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Comparison in Accumulation of Lanthanide Elements Among Three Brassicaceae Plant Sprouts

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Abstract Three kinds of sprouts in the Brassicaceae family of plants, namely, pink kale, radish and mustard were evaluated for the possibility of phytoremediation of lanthanides. The mustard sprout more efficiently accumulated lanthanides (e.g. 0.26 nmol La/g) than other Brassicaceae family plant sprouts (0.16 nmol La/g in the radish), however the radish sprout showed the fastest growth among three sprouts. Faster growth compensated for less efficiency in lanthanide accumulation (28 pmol La in the radish vs. 12 pmol La in the mustard) indicating that the radish is the most preferable sprout for the phytoremediation of lanthanides.

Keywords Lanthanides · Brassicaceae · Sprout · Phytoremediation

Rare earth elements (REEs) are comprised of 16 transition metals, i.e., scandium (Sc), yttrium (Y), and 14 lanthanides, including lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Ne), samarium (Sa), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu) except promethium (Pr) which has no naturally occurring isotopes. REEs possess similar chemical properties and are widely used in various products, such as high refractive index glass (La), rare-earth magnets (Nd, Sm, Gd, and Dy), and an MRI contrast agent (Gd). Clearly, the industrial demand for REEs has increased (Du and Graedel 2011). REEs are also used in agriculture as fertilizer to improve crop growth and production, and this has resulted in an increase in the concentrations of REEs in soil. In addition, Gd is frequently used in medicine as an MRI contrast agent, such as (-)-1-deoxy-1-(methylamino)-D-glucitol dihydrogen [*N*,*N*-bis[2-[bis- (carboxymenthyl)amino]ethyl]glycinato (5-)] gadolinate (2-) (1:1) (meglumine gadopentetate) and (±)-10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetatogadolinium [III] (gadoteridol) (Spinosa et al. 2002). However, it was reported that Gd used in urban areas is not completely treated in sewage plants and released into rivers (Verplanck et al. 2010). Consequently, the widespread use of REEs has led to their accumulation in the environment.

Phytoremediation, which is the remediation of environmental contaminants, such as toxic metals and metalloids, by plants, has been reported. *Solanum nigrum* L. and *Pteris vittata* L. are well-known hyperaccumulators of cadmium and arsenic, respectively, and have been used for phytoremediation (Ji et al. 2011; Natarajan et al. 2011). It was reported that some pteridophytes, such as *Dicranopteris dichotoma* and *Pronephrium simplex*, can hyperaccumulate REEs in an amount equivalent to 0.1 % of leaf dry weight (Wang et al. 2003; Lai et al. 2006).

Brassicaceae family plants, such as radish and Indian mustard, are known as accumulators of selenium (Se) and used for the phytoremediation of Se (Bañuelos and Lin 2005; Bañuelos et al. 2007). These plants accumulate large amounts of sulfur in the various forms of sulfur-containing metabolite, such as isocyanate and thioamino

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acids (Lampe and Peterson 2002), and hence, may be also able to accumulate Se, which belongs to the same group as sulfur in the periodic table. Indeed, unique Se-containing amino acids, such as Se-methylselenocysteine, selenomethionine, methylselenomethionine selenonium, and selenohomolanthionine, have been identified in Brassicaceae family plants (Grant et al. 2004; Ogra et al. 2007; Yathavakilla et al. 2005). In addition, thioamino acids present in large amounts are expected to be an accumulator of heavy metals, such as REEs, because of their ability to sequester heavy metals.

In this study, the sprouts of three Brassicaceae family plants, i.e., pink kale (*Brassica oleracea* var. *acephala*), Indian mustard (*Brassica juncea*), and Japanese radish (*Raphanus sativus*), were evaluated for their tolerance to lanthanides and lanthanide accumulating ability. The sprouts grow rapidly and can be hydroponically cultivated; thus, they are appropriate for the remediation of contaminants from soil and water. The accumulation profiles of lanthanides were compared among the three kinds of Brassicaceae sprouts.

