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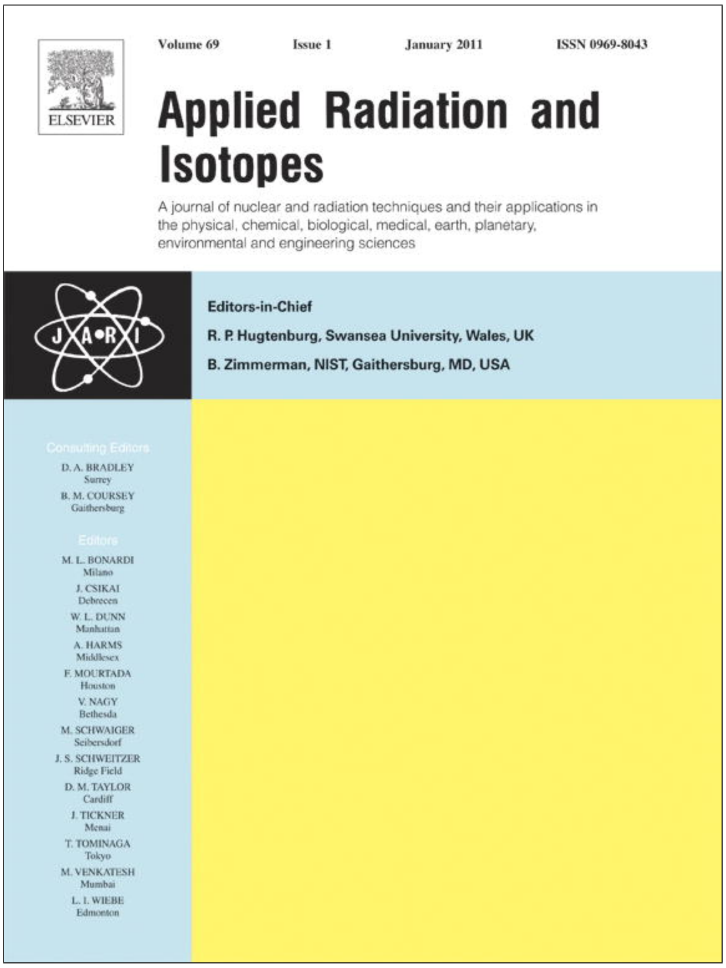

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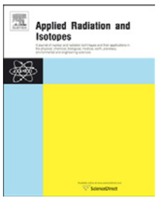

Accumulation of radium in relation to some chemical analogues in *Dicranopteris linearis*

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ABSTRACT

This study elucidates the uptake and accumulation of radium in the field-growing fern *Dicranopteris linearis* by relating the radium concentration to some potential chemical analogues, including alkaline earth metals, rare earth elements, and some important heavy metals. Time-dependent accumulation of radium and these chemical analogues for *D. linearis* were described by the ^{228}Th/^{228}Ra activity ratio, an index for inferring plant age. The correlation between radium and these elements was assessed by statistical analysis and used as a reference to elucidate the uptake and accumulation of radium in relation to the chemical analogues. Analytical and statistical results showed that the concentrations of alkaline earth metals (except for Mg) rare earth elements and some heavy metals in *D. linearis* increased linearly with plant age. These elements, exhibiting a similar accumulation pattern to radium and significant correlation coefficients with radium, were considered as the chemical analogues to radium. Additionally, the plant/soil concentration ratios (CRs) for radium and most of these analogues in *D. linearis* exceeded 1, consistent with the definition of hyper-accumulator plants.

1. Introduction

Naturally occurring and artificial radionuclides can be taken up by plants. The uptake by crop plants of an important artificial radioactive cesium (^{137}Cs) has been noted; ^{137}Cs has been spread widely and delivered radiation to humans during the past decades due to nuclear accidents or nuclear weapon tests (UNSCAR, 2000). Conversely, naturally occurring radionuclides that belong to the thorium (^{232}Th) and uranium (^{238}U) series can be taken up by plants growing in normal and contaminated areas. Plants growing in uranium mining areas contained elevated levels of ^{232}Th, ^{238}U, and radium (^{226}Ra and ^{228}Ra). The associated plant/soil concentration ratios (CRs) were also higher than those for a normal background (Ibrahim and Whicker, 1988a; Ibrahim and Whicker, 1988b). Using the CR values, several transport models have been developed to predict the radiation dose to humans from radionuclides released in the biosphere (Sheppard, 1985; Sheppard and Sheppard, 1985). These studies focused on assessing human radiation dose from nuclear and U-mining activities. However, little information exists concerning the transport of radionuclides by plants, which can accumulate naturally occurring radionuclides even when growing in a normal radiation background.

Alkaline earth metals and/or rare earth elements (REEs) were commonly considered to accumulate in plants together with naturally occurring radionuclides. Brazil nuts are known to contain higher levels of radium isotopes than normal plants even though *Bertholletia excelsa* (Brazil nut tree) grows in a background environment with normal radiation levels (Penna-Franca et al., 1968; Smith, 1971). Brazil nuts also contain high levels of barium (0.1–0.3%), which is considered chemically analogous to radium. The level of alkaline earth metals Ra, Th, and lanthanum (La) are closely associated with the level of calcium (Ca) in plants (Million et al., 1994; Linsalata et al., 1989). All these elements are considered immobile and accumulate in plants during growth.

