Soil Bioavailability of Rare Earth Elements and Their Effects on Tree Growth

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Abstract

The application of rare earth elements (REEs) for industrial and agricultural uses has been increased in the last decades. The more commonly used ones are the light REEs such as lanthanum (La), cerium (Ce), praseodymium (Pr) and neodymium (Nd). The ecological studies on REEs mainly focus on crops. It was claimed that the crop yields and qualities were improved after being exposed to REEs. However, the phytotoxicity data of REEs are scarce and their influence on forestry plants have received less attention. The ecosystem and the forestry industry would benefit if the positive effects were exhibited by trees. Moreover, edaphic factors may affect the amount of REEs being absorbed when the plants are exposed to REEs in soil. There is a paucity of information on REE bioavailability in soil. The present study aimed to investigate the effects of REEs on plant growth and their bioavailability in soil. Results obtained could contribute to a safer application of REEs to plants.

The first experiment examined the phytotoxicity of La, Ce, Pr and Nd. REEs reduced the seed germination rate and root elongation of *Brassica chinensis* and *Lolium perenne* within the tested concentration range. Their toxicity to plants was lower when compared with heavy metals. The median effective concentrations (EC50s) of REEs ranged from 9.52 to 20.1 mg/L. The EC50s of La and Ce were significantly lower than those of Pr and Nd. The sensitivity of *B. chinensis* to REEs was similar to that of *L. perenne*. *B. chinensis* may be proposed to be used in the future phytotoxicity bioassay as a standard.

The effects of REEs on the seedlings of *Acacia auriculiformis* and *Eucalyptus citriodora* were investigated. Significant growth stimulation in height, standing leaf

number and biomass production were observed in seedlings grown in soil added with low concentration REEs (1-5 mg/kg). The effects diminished when the REEs application rate was 25 mg/kg and were not significantly different to the control. The difference in the performance of seedlings may be attributed to an increase in absorption at high application rates. The uptake of P was influenced by the presence of REEs, but the uptake of N and mineral elements were similar to the control. The tree seedlings preferred La to the other three REEs, which may be due to their differences in ionic radii. N-fixing species *A. auriculiformis* was expected to grow better than non N-fixing species *E. citriodora*. However, the difference narrowed in the presence of REEs.

Soil pH significantly affected the fractionation of La in soil, while the effect of organic matter content was not significant. There was more La in the exchangeable and carbonated bound fraction (B1 fraction) when the soil was acidic rather than alkaline. The tissue La content had a significant positive relationship with the concentration in B1 fraction. However, the relationships were weak with concentrations in Fe-Mn oxide bound fraction (B2 fraction) and organic and sulphide bound fraction (B3 fraction). The results revealed that REEs in B1 fraction would be the more bioavailable form and REEs were more available under acidic condition.

Overall, the present study provided new information on the toxicity of REEs and their effects on plants and the effects of soil properties on the bioavailability of REEs. REEs should not pose great hazard to plant and they will promote plant growth when they are applied at appropriate concentration and soil conditions.

摘要

稀土元素在工農業的應用日益廣泛，當中以輕元素如鑭(La)、鈰(Ce)、鐠(Pr)及釹(Nd)最爲普遍。過往的研究主要集中在稀土元素對農作物的生態影響，部份研究指出施用稀土元素能增加作物產量及品質，但有關它們對植物的毒性研究卻相當有限，有關林業用品種的研究更少。倘稀土元素能促進樹木生長，對林業以至生態保育將有莫大裨益。另外，由於植物大多透過土壤吸收稀土元素，土壤特性將影響稀土元素的植物吸收及利用，惟相關資料亦欠完整。本研究旨在探討稀土元素對植物的毒性、對林木品種生長的影響及其生物可利用率。並爲稀土元素安全地應用提供科學跟據。

首個實驗透過種子萌芽測試評估鑭、鈰、鐠及釹四種稀土元素的植物毒性。在測試的濃度範圍內，各元素的溶液均抑制了小白菜(*Brassica chinensis*)及多年生黑麥草(*Lolium perenne*)種子的發芽率及胚根生長。與重金屬相比，稀土元素對植物的毒性較低。其半數效應濃度介乎 9.52 至 20.1 mg/L，當中鑭及鈰的半數效應濃度顯著低於鐠及釹。雖然小白菜用於植物毒性測試未受普及，實驗結果顯示小白菜和多年生黑麥草對稀土元素的敏感度相若，因而小白菜也可以作爲模式植物用於毒理性研究。

第二個項目研究稀土元素對耳果相思(*Acacia auriculiformis*)及檸檬桉(*Eucalyptus citriodora*)樹苗生長之影響。於土壤加入低濃度 (1-5 mg/kg)的稀土元素能顯著地促進樹苗生長，在樹高、葉片數目及生物量上都顯著增加。但當投放量增加至 25 mg/kg 時，促進生長的影響則不及投放低濃度之組別，各項生長指標與沒添加稀土元素的對照組沒有明顯差異。這或與稀土元素攝取量隨著添加濃

度上升而增加有關。研究結果還顯示，稀土元素對磷的吸收有影響，但對氮和礦物質的吸收則沒有顯著影響。在四種稀土元素中，樹苗優先吸收鑭，這或與元素的離子半徑有關。另一方面，添加稀土元素縮窄了非固氮植物(檸檬桉)與固氮植物(耳果相思)之間生長的差異。

第三項實驗利用連續提取方法進一步研究土壤特性在稀土元素攝取過程所扮演的角色。土壤的酸鹼度對鑭在土壤中的賦存形態有顯著性影響，但有機質含量對其並無顯著影響。相比於鹼性土壤，酸性土壤中有較多可交換態及碳酸鹽結合態的鑭。鑭在植物組織中的含量與土壤中可交換態及碳酸鹽結合態的鑭含量呈顯著性的正線性關係，但與鐵錳氧化物結合態及有機結合態的含量無顯著相關性。結果顯示，可交換態及碳酸鹽結合態較易被植物吸收利用，而在酸性土壤中，稀土元素的生物可利用的形態較多，生物可利用率也較高。

總的來說，本論文研究了稀土元素的毒性、對植物生長的影響及土壤特性對稀土元素的生物可利用率的影響。稀土元素對植物不構成嚴重的威脅，在合適的應用分量及土壤特性下，稀土元素更可促進植物生長。

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Chapter 1 Introduction

1.1 Definition of rare earth elements

Rare earth elements (REEs) are members of Group IIIA elements in the periodical table (Figure 1.1). The definition of elements being included as REEs has different versions. The term lanthanide is used for the 14 elements with atomic number 58-71 in the periodic table. Lanthanons (Ln) is applied to the 16 elements in the group lanthanum (La) to lutetium (Lu) plus yttrium (Y). Lanthanum is the element before cerium (Ce) having atomic number of 57 and occupancy of the 4*f* electron shell, thus La is usually included as a member of REEs. Yttrium is also a member of Group IIIA and has similar chemical properties to those of REEs. The lightest element in Group IIIA scandium (Sc), shows a sufficiently distinct chemistry, owing to the relatively small radius of its 3+ ion, to warrant separate description. In current ecological usages, REEs refer to La, Y and 14 lanthanides. The symbol, atomic number and atomic weight of REEs are shown in Table 1.1.

REEs are classified into two sub-groups, light rare earth elements (LREEs) and heavy rare earth elements (HREEs) (Table 1.1) (Topp, 1965). The classification is defined by the atomic number and atomic mass of the elements. The elements from La to Eu, with lower atomic numbers and masses, are referred as LREE or called cerium group, while those from Gd to Lu plus Y (higher atomic numbers and masses) are referred as HREE or yttrium group. Occasionally, one more group named middle rare earth elements (MREEs) are used to represent the elements from Sm to Ho.

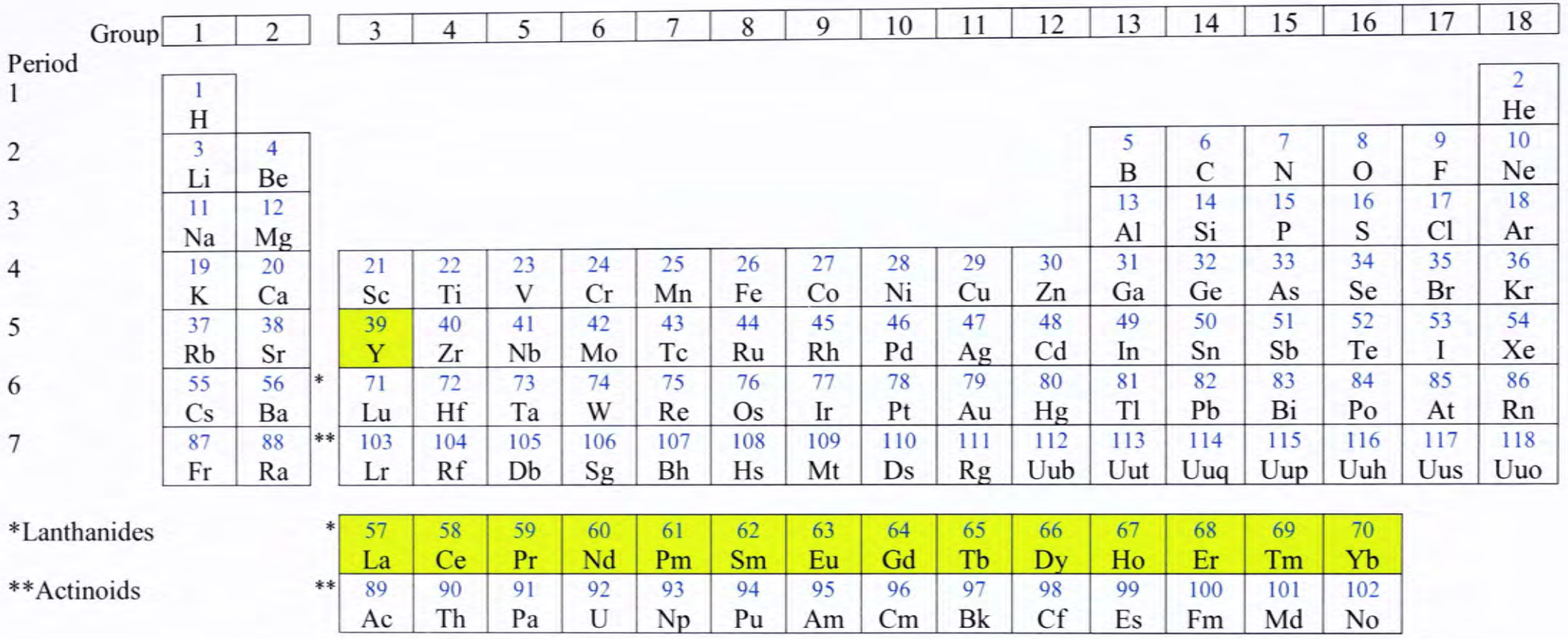


Figure 1.1 Periodic table. Shaded elements are classified as REEs.

Table 1.1 Atomic numbers, atomic weights and descriptive classification of REEs (Topp, 1965).

Element	Symbol	Atomic number	Atomic weight	Descriptive classification	
Lanthanum	La	57	138.91	Light rare earth elements	Light rare earth elements
Cerium	Ce	58	140.12		
Praseodymium	Pr	59	140.91		
Neodymium	Nd	60	144.24		
Promethium	Pm	61	145.00		
Samarium	Sm	62	150.35		Middle rare earth elements
Europium	Eu	63	151.96		
Gadolinium	Gd	64	157.25	Heavy rare earth elements	
Terbium	Tb	65	158.92		
Dysprosium	Dy	66	162.50		
Holmium	Ho	67	164.93		Heavy rare earth elements
Erbium	Er	68	167.26		
Thulium	Tm	69	168.93		
Ytterbium	Yb	70	173.04		
Lutetium	Lu	71	174.97		
Yttrium	Y	39	88.91		

The name, rare earth element, leads to several common misconceptions to the elements. The first most widespread misconception is that they are rare. REEs are not particularly rare comparing with other elements. All REEs are more abundant than Ag, Pt and Au (Topp, 1965; Evan, 1990). Ce is more abundant than Zn, while La, Ce and Nd are more abundant than Pb (Table 1.2). The elements were considered to be rare because pure forms of the element are difficult to be discovered. Due to similarity in their properties, they exist in ores together.

Table 1.2 Comparison of abundance of REEs and other elements in igneous rocks (Topp, 1965).

Element	Atomic number	Abundance (g/tonne)	Element	Atomic number	Abundance (g/tonne)
La	57	19	Be	4	6
Ce	58	44	Co	27	40
Pr	59	5.6	Ni	28	100
Nd	60	24	Cu	29	100
Pm	61	-	Zn	30	40
Sm	62	6.5	As	33	5
Eu	63	1	Mo	42	15
Gd	64	6.3	Ag	47	0.1
Tb	65	1	Cd	48	0.5
Dy	66	4.3	Pt	78	0.005
Ho	67	1.2	Au	79	0.005
Er	68	2.4	Hg	80	0.5
Tm	69	0.3	Pb	82	16
Yb	70	2.6			
Lu	71	0.7			
Y	39	31			

The second misconception is that REEs are regarded as earth elements. Earth elements are substances which possess properties of alkalis, do not float and do not change on heating, are almost insoluble in water and evolve gas bubbles during reaction with water (Evan, 1990; Barrett and Dhesi, 2001). Yttria (yttrium rich mineral) was discovered in certain rare Swedish ores in 1794. Scientists tried to melt yttria and observe any change occurring in them when heated but failed.