Materials and Methods

Standard solutions of $La(NO_3)_3$, $Ce(NO_3)_3$, $Pr(NO_3)_3$, $Nd(NO_3)_3$, $Sm(NO_3)_3$, $Eu(NO_3)_3$, $Gd(NO_3)_3$, $Tb(NO_3)_3$, $Dy(NO_3)_3$, $Ho(NO_3)_3$, $Er(NO_3)_3$, $Tm(NO_3)_3$, $Yb(NO_3)_3$, and $Lu(NO_3)_3$, potassium hydroxide (KOH), and nitric acid were purchased from Wako (Osaka, Japan). The stock mixture containing the 14 REEs at equimolar concentrations was made from the standard solutions and then adjusted to neutral pH with 0.1 M KOH. It was then diluted with 0.01 M KNO_3 to prepare exposure solutions at the concentrations of 1 μM and 5 μM. All reagents were of analytical grade. Deionized water (18.3 MΩ cm) was used throughout.

Sprouts of three Brassicaceae plants, pink kale (*B. oleracea* var. *acephala*), Indian mustard (*B. juncea*), and Japanese radish (*R. sativus*), were used in this study. Sterilized seeds were sown on rock wool in a plastic tray and treated with a 30 mL portion of the REE mixture at the concentration of 1 or 5 μM. Another set of sterilized seeds were treated with 30 mL of 0.01 M KNO_3 and used as control. The plants were grown for 24 h in the dark in a growth chamber (LH-55-RDS; Nihonikakikai, Osaka, Japan) set at 25°C. After 1 week, the sprouts were harvested and the roots removed. The ground parts were weighed and lyophilized.

A 0.1 g portion of a dried sample was digested in a microwave oven with nitric acid in a Teflon PTFE tube. The concentrations of La (m/z 139), Ce (140), Pr (141), Nd (146), Sm (147), Eu (153), Gd (157), Tb (159), Dy (163), Ho (165), Er (166), Tm (169), Yb (172), and Lu (175) were measured with an ICP-MS (Agilent 7500ce, Agilent Technologies, Hachiouji, Japan).

Data are shown as means ± standard deviation (SD). Differences in plant mass between control and exposed plants were statistically analyzed by the Dunnett test. Differences in the metal concentrations among three sprouts were tested using the Student's *t* test. A *p* value less than 0.05 was considered to be statistically significant.

Results and Discussion

Control Japanese radish sprout showed better growth than the other two control sprouts, i.e., its weight was approximately five times greater than those of the other two sprouts (Table 1). Growth of Indian mustard sprout was significantly stimulated by the lanthanide treatment. Growth of pink kale and Japanese radish sprouts tended to increase with lanthanide treatment at either concentration, but the increase was not significant. It has been reported that light lanthanides, in particular, La and Ce, stimulate the growth of maize and *Arabidopsis thaliana* at concentrations of several μM (Boyko et al. 2011; Gong et al. 2011). Although it was unclear which lanthanide ion(s) stimulated the growth of mustard sprout, the lanthanide mixture showed a positive effect on the growth. On the other hand, it has also been demonstrated that treatment with La and Ce at sub-mM concentrations reduced the growth of wheat (*Triticum aestivum L.*) (Hu et al. 2002). As the lanthanide mixture containing lanthanides each at a concentration of 5 or 1 μM did not affect the growth of the three Brassicaceae sprouts, those concentrations were used in further experiments.

Lanthanide concentrations in control sprouts were below their detection limits (data not shown). In pink kale sprout treated at the concentration of 1 μM, Ce concentration tended to be higher than the concentrations of the other lanthanides (Table 2). In mustard and radish sprouts treated at the concentration of 1 μM, the concentrations of light

Table 1 Weights (mean ± SD, g) of three Brassicaceae sprouts treated with lanthanide mixture at different concentrations

	Control	1 μM	5 μM
Pink kale	0.029 ± 0.011	0.037 ± 0.011	0.039 ± 0.012
Indian mustard	0.032 ± 0.009	0.046 ± 0.010*	0.042 ± 0.008*
Japanese radish	0.158 ± 0.031	0.175 ± 0.076	0.171 ± 0.047

* $p < 0.01$ versus control group

lanthanides (La, Ce, Pr, Nd, Sm, and Eu), in particular, La and Ce, were apparently higher than those of the other lanthanides. It has been reported that some species of ferns preferably accumulate light lanthanides rather than heavy lanthanides (Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) (Fu et al. 1998; Lai et al. 2006). This could be accounted for by the fact that the ionic radii of light lanthanides are similar to that of calcium. The ionic radii of La^{3+}, Ce^{3+}, Lu^{3+}, and Ca^{2+} are 117, 115, 100, and 114 pm, respectively. Hence, light lanthanides are incorporated into the roots via a calcium transporter. However, these tendencies were not obvious in pink kale and radish sprouts treated at the concentration of 5 µM, suggesting that the incorporation of light lanthanides was saturated at that concentration. On