Some ferns, such as *Gleichenia japonica*, *Dicranopteris dichotoma*, and *Struthiopteris niponica*, were identified as accumulators of barium (Ba), actinium (Ac), Ra, and REEs (Kabashi and Tominaga, 1985; Koyama et al., 1987). The fern *D. dichotoma* reportedly accumulated REEs, ranging 0.3–0.7% of its dry leaf biomass (Wang et al., 1997; Shan et al., 2003). Another fern *Dicranopteris linearis* has also been identified as a potential accumulator plant for REEs, which can be as high as 0.1% of its dry biomass (Wei et al., 2005).

A remarkable accumulation of radium isotopes ^{224}Ra, ^{226}Ra, and ^{228}Ra in the fern *D. linearis* was reported by Chao et al. (2006, 2007). The radium concentration in *D. linearis* was estimated to be 10–100 times higher than the reported values for normal plants (Kabata-Pendias and Pendias, 2001). Notably, the concentrations of radium and alkaline earth metals Ca, strontium (Sr), and Ba in *D. linearis* increased with growing time (Chao et al., 2007).

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We suspect this species is a hyper-accumulator of alkaline earth metals, REEs and radium isotopes; these elements/nuclides have similar chemical behaviors.

Plants capable of accumulating heavy metals when growing in non-polluted environments are endemic to metalliferous soils typically found in an edaphically taxon (Wenzel and Jockwer, 1999; Zu et al., 2004). However, few studies investigated that plants accumulate radionuclides naturally occurring in the environment. In this study, concentrations of naturally occurring radionuclides, namely ^{232}Th, ^{238}U and radium isotopes ^{226}Ra and ^{228}Ra, alkaline earth metals, REEs, as well as some heavy metals of environmental interest in *D. linearis* were determined and compiled for statistical analysis. The bioavailability of Ra and some chemical analogues from soil to plant were expressed as a CR, which describes the transfer from soils to plants. First, time-dependent concentrations of these elements/nuclides were determined using the ^{228}Th/^{228}Ra dating technique (Chao et al., 2007), and the corresponding concentrations of these elements in plant samples with different tissue ages were determined. In terms of plant uptake, the correlation between radium concentrations and those of other elements were determined by statistical analysis. Both metal concentrations and corresponding CRs were utilized to identify the elements hyper-accumulated by *D. linearis*.

2. Materials and methods

2.1. Sample collection

Fresh *D. linearis* fronds weighing 200–500 g were sampled during 2002 and 2003 at Shihlin, which is located on the footslopes of Yangmingshan National Park in suburban Taipei, Taiwan. The sampling locality was confined to a 4000 m^2 area [200 m $(L) \times 20$ m (W)], where the population of *D. linearis* is dense. To trace the chronosequential change and accumulation of some elements, fronds growing at different stages were collected for this experiment (Chao et al., 2007). Soils were sampled at a depth of <10 cm, which is commonly assumed to cover the root zone of plants. The pH of the soil was roughly 4.5. For radionuclide and elemental analyses, soil samples were dried at 80 °C and filtered through a 2-mm mesh.

2.2. Element analysis

Sub-samples of soils and plants in ash were dissolved and digested with concentrated hydrofluoric acid (HF) and nitric acid (HNO_3) in a microwave oven (Mars-5X, CEM, USA). The clear solutions were diluted, and concentrations of alkaline earth metals, REEs, heavy metals, and naturally occurring radionuclides (^{232}Th and ^{238}U) were analyzed using an inductively coupled plasma atomic emission spectrometer (ICP-AES) (JY 2000, Horiba Jobin Tvon Inc., France) and mass spectrometer (ICP-MS) (7500ce, Agilent, Japan). Standard solutions (E. Merck, Darmstadt, Germany) were used for determining elemental concentrations.

2.3. Radioactivity measurement

Radioactivity measurements of ^{226}Ra and ^{228}Ra, two long-lived radium isotopes, and ^{228}Th in soil and plant samples were conducted using a 30% germanium detector. The detailed procedures for sample treatment and gamma-ray measurements were described in our previous studies (Chao et al., 2006, 2007). The radioactivities of the naturally occurring radionuclides in this study were converted to concentrations (by weight). Table 1 lists these concentrations and related nuclear data.

Table 1
Relationship of concentrations (by weight) and radioactivity for the naturally occurring radionuclides.

Radionuclide	Decay constant (yr^{-1})	Half-life ($t_{1/2}$) (yr)	Concentration ($g\,g^{-1}$) equivalent to radioactivity of 1 Bq kg^{-1}
^{232}*Th-series*			
^{232}Th	4.91×10^{-11}	1.41×10^{10}	2.50×10^{-7}
^{228}Ra	0.121	5.75	9.91×10^{-17}
^{228}Th	0.363	1.91	3.29×10^{-17}
^{238}*U-series*			
^{238}U	1.55×10^{-10}	4.47×10^{9}	8.00×10^{-8}
^{226}Ra	4.33×10^{-4}	1.60×10^{3}	2.73×10^{-14}

2.4. Plant age determination

The radioactivities of endogenous radionuclide ^{228}Ra and its descendant nuclide ^{228}Th in plants were determined, and the derived ^{228}Th/^{228}Ra ratios were used to infer plant tissue age. Plant tissue age of each sample was estimated using the activity ratio, which was established by Chao et al. (2007). The ^{228}Th/^{228}Ra activity ratio for each sample was measured and corrected to its collection date (Chao et al., 2007). Therefore, concentrations of Ra and its chemical analogues in plants can be associated with tissue age.