The above misconceptions were due to the limitation of separation technology when REEs were discovered. Scientists cannot separate each element from the mixture using old technology. As the extraction and distillation technology

developed, pure REEs can be extracted and so their real properties have been concluded.

1.2 Discovery of REEs

REEs were first discovered in 1794 by J. Gadolin (Greenwood and Earnshaw, 1997; Barrett and Dhesi, 2001). Gadolin isolated yttria which was thought to be an oxide of a single new element. In fact it is a mineral containing predominantly Y and other heavy rare earths. About ten years later, another group of elements named ceria was isolated. Between 1839 and 1843, these two 'elements' were shown by the Swedish surgeon C.G. Mosander to be mixtures of oxides of Sc, Y, La and the fourteen lanthanides (Greenwood and Earnshaw, 1997). Mosander found that the components of the mixtures can be separated by a series of fractional precipitations. The two new oxides found in yttria were named erbia and terbia, and those found in ceria were named lanthana and didymia. The subsequent hundred years were spent in isolating new rare earth from their mixtures. The classical methods separated REEs based on differences in the basicity and solubility of salts. However, the differences were too small to be distinguished easily; these classic methods required tedious repetition of processing (Miranda Jr. *et al.*, 2002). Some of the elements required thousands of times to be isolated. Tm required 15000 fractional crystallizations to be purified out. As the separation technology advanced, many compounds previously considered pure actually contained a mixture of more than one REEs. Didymia was shown to be a mixture of the oxides of Sm, Pr, Nd and Eu. Erbia and terbia were mixtures of the

oxides of Yb, Ho, Tm, Dy and Lu. The discoveries of individual REE are shown in Table 1.3.

1.3 Physical and chemical properties of REEs

REEs appear as typical metals. They are metallic lustre and silvery in appearance (except Eu and Yb which are pale yellow). They are rather soft but become harder across the series, in the order of increasing hardness are Ce, Nd, Pr and Sm. Ce is as hard as Sn, while Sm has approximately the hardness of Fe.

The number of isotopes which was occurring naturally alters between few and several for odd and even atomic number respectively (Table 1.4). It has the same pattern as the natural abundance of the elements that elements with even atomic number were more abundant.

The REEs are highly electropositive. The predominant ionic form is the trivalent cation Ln^{3+}. The prevalence of the +3 oxidation state is a result of the stabilizing effects exerted on various orbitals by increasing ionic charge. The stabilizing effect on the orbitals is in the order of $4f > 5d > 6s$. When an ionic charge of +3 is reached, the $6s$ and $5d$ orbitals are emptied. The electrons remaining in the $4f$ orbitals are themselves so far less extent so that allowing loss of a further electron. Most of the ions at divalent and tetravalent states are not stable enough to exist for a long time; only Ce^{4+} and Eu^{2+} are stable enough to exist in aqueous solution. Ce^{4+} has emptied $4f$ orbital while Eu^{2+} has fully filled the $4f$ orbital that both are stable states.

Table 1.3 Discovery of the REEs (Greenwood and Earnshaw, 1997; Barrett and Dhesi, 2001).

Element	Year of identification	Discoverer	Origin of name
From ceria			
Lanthanum	1839	C.G. Mosander	Lanthanein: to lie hidden
Cerium	1803	C.G. Mosander	Ceres: the asteroid
Praseodymium	1885	C.A. von Welsbach	Prasios: green; dymium: twin
Neodymium	1885	C.A. von Welsbach	Neo: new; Dymium=twin
Promethium	1947	M.G. Coryell	Prometheus: the Greek god who stole fire from heaven for men's use
Samarium	1879	L. de Boisbaudran	Samarskite: the mineral
Europium	1889	E.A. Demarcay	Europe
From yttria			
Gadolinium	1880	J.C.G. de Marignac	Finnish chemist, J. Gadolin
Terbium	1843	C.G. Mosander	After the town Ytterby in Sweden
Dysprosium	1886	L. de Boisbaudran	Dysprositos: hard to get at
Holmium	1879	P.T. Cleve	Holmia, Latin of Stockholm
Erbium	1843	C.G. Mosander	After the town Ytterby in Sweden
Thulium	1878	P.T. Cleve	After Thule, the Roman name for the northern-most region of the inhabitable world
Ytterbium	1878	J.C.G. de Marignac	After the town Ytterby in Sweden
Lutetium	1907	C.A. von Welsbach	Lutetia, Latin for Paris
Yttrium	1794	C.G. Mosander	After the town Ytterby in Sweden

Table 1.4 Some properties of REEs.

Properties	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Y
Atomic number	57	58	59	60	61	62	63	64	65	66	67	68	69	70	81	39
Number of isotopes	2	4	1	7	-	7	2	7	1	7	1	6	1	7	2	1
Outer electron configuration	$5d^1$ $6s^2$	$4f^2$ $6s^2$	$4f^3$ $6s^2$	$4f^4$ $6s^2$	$4f^5$ $6s^2$	$4f^6$ $6s^2$	$4f^7$ $6s^2$	$4f^7$ $5d^1$ $6s^2$	$4f^9$ $6s^2$	$4f^{10}$ $6s^2$	$4f^{11}$ $6s^2$	$4f^{12}$ $6s^2$	$4f^{13}$ $6s^2$	$4f^{14}$ $6s^2$	$4f^{14}$ $5d^1$ $6s^2$	$4d^1$ $5s^2$
Melting point (°C)	920	798	931	1021	1042	1074	822	1313	1365	1412	1474	1529	1545	819	1663	1552
Boiling point (°C)	3469	3433	3520	3074	3000	1794	1429	3273	3230	2567	2700	2868	1950	1196	3402	3030
Ionization energies ($kJmol^{-1}$)																
1st	538	541	522	530	536	542	547	595	569	567	574	581	589	603	513	616
2nd	1067	1047	1018	1034	1052	1068	1085	1172	1112	1126	1139	1151	1163	1175	1341	1181
3rd	1850	1940	2090	2128	2140	2285	2425	1999	2122	2230	2221	2207	2305	2408	2054	1980
Density (25°C)/gcm^{-3}	6.70	6.77	6.77	7.00	6.47	7.52	5.23	7.90	8.23	8.55	8.80	9.07	9.32	6.97	9.84	4.47
Unpaired 4f electrions	0	1	2	3	4	5	6	7	6	5	4	3	2	1	0	0
Magnetic moment	0.00	2.56	3.62	3.68	2.83	1.60	3.45	7.94	9.70	10.6	10.6	9.60	7.60	4.50	0.00	0.00
Colour of Ln^{3+}	-	-	Yellowish -green	Reddish	Pink; yellow	Yellow	-	-	-	Yellow	Pink; yellow	Reddish	Pale green	-	-	-

-: Colourless

The ionic radii of ions decline as the atomic number of the elements increase. Such phenomenon is called lanthanide contraction. It occurs because electrons are progressively added to the inner orbital, the directional characteristics of the 4*f* orbitals cause the 4*f* electrons incompletely shield themselves and other 4*f* electrons from the nuclear charge. Number of proton increases with the atomic number. Each unit increase in nuclear charge produces a net increase in attraction for the whole extranuclear electron charge cloud. The entire ionic structure therefore contracts.

The melting points as well as the densities of REEs generally increase with the atomic number. Marked anomalies are shown by Eu and Yb for melting point because they can exist as bipositive metals.

The magnetic moments of REEs arise from the presence of 4*f* electron with unpaired spins. Gd^{3+} has more unpaired 4*f* electrons than other REE ion, but the magnetic moment is not the highest. It is because the 4*f* electrons are sufficiently well screened that both their spins and their orbital motions about their nuclei contribute to the magnetic moment. The REEs contains two magnetic maxima. Tb^{3+}, Dy^{3+}, Ho^{3+} and Er^{3+} are among the strongest paramagnetic ions. Such property allows REEs to be a great material to make magnet.

REEs form insoluble carbonates, fluorides, hydroxides, oxalates and phosphates (Todorovsky *et al.*, 1997). They form soluble chlorides, nitrates and perchlorates.

Solubility generally decreases down the series while opposite trend is observed for salts of strong-acid anions, such as sulphate.

Many REE ions give out beautiful colours. Electronic transitions in the 4*f* orbitals give rise to characteristic absorption spectra. Nd^{3+}, Sm^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+} and Tm^{3+} absorb light in the visible region and are coloured (Table 1.4).

1.4 Abundance of REEs on earth

REEs are always found as associated groups in minerals and rocks, in no case is one of the REEs found in complete isolation (Aide and Pavich, 2002; Parsons *et al.*, 2005). There are more than 200 minerals in the world containing REEs. These ores may be classified in one of three groups according to their total REE content:

(1) Minerals with major and usually essential contents of the REEs. This group includes more than 70 minerals which are composed of all REE species and some lanthanide-rich equivalents of low-REE minerals.

(2) Minerals with minor but not essential contents of the REEs. Around 200 minerals fall in this class.

(3) Minerals with very low concentrations of REEs. Common rock-forming minerals are members of this category. Relative REE abundance may be inferred from distribution coefficient values.

The main factor affecting distribution of REEs in minerals is the structure of the minerals (Humphris, 1984; Greenwood and Earnshaw, 1997; Ramesh *et al.*, 2000).

Minerals with high coordination number (10-12) prefer to select light REEs; those with low coordination number (as low as 6) are yttrium-selective; and those with intermediate number (7-9) have complex composition, with both light and heavy REEs present. Ionic radius of the element also affects the distribution. Only elements with suitable ionic radius can bind to the minerals. Minerals usually modify their structure to accommodate the REEs. Xenotime is tetragonal composed of yttrium phosphate. This structural modification is required to accommodate the heavier but smaller atoms of the yttrium group. Table 1.5 lists the major mineral-containing REEs. Among the minerals listed, bastnasite, monazite and xenotime are the most commonly known minerals.

1.4.1 Bastnasite

Bastnasite is a kind of carbonates with fluoride mineral, which is greasy, wax-yellow to reddish-brown. There are several varieties of bastnasite: bastnasite-(Ce) (with formula of (Ce, La)CO_3F), bastnasite-(La) (with formula of (La, Ce)CO_3F) and bastnasite-(Y) (with formula of (Y, Ce)CO_3F). The former two highly prefer to select light REE (Figure 1.2), while the latter one is an exception. Bastnasite-(Y) selects yttrium which is classified as a heavy REE, rather than other light REEs.

Bastnasite is made up of stacks of carbonate ion layers and cerium fluoride layers. The carbonate layers are complex with angled carbonate triangular groups. The CeF layers form flat hexagonal sheets with each cerium bonded to three fluorines.

Table 1.5 Major minerals containing REEs (Clark, 1984).

Mineral	Formula	Crystal system
Aeschynite	$(Ce,Ca,Fe,Th)(Ti,Nb)_2(O,OH)_6$	orthorhombic (metamict)
Agardite	$(Y,Ca)Cu_6(AsO_4)_3(OH)_6.3H_2O$	hexagonal
Agrellite	$NaCa_2Si_4O_{10}F$	triclinic
Allanite	$(Ce,Ca,Y)_2(Al,Fe^{2+},Fe^{3+})_3(SiO_4)_3OH$	monoclinic
Ancylite	$SrCe(CO_3)_2OH.H_2O$	orthorhombic
Apatite	$Ca_5(PO_4)3_F$	hexagonal
Ashcrpftine	$KNa,CaY_2Si_6O_{12}(OH)_{10}.4H_2O$	tetragonal
Bastnasite	$(Ce,La)(CO_3)F$	hexagonal
Belovite	$(Sr,Ce,Na,Ca)_5((PO_4)_3OH$	hexagonal
Braitschite	$(Ca,Na_2)_7(Ce,La)_2B_{22}O_{43}.7H_2O$	hexagonal
Brannerite	$(U,Ca,Ce)(Ti,Fe)_2O_6$	Monoclinic (metamict)
Britholite	$(Ce,Ca)_5(SiO_4,PO_4)_3(OH,F)$	hexagonal
Brockite	$(Ca,Th,Ce)PO_4.H_2O$	hexagonal
Burbankite	$(Na,Ca,Sr,Ba,Ce)_6(CO_3)_5$	hexagonal
Calkinsite	$(Ce,La)_2(CO_3)_3.4H_2O$	orthorhombic
Cappelenite	$BaY_6B_6Si_3O_{25}$	hexagonal
Carbocernaite	$(Ca,Ce,Na,Sr)CO_3$	orthorhombic
Caysichite	$(Y.Ca)_4Si_4O_{10}(CO_3)_3.4H_2O$	orthorhombic
Cerianite	$(Ce^{4+},Th)O_2$	cubic
Cerite	$(Ce,Ca)_9(Mg,Fe^{2+})Si_7(O,OH,F)_{28}$	trigonal
Cerotungstite	$CeW_2O_6(OH)_3$	monoclinic
Chevkinite	$(Ca,Ce,Th)_4(F^{e2+},Mg)_2(Ti,Fe^{3+})_3Si_4O_{22}$	monoclinic
Chukhrovite	$Ca_3(Y,Ce)Al_2(SO_4)F_{13}.10H_2O$	cubic
Churchite	$YPO_4.2H_2O$	monoclinic
Cordylite	$(Ce,La)_2Ba(CO_3)_3F_2$	hexagonal
Davidite	$(La,Ce)(Y,U,Fe^{2+})(Ti,Fe^{3+})_{20}(O,OH)_{38}$	trigonal (metamict)
Donnayite	$Sr_3NaCaY(CO_3)_6.3H_2O$	triclinic
Dysanalyte	$(Ca,Ce,Na)(Ti,Nb,Ta)O_3$	cubic
Eudialyte	$(Ca,Na,Ce)_5(Zr,Fe)_2Si_6(O,OH,Cl)_{20}$	trigonal
Euxenite	$(Y,Ca,Ce,U,Th)(Nb,Ta,Ti)_2O_6$	orthorhombic (metamict)
Ewaldite	$Ba(Ca,Y,Na,K)(CO_3)_2$	hexagonal
Fergusonite	$(Y,Er)(Nb,Ta)O_4$	tetragonal (metamict)
Fersmite	$(Ca,Ce,Na)(Nb,Ti,Fe,Al)_2(O,OH,F)_6$	orthorhombic
Florencite	$CeAl_3(PO_4)_2(OH)_6$	trigonal
Fluocerite	$(Ce,La)F_3$	hexagonal
Formanite	$(Y,Er)(Ta,Nb)O_4$	tetragonal (metamict)
Gadolinite	$(T,Ce)_2Fe^{2+}Be_2Si_2O_{10}$	monoclinic
Gagarinite	$NaCaY(F,Cl)_6$	trigonal
Hellandite	$(Ca,Y)_6(Al,Fe^{3+})Si_4B_4O_{20}(OH)_4$	monoclinic
Hibonite	$(Ca,Ce)(Al,Ti,Mg)_{12}O_{19}$	hexagonal

Table 1.5 Major minerals containing REEs (continued).

