After measuring dry weight, the plants were ground. Approximately 0.5 g (where available) of each plant sample was placed in a beaker with 15 ml high-purity HNO_3–$HClO_4$ (3:1) acid mixture, allowed to stand at room temperature overnight, and then heated to 140–180°C for complete digestion. Reagent blanks were processed to ensure that Cd was not added during sample preparation. The digested solutions were analysed for Cd using atomic absorption spectrophotometer (170–30 Hitachi, Tokyo, Japan). Results are presented on dry weight (DW) basis.

2.3 Statistical Analysis

Analysis of variance, Tukey simultaneous tests for dry matter and Cd concentration in plant tissue were performed using Minitab. The level of significance was $p<0.05$, unless mentioned otherwise in the text.

3 Results

3.1 Visible Toxic Symptoms and Plant Growth

Visible Cd toxicity symptoms on young leaves were chlorosis and white spots, and old leaves twisted when radish and water spinach were grown at any level of Cd treatments (1.5 to 10 μM). These symptoms did not appear in arum at 10 μM Cd, but at the highest Cd treatment (50 μM Cd) growth was decreased substantially (Tables 1 and 2). Growth of radish and water spinach decreased significantly at all Cd concentrations in the nutrient solution, while for arum, a decrease of plant parts was found only at the highest level of 50 μM Cd as compared to the control. The growth of arum was unaffected at 10 μM Cd, suggesting the possibility that this plant is a good phytoextractor for low Cd contaminated soils (Tables 1 and 2).

3.2 Cadmium Concentration and Accumulation

The concentration of Cd in different parts of arum, radish and water spinach increased with increasing Cd levels in the nutrient solution. Concentration of Cd in the control plants was below the detection limit (0.03 mg kg^{-1}). Cadmium concentrations in arum at 10 μM Cd were 158 in the dead leaves, 37 in the normal leaves, 108 in the stems, 42 in the bulbs and 1,195 mg kg^{-1} in the roots, but these values increased to 1,060, 280, 715, 290, and 3,840 mg kg^{-1} in the dead leaves, normal leaves, stems, bulbs and roots, respectively, at 50 μM Cd in the nutrient solution

Table 1 Effect of Cd application rates on the dry weight, Cd accumulation and distribution of arum grown in hydroponics

Treatment μM Cd	Dead leaves	Leaves	Stems	Bulbs	Roots
Dry weight (g)					
0	4.85 a	43.67 a	58.95 a	49.78 a	11.62 a
10	3.94 a	40.67 a	52.40 a	41.12 a	13.39 a
50	2.81 a	19.34 b	21.00 b	28.57 b	6.31 b
Cd accumulation mg plant^{-1}					
0	nd	nd	nd	nd	nd
10	0.63 b	1.51 b	5.63 b	1.73 b	15.72 b
50	2.96 a	6.55 a	14.53 a	8.08 a	24.38 a
Distribution (%)					
0	nd	nd	nd	nd	nd
10	2.5 b	4.41 b	22.7 a	7.0 b	62.3 a
50	5.3 a	11.6 a	25.7 a	14.3 a	43.2 b

Means with the same letter are not significantly different at $p<0.05$ level in each plant parts of arum.

nd Not detected.

(Fig. 1a). In the whole plant, Cd concentration was 166 and 724 mg kg^{-1} at the respective 10 and 50 μM Cd supply levels. The ratio of Cd concentration between leaves and bulbs remained similar (close to 1.0) at both levels of Cd addition (10 and 50 μM Cd). In radish, cadmium concentrations increased from 83 to 351 mg kg^{-1} in the shoots and 27 to 140 mg kg^{-1} in the roots when Cd concentration in the nutrient solution increased from 1.5 to 10 μM Cd (Fig. 1b). The corresponding increases in water spinach were from 1,984 to 2,380 mg kg^{-1} in roots, from 160 to 336 mg kg^{-1} in stems and from 55 to 104 mg kg^{-1} in leaves (Fig. 1c).

Similar to Cd concentration, the accumulation (concentration × DW of plant) of Cd increased in different parts of arum with Cd application rates in the solution, but in radish and water spinach, accumulation was not significantly different at Cd application rates except at the highest Cd application rate in radish. At 10 μM Cd level, total accumulation was 25, 0.23, 0.44 mg Cd plant^{-1} in arum, radish and water spinach, respectively. Cadmium accumulation