2.5. Expression of concentration ratio

Since the elemental concentrations in *D. linearis* varied with growing time, the CR of each element can be expressed as a function of age (t). Because the life span of *D. linearis* is roughly 1 yr (Chao et al., 2007), one can adopt CRs at $t=1$ yr, denoting the time at which the shoots reached maturity, which is when the maximum transfer from soil to plant for linearly accumulating elements occurs.

2.6. Statistical analysis

Statistical analysis was conducted using the statistical package SPSS 13.0. All experiments were performed independently in triplicate, and the coefficient of variation (CV) was used to confirm the accuracy and validity of output data. To understand an index of uptake and metabolism of Ra among the related elements, a multiple regression analysis based on the stepwise method was performed to evaluate the contribution of each chemical analogue to radium accumulation according to plant age after controlling other potential chemical analogues (e.g., alkaline earth metals, REEs, and heavy metals). The analysis of Spearman correlation was conducted to elucidate the correlation between radionuclides and chemical elements. A two-tailed value of $p<0.05$ was considered statistically significant.

2.7. Identification of D. linearis as a hyper-accumulator plant

Some criteria have been proposed to assess plants as hyper-accumulators. Commonly, a hyper-accumulator plant can be defined as follows: (1) the metal concentration in shoots must exceed 1% for Zn and Mn, 0.1% for Al, As, Se, Ni, Co, Cr, Cu, and Pb, and 0.01% for Cd (Baker and Brooks, 1989; Baker et al., 2000); (2) the metal concentration in the above-ground portion of a plant must be 10–500 times higher than in those growing in non-polluted environments (Zu et al., 2005); (3) the ratio of shoot to root concentration must exceed 1 (McGrath and Zhao, 2003;

Zu et al., 2005); (4) concentration ratio (CR) of shoot to root must exceed 1 (McGrath and Zhao, 2003; Zu et al., 2005).

In this study, two of above-mentioned criteria were used to identify *D. linearis* as a hyper-accumulator plant: (I) Metal concentration higher than the threshold concentrations relevant to the potential toxic elements (e.g., Al, As, and Se), which are associated with phyto-remediation techniques for removing contaminated metals from soils. (II) The CR of metal must exceed 1. CR is a measure of the capacity of a plant to take up and transport metals to the shoots. Most plants had CRs of <1 for heavy metals and metalloids. Thus, criterion II was suitable for determining whether *D. linearis* had the ability to uptake naturally occurring radionuclides, alkaline earth metals, and REEs in a non-polluted environment.

3. Results and discussion

3.1. Elemental concentrations in the plant and soil samples

Table 2 lists the concentrations and average values of elements/nuclides for 50 plants and 12 soil samples. Both the concentrations of radionuclides and elements in soils are in normal ranges (Kabata-Pendias and Pendias, 2001), implying that the soils in this area are not metal/radio-contaminated by both industrial and human activities. The CV (%) exhibited the degree of dispersion in concentration. For most elements, the metal concentrations in plants varied more than those in soils, namely $(CV)_p/(CV)_s > 1$, indicating that these element concentrations in shoots varied significantly during plant growth. Notably, $(CV)_p/(CV)_s$ ratios for alkaline earth metals and REEs were higher than those of other elements, likely due to variation in the growing season. On the contrary, elements such as Mg, Al, Cr, Mn, Fe, Co, Ni, Cu, and Zn had smaller concentration fluctuations than other elements; most of these elements are considered nutrient elements to plants (Gardner et al., 1985). The time-dependent variations of these elements are described in detail in the following section.

3.2. Element accumulation pattern with time

Chao et al. (2007) and Wyttenbach et al. (1995) demonstrated that concentrations of Ra (^{226}Ra and ^{228}Ra) and alkaline earth elements Ca, Sr, and Ba increased linearly over time during the growing period. Since the ^{228}Th/^{228}Ra ratio can be used as a measure of plant tissue age, time-dependent variations of all elements in this study were depicted as regression equations

Table 2
Concentrations and distribution of the plant (*D. linearis*) and soil samples.