Huanghoite	$CeBa(CO_3)_2F$	hexagonal
Iimoriite	$Y_5(SiO_4)_3(OH)_3$	triclinic
Ilimaussite	$Ba_2Na_4CeFeNb_2Si_8O_{28}.5H_2O$	monoclinic
Ilmajokite	$(Na,Ce,Ba)_2TiSi_3O_5(OH)_{10}.nH_2O$	monoclinic
Iraqite	$(K,La,Ce,Th)(Ca,Na,La)_2Si_8O_20$	hexagonal
Joaquinite	$Ba_2NaCe_2Fe^{2+}(Ti,Nb)_2Si_8O_{26}(OH,F)\cdot H_2O$	monoclinic
Kainosite	$Ca_2(Y,REE)_2(Si_4O_{12})CO_3\cdot H_2O$	orthorhombic
Karnasurtite	$(Ce,La,Th)(Ti,Nb)(Al,Fe^{3+})(Si,P)_2O_7$	hexagonal
Keilhauite	$(Ca,Y,Ce)(Ti,ALFe^{3+})SiO_5$	monoclinic
Knopite	$(Ca,Ce)TiO_3$	cubic
Lanthanite	$(La,Ce)_2(CO_3)_3.8\ H_2O$	orthorhombic
Laplandite	$Na_4CeTiPSi_7O_{22}.5H_2O$	orthorhombic
Loparite	$(Ce,Na,Ca)(Ti,Nb)O_3$	orthorhombic
Loveringite	$(Ca,Ce)(Ti,Fe^{3+},Cr,Mg)_{21}O_{38}$	trigonal (metamict)
Mckelveyite	$Na_2Ba_4(Y,Ca,Sr,U)_3(CO_3)_9\cdot 5H_2O$	triclinic
Melanocerite	$Ce_4CaBSi_2O_{12}(OH)$	hexagonal (metamict)
Monazite	$(Ce,La)PO_4$	monoclinic
Mosandrite	$(Na,Ca,Ce)_3(Ti(SiO_4)_2F$	monoclinic (metamict)
Nordite	$Na_3Ce(Sr,Ca)(Mn,Mg,Fe,Zn)_2Si_6O_{18}$	orthorhombic
Okangoanite	$(Na,Ca)_3(Y,Ce,Nd,La)_{12}Si_{12}B_2O_{27}F_{14}$	hexagonal
Parisite	$(Ce,La)_2Ca(CO_3)_3F_2$	hexagonal
Perrierite	$(Ca,Ce,Th)_4(Mg,Fe^{2+})_2(Ti,Fe^{3+})_3Si_4O_{22}$	monoclinic
Phosinaite	$H_2Na_3(Ca,Ce)(SiO_4)PO_4$	orthorhombic
Polycrase	$(Y,Ca,Ce,U,Th)(Ti,Nb,Ta)_2O_6$	orthorhombic (metamict)
Polymignite	$(Ce,Fe,Y,Th)(Nb,Ti,Ta)O_4$	orthorhombic (metamict)
Priorite	$(Y,Ca,FeTh)(Ti,Nb)_2(O,OH)_6$	orthorhombic (metamict)
Pyrochlore	$(Na,Ca,Ce)_2Nb_2O_6(OH,F)$	cubic
Retzian	$Mn_2Y(AsO_4)(OH)_4$	orthorhombic
Rhabdophane	$(Ce,La)PO_4.H_2O$	hexagonal
Rontgenite	$(Ce_3,La)_3Ca_2(CO_3)_5F_3$	hexagonal
Sahamalite	$(Mg,Fe)(Ce,La)_2(CO_3)_4$	monoclinic
Samarskite	$(Y,Ce,U,Fe)(Nb,Ta,Ti)_2(O,OH)_6$	orthorhombic (metamict)
Saryarkite	$Ca(Y,Th)Al_5(SiO_4)_2(PO_4,SO_4)_2(OH)_7.6H_2O$	hexagonal
Sazhinite	$Na_3CeSi_6O_{15}\cdot 6H_2O$	orthorhombic
Scheteligite	$(Ca,Fe,Mn,Bi,Y)_2(Ti,Ta,Nb,W)_2(O,OH)_7$	orthorhombic
Semenovite	$(Ca,Ce,La,Na)_{10\text{-}12}(Fe^{2+},Mn)(Si,Be)_{20}(O,OH,F)_{48}$	orthorhombic
Spencite	$(Y,Ca,La,Fe)_5(Si,B,Al)_3(O,OH,F)_{13}$	hexagonal (metamict)
Steenstrupine	$(Ce,cLa,Na,Mn)_6(SiP)_6O_{18}OH$	trigonal
Stillwellite	$(Ce,La,Ca)BSiO_5$	trigonal
Synchysite	$(Ce,La)Ca(CO_3)_2F$	hexagonal

Table 1.5 Major minerals containing REEs (continued).

Tengerite	$CaY_3(CO_3)_4(OH)_3 \cdot 3H_2O$	monoclinic
Thalenite	$Y_3Si_3O_{10}(OH)$	monoclinic
Thortveitite	$(Sc,Y)_2Si_2O_7$	monoclinic
Tombarthite	$Y_4(Si,H_4)_4O_{12-x}(OH)_{4+2x}$	monoclinic
Tornebohmite	$(Ce,La)_3Si_2O_8OH$	hexagonal
Tranquillityite	$Fe_8(Zr,Y)_2Ti_3Si_3O_{24}$	monoclinic
Tritomite	$(Ce,La,Y,Th)_5(Si,B)_3(O,OH,F)_{13}$	hexagonal (metamict)
Tundrite	$Na_3(Ce,La)_4(Ti,Nd)_2(SiO_4)_2(CO_3)O_{34}(OH) \cdot 2H_2O$	triclinic
Tveitite	$Ca_{1-x}Y_xF_{2+x}$	monoclinic
Vitusite	$Na_3(Ce,La,Nd)(PO_4)_2$	orthorhombic
Xenotime	YPO_4	tetragonal
Yttrocerite	$(Ca,Ce)F_2$	cubic
Yttrocrasite	$(Y,Th,Ca,U)(Ti,Fe^{3+})_2(O,OH)_6$	orthorhombic (metamict)
Yttrofluorite	$(Ca,Y)F_2$	cubic
Yttrotantalite	$(Y,U,Fe)(Ta,Nb)O_4$	monoclinic (metamict)
Yttrotungstite	$YW_2P_6(OH)_3$	monoclinic
Zhonghuacerite	$Ba_2Ce(CO_3)_3F$	trigonal
Zircon	$(Zr,Y)(Si,P)O_4$	tetragonal

1.4.2 Monazite

Monazite is a kind of phosphate minerals with formula of (Ce, La)PO_4. It is yellow to reddish-brown colour. The mineral composed of orthophosphates of La, Ce, Pr, Nd, Sm and Eu. Monazite has strongly selectivity on light REE on its composition (Figure 1.2). The monazite structure will accept REE ions with ionic radii among those of La and Eu (Clark, 1984). However, heavy REE can also be found in monazite that Th and U were observed in monazite from a bay in Brazil (Wasserman *et al.*, 2001)

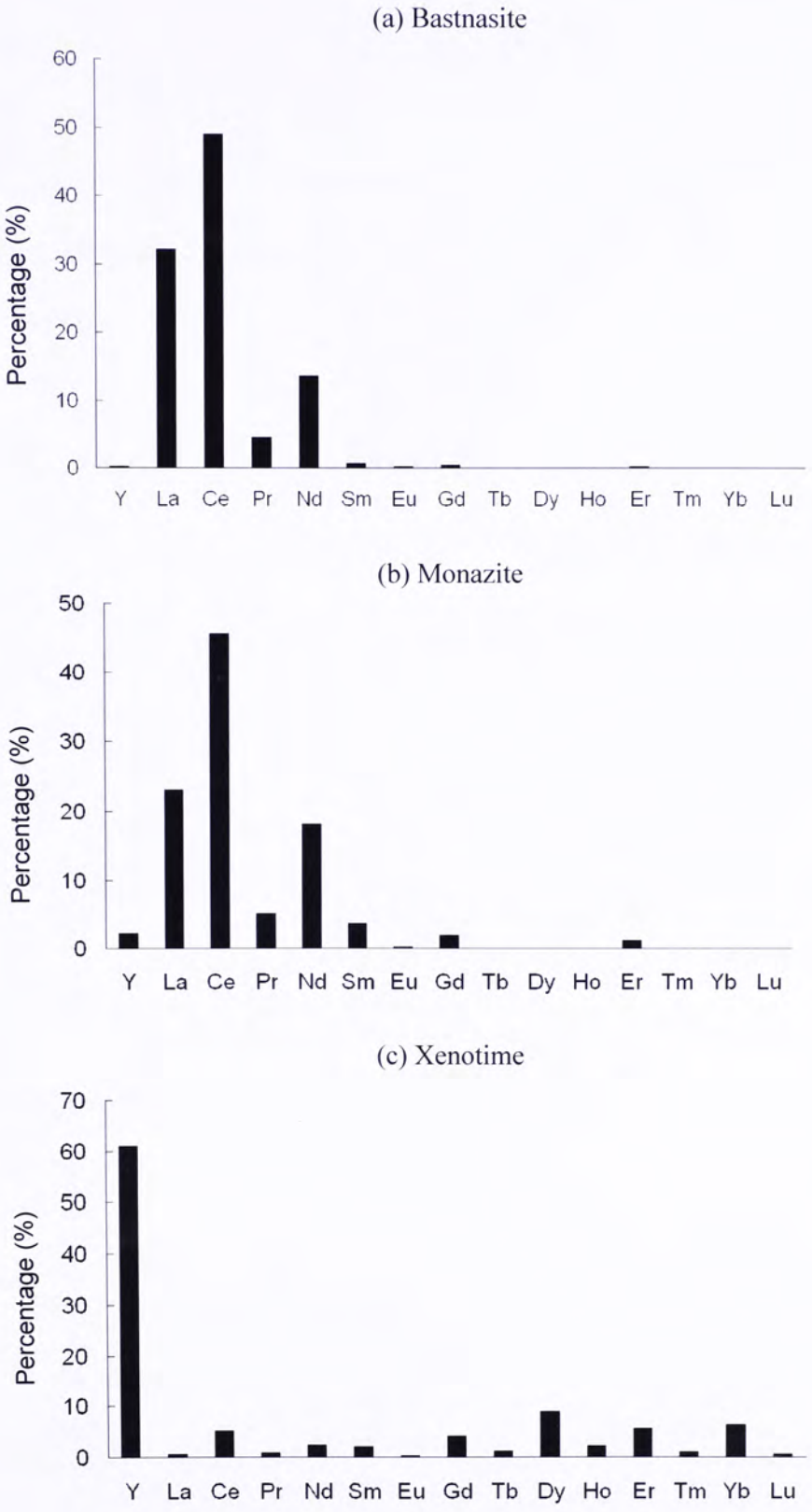


Figure 1.2 Rare earth contents of principal minerals, as percentage of total rare earth oxides (Doyle *et al.*, 2000).

Monazite is a very widespread form in phosphatic pegmatites. It is a standard trace constituent in many ordinary igneous, metamorphic and vein filling rocks. Monazite is radioactive. The radiation may produce destructive effects on its crystal lattice structure completely but leaving the outward appearance of the crystal unchanged.

1.4.3 Xenotime

Xenotime is also a phosphate mineral composed of yttrium whose formula is YPO_4 (Figure 1.2). The crystal of mineral is tetragonal and typically translucent to rarely transparent in shades of brown to yellowish brown, but sometimes greenish brown, muted red and yellow.

Xenotime is a widespread REE mineral occurring in acid and alkaline igneous rocks, metamorphic rocks and pegmatites. It is a major source of Y, though U or other REEs (such as erbium and thorium) may replace some Y. Xenotime is often slightly radioactive since the mineral contains radioactive elements.