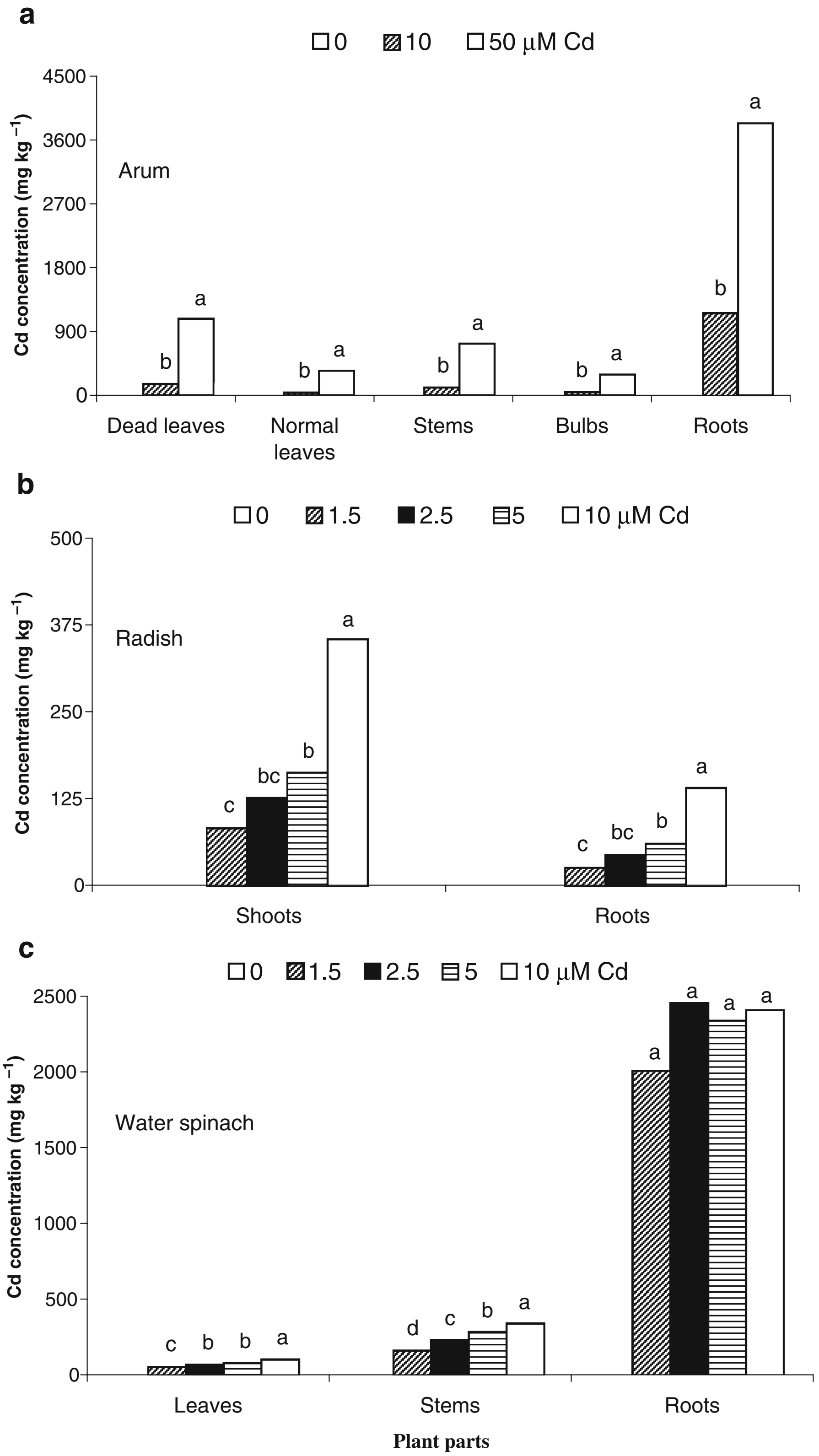
Table 2 Effect of Cd application rates on the dry weight, Cd accumulation and distribution of radish and water spinach grown in hydroponics

Rate	Radish		Water spinach		
Treatment μM Cd	Shoots	Roots	Leaves	Stems	Root
Dry weight (g)					
0	0.92 a	1.64 a	0.81 a	0.25 a	0.33 a
1.5	0.47 b	0.96 b	0.44 b	0.14 b	0.25 b
2.5	0.50 b	0.78 b	0.37 b	0.12 bc	0.20 bc
5	0.42 b	0.87 b	0.25 c	0.08 c	0.15 c
10	0.41 b	0.55 b	0.23 c	0.08 c	0.16 c
ANOVA	S	S	NS	NS	NS
Cd accumulation (mg plant^{-1})					
0	nd	nd	nd	nd	nd
1.5	0.04 b	0.03 b	0.02	0.02	0.50
2.5	0.06 b	0.04 b	0.03	0.03	0.47
5	0.07 b	0.05 b	0.02	0.02	0.04
10	0.15 a	0.08 a	0.02	0.03	0.04
ANOVA	S	S	NS	NS	NS
Distribution (%)					
0	nd	nd	nd	nd	nd
1.5	58	38	4.5	4.4	91
2.5	62	34	5.0	5.3	90
5	54	43	5.2	6.5	89
10	63	35	5.4	7.0	88
ANOVA	NS	NS	NS	NS	NS

Means with the same letter are not significantly different at $p<0.05$ level in each plant parts.

nd Not detected; *S* significant; *NS* not significant.