Elements	Plant concentration (ng g^{-1})			(CV)$_p$ (%)	Soil concentration (ng g^{-1})			(CV)$_s$ (%)	$\frac{(CV)_p}{(CV)_s}$
	Mean	Minimum	Maximum		Mean	Minimum	Maximum		
Naturally occurring radionuclides									
^{226}Ra	1.60×10^{-3}	4.10×10^{-5}	4.53×10^{-3}	83.2	9.15×10^{-4}	4.10×10^{-4}	2.03×10^{-3}	44.6	1.865
^{228}Ra	1.10×10^{-5}	1.16×10^{-6}	2.80×10^{-5}	70.9	5.67×10^{-6}	2.68×10^{-6}	7.35×10^{-6}	22.9	3.096
Th (^{232}Th)	44.5	3.9	447	155	13200	5390	18500	24.8	6.238
U (^{238}U)	13.3	4.8	39.0	56.3	1610	1230	2070	15.6	3.609
Alkaline earth metals									
Mg	565000	348000	868000	19.3	1420000	598000	2660000	50.8	0.380
Ca	373000	22200	1930000	129.1	387000	224000	565000	31.3	4.125
Sr	34000	2320	98800	78.3	95900	42100	143000	29.1	2.691
Ba	320000	24800	1230000	88.2	353000	188000	580000	35.4	2.492
Rare earth elements									
Y	13900	532	69600	109.6	6950	3170	17500	54.9	1.996
La	26400	1000	127000	102.8	25100	9050	84300	78.4	1.311
Ce	49200	1430	281000	109.2	56600	19800	186000	76.3	1.431
Pr	4230	106	24700	114.3	6020	2740	14500	51.0	2.241
Nd	16800	333	96200	121.0	22100	9350	52500	50.1	2.415
Sm	2960	50.0	17700	127.1	4260	2080	8710	41.2	3.085
Eu	707	14.6	3650	119.6	1100	654	1780	32.8	3.646
Gd	3940	81.5	23300	122.0	3790	1750	8840	48.0	2.542
Tb	608	12.7	3900	127.4	704	449	1060	27.2	4.684
Dy	3330	77.5	22700	130.3	2220	1190	4560	40.1	3.249
Ho	587	13.7	4110	131.3	612	340	830	26.6	4.936
Er	1400	28.7	10300	135.0	1170	714	1920	30.2	4.470
Tm	157	4.1	1180	137.0	421	106	611	48.1	2.848
Yb	784	14.1	6030	140.8	975	594	1370	26.5	5.313
Lu	96.3	0.8	741	142.6	359	102	529	48.1	2.965
ΣREE	125000	3710	692000	108.0	13200	53700	385000	64.5	1.674
Heavy metals									
Al	1810000	639000	3830000	45.6	40100000	11200000	70100000	45.6	1.000
Cr	235	43.4	442	40.2	24000	5080	47900	56.5	0.712
Mn	734000	52700	2220000	83.9	84800	35200	218000	68.2	1.230
Fe	71700	37300	141000	36.3	18800000	9850000	34300000	36.1	1.006
Co	93.9	16.6	298	78.9	2220	1200	5920	62.7	1.258
Ni	336	79.6	905	53.4	5840	1520	9680	45.6	1.171
Cu	3440	2000	5700	22.2	9300	4170	14400	28.8	0.771
Zn	18900	9600	36600	34.1	27900	14400	70400	54.2	0.629
As	863	17.4	4390	115.5	9990	4280	13300	24.3	4.753
Se	2770	29.1	16000	122.7	1570	684	3540	50.5	2.430
Cd	101	32.2	270	62.3	80.3	44.0	162	43.4	1.613
Pb	27600	2750	131000	117.4	33700	17900	60900	34.8	3.374

Table 3
Elemental concentrations and CRs in *D. linearis* as the functions of time.

Element	Regression equation of concentration (ng g^{-1})	Regression equation of CR	r	Group
Naturally occurring radionuclides				
^{226}Ra	$C=3.44\times10^{-3}t$	CR=3.763t	0.830	A
^{228}Ra	$C=2.25\times10^{-5}\,t$	$CR=3.978t$	0.821	A
Th (^{232}Th)	$C=75.0t$	$CR=0.005t$	0.141	B
U(^{238}U)	$C=25.4t$	$CR=0.016t$	0.778	A
Alkaline earth metals				
Mg	$C=852934t$	$CR=0.6t$	0.215	B
Ca	$C=789664t$	$CR=2.038t$	0.510	A
Sr	$C=70204t$	$CR=0.732t$	0.779	A
Ba	$C=664791t$	$CR=1.883t$	0.707	A
Rare earth elements				
Y	$C=30188t$	$CR=4.342t$	0.647	A
La	$C=58802t$	$CR=2.341t$	0.751	A
Ce	$C=109185t$	$CR=1.931t$	0.702	A
Pr	$C=9415t$	$CR=1.564t$	0.676	A
Nd	$C=37558t$	$CR=1.701t$	0.649	A
Sm	$C=6613t$	$CR=1.554t$	0.617	A
Eu	$C=1568t$	$CR=1.421t$	0.637	A
Gd	$C=8752t$	$CR=2.306t$	0.630	A
Tb	$C=1354t$	$CR=1.924t$	0.608	A
Dy	$C=7456t$	$CR=3.359t$	0.600	A
Ho	$C=1314t$	$CR=2.148t$	0.597	A
Er	$C=143t$	$CR=2.693t$	0.586	A
Tm	$C=352t$	$CR=0.835t$	0.574	A
Yb	$C=1758t$	$CR=1.803t$	0.560	A
Lu	$C=216t$	$CR=0.603t$	0.556	A
$\sum$REE	$C=277674t$	$CR=30.524t$	0.709	
Heavy metals				
Al	$C=3279976t$	$CR=0.082t$	0.770	A
Cr	$C=336t$	$CR=0.014t$	−0.225	B
Mn	$C=1476753t$	$CR=17.417t$	0.661	A
Fe	$C=126259t$	$CR=0.007t$	0.823	A
Co	$C=165t$	$CR=0.075t$	0.377	B
Ni	$C=564t$	$CR=0.097t$	0.400	B
Cu	$C=4620t$	$CR=0.497t$	−0.571	B
Zn	$C=32936t$	$CR=1.182t$	0.821	A
As	$C=1915t$	$CR=0.192t$	0.663	A
Se	$C=5875t$	$CR=3.730t$	0.581	A
Cd	$C=178t$	$CR=2.306t$	0.481	B
Pb	$C=60274t$	$CR=1.790t$	0.620	A

C: concentration of individual element; CR: concentration ratio of individual element (plant concentration/mean soil concentration); t: growth time (year) of *D. linearis*; r: Pearson correlation between CR of individual element and growth time (t) of *D. linearis* underlying the multiple regression analysis controlling other potential elements.