1.5 Reserves and resources

1.5.1 World reserves

Ores containing REEs are distributed worldwide such as in Asia, South Africa and Australia (Haxen *et al.*, 2005). The ores in these regions provide most of the world's La and lanthanides. The major ore types in different countries (except China) were identified in the assessment by the US Bureau of Mines and US Geological

Survey (Table 1.6). In 1949, a vast deposit of bastnasite has been discovered in the Sierra Nevada Mountains in the US (Greenwood and Earbshaw, 1997). The total reserves of rare earth ore deposits in the world are over 43.5 million tonnes in 1989 (Wang and Dou, 1996) and 100 million tonnes in 1993 (Chengdu Beyond Chemical Co. Ltd., 2001).

Table 1.6 The major ore type in different countries (Neary and Highley, 1984).

Countries	Major ore type
Australia	Heavy mineral placers (monazite)
Brazil	Beach placers, carbonatite and alkaline rocks
Canada	Uranium ores
Egypt	Fluviatile placers (monazite)
Finland, Norway, Sweden	Carbonatite, alkaline rocks
India	Beach placers (monazite)
Korea	Placers (monazite)
Madagascar	Beach placers (monazite)
Malawi	Carbonatite
Malaysia	Alluvial tin placers (monazite and xenotime)
Sri Lanka	Beach placers (monazite)
South Africa	Monazite vein
US	Bastnasite-bearing carbonatite
Former U.S.S.R.	By-product of apatite processing

The data did not include the reserves of REE deposits in China and in southern Australia.

Besides China, the Russian Federation has possessed the largest reserves of REEs (Table 1.7). The US Geological Survey estimated the country's reserves as 19 million tonnes, represented around 19% of the world's total reserves. The major mineral types were loparite and apatite.

Table 1.7 World reserves of rare earth oxides (Wang and Duo, 1996; Chengdu Beyond Chemical Co. Ltd., 2001).

Countries	Reserves	
	1989 (%)	1993 (%)
China	80.0	43.0
Russian Federation	1.00	19.0
US	12.3	13.0
Australia	1.50	5.20
India	4.00	1.10
Canada	0.36	0.94
South Africa	-	0.39
Brazil	0.04	0.28
Malaysia	0.07	0.03
Srilanka	-	0.01
Others	0.39	21.0
Total	45.0 million tonnes	100 million tonnes

The US is estimated to have 13% of the world's total reserves in 1993. The largest deposit of REE is found in Mountain Pass, California, in which the major mineral resource is bastnasite. For monazite, the largest deposits are found in Florida and in the Atlantic continental shelf sediments.

1.5.2 REE resources in China

Since the 1930s, ore deposits in China have been discovered. However, major deposits were identified after 1950s. China is suggested the world's largest reserve of rare earth ores at present. The reserve amounts to 36 million tonnes of rare earth ores, making up nearly 80% of the world's total reserves in 1989 (Table 1.7). Even though there have been discoveries of rare earth deposits in Australia, the US and Canada, China still has the largest country reserves of REEs in the world. The type,

distribution and date of discovery of China's major rare earth deposits are listed in Table 1.8.

Table 1.8 Major rare earth deposits in China (Wang and Dou, 1996).

Type	Average rare earth ore content	Date of discovery
Mixed rare earth ores	6%	1935
Bastnasite	2.75%	1960s
Monazite	750 g/m^3	Early 1950s
Xenotime	140 g/m^3	Early 1950s
Rare earth-containing collophanite	0.095%	Early 1980s

The largest rare earth deposits in China are deposits in Baiyunebo located 135 km north of Baotou (Chengdu Beyond Chemical Co. Ltd., 2001). It is also the world's largest rare earth deposit. The recoverable reserves amount to 32.2 million tonnes. It is believed that the total deposit may exceed 91 million tonnes. Baiyunebo ore mine is a mixed rare earth ore containing mainly light REEs. The two major minerals contained in Baiyunebo deposit are bastnasite and monazite.

Bastnasite [$(Ce,La)(CO_3)F$] is dispersed in Weishan County, Shandong Province, and Mianning Conty, Sichuan Province. The deposit has been proved to be of high purity and coarse simple mineral bastnasite, and thus favours the separation of REEs from the associated minerals that contain lead, molybdenum and bismuth.

Monazite [(Ce,La,Nd,Th)(PO_4)] and xenotime [YPO_4] are discovered in the Guangdong, Guangxi, Hainan and Hunan provinces. The industrial reserves of REEs contained in monazite amount to 435000 tonnes. Nanshanhai mine located in Guangdong is the main place for extracting monazite and xenotime. The mines contain 1084 g/m^3 monazite and 200 g/m^3 xenotime. High-grade REEs were obtained in the minerals generated from the mine.

1.6 Production and demand of REEs

REEs are applied in a wide aspect nowadays. Their demand is high, with an annual figure of 63000 tonnes in the world (Han *et al.*, 1999). Figure 1.3 shows the countries and regions that consumed REEs. The largest consuming country is the US which accounts for 27% of the total demand (Great Western Minerals Group Ltd., 2006).

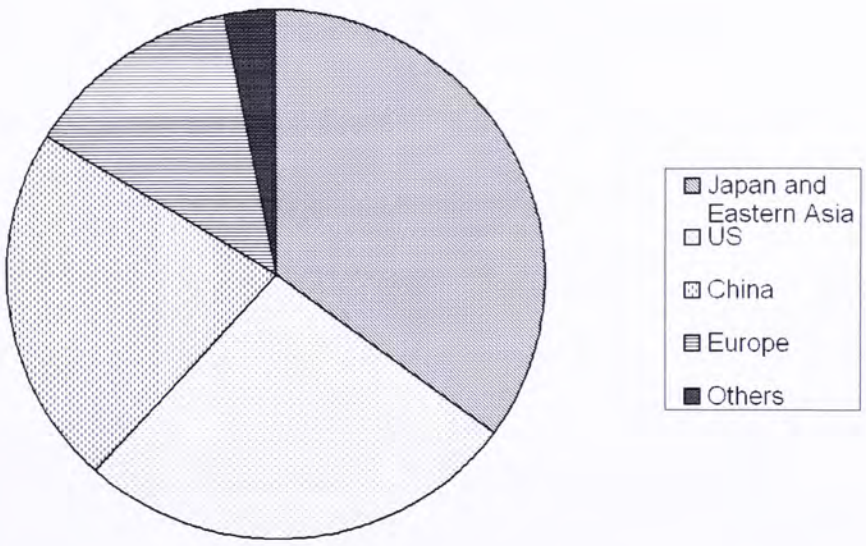


Figure 1.3 Distribution of the REE demand (by region) (Han *et al.*, 1999).

1.6.1 Production and demand in the US

In 1965, a large rare earth deposit was discovered in the Mountain Pass, California of the US. The US Bureau of Mines identified that the light-brown heavy mineral was bastnasite (Hedrick, 1997). Such discovery tremendously rose the quantity of REE produced in the US (Neary and Highley, 1984). Production of REEs in the US became the most important around the world in a very short time (Figure 1.4). The production of REE minerals was nearly 17000 tonnes in 1980 and 20000 tonnes in 1996 (Table 1.9). Destinations of REEs exported from the US included more than 30 countries and cities. Most of the REEs were exported to developed countries, such as Canada, Germany, France and Japan.

The US is not only one of the largest producers of REEs, but also one of the greatest consumers (Great Western Minerals Group Ltd., 2006). The country consumes 18200 tonnes REEs each year. Even the production quantity of REEs in the US is dominant in the world, the quantity of imported REEs exceeds that of production. In 1996, the net import weight increased to 9352 tonnes, which was almost 70% more than that in 1995 (Table 1.9). The major countries that export REEs to the US are China, France, India and Japan. China has become the greatest export country to the US since 1995. More than 90% of the REEs needed by the US were imported from China in 1990 (Haxel *et al.*, 2005).

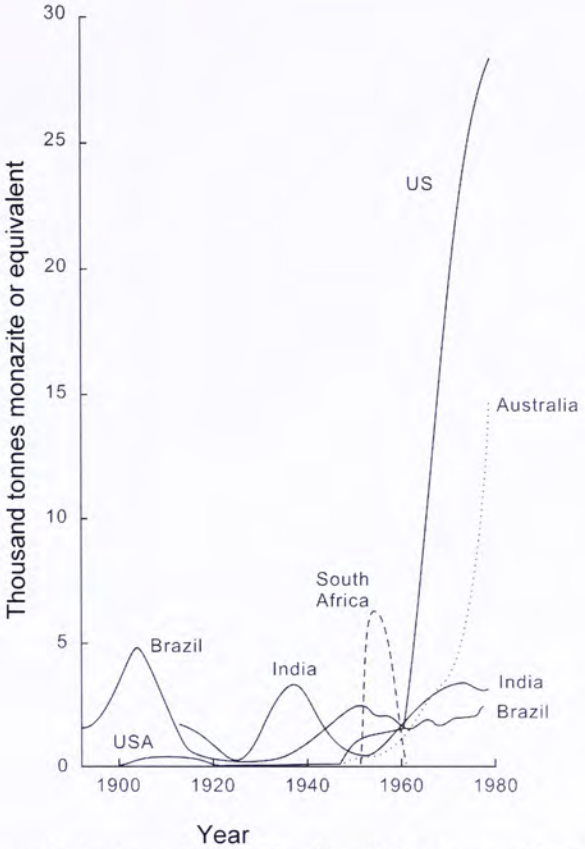


Figure 1.4 Major producing countries of REEs since 1895 (Neary and Highley, 1984).

Table 1.9 Import and export of REEs in the US (Han *et al.*, 1999).

Year		1994	1995	1996	1996/1994
Weight (tonnes)	Import	9160	15400	21600	2.4
	Export	9520	9890	12200	1.3
	Net import	-363	5530	9340	-
Value (million US dollars)	Import	79	110	126	1.6
	Export	46	61	72	1.6
	Net import	33	49	54	1.6

1.6.2 Production and export in China

China started production of REE in the mid 1950s. At the beginning, monazite was produced in limited quantity since the extraction technology is less advanced and demand of REE was low. With the world's increasing demand, production consequently rose (Karayannopoulos, 2004). Production of REEs in China increased on average 40% annually since 1980s (Table 1.10) (Wang and Dou, 1996). Demand for individual REE also increased sharply.

Table 1.10 REE production and export in China (Wang and Dou, 1996; Han *et al.*, 1999).

Year		1993	1994	1995	1996	1997	Average annual increase (%)
Export	Amount (tonnes)	11600	14500	24500	27900	28500	+25.3
	Value (million US dollars)	108	150	271	293	320	+31.1
Production	Amount (tonnes)	20600	25400	36300	41100	42200	+19.6

Most of the REE produced are exported. China cumulatively exported 106984 tonnes in the period 1993-1997 (Han *et al.*, 1999). The quantity increased at a rate of 25.3% (by weight) and 31.1% (by value) (Table 1.10). The major markets for China REEs are the US and Japan (Haxel *et al.*, 2005). The quantities of REE exported to the US were 1633 tonnes in 1994 and increased 9 times to 14512 tonnes in 1996.

China exported 11103 tonnes (56.5% of total imported REE in Japan) mixed REEs to Japan in 1997 (Han *et al.*, 1999). The weight of REEs exported to Japan increased at a rate of 24.9% from 1993-1997.

1.6.3 Production in other countries

India, which produced the largest amount of REEs until 1940, has reduced the production of REEs in the recent years. India produces 1814 tonnes each year. France has the world's largest manufacturer of purified REEs. The REE products are exported to Japan, the US and Europe. Companies in Norway produce 1179 tonnes REEs as by-product of apatite extraction.

1.7 Separation of REEs

REEs are found in natural ores as mixture of different elements. REEs must be extracted from the minerals and other elements so that they can be utilized commercially. Methods of separation vary considerably as the details depend on the ore being used and the extent to which the metals are to be separated from one another. Separation methods can be classified into two categories: classical methods and modern methods.

1.7.1 Classical methods

Classical methods consist of fractional precipitation and fractional crystallization. Fractional precipitation involves the addition of a precipiant to a mixture in an amount insufficient for complete precipitation. For fractional crystallization, individual REE

is separated based on the difference in solubilities of the salts in different aqueous solution (Evan, 1990). The process of evaporation and recrystallization was continued until the target REE is separated. This method is still used since it can separate REEs in large quantity that satisfies economical production (Healy and Kremers, 1961).

1.7.2 Modern methods

Classical methods have the advantage that a large quantity of REEs can be separated in a short time. However, if the requirement is to completely separate adjacent REEs, the process needs to repeat numerous times to achieve a desirable purity. Modern methods which are able to separate REEs in high purities (99.99%) are developed to achieve the requirement (Hedrick, 1997). Modern methods include ion-exchange separation and solvent extraction.

1.7.2.1 Ion exchange separation

To produce high-purity REEs, ion-exchange technique is applied. It is a technique based on the sorption ability of REE on a suitable ion-exchange medium followed by differential displacement of the individual ions with an eluting solution, such as EDTA with ferric and cupric ions (Powell, 1961).

1.7.2.2 Solvent extraction

REEs are separated from each other based on the variation in solubility in the extractants. An organic phase, such as di-2-ethylhexyl phosphoric acid and tributylphosphate is in contact with an acidic solution containing REEs (Doyle, 2000;

Miranda, Jr. *et al.*, 2002). REEs are then preferentially separated into the organic phase. The organic phase is required to consist of two miscible phases since the extractant is highly viscous (Fray, 2000). REEs with different atomic masses are dissolved in various stages and collected.