Fig. 1 Effect of Cd levels (µM) on Cd concentrations in plant parts of arum (**a**), radish (**b**) and water spinach (**c**) grown in hydroponics. Means with the same letter within the plant parts are not significantly different at $p <$ 0.05 level



in different parts of arum decreased in the order: root > stems > bulbs > normal leaves > dead leaves (Tables 1 and 2).

3.3 The Distribution of Cadmium in Plant Parts

Cadmium distribution (percent of the total uptake) in plant parts of three different species varied significantly among the Cd treatments. In arum, 3% and 5% of total Cd was found in the dead leaves, 4% and 12% in the normal leaves, 22% and 26% in the stems and 7% and 14% in the bulbs, at 10 and 50 μM Cd in the solution, respectively. In the arum roots, the distribution of Cd was opposite to that found in other plant parts at the two rates of Cd used. At 10 μM Cd in the solution, a higher percentage (62%) was found in roots, but the proportion went down to 43% at 50 μM Cd in the nutrient solution (Table 1). In radish, 54% to 63% of total Cd was in the shoots, 35% to 43% in the roots, while in water spinach, most of the absorbed Cd (>88%) was accumulated in the roots. The distribution of Cd among plant parts was not affected by the rate of Cd addition in radish and water spinach (Tables 1 and 2).

4 Discussion

Growth of radish and water spinach was decreased significantly with Cd treatments, even at a low concentration of Cd (1.5 μM) in the nutrient solution. This suggested a toxic effect of Cd on radish and water spinach. Such a deleterious effect was not seen in arum up to 10 μM Cd in the growth medium. No significant difference in dry weight of arum between control and 10 μM Cd treatments indicates that the arum plant has higher tolerance to Cd than the other two plant species tested (Tables 1 and 2). Cadmium concentration in all three plants increased with Cd levels in the nutrient solution but the magnitude of Cd increase varied among the plant parts and species used, showing a differential accumulation and tolerance pattern among plant species (Fig. 1a–c).

Growth of arum plant was unaffected at 10 μM Cd in the nutrient solution. At this level total accumulation was 25 mg plant^{-1}. Total accumulation of Cd in arum at 50 μM Cd was 56.5 mg plant^{-1} in spite of slight growth retardation. Among the plant parts, about 50% and 25% of total Cd was accumulated in the roots and in stems of arum on average, indicating that roots and stems are the major sinks of Cd accumulation. The highest proportion of Cd in the roots of arum and water spinach may be due to immobilization of Cd through precipitation and/or adsorption on the root surface and within the symplasm of root cells as well as due to sequestration of Cd by phytochelatins in the vacuoles of root cells (Shute and Macfie 2006).

Researchers are trying to find new plants for the purpose of phytoremediation. Most accumulator plants are small in size. *Arabidopsis halleri gemmifera* is a new heavy metal accumulator plant found in Japan, which has phytoextraction capacity almost similar to *Thlaspi caerulescens* (Kashem et al. 2007; Kubota and Takenaka 2003; Lombi et al. 2000). Kashem et al. (2007) measured Cd concentrations of 820, 590 and 400 mg kg^{-1} Cd in leaves, stems and roots of *Arabidopsis halleri gemmifera*, respectively, at 10 μM Cd in the nutrient solution with a total accumulation of 2.8 mg plant^{-1}. In this study, arum plants showed a nine-fold higher Cd accumulation than that of *Arabidopsis halleri gemmifera* at the same Cd treatment in hydroponics because of the enormous biomass production of arum. Using *Thlaspi caerulescens* (Lombi et al. 2000), *Arabidopsis halleri gemmifera* (Kashem et al. 2007) and *Sedum alfredii* hance (Yang et al. 2004) hyperaccumulators for phytoextraction of Cd contaminated soils may not be always suitable for large-scale remediation efforts because the plants are small and grow slowly.

It has been shown in several studies that Cd tolerant plants must be able to prevent the absorption of excess Cd, or detoxify the Cd after it has been absorbed (Jiang et al. 2001). It is important to select a phytoextraction plant with high Cd accumulation capability that is also compatible with mechanized cultivation practice and local weather conditions. This type of plant may yield more immediately practical outputs than selection based solely on high tolerance to Cd. Arum with many roots can accumulate substantial amounts of Cd, and it is possible to harvest the entire plant including roots. It is fast growing, easily propagated, easy to manage and capable of growing in both dry and marshy conditions. In this study, arum plants appear to possess the potential to provide a novel technique for the removal of Cd from industrially contaminated waters and soil. Imamul Huq et al. (2005) found a significant amount of arsenic (As) in the shoots of arum and proposed that arum may be a potential candidate for phytoremediation of As and other toxic metals.

5 Conclusions

The results indicate that arum has an excellent potential for Cd phytoremediation because of high biomass production and high tolerance to Cd as compared to the other two plant species tested in this study. If plant uptake under field soil conditions is similar to that observed in this experiment, then this plant could be used to decontaminate moderately Cd contaminated soils. Future research on soil culture needs to be conducted to verify the potential of Cd accumulation in arum.

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