Table 2 Concentrations and amounts (mean ± SD) of lanthanides in Brassicaceae sprouts treated with lanthanide mixture at different concentrations

	La	Ce	Pr	Nd	Sm	Eu	Gd
Concentration (nmol/g wet wt.)							
1 µM							
Pink kale	0.08 ± 0.05^{c}	0.18 ± 0.06^{b}	0.12 ± 0.04^{b}	0.14 ± 0.05^{ab}	0.11 ± 0.03^{ab}	0.11 ± 0.03^{ab}	0.12 ± 0.04^{ab}
Indian mustard	0.26 ± 0.01^{a}	0.30 ± 0.03^{a}	0.17 ± 0.01^{a}	0.20 ± 0.01^{a}	0.13 ± 0.001^{a}	0.12 ± 0.004^{a}	0.15 ± 0.01^{a}
Japanese radish	0.16 ± 0.03^{b}	0.18 ± 0.04^{b}	0.08 ± 0.02^{b}	0.10 ± 0.03^{b}	0.06 ± 0.02^{b}	0.06 ± 0.02^{b}	0.07 ± 0.03^{b}
5 µM							
Pink kale	0.32 ± 0.02^{b}	0.32 ± 0.00^{b}	0.28 ± 0.01^{b}	0.30 ± 0.01^{b}	0.26 ± 0.01^{b}	0.26 ± 0.02^{b}	0.32 ± 0.02^{b}
Indian mustard	1.3 ± 0.3^{a}	1.1 ± 0.2^{a}	0.89 ± 0.19^{a}	0.94 ± 0.19^{a}	0.75 ± 0.14^{a}	0.73 ± 0.14^{a}	0.92 ± 0.18^{a}
Japanese radish	0.56 ± 0.01^{b}	0.46 ± 0.01^{b}	0.36 ± 0.04^{b}	0.36 ± 0.05^{b}	0.31 ± 0.04^{b}	0.30 ± 0.05^{b}	0.36 ± 0.06^{b}
Amount (pmol/plant)							
1 µM							
Pink kale	3.0 ± 1.7	6.6 ± 2.2	4.2 ± 1.3	5.1 ± 1.7	3.9 ± 1.2	3.9 ± 1.1	4.5 ± 1.3
Indian mustard	12 ± 0.5	14 ± 2	7.6 ± 0.3	9.3 ± 0.5	6.0 ± 0.1	5.6 ± 0.2	6.8 ± 0.3
Japanese radish	28 ± 6	32 ± 7	14 ± 4	17 ± 5	11 ± 3	11 ± 4	13 ± 4
5 µM							
Pink kale	12 ± 1	13 ± 0.1	11 ± 1	12 ± 0.3	10 ± 0.4	10 ± 1	12 ± 1
Indian mustard	57 ± 13	46 ± 8	38 ± 8	40 ± 8	32 ± 6	31 ± 6	39 ± 7
Japanese radish	95 ± 2	78 ± 2	61 ± 8	62 ± 8	52 ± 8	51 ± 9	61 ± 11