1.8 Applications

1.8.1 Alloys

REEs are used in metal industries to make different kinds of alloys, mainly iron and steel (Pedreira *et al.*, 2001). The application quantity of REEs in making alloys has been increasing since 1986 (Figure 1.5). They are in the form of pure REEs or mischmetal, which is an alloy mixture of La, Ce and other REEs (Lundin and Wilson, 2000). Barrett and Dhesi (2001) suggested mischmetal comprising 50% Ce, 25% La, 15% Nd and 10% other REEs and Fe. The old method that added michmetal to steel and iron was by pressing it into molten iron, while modern advanced methods are injection and converting ladle (Wang and Dou, 1996).

When the steel or iron is rolled or forged at high temperature, melting of inclusions of iron sulphide compound will occur, and as a result cracks develop. Addition of REEs can control the content and shape of sulphide inclusion in cast irons and steels. REEs combine with sulphide inclusions to form particles with a more rounded morphology which is more stable because they do not deform significantly even at high temperature (Lundin and Wilson, 2000). Mechanical properties, like ductility, corrosion, oxidation resistance and creep resistance, of cast iron and steel are

improved after modification of inclusion morphology. The effect is important to applications in heavy industries including pipes for oil and gas transmission. REEs modified cast Fe can also be applied in automobile industry to produce lighter vehicles, and cast pipes for water.

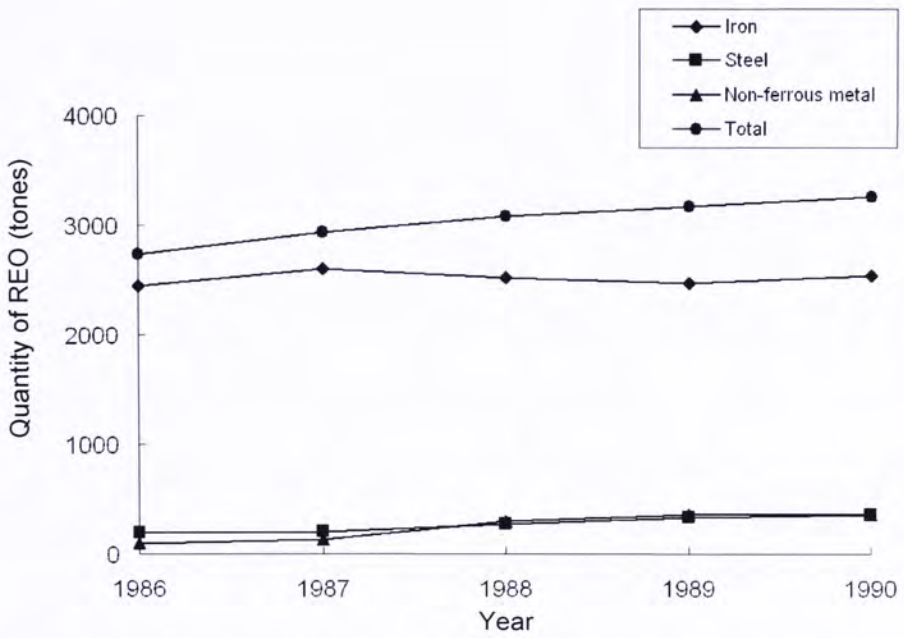


Figure 1.5 Consumption of REEs in metallurgy from 1986 to 1990 (Zhang *et al.*, 2006).

Non-ferrous alloys that frequently added with REEs are Al and Mg alloy. REEs are added as minor additions to the alloys by directly adding REE compounds to aluminium and magnesium electrowinning cell. Although the quantity is less that 1%, the effects are significant. Addition of REEs is effective in enhancing the mechanical properties of high-strength Al alloys. The mechanism for improving the mechanical properties is still not clear. Lundin and Wilson (2000) proposed that morphology of intermetallics was shifted from platelike toward spherical in shape, which reduced their

impact as crack initiators. When Ce was added to Al-Si alloy, size and the fraction of primary silicon (Si) decreased markedly with the increasing Ce content, while the roundness of primary Si increased (Zhang *et al.*, 2006). Non-ferrous alloys added with REEs are used in helicopter gearboxes, aircraft landing wheels and satellites. La-Ni alloys are developed to make low-temperature fuel cells for storage of hydrogen (Fray, 2000). Al-Mg-Si alloys added with REE were used for cables, and that for Fe-Cr-Al alloys are for electric heating wire.

1.8.2 Permanent magnets

Nd and Sm have been the most commonly applied materials to produce permanent magnet, in which Y, La, Ce, Pr or mischmetal can also be used. Dy and Tb are used in the last few years in new NdFeB alloys for magnets with higher coercivity and temperature stability, primarily for the automotive industry. La is added in small amount in some ferrite applications to make compounds like $Ba_{1-x}La_xFe_{12-x}Co_xO_{19}$ and $Sr_{1-x}La_xFe_{12-x}Co_xO_{19}$ (where x = 0, 0.1, 0.2, 0.3, 0.4) which improve magnetization, and the coercivity and anisotropy field of the produced magnets (Grössinger *et al.*, 2003). The maximum energy can exceed 800 kJm^{-3} for the magnet (Pang, 2006). Efforts have been paid in improving the magnetic properties of REE compounds (Valcanover, 2005). An example is that the anticorrosion property is improved by coating a layer of Zn-Cr on the surface of NdFeB permanent magnet (Figure 1.6). Due to reduction of weight compared with traditional magnet, REE magnets are favourable to be used in portable devices, such as electric sewing machines, motors in automobiles and heater fans. Other applications include used in anti-wax devices and dehydrators in oil mining.

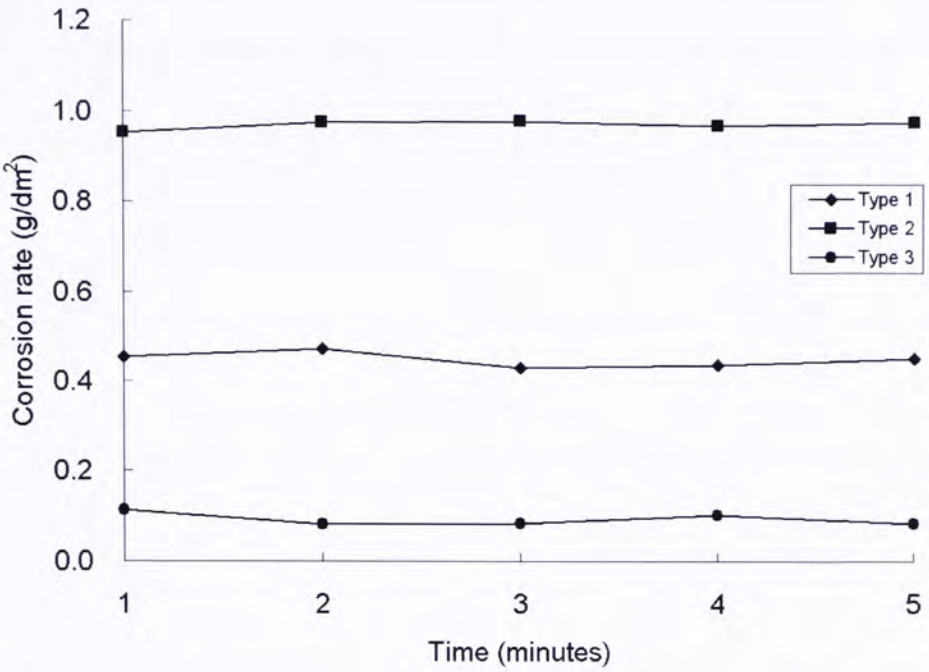


Figure 1.6 Corrosion rates of various coatings on NdFeB magnet in 3.5% sodium chloride solution immersion test (g/dm^2) (Yu and Chen, 2006)
Notes: Type 1- Coating of zinc plating; Type 2- Coating of nickel plating; Type 3- Zinc chromium coating.

1.8.3 Catalysts

Petroleum industry is the major market of catalysts made with REEs. Mixture of rare earth oxides are used, rather than certain individual REE to reduce the production cost, as catalysts for the cracking of crude petroleum (Pedreira *et al.*, 2004). Almost all catalysts used in oil refineries have been substituted by REE-containing catalysts. Zeolite promoted catalysts were stabilized thermally and hydrothermally by the added REEs. The catalysts consist of 5% to 25% zeolite which composed of 4% rare earth oxide. The catalyst is also employed in various other organic reactions, including hydrogenation of ketones to form secondary alcohols, dehydrogenation of

alcohols and butanes and formation of polyesters. Controlling air pollution may be a potential use of the REE-containing catalysts. The catalyst provide a platform for converting oxides of nitrogen and unburnt hydrocarbons exhausted from motor vehicles into nitrogen, carbon dioxide and water vapour which is less environmentally polluted.

1.8.4 Glass additives

REEs are used in glass industries in two aspects, glass polishing compounds and colouring. Over the last 10 years, REE compounds have almost replaced red iron oxide rouges, in which cerium oxide is used extensively because it gives high polishing rates and low surface roughness (Osseo-Asare and Suphantharida, 2000). Technical grade rare earth oxides powders contain about 40-45% cerium oxide are commonly used. The powders are diluted with other materials when used. The polished glasses are used in the production of lenses for spectacles, cameras and binoculars.

Fe (II) ion generates a blue colour to glasses. REEs are used to decolourize the undesirable original colour, because of the presence of iron, of glasses. Light rare earth oxide is used as an oxidizing agent to control the decolourizing function of Se which is the usual decolourizing agent. The oxide controls the oxidation state of Se, maintaining a pink colour to complement the green to blue colour of Fe. Cerium oxide can decolourize satisfactorily when iron contents are up to 0.1% Fe_2O_3.

Many REE oxides have attractive and beautiful colour that makes them suitable to colour glass. Er makes glass pink, Nd makes glass blue to wine red, Ho gives blue and Pr produces a green colour.

1.8.5 Phosphors in television screens and similar fluorescent surfaces

High-purity rare earth oxides are used in colour television tubes, fluorescent lighting tubes and X-ray intensifying screens as phosphors (Pedreira *et al.*, 2004). Silver-activated zinc cadmium was used as red phosphors in the past. However, the colour generated by the phosphors cannot match the emission available from green and blue phosphors and subsequently they are replaced by the rare earth phosphors. High-purity oxide of Eu and Y has been used as the phosphors for colour-television picture tubes since the mid 1960s (Barrett and Dhesi, 2001). A mixture of Eu and Y oxides provide a brilliant-red phosphor. Europium-doped oxides and oxysulphides, particular Y_2O_3(Eu), Gd_2O_3(Eu) and Y_2O_3S(Eu) can give high intensity, good colour balance among the three types of phosphors as well as a better overall tube brightness.

Medical X-ray radiography has applied high-purity rare earth oxides as X-ray phosphors (Neary and Highley, 1984). An intensifying screen is used with a matching film. Every X-ray photon that is absorbed by a phosphor screen is amplified into hundreds of visible or ultra-violet radiation photons which are then recorded by a detector like photographic film. Such system can increase film exposure and hence decrease the X-ray dosage to patients. In recent years, compounds of REEs and

alkaline-earth borates such as $RECa_4O(BO_3)_3$, $RE_2CaO(BO_3)_2$, $REBaB_9O_{16}$ and $REBa_3(BO_3)_3$ have attracted attention to be developed in this aspect (Duan *et al.*, 2005).

1.8.6 Fertilizers and feed additives

REEs have been added to fertilizer, in the form of soluble salts like nitrate. The REE-containing fertilizers have been widely used in more than 20 provinces in China since 1972 (Zhu *et al.*, 1997). REE-containing fertilizers have been applied to different plants, for instance, crops, vegetables, fruit and grasses. Crops added with the fertilizers were claimed to have great yield and improved quality. Crop yield was reported to increase by 8-50% (Brown *et al.*, 1990). The yields of wheat, corn, bean and peanut were increased by 6-15%, 7-14%, 6-12% and 8-15% respectively (Xu *et al.*, 2002). The quality of agricultural crops is also improved. Foliages have darker green colour. Amino acids in wheat increased 2-12% after being irrigated with REEs. Strength of cotton fibre was improved 0.33-6.70% (Xiong *et al.*, 2000). Sucrose in sugar cane treated with REEs was 0.61% more than those without REEs. Starch in potato was increased by 1.2%. REEs also increase the resistance to disease of crop. Relative resistance of REE-treated cotton to fungi was 20-40% higher than the control.

REEs have been used as feed additives for pig and sheep (Hu *et al.*, 1998). Pigs fed with REEs are red in skin and have good stomach. Their weights gained by 10-20%.

1.8.7 Dyeing auxiliary

REEs have been used as a dyeing auxiliary in China since 1977 (Wang and Dou, 1996). The REE-added dye can give a bright and lustrous colour to the fibre. Dyes added with REEs are applied to different kinds of fibre like cotton, silk, nylon, nitrilon and wool.

1.9 REEs in the environment

REEs are present in the earth crust. They also exist in soil and plants in the environment. In the natural environment, the concentration of REEs in plants highly depends on their concentrations in the surrounding soil.

1.9.1 REEs in soil

REEs in natural soil mainly come from the weathering of the soil parent materials (Zhu, *et al.*, 1998a). However, extra REEs would be added to the soil as a result of human activities on near mining sites or cultivated lands (Zhu *et al.*, 1997; Ramanaaiah, 1998; Diegor *et al.*, 2001). Concentration of REEs varied widely, ranging from below detection limit to over 120 mg/kg. Abundance of REEs in soil varied among soil types, soil depths and countries. Forest soil in Bulgaria contained 17.9-72.0 mg/kg La, while La in a sandy soil from Sweden was below detection limit (Kabata-Pendias and Pendias, 1992). In a mangrove area in Brazil, the La contents increased along the soil depth (Wasserman *et al.*, 2001).