(Table 3) for each element based on the assumption that all elements accumulated linearly over time. The correlation coefficients of these regression equations were compiled to varying degrees with this assumption. The elements with good linearity ($r > 0.5$) were considered element candidates to be accumulated over time (Group A), while the others (Group B) were not ($r < 0.5$). Elements in Group A were radium, alkaline earth metals (except for Mg), all REEs, and heavy metals Al, Mn, Fe, Zn, As, Se, and Pb. Overall, the $(CV)_p/(CV)_s$ ratios of Group A elements were relatively higher than element ratios of Group B (Table 2), implying that concentrations of Group A elements were age-dependent, and increased with the increase in the growing time.

3.3. *Concentration ratios*

The variations in CRs of these elements are presented as functions of time (Table 3). The concentrations of Group A elements accumulated linearly over time and peaked at the end of 1 year ($t=1$ yr). Most elements in Group A, accumulated linearly during the growth period, are immobile and are similar to calcium. The CRs of most Group A elements exceed 1 (at $t=1$ yr), fulfilling Criterion II for hyper-accumulator plants. Conversely, except for Cd, no element in Group B had a CR >1. Notably, the Al concentration can be as high as 0.328% by weight, exceeding the threshold level of 0.1% for a hyper-accumulator plant (Criterion I); however, the CR of Al was <1. This uptake characteristic for Al was similar to that of the rare and endangered species *Plantago almogravensis* (Branquinho et al., 2007). On the other hand, the Mn concentration (0.15%) was lower than the threshold level of 1.0% in Criterion I, even though it had the highest CR of 17.4.

3.4. *Uptake of radium relative to that of other elements*

3.4.1. *Naturally occurring radionuclides*

Most elements in plants can be readily analyzed by ICP-AES/-MS, while Ra (^{226}Ra and ^{228}Ra) should be determined by the radiometric method using gamma-ray spectrometry because its concentrations in weight were quite low, far below 10^{-11} g/g (Table 1). Radium is an alkaline earth metal and a naturally occurring radionuclide. The concentrations of ^{226}Ra and ^{228}Ra accumulated linearly to 126 Bq/kg (3.44×10^{-12} g) and 228 Bq/kg (2.25×10^{-14} g), respectively, and their CRs were 3.76 and 3.98, respectively (Table 3). Their concentrations (in weight or radioactivity) were remarkably higher than those in normal plants by a factor of 100 (Kabata-Pendias and Pendias, 2001).

The concentrations of ^{232}Th and ^{238}U, the respective parent radionuclides of ^{228}Ra and ^{226}Ra, in *D. linearis* and their CRs were quite low, although ^{238}U had a good correlation with ^{226}Ra ($r=0.810$; $p<0.001$) and ^{228}Ra ($r=0.832$; $p<0.001$) (Table 4). For some crop plants grown in an area with an elevated radiation background, ^{232}Th was considered a chemical analogue of Ca and some transuranic elements in the plant uptake process and a CR value around 10^{-4} was reported (Linsalata et al., 1989). Such a low transfer for Th may be responsible for its associated low distribution coefficient (Sheppard, 1985). The CR of ^{232}Th for *D. linearis* was 5×10^{-3}, the lowest among all elements (Table 3). Overall, the concentrations of ^{232}Th and ^{238}U were comparable to those plants grown in a normal background (Kabata-Pendias and Pendias, 2001). However, ^{232}Th and ^{238}U can be highly concentrated by plants growing in mining areas (Ibrahim and Whicker, 1988a).

3.4.2. *Alkaline earth metals*

Magnesium, an essential element for plants, is at the center of the chlorophyll molecule and an activator of photosynthesis and respiration enzymes (Gardner et al., 1985). Magnesium is somewhat mobile and redistributed from older to younger tissues; its concentration in this study varied little during growth compared to those of other alkaline earth metals (Table 2). With the exception of Mg, a good correlation existed between the other alkaline earth metals Ca, Sr, Ba, and Ra ($r=0.637$–0.838; $p<0.001$) (Table 4). Notably, Ra, Ba, and Sr are chemically similar to Ca, one of the most immobile essential elements in plants (Hanson, 1984; Kirkby and Pilbeam, 1984). In comparison, Ba concentrations in *D. linearis* (24.8–1230 μg g^{-1}) were higher than those in normal plants, while Sr concentrations (2.3–99 μg g^{-1}) did not differ from those in normal plants (Kabata-Pendias and Pendias, 2001). The CRs of Ca and Ba were around 2, and the Ca concentrations in *D. linearis* were in the adequate range (Gardner et al., 1985). Previous studies indicated that Ra-accumulating plants usually contained high amounts of barium. For instance, Ba can be as high

Table 4
Spearman correlation coefficient between naturally occurring radionuclides and alkaline earth metals.