	Tb	Dy	Ho	Er	Tm	Yb	Lu
Concentration (nmol/g wet wt.)							
1 µM							
Pink kale	0.11 ± 0.03^{ab}	0.11 ± 0.03^{a}	0.12 ± 0.03^{a}	0.13 ± 0.03^{a}	0.12 ± 0.03^{a}	0.12 ± 0.03^{a}	0.13 ± 0.03^{a}
Indian mustard	0.12 ± 0.01^{a}	0.12 ± 0.003^{a}	0.12 ± 0.01^{a}	0.13 ± 0.004^{a}	0.11 ± 0.004^{a}	0.11 ± 0.002^{a}	0.12 ± 0.004^{a}
Japanese radish	0.07 ± 0.02^{b}	0.08 ± 0.03^{a}	0.09 ± 0.03^{a}	0.10 ± 0.03^{a}	0.09 ± 0.03^{a}	0.10 ± 0.03^{a}	0.10 ± 0.03^{a}
5 µM							
Pink kale	0.29 ± 0.02^{b}	0.30 ± 0.02^{b}	0.34 ± 0.02^{b}	0.35 ± 0.02^{b}	0.32 ± 0.02^{b}	0.31 ± 0.02^{b}	0.33 ± 0.02^{b}
Indian mustard	0.76 ± 0.14^{a}	0.74 ± 0.13^{a}	0.79 ± 0.14^{a}	0.78 ± 0.12^{a}	0.67 ± 0.10^{a}	0.59 ± 0.08^{a}	0.63 ± 0.08^{a}
Japanese radish	0.33 ± 0.07^{b}	0.34 ± 0.07^{b}	0.37 ± 0.09^{b}	0.39 ± 0.10^{b}	0.36 ± 0.09^{b}	0.34 ± 0.08^{b}	0.36 ± 0.08^{b}
Amount (pmol/plant)							
1 µM							
Pink kale	4.1 ± 1.2	4.1 ± 1.2	4.4 ± 1.2	4.7 ± 1.2	4.5 ± 1.1	4.3 ± 1.2	4.8 ± 1.1
Indian mustard	5.5 ± 0.2	5.6 ± 0.1	5.7 ± 0.2	5.9 ± 0.2	5.2 ± 0.2	5.0 ± 0.1	5.3 ± 0.2
Japanese radish	13 ± 4	15 ± 5	15 ± 5	17 ± 6	16 ± 5	17 ± 6	17 ± 5
5 µM							
Pink kale	12 ± 1	12 ± 1	13 ± 1	14 ± 1	13 ± 1	12 ± 1	13 ± 1
Indian mustard	32 ± 6	31 ± 6	34 ± 6	33 ± 5	29 ± 4	25 ± 3	27 ± 4
Japanese radish	57 ± 11	59 ± 13	64 ± 15	67 ± 16	62 ± 15	58 ± 13	61 ± 14

Values marked by different letters in the same column are significantly different

Table 3 Ratios of lanthanide concentrations in sprouts treated with 5 μM lanthanide mixture to those treated with 1 μM lanthanide mixture

	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Pink kale	3.9	1.8	2.4	2.2	2.4	2.5	2.6	2.7	2.7	2.8	2.8	2.7	2.6	2.5
Indian mustard	5.1	3.6	5.4	4.6	5.6	6.0	6.1	6.3	6.1	6.4	6.1	5.9	5.4	5.4
Japanese radish	3.5	2.5	4.5	3.7	4.8	4.8	4.9	4.6	4.1	4.3	4.1	4.0	3.6	3.6

the other hand, more light lanthanides were accumulated in mustard sprout than heavy lanthanides even at the higher concentration, despite the decrease in the ratio of light lanthanides to heavy lanthanides. Although there were no apparent differences in the accumulation of heavy lanthanides among the three sprouts, mustard sprout more efficiently incorporated light lanthanides than pink kale and radish sprouts. This tendency became more evident at the higher concentration of lanthanides, i.e., 5 μM. The ratios of lanthanide concentrations in the sprouts treated with lanthanides at the concentrations of 1 and 5 μM were calculated (Table 3). The ratios of lanthanides in mustard sprout were almost 5 in all lanthanides except Ce. This indicates that mustard sprout incorporated all lanthanide elements in a dose-dependent manner. In contrast, the ratios in pink kale and radish sprouts were less than 3 and 5, respectively. This indicates that the uptake of lanthanides by these two sprouts may be saturated at a high concentration of lanthanides. Therefore, mustard sprout most efficiently incorporated lanthanides among the three sprouts from the viewpoint of lanthanide concentration in plant. At present, there is no reasonable explanation as to why mustard sprout shows better accumulation efficiency than the two other Brassicaceae sprouts. The chemical species of the accumulated lanthanides may be relevant to the accumulation efficiency; thus, the speciation of lanthanides accumulated in the sprouts should be performed in future studies.

The amounts of lanthanides accumulated in the sprouts were also calculated (Table 2). Because radish sprout showed the fastest growth and the largest biomass among the three sprouts, it was able to accumulate the largest amount of lanthanides. Mustard sprout can accumulate lanthanides more efficiently than radish sprout, and its growth is stimulated by lanthanide treatment. However, the rapid growth of radish sprout overcame its low accumulation efficiency. Considering the concentrations of accumulated lanthanides, the growth rate, and the biomass, radish sprout was the most preferable for the phytoremediation of lanthanides among the three Brassicaceae sprouts.

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