REEs differ in their chondritic normalized concentrations. Light REEs are more abundant than heavy REEs (Henderson, 1984). Kabata-Pendias and Pendias (1992) found that concentrations of La, Ce, Tm and Lu were 26.1, 48.7, 0.46 and 0.34 mg/kg respectively. Chondritic normalized La/Lu ratio was 10 in soil. Some terrestrial rocks show great negative Eu anomalies, while some others are abundant in Ce (Sen Gupta and Bertrand, 1995; Diatloff *et al.*, 1996a; Wang *et al.*, 2000).

REEs are more abundant as soil depth increases. Abundance of La, Ce and Nd in 0-5 cm soil were 23, 47 and 21 mg/kg respectively, while those concentrations at depth of 25-30 cm were 35, 74 and 30 mg/kg in the same soil (Laul *et al.*, 1979).

REEs exist in soil in various forms. Most of them are dressed strongly in the solid phase of soil and the rest remained as free ionic form. Plant cannot assimilate all kinds of REEs. Only those can be taken up by plants are said to be bioavailable. In order to understand more about the bioavailability of REEs to plants, methods were developed to extract REEs in various fractions of soil. The method modified by the Community Bureau of Reference has been the one of the most widely used method for the fractionation of chemicals in soil (Cao *et al.*, 2002; Mossop and Davidson, 2003). By finding the relationship between the abundance of REEs in plants and in different fractions, the bioavailability of REEs to plants could be assessed.

1.9.2 REEs in plants

Contents of REEs are usually very low in plants but still exist in a wide range (from $1x10^{-3}$ to $1x10^{-1}$ mg/kg). La content in corn and potato were 5 μg/kg and 2 μg/kg respectively, while Pr abundance was less than 1 and 2 μg/kg respectively (Laul *et al.*, 1979). In the same experiment, heavy REEs in plant were less abundant than light REEs; levels of Lu in vegetables were 0.02-0.04 μg/kg. In *Pinus sylvestris* and *Vaccinium vitis-idaea*, REE concentrations ranged from 0.0035 to 0.38 mg/kg (Markert and Li, 1991). Although most plants have very few REEs, some were recorded to be accumulators of REEs. The concentration accumulated in *Euglena gracilis* was 10^5 times more than other green algae (Shen *et al.*, 2002). Total REE content in fern was 97 mg/kg, which was two orders of magnitude higher than in other species (Chiarenzelli *et al.*, 2001).

Patterns of REEs in plants are usually very similar to that of the host soil since plants absorb REEs mainly from soil (Djingova and Ivanova, 2002). This implies that fractionation of REEs by plants is less likely. However, contradictory results have been obtained. Ce contents in fern (*Dryopteris filix-mas*) and spruce (*Picea abies*) were much smaller than values calculated from the contents of La and Pr (Wyttenbach *et al.*, 1998). Samphire showed significant discrimination in the uptake of REEs (Pais and Jones, 1997). Markert and Li (1991) reported that Dy, Er and Eu concentration ranges were ten times lower than other REEs. More researches are required to get a better understanding of the REEs patterns in plants.

1.10 Overview of toxicological studies of REEs

Although the application of REEs is so popular that they are used in many aspects, studies on the toxicology of REEs are rare. Most of the studies focus on the toxicity of REEs to several species of terrestrial animals (Tu *et al.*, 1994). Table 1.11 shows the median lethal dose (LD50) of several REEs using rats, mice and guinea pig. Rats and guinea pigs are more sensitive than mice. When the route of administration was different, the LD50s of REEs also varied. It was found that intraperitoneal or intravenous injection would cause a lower LD50s than oral administration (Xiong *et al.*, 2000). The toxicity ranges are reduced by factors of 100 and 1000 for intraperitoneal or intravenous injection respectively (Sigel and Sigel, 2003). According to the review by Haley (1965), the acute toxicity of REEs was very low that they would not pose a threat to the health of human.

Despite these studies shows that REEs have a very low toxicity to animals, toxicity of REEs to plants is still not well understood. Some reports demonstrated that plant growth was inhibited by REEs. The elongation of oat (*Avena sativa*) coleoptile was inhibited when coleoptile was soaked in solution of La^{3+}, Pr^{3+} and Nd^{3+} (Pickard, 1970). The inhibitory effects brought by La^{3+} and Nd^{3+} were greater than the effect by Pr^{3+}. Root elongation of corn (*Zea mays*) and mungbean (*Vigna radiata*) were reduced by half when they were immersed in La and Ce solution (Diatloff *et al.*, 1995a). Hu *et al.* (2002) showed similar results that root elongation of wheat (*Triticum aestivum*) was inhibited by 50% when exposed to 5 mg/L La or Ce.

Table 1.11 Median lethal doses of REEs on rats, mice and guinea pig (Hirano and Suzuki, 1996; Xiong *et al.*, 2000).

REE	Route	animal	LD50 (mg/kg)
$LaNO_3$	ip	mouse	150
CeCl3	iv	rat	55
$CeNO_3$	po	mouse	1178
$PrNO_3$	po	rat	3500
$NdNO_3$	po	rat	2750
$SmNO_3$	po	rat	2900
$EuNO_3$	ip	rat	210
$EuCl_3$	po	rat	5000
$DyCl_3$	ip	mouse	585
$DyCl_3$	po	mouse	7650
$HoNO_3$	ip	mouse	560
$DoNO_3$	po	mouse	7200
$ErCl_3$	ip	mouse	535
$ErCl_3$	po	mouse	6200
$TmNO_3$	ip	rat	285
$YbNO_3$	po	rat	3100
$ScCl_3$	iv	mouse	24
$ScCl_3$	ip	mouse	440
$YbNO_3$	ip	rat	255
$YbNO_3$	po	rat	3100
$LuNO_3$	ip	rat	325
$RE(NO_3)$	po	mouse	1876
$RE(NO_3)$	po	rat	1832
$RE(NO_3)$	po	guinea pig	1397

Notes: ip: intraperitoneal injection; iv: intravenous injection; po: oral administration

Some other studies indicated that plants exposed to REEs had a stimulated growth. Chang (1991) found that seedling development was promoted by REEs. Biomass, leaf area and height of sugar beet (*Beta vulgaris*) were increased after application of REE mixture (Tian *et al.*, 1990). Dry weight of roots of wheat (*Triticum aestivum*) seedlings treated with Eu was increased by 64% (Shtangeeva and Ayrault, 2006). Twice more flowers of *Arabidopsis thaliana* bolted when they were growing in 2.5 μM Ce nitrate, while increase in the length of primary roots was observed in 50 μM La nitrate and 10 μM Ce nitrate (He and Loh, 2000).

The results of the toxicity studies were contradictory. One possible reason may be differences in the methods being used. Further investigation about the effects of REEs should be carried out to get more information.

1.11 Current study

1.11.1 Thesis outline

This project consisted of three experiments. Seed germination and root elongation test was carried out to reveal the phytotoxicity of REEs. Forestry plants, in which tree was the representative in this study, was employed to investigate the effect of REEs by assessing their growth performance. Besides, relationship between the bioavailability of REEs and soil pH and organic matter content was studied. Relationship between bioavailability of REEs and growth performance was also determined.

1.11.2 Objectives

The present study aimed to provide more information on the effects of REEs to forestry plants. The objectives are as follows:

(1) To study the potential toxicity of REEs on terrestrial plants,

(2) To assess the effects of REEs on growth performance of forestry plants and the distribution of REE in plants,

(3) To investigate the bioavailability of REEs in soil, and

(4) To examine the effect of pH and organic matter on the availability of REEs to plants.

1.11.3 Significance

Although REEs are widely used for agricultural crops, there is a paucity of toxicity data on terrestrial plants. This study could provide phytotoxicity information on REEs by standard phytotoxicity test using sensitive terrestrial plant seeds.

In recent years, industrial and agricultural application of REEs has been developed. For agricultural uses, 50-100 million tonnes of REEs enter the agroecosystem every year (Liang *et al.*, 2005). Since 1990s, REE-containing fertilizers have been applied in China and the rate has increased drastically. The area applied with fertilizer was approximately 3.7×10^6 ha in 1993 but increased to 2.0×10^7 ha in 1995 (Diatloff *et al.*, 1996a). Although REEs exist in the earth crust, application of REEs will release extra REEs into the environment. Ecological and toxicological consequence of adding extra REEs into the environment is still not clear, and thus more research efforts should be put on this. Studies on the effects of REEs have focused on agricultural crops, such as wheat and maize, and the results showed that REEs can increase the crop yield and improve product quality at low concentration. However, there is very limited literature and thorough research concerning the effects of REEs on forestry plants. Forests make up a major part of the natural environment. Forests are significant for exchange of materials and energy in the biosphere. Many resources, such as food, wood, cotton and medicine, used in human daily life come from forests. If the beneficial effects exhibited by the crops are also demonstrated in forestry trees, REEs can be applied to forests, and it will be a great contribution to ecosystem and forestry industry. The present study puts great emphasis on the effects of REE on

growth of forestry species which gives useful information on the influences of REE on environment, and as a result, managing the use of REEs in agricultural and industrial aspects.

REE-containing fertilizers can be applied to plants through foliar sprays, seed treatments or as additives to soils (Xie *et al.*, 2002). Among these methods, addition to soil is most frequently used due to lower cost and higher efficiency. Another experiment using tree seedlings in soil was carried out to investigate effect of REEs on the soil-plant system, which is a major part of the ecosystem. The result can give more information about the effect of REEs on the ecosystem and application of REEs to soil-plant system. Plant absorbs REEs in soil through its roots. The quantity of REEs that plant can absorb depends on the availability of REEs in soil. REEs react with soil and are converted into different fractions which vary considerably in their bioavailability. Soil properties such as pH and organic matter affect the reaction as well as bioavailability of REEs. Therefore, an experiment with different soil properties was carried out to study the influence of pH and organic matter on REE bioavailability and their effects on plant growth.

Chapter 2 Phytotoxicity of rare earth elements

2.1 Introduction

2.1.1 Ecotoxicity of REEs

Nevertheless the widespread use of REEs in industry and agriculture, very limited information is available on their toxicity to plants. Since REEs are used as medical tracer, toxicity to mammals received much attention. Many studies focus on several terrestrial mammals, such as rats, mice, dogs and guinea pigs (Tu *et al.*, 1994). The toxicity of REEs on these animals depends on the routes of exposure. The toxicity was the highest when animals were exposed through intravenous injection (Ding and Ma, 1984). The median lethal doses (LD50s) were 6 and 7 mg/kg when rats were injected intravenously with of $NdNO_3$ and $PrNO_3$ solution respectively (Xiong *et al.*, 2000). The toxicity was 100 times reduced when REEs were administrated orally which may be because of the low rate of REEs absorption in the intestinal tract (Brown *et al.*, 1990). The toxicity of REEs to mammals was reported to occur mainly in the pulmonary system (Hirano and Suzuki, 1996; Sigel and Sigel, 2003). Prolonged inhalation of REEs by guinea pigs caused bronchitis and pulmonary fibrosis. However, Haley (1991) pointed out that the toxicity may be brought from the radioactive materials rather than the stable elements.

Some studies worked on the toxicity of REEs to aquatic organisms. Most of the exogenous REEs were adsorbed by sediment (Yang *et al.*, 1999b). The adsorbed REEs may be desorbed at a later time. Thus aquatic organisms are potentially influenced by REEs. Fish is one of the most studied aquatic organisms.

Most of the absorbed REEs accumulated in the internal organs and gills of *Cyprinus carpio* (Tu *et al.*, 1994). The major edible part, muscle, contained the least amount of REEs. Among the four fish species tested, *Hypophthalmichthys molitrix* (silver carp) was the most sensitive species to REEs (Hu and Luo, 1980). The 48-hour LC50 was 12.2 mg/L in silver carp, while the LC50s in *Ctenopharyngodon idellus* and *Carassius auratus* were 32.4 and 36.0 mg/L respectively. Yang *et al.* (1999b) reported that daphnia was more sensitive to REEs than fish. The age at maturity was delayed by La at concentrations higher than 39 μg/L. The 48-hour EC50 of La to *Daphnia carinata* was 1.18 mg/L (Barry and Meehan, 2000).

REEs were reported to have inhibitory effect to plant. The root length was reduced almost 50% when seedlings of *Triticum aestivum* were immersed in 5 mg/L La and Ce solution (Hu *et al.*, 2002). The growth of oat coleoptile in La, Pr and Nd solution was inhibited due to the alternation of permeability of cell to K^+ ions (Pickard, 1970). Shoot dry weights of maize and mungbean were reduced when they were grown in > 0.7 mg/L La and Ce solution culture (Diatloff *et al.*, 1995a). The shoot dry weight of *Vigna radiata* was reduced by 27% when the plants were sprayed with La and Ce solution (Diatloff *et al.*, 1996b). However, some other information showed that low concentration can promote the growth of plant. Germination rate of sugar beet (*Beta vulgaris*) was increased by 1.6-6.9% (Tian *et al.*, 1990). The productions of rice and wheat were enhanced by up to 10 and 13% respectively (Ding and Ma, 1984). Solution of low concentration of La increased the root growth of corn by 36% and mungbean by 21% (Diatloff *et al.*, 1995b).

Since the results were controversial, this study aimed at investigating toxicity of REEs so that REEs can be used more safely.

2.1.2 Toxicity tests using higher plants

Toxicity testing of REEs mainly focused on animals. Since REEs was applied increasingly to plants, getting more information about the toxicity of REEs on higher plants is necessary for the safe application of REEs. However, previous tests have scarcely used higher plants that have been commonly used in toxicity assays for heavy metals and other compounds.