	^{226}Ra	^{228}Ra	Th(^{232}Th)	U(^{238}U)	Mg	Ca	Sr	Ba
^{226}Ra	1.000 –	0.898* $p<0.001$	0.426* $p=0.004$	0.810* $p<0.001$	0.203 $p=0.182$	0.637* $p<0.001$	0.838* $p<0.001$	0.811* $p<0.001$
^{228}Ra	0.898* $p<0.001$	1.000 –	0.374* $p=0.011$	0.832* $p<0.001$	0.295* $p=0.049$	0.678* $p<0.001$	0.835* $p<0.001$	0.706* $p<0.001$
Th(^{232}Th)	0.426* $p=0.004$	0.374* $p=0.011$	1.000 –	0.426* $p=0.004$	0.251 $p=0.096$	0.294* $p=0.050$	0.366* $p=0.014$	0.487* $p<0.001$
U(^{238}U)	0.810* $p<0.001$	0.832* $p<0.001$	0.426* $p=0.004$	1.000 –	0.485* $p<0.001$	0.506* $p<0.001$	0.782* $p<0.001$	0.713* $p<0.001$
Mg	0.203 $p=0.182$	0.295* $p=0.049$	0.251 $p=0.096$	0.485* $p<0.001$	1.000 –	0.058 $p=0.705$	0.292 $p=0.052$	0.126 $p=0.411$
Ca	0.637* $p<0.001$	0.678* $p<0.001$	0.294* $p=0.050$	0.506* $p<0.001$	0.058 $p=0.705$	1.000 –	0.749* $p<0.001$	0.711* $p<0.001$
Sr	0.838* $p<0.001$	0.835* $p<0.001$	0.366** $p=0.014$	0.782* $p<0.001$	0.292 $p=0.052$	0.749* $p<0.001$	1.000 –	0.767* $p<0.001$
Ba	0.811* $p<0.001$	0.706* $p<0.001$	0.487* $p<0.001$	0.713* $p<0.001$	0.126 $p=0.411$	0.711* $p<0.001$	0.767* $p<0.001$	1.000 –

* Correlation is significant at the 0.05 level (2-tailed).

Table 5
Spearman correlation coefficient between rare earth elements and radium.

	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	^{228}Ra	^{226}Ra
Y	1.000	0.927	0.928	0.913	0.913	0.916	0.920	0.935	0.936	0.938	0.941	0.942	0.940	0.926	0.919	0.680	0.707
La	0.927	1.000	0.981	0.950	0.944	0.919	0.925	0.928	0.906	0.903	0.907	0.911	0.907	0.894	0.885	0.776	0.838
Ce	0.928	0.981	1.000	0.978	0.977	0.957	0.957	0.962	0.943	0.940	0.943	0.946	0.943	0.937	0.930	0.728	0.812
Pr	0.913	0.950	0.978	1.000	0.992	0.979	0.983	0.982	0.974	0.972	0.974	0.976	0.971	0.962	0.958	0.702	0.804
Nd	0.913	0.944	0.977	0.992	1.000	0.991	0.992	0.990	0.978	0.976	0.977	0.978	0.975	0.970	0.965	0.677	0.780
Sm	0.916	0.919	0.957	0.979	0.991	1.000	0.995	0.996	0.988	0.985	0.985	0.985	0.982	0.979	0.974	0.640	0.733
Eu	0.920	0.925	0.957	0.983	0.992	0.995	1.000	0.996	0.989	0.988	0.988	0.988	0.987	0.982	0.979	0.646	0.749
Gd	0.935	0.928	0.962	0.982	0.990	0.996	0.996	1.000	0.994	0.992	0.992	0.992	0.990	0.983	0.979	0.651	0.744
Tb	0.936	0.906	0.943	0.974	0.978	0.988	0.989	0.994	1.000	0.999	0.999	0.998	0.994	0.986	0.982	0.637	0.719
Dy	0.938	0.903	0.940	0.972	0.976	0.985	0.988	0.992	0.999	1.000	0.999	0.998	0.994	0.987	0.982	0.633	0.715
Ho	0.941	0.907	0.943	0.974	0.977	0.985	0.988	0.992	0.999	0.999	1.000	0.999	0.996	0.988	0.984	0.634	0.719
Er	0.942	0.911	0.946	0.976	0.978	0.985	0.988	0.992	0.998	0.998	0.999	1.000	0.997	0.990	0.986	0.640	0.729
Tm	0.940	0.907	0.943	0.971	0.975	0.982	0.987	0.990	0.994	0.994	0.996	0.997	1.000	0.995	0.992	0.634	0.728
Yb	0.926	0.894	0.937	0.962	0.970	0.979	0.982	0.983	0.986	0.987	0.988	0.990	0.995	1.000	0.997	0.609	0.714
Lu	0.919	0.885	0.930	0.958	0.965	0.974	0.979	0.979	0.982	0.982	0.984	0.986	0.992	0.997	1.000	0.605	0.713
^{228}Ra	0.680	0.776	0.728	0.702	0.677	0.640	0.646	0.651	0.637	0.633	0.634	0.640	0.634	0.609	0.605	1.000	0.898
^{226}Ra	0.707	0.838	0.812	0.804	0.780	0.733	0.749	0.744	0.719	0.715	0.719	0.729	0.728	0.714	0.713	0.898	1.000

All correlation coefficients are statistically significant.

as 5890 μg g^{-1} in the endosperm of Brazil nuts (Smith, 1971); the Ba varied at 300–2530 μg g^{-1} in the fern *G. japonica* (Kobashi and Tominaga, 1985).