Seed is an important stage in the life cycle of higher plant. A seed is a fertilized mature ovule consisting of embryo, stored food material and protective coat. A series of events, such as cell expansion, reconstitution of lipid membranes, activation of proteins, repair of DNA damage and protein incurred, replacement of extensively damaged membranes, resumption of transcription and translation, activation of respiration and reserve mobilization, occur after seeds have imbibed a sufficient quantity of water (Kapustka, 1997; Soeda *et al.*, 2005). Impact of chemical applied to seeds not only influence germination process, but also the subsequent reactions as the plant grows. The effect has direct impact on the plant's survival (Marchiol *et al.*, 1999). Seedlings are more sensitive than older plants; the concentration or dose that causes effect on seeds may not be sufficient to give observable effect on older plants (Misra *et al.*, 1994). Seed germination and root elongation test is the most commonly applied technique in assessing the effect of

heavy metals and trace elements on plants (Verkleij, 1993). Comparing the rates of seed germination and the length of primary root can find out the effect of chemicals on plants.

2.1.3 Advantages of seed germination and root elongation test

Germination test as a method of phytotoxicity assessment has several advantages. Seeds can be stored dry and remain viable for a long time. Most seeds remain viable for at least three to five years, or even longer. Cost of storage is very low because seeds are simply stored in plastic bags at room temperature. Germination test can be conducted at any time. It is an advantage over methods that involve seedlings that are available seasonally. Seed germination test can be carried out in the seasons when the seedlings are not available. Seeds are activated by simple treatment such as soaking in water without complex pretreatment. Seeds of some aquatic macrophytes exhibit periods of natural dormancy and require bleach or acid treatments, heating, or scarification to facilitate germination (Muller *et al.*, 2001).

Germination test is simple and easy to conduct (Zucconi *et al.*, 1981). The most commonly used equipment includes Petri dish, pots, soil and filter paper, which is generally available in most laboratories.

Results of germination test are obtained after a short time between one to ten days. Toxicity of Cu was determined by exposing *T. Typha latifolia* to copper

sulphate for 7 days (Muller *et al.*, 2001). Seed of *Brassica parachinensis*, *Lactuca sativa*, *Azukia mungo* and *Lycopersicon esculentum* germinated after 5 days and they were used to evaluate the toxicity of spent litter (Tam and Tiquia, 1994). Root lengths of *Brassica rapa* and *Lepidium sativum* were measured after a 72 hour germination period to monitor landfill leachate (Devare and Bahadir, 1994). The germination percentage and root length of *Hordeum vulgare* immersed in urban organic waste was measured after 5 days (Pascual *et al.*, 1997)

A large amount of seeds can be placed in a small container during germination test. This can save the space for the experiment (Gorsuch *et al.*, 1990). Volume of test sample to which the seeds are exposed is small so that large container is not required. The volume of sample preparation and waste disposal are minimized. Such advantage is significant when the test chemical is hazardous.

Chemicals may not appear individually in the environment. The effect of the mixture may be synergistic or antagonistic. Seed is a good tool to test the synergistic and antagonistic effects by exposing to chemical mixture. Chemical analysis alone is not able to show the effects.

Standardized protocols were established by authoritative organizations, such as the Organization for Economic Cooperation and Development (OECD) and the United States Environmental Protection Agency (USEPA). Seed germination test has been a representative and extensively used method to test the toxicity of chemicals.

2.1.4 Selection of species

Current databases, such as PHYTOTOX, provide information of more than 1500 different species that have been exposed to a variety of test chemicals (Fletcher, 1988; Powell, 1997).

The selection of species should be based on the ecological relevance of species, species specific life-cycle characteristics and region of natural occurrence (OECD, 2003). The species selected in the germination test should be sensitive to the suspected toxic compounds in order to get an index to protect other non-tested species.

An ideal species should be endemic to the area of concern (Powell, 1997). They are economically important and constitute major cash crops to the area. They should be easily available and can be used at any time in a year. No special pretreatment such as chilling, prewashing or scarification is required before the test.

Seeds should have relatively high and uniform germination rates in order to ensure the germination rate obtained is due to the stress applied rather than due to the health and viability of seeds (Powell, 1997; OECD, 2003). The species should germinate in a short time to give fast, measurable responses (Al-Farraj *et al.*, 1984).

Various plant species have been recommended by the OECD, USEPA and United States Food and Drug Administration (USFDA) for germination testing

(Tables 2.1-2.3). Besides the crop species used in the existing plant tests, OECD also proposed more than 120 non-crop species for the evaluation of herbicides. These species are highly recommended since they are recognized as important to wildlife as food sources, though some of them are weed species. Serious problems to organisms in higher trophic levels may be avoided if possible impacts on plant are monitored and early action is taken.

2.1.5 Endpoint of test

A variety of test endpoints have been developed by different organizations. Qualitative endpoints include appearance of seed and root. Quantitative measurement endpoints are survival, germination rate, seedling growth, shoot length, root diameter, root length, root biomass and photosynthesis rates. USEPA relies on tests of seed germination, root elongation and growth reduction for evaluation of toxicity of chemicals on plants (SCOPE, 1995). The most commonly adopted endpoints are percentage of germination, root length and germination index. Germination index is obtained by combining seed germination and root length according to the following equation (Zucconi *et al.*, 1981):

$$\text{Germination index (GI)} = \frac{G_i}{G_0} \times \frac{R_i}{R_0} \times 100$$

where G_i = Germination rate in treatment (%)
G_o = Germination rate in control (water) (%)
R_i = Root length in treatment (mm)
R_o = Root length in control (water) (mm)

Table 2.1 Test species recommended by OECD used in plant tests guidelines (OECD, 2003).

Family	Species	Common names
Chenopodiaceae	*Beta vulgaris*	Sugar beet
Compositae (Asteraceae)	*Lactuca sativa*	Lettuce
Cruciferae (Brassicaceae)	*Sinapis alba*	Mustard
	Brassica chinensis	Chinese cabbage
	Brassica napus	Oilseed rape
	Brassica oleracea var. *capitata*	Cabbage
	Brassica rapa	Turnip
	Lepidium sativum	Garden cress
	Raphanus sativus	Radish
Cucurbitaceae	*Cucumis sativa*	Cucumber
Leguminosae (Fabaceae)	*Glycine max (G. soja)*	Soybean
	Phaseolus aureus	Mung bean
	Pisum sativum	Pea
	Trigonella foenum-graecum	Fenugreek
	Lotus corniculatus	Birdsfoot trefoil
	Trifolium pratense	Red clover
	Vicia sativa	Vetch
Solanaceae	*Lycopersicon esculentum*	Tomato
Umbelliferae (Apiaceae)	*Daucus carota*	Carrot
Gramineae (Poaceae)	*Avena sativa*	Oats
	Hordeum vulgare	Barley
	Lolium perenne	Perennial ryegrass
	Oryza sativa	Rice
	Secale cereale	Rye
	Sorghum vulgare	Grain sorghum
	Triticum aestivum	Wheat
	Zea mays	Corn
Liliaceae (Amarylladaceae)	*Allium cepa*	Onion

Table 2.2 Test species recommended by USEPA (USEPA, 1996).

Family	Species	Common names
Compositae	*Lactuca sativa*	Lettuce
Cruciferae	*Brassica oleracea*	Cabbage
Cucurbitaceae	*Cucumis sativus*	Cucumber
Leguminosae	*Glycine max*	Soybean
Liliaceae	*Allium cepa*	Onion
Poaceae	*Avena sativa*	Oat
	Lolium perenne	Perennial ryegrass
	Zea may	Corn
Solonaceae	*Lycopersicon esculentum*	Tomato
Umbelliferae	*Daucus carota*	Carrot

Table 2.3 Test species recommended by USFDA (Kapustka, 1997; Kapanen and Itävaara, 2001).

Family	Species	Common names
Compositae	*Lactuca sativa*	Lettuce
Cruciferae	*Brassica oleracea*	Cabbage
Cucurbitaceae	*Cucumis sativus*	Cucumber
Leguminosae	*Glycine max*	Soybean
	Phaseolus vulgaris	Bean
Poaceae	*Avena sativa*	Oat
	Lolium perenne	Perennial ryegrass
	Triricum aestivum	Wheat
	Zea mays	Corn
Solonaceae	*Lycopersicon esculentum*	Tomato
Umbelliferae	*Daucus carota*	Carrot

The definition of germination differs among different organizations and authors. American Public Health Association (APHA) and USEPA define germination as when radicle attains a length of 5 mm or longer while USFDA sets at 3 mm. In some cases, germination is considered to be completed when seed coat is penetrated by the elongated embryo (Kapustka, 1997).

2.1.6 Median effect estimates

Based on the definition proposed by USEPA, median effective concentration (EC50) refers to the chemical concentration that affects 50% of the test criterion. The EC50 has often been used for comparison because it has the smallest confidence interval (Kapustka, 1997).

2.1.7 Objective

Most of the toxicity test concerning rare earth elements (REEs) involved aquatic system or mammals. The effect of REEs on terrestrial plants is not well known. The objective of this experiment is to assess the toxicity effect of REEs on seeds of terrestrial plants using germination rate and root length as endpoints.

2.2 Materials and methods

The procedure was modified from the Seed Germination/Root Elongation Toxicity Test (OPPTS 850.4200) in the Ecological Effects Test Guidelines developed by the Office of Prevention, Pesticides and Toxic Substances, USEPA

(USEPA, 1996).

2.2.1 Test species

The species chosen in the test were *Brassica chinensis* (Chinese white cabbage) and *Lolium perenne* (perennial ryegrass). *B. chinensis* is a common species in England and one of the most important vegetables consumed in Hong Kong (Wong and Leung, 1989). *L. perenne* is widely used in many phytotoxicity tests proposed by USEPA, OECD and USFDA since it is sensitive to many chemicals (Gorsuch *et al.*, 1990). The seeds were purchased from local seed suppliers. Seeds of similar sizes and without superficial damage were chosen.

2.2.2 Test chemicals

Four REEs were used in this experiment, and they were lanthanum (La), cerium (Ce), neodymium (Nd) and praseodymium (Pr). All of them are classified as light REEs. These REEs occupy the largest proportion in commercial fertilizer (Zhang and Shan, 2001). La was purchased from Fluka, Ce was supplied by Riedel-de Haën (RdH) while Nd and Pr were provided by Aldrich. The purity of the chemicals were no less than 99.9%. All chemicals were in their nitrate forms which is the form used in commercial fertilizers.

2.2.3 Range finding test

A piece of Whatman #42 filter paper was placed in a Petri dish (9 cm diameter). The filter paper was soaked with 5 mL REE solution of different concentrations,

ranging 0 (control), 0.01, 0.10, 1.00, 10.0, 100 and 1000 mg/L. The REE solutions were prepared by dissolving REE nitrate in Milli-Q water at the same day of phytotoxicity test. Twenty seeds were distributed evenly on the filter paper (Plate 2.1-2.2) and there were three replicates for each concentration. The Petri dishes were incubated in a controlled environment chamber at 25°C in total darkness for 4 days and 6 days for *B. chinensis* and *L. perenne* respectively (USEPA, 1996). The number of seeds germinated was counted at the end of the incubation period. Penetration of the radicle out of the seed coat was used as a sign of seed germination (Kapustka, 1997).

2.2.4 Definitive test

The test species were *B. chinensis* and *L. perenne*. The concentrations of REE solution were 0, 0.16, 0.31, 0.63, 1.25, 2.50, 5.00, 10.0 mg/L. The incubation condition was the same as in range finding test. There were three replicates for each treatment. The number of seeds germinated was counted and the length of primary root was measured by a caliper.

2.2.5 Statistical analyses

The means and standard deviations were calculated. The median effective concentration (EC50) was calculated from the dose-response relationship between percentage of root length of control and REE concentrations by probit analysis using SigmaPlot. The criterion of non-overlapping 95% confidence intervals was used to determine the significant difference ($p = 0.05$) between EC50s (APHA, 1995).

2.3 Results

2.3.1 Range finding test

Germination rate as a more convenient endpoint was measured in the range finding test to find out the concentration range applied in the definitive test. Seeds of both *B. chinensis* and *L. perenne* in pure distilled water (control) had germination rate of 92% and 95% at the end of incubation period. This met the criteria set by USEPA as test species for phytotoxicity test.

Germination rates decreased as concentration of REE solution increased. The germination rate of both species decreased slightly when concentration of La was below 1 mg/L. A rapid decline was found when concentration was higher than 10 mg/L. Some roots had brownish tips at high concentration. No seeds germinated when the concentration was at or higher than 100 mg/L.

2.3.2 Definitive test

2.3.2.1 Germination rate

Germination rates of seeds in solution without REE were 95% for both *B. chinensis* and *L. perenne*. Germination rates were hereafter expressed as percentage of germination of control.

A general decreasing trend was observed in all treatments for *B. chinensis* (Figure 2.1). A quite even decrease rate was shown by *B. chinensis* treated with most REEs except Pr. The addition of La, Ce, Pr and Nd reduced germination

significantly ($p < 0.05$) at concentrations of 0.63, 2.50, 0.63 and 1.25 mg/L respectively. The germination rate decreased to a range from 30.5 to 65.4% at the highest concentration of 10 mg/L for the four REEs. The slope of the dose-response curve of *B. chinensis* in Nd solution was relatively smaller. It showed that Nd at high concentration had less influence on percentage of germination of *B. chinensis*.