3.4.3. *Rare earth elements*

Almost all REEs in *D. linearis* had remarkably high concentrations, exceeding reported values for other plants (Kabata-Pendias and Pendias, 2001). All REE concentrations were positively correlated with those of ^{226}Ra ($r=0.707$–0.838; $p<0.001$) and ^{228}Ra ($r=0.605$–0.776; $p<0.001$) (Table 5). The REEs have similar ionic radii and electronic configurations, resulting in their chemical characteristics in biogeochemical processes. Notably, the correlation coefficients between REEs (except for Y) and Ra roughly decreased as REEs atomic number increased. The concentrations of REEs in plant tissue usually do not reflect their distribution patterns in host soil due to fractionation (Liang et al., 2001; Ding et al., 2006; Li et al., 1998). Almost all correlation coefficients between the 15 REEs exceeded 0.9 ($p<0.001$), exhibiting a marked similarity in the uptake process by *D. linearis.*

Significant correlations existed between Ba, La, and Ce in some mushroom species, even though their concentrations were in normal ranges (Aruguete et al., 1998). No biological function was associated with REEs and Ba in plants; however, REEs and Ba are considered chemically similar to Ca, and can substitute each other in calcium-coordinating molecules (Martin, 1983; Evans, 1990). The structure of REE-binding biological molecules in both *D. dichotoma* and *D. linearis* has been identified, elucidating the roles REEs play and their distribution in plant tissue (Guo et al., 1996; Shan et al., 2003; Wei et al., 2005). No literature focuses on the role of radium isotopes (^{226}Ra and ^{228}Ra) in these plants. We anticipate that radium can replace REEs in the REE-binding biological molecules despite its low concentrations by weight (Table 1).

The CRs of REEs varied at 0.6–4.34 (Table 3). Most CRs exceeded 1, except for those of thulium (Tm) and lutetium (Lu),

Table 6
Spearman correlation coefficient between the heavy metals and radium.

	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Pb	^{228}Ra	^{226}Ra
Al	1.000	−0.184	0.638*	0.829*	0.407*	0.141	−0.484*	0.871*	0.670*	0.614*	0.680*	0.697*	0.837*	0.785*
Cr	−0.184	1.000	−0.279	−0.062	−0.034	0.068	0.381*	−0.255	−0.363*	−0.358	−0.054	−0.494*	−0.310	−0.240
Mn	0.638*	−0.279	1.000	0.634*	0.72*8	0.443*	−0.645*	0.699*	0.877*	0.908*	0.242	0.609*	0.617*	0.709*
Fe	0.829*	−0.062	0.634*	1.000	0.381*	0.218	−0.482*	0.806*	0.635*	0.570*	0.641*	0.711*	0.813*	0.749*
Co	0.407*	−0.034	0.728*	0.381*	1.000	0.695*	−0.357*	0.439*	0.686*	0.709**	0.088	0.315*	0.296*	0.401*
Ni	0.141	0.068	0.443*	0.218	0.695*	1.000	−0.103	0.293	0.415*	0.384*	−0.090	0.167	0.088	0.225
Cu	−0.484*	0.381*	−0.645*	−0.482*	−0.357*	−0.103	1.000	−0.501*	−0.715*	−0.793*	−0.161	−0.573*	−0.609*	−0.611*
Zn	0.871*	−0.255	0.699*	0.806*	0.439*	0.293	−0.501*	1.000	0.741*	0.684*	0.709*	0.737*	0.832*	0.871*
As	0.670*	−0.363*	0.877*	0.635*	0.686*	0.415*	−0.715*	0.741*	1.000	0.970*	0.259	0.632*	0.663*	0.749*
Se	0.614*	−0.358	0.908*	0.570*	0.709*	0.384*	−0.793*	0.684*	0.970*	1.000	0.230	0.558*	0.592*	0.682*
Cd	0.680*	−0.054	0.242	0.641*	0.088	−0.090	−0.161	0.709*	0.259	0.230	1.000	0.430*	0.612*	0.607*
Pb	0.697*	−0.494*	0.609*	0.711*	0.315*	0.167	−0.573*	0.737*	0.632*	0.558*	0.430*	1.000	0.782*	0.778*
^{228}Ra	0.837*	−0.310	0.617*	0.813*	0.296*	0.088	−0.609*	0.832*	0.663*	0.592**	0.612*	0.782*	1.000	0.898*
^{226}Ra	0.785*	−0.240	0.709*	0.749*	0.401*	0.225	−0.611*	0.871*	0.749*	0.682*	0.607*	0.778*	0.898*	1.000

* Correlation is significant at the 0.05 level (2-tailed).

which were close to the findings in South-Jiangxi Region, in which the CRs for REEs were 0.72–2.47 in a mining area and as high as 10 in the non-mining area (Wei et al., 2001). We believe that the CRs of REEs in *D. linearis* varied with REE concentrations in surrounding soils; the CRs increased when REE concentrations in the environment were relatively low. This uptake behavior resembles that of *Thlaspi caerulescens*, a hyper-accumulator plant that removes Zn and Cd from soils (Zhao et al., 2003).

3.4.4. Heavy metals

As a whole, the heavy metals had more complex uptake patterns by *D. linearis* compared to those of alkaline earth metals and REEs. Among the heavy elements, Al, Mn, Fe, Zn, As, Se, Cd, and Pb had strongest correlations with ^{226}Ra (r=0.607–0.871; $p<0.001$) and ^{228}Ra (r=0.612–0.837; $p<0.001$) (Table 6); all, except for Cd, are members of Group A. The concentration of Al in *D. linearis* was much higher than those of other elements in this study. Aluminum was the only element with a concentration exceeding the threshold concentration (0.1%) for defining a hyper-accumulator plant (Criterion I). Such a high Al concentration in plant tissue can be explained in that for acid soils with low pH (<5.5), a high proportion of cation exchange sites in minerals is occupied by Al, which may replace other polyvalent cations, such as Mg and Ca (Marschner, 1995). The CR of Mn was 17.4, the highest among all elements. However, its concentration did not meet the threshold concentration (1%), as defined by Criterion I. For the Norway spruce, high concentrations of Mn had similar accumulation patterns as Ca, Sr, and Ba (Wyttenbach et al., 1995), even though the Mn concentrations were much less than that of Ca in this study. The CRs of Fe and As were lower than those of other elements in Group A. Although the CRs of Zn, Se, and Pb exceeded 1 and were accumulated by *D. linearis*, their concentrations were in normal ranges (Gardner et al., 1985; Kabata-Pendias and Pendias, 2001). Few studies have dealt with the accumulation of heavy metals related to the Ra and REE-accumulator plants.

3.5. Identification of *D. linearis* as a hyper-accumulator plant

Based on the uptake pattern over time, the elements in this study were classified into Group A (linear accumulation) and Group B (non-linear accumulation). Generally, elements in Group A had higher CVs and CRs, and better correlations ($r>0.5$) with radium than those of elements in Group B, i.e., the elements in Group B had lower CVs and CRs, and weaker correlations ($r<0.5$) with radium. Based on the two criteria for identifying *D. linearis* as a hyper-accumulator plant, Table 7 summarizes the elements hyper-accumulated in *D. linearis*. Only Al exceeded the threshold concentration and thereby met Criterion I. Based on the definition of Criterion II, 22 elements/nuclides, including radium, with CRs >1 were considered hyper-accumulated by *D. linearis*. With the exception of Cd, all these elements are in Group A. This implies

Table 7
Indentification of *D. linearis* as a hyper-accumulator plant.

Element	Group A	Criterion I	Criterion II
Naturally occurring radionuclides			
^{226}Ra	√		√
^{228}Ra	√		√
Th (^{232}Th)			
U(^{238}U)	√		
Alkaline earth metals			
Mg			
Ca	√		√
Sr	√		
Ba	√		√
Rare earth elements			
Y	√		√
La	√		√
Ce	√		√
Pr	√		√
Nd	√		√
Sm	√		√
Eu	√		√
Gd	√		√
Tb	√		√
Dy	√		√
Ho	√		√
Er	√		√
Tm	√		
Yb	√		√
Lu	√		
Heavy metals			
Al	√	√	
Cr			
Mn	√		√
Fe	√		
Co			
Ni			
Cu			
Zn	√		√
As	√		
Se	√		√
Cd			√
Pb	√		√

Criterion I: shoot concentrations > the threshold values of heavy metals.
Criterion II: CR > 1.

that metals be continuously accumulated with time in hyper-accumulator plants during growth.

Most hyper-accumulator plants are metallophytes growing in mineralized areas and have developed mechanisms that allow them to limit uptake of metal concentrations toxic to most plants. Notably, although *D. linearis* is not a metallophyte, it hyper-accumulated radium and some chemical analogues even when grown under a normal radiation condition. Thus, *D. linearis* has potential for use to remove radium and REEs from contaminated soils, in such areas as uranium mining areas or area contaminated with REEs.

4. Conclusions

In this study, the accumulation of radium and its potential chemical analogues by *D. linearis* was investigated in an uncontaminated environment. Radium and the other 21 elements, including most alkaline earth metals, REEs and some heavy metals, were accumulated by *D. linearis*. These elements have the following characteristics in common: (1) linear accumulation over growth time, (2) high soil-to-plant transfer (CR $>$ 1), and (3) strong correlations between these elements and radium. Therefore, these elements are considered to be chemical analogues for radium.

To date, the uptake mechanism for non-essential elements by plants remains unclear. Some of these elements in trace amounts can be assimilated together with their corresponding essential analogues. Little information exists about plants capable of concentrating naturally occurring radionuclides, especially radium, which may be readily taken up by crop plants, and further pose a radiological impact to humans. Barium and REEs have strong correlations with radium and comparable CRs to that of radium. They seem to be meaningful to infer the corresponding radium content in plants. This suggests that a hyper-accumulator plant for Ba and/or REEs can also concentrate radium; all of these elements/nuclides have similar behaviors in the biogeochemical cycle.

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