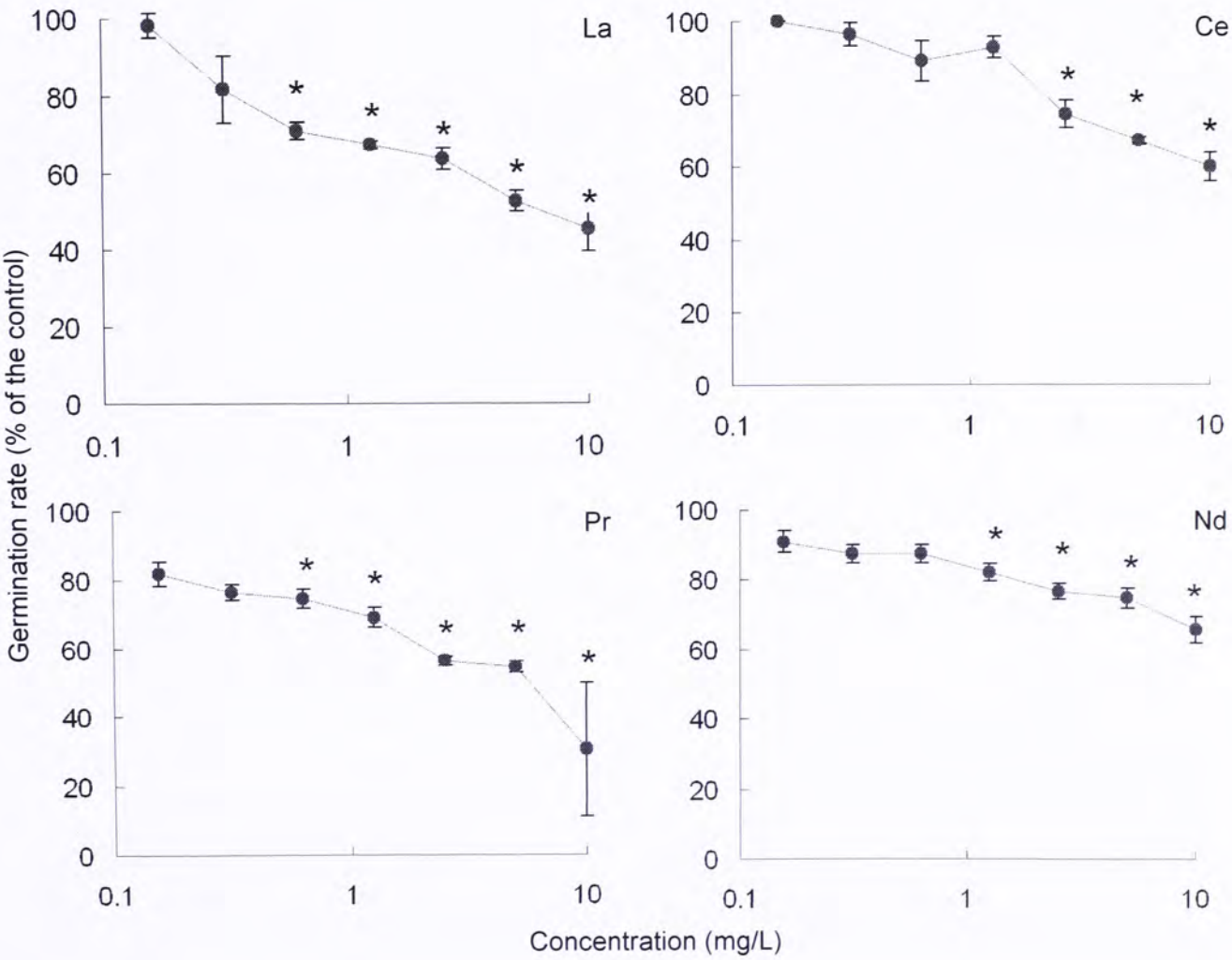


Figure 2.1 Germination rate of *B. chinensis* in solutions of different concentrations of REEs. Error bar represents standard deviation (n=3). Asterisk indicates significant difference ($p < 0.05$) when compared with the control.

A similar trend was observed for *L. perenne* (Figure 2.2). Germination rates of *L. perenne* are not greatly influenced by REEs when concentration of solution was below 10 mg/L, with the percentage of germination ranged from 71.9 to 82.5%. *L. perenne* was less sensitive to increasing concentration of REEs than *B. chinensis* as shown by the slopes of the dose response curves. This implies germination of *L. perenne* was less inhibited when subject to the same concentration of REE. For instance, germination rate of *B. chinensis* exposed to 0.63 mg/L La was 71.0%, while that of *L. perenne* was 89.6%. The lowest concentration giving significant inhibition to germination of *L. perenne* was greater than those of *B. chinensis*. For La, concentrations lower than 1.25 mg/L did not significantly ($p > 0.05$) inhibit the germination of *L. perenne*. For Pr, the lowest concentration that caused significant reduction in germination was 5 mg/L.

2.3.2.2 Root length

Root elongation in general decreased with increasing REE concentration. Figure 2.3 shows the relationship between root length of *B. chinensis* and REE concentration. Significant inhibitory effects of REEs on root length were observed, but at different concentrations for the various REEs. The concentration that gave significant inhibition to the root elongation for *B. chinensis* was lower than the concentration that significantly affected germination rate. The concentration causing significant reduction in root length was 0.16 mg/L for La and Ce, which reduced root elongation for 15.8 and 24.3% respectively. The lowest Pr concentration that produced significant inhibitory effect to root length was 0.63

mg/L, which was higher than those of La and Ce. This concentration was the highest for Nd (1.25 mg/L), showing that Nd was the least toxic to *B. chinensis* among the four REEs studied.

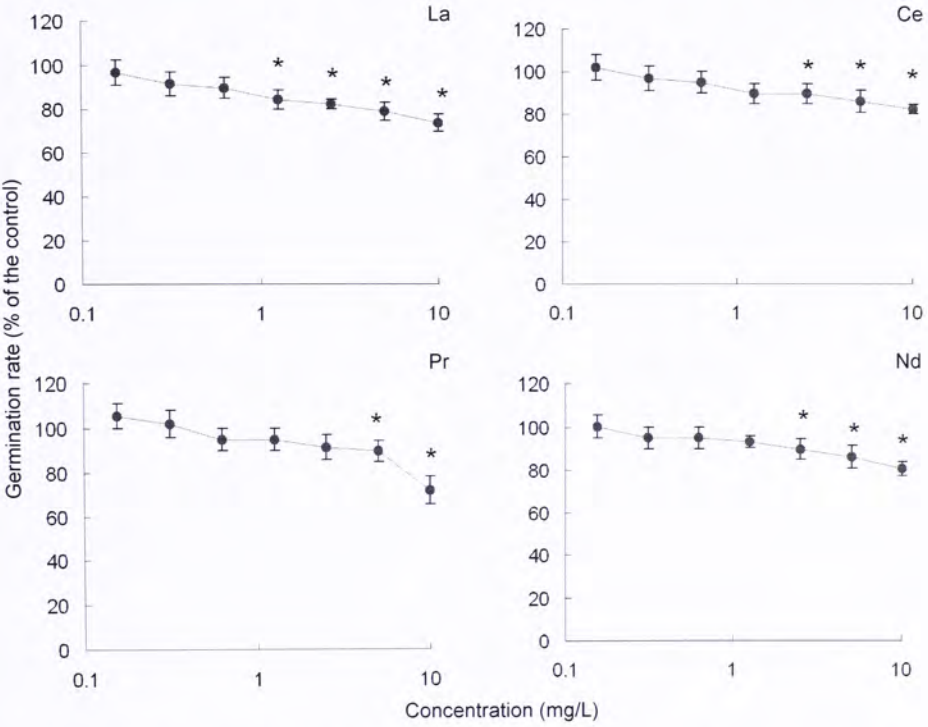


Figure 2.2 Germination rate of *L. perenne* in solutions of different concentrations of REEs. Error bar represents standard deviation (n=3). Asterisk indicates significant difference ($p < 0.05$) when compared with the control.

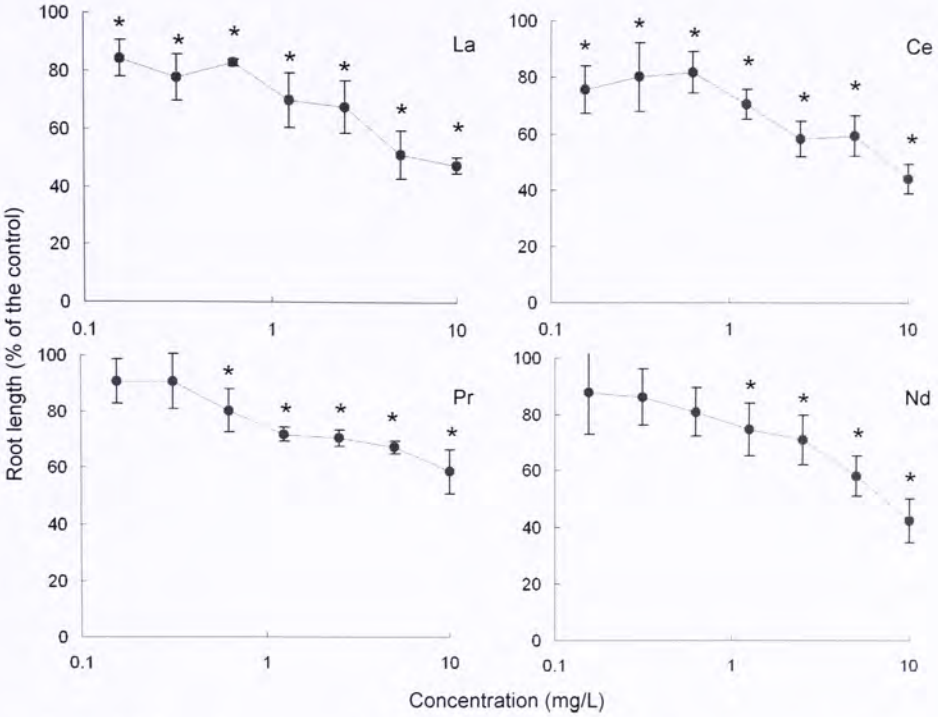


Figure 2.3 Root elongation of *B. chinensis* in solutions of different concentrations of REEs. Error bar represents standard deviation (n=3). Asterisk indicates significant difference ($p < 0.05$) when compared with the control.

REEs at concentration < 0.63 mg/L were not significantly inhibitory to root elongation in *L. perenne* (Figure 2.4). Root elongations at concentration of 0.63 mg/L were 80-89% of the control for the four REEs. Root growth of *L. perenne* treated with Nd solution was significantly affected when concentration was higher than 2.5 mg/L. However, at the same concentration, root length of *B. chinensis* was lower than that of *L. perenne*. Root growth of *B. chinensis* was significantly inhibited ($p < 0.05$) at lower concentration than *L. perenne*. Root length of *B.*

chinensis was 11.7% lower than that of *L. perenne* for La at a concentration of 2.5 mg/L. In cases of Pr and Nd, *B. chinensis* was slightly more inhibited than *L. perenne*. *B. chinensis* may be more sensitive to REEs than *L. perenne*.

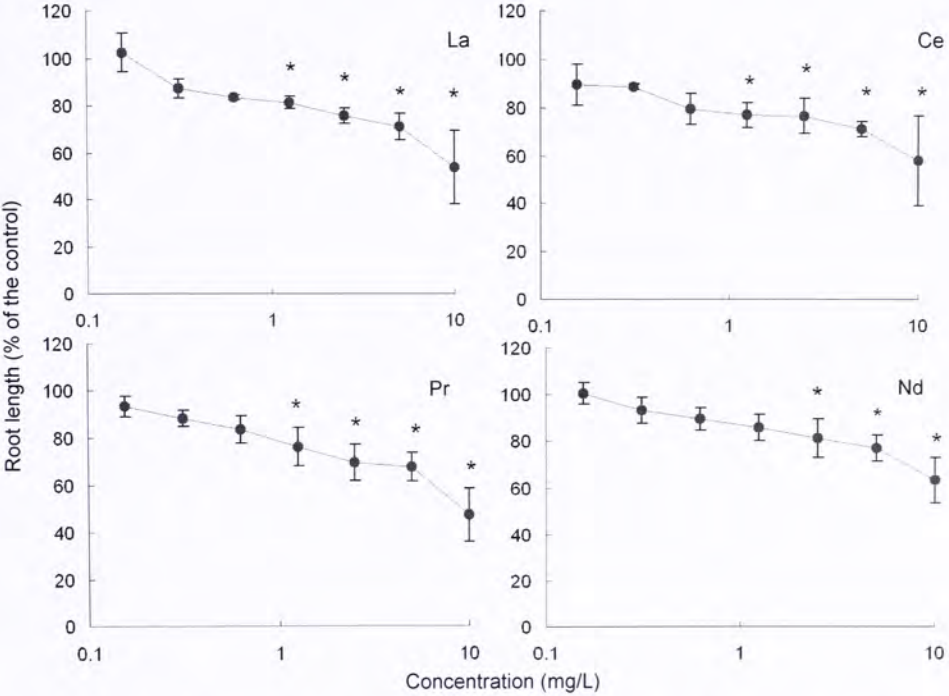


Figure 2.4 Root elongation of *L. perenne* in solutions of different concentrations of REEs. Error bar represents standard deviation (n=3). Asterisk indicates significant difference ($p < 0.05$) when compared with the control.

2.3.2.3 Germination index

Germination index (GI) was obtained by multiplying germination rate and root length, both expressed as percentage of control. The germination index

decreased as a function of REE concentration for both species (Figures 2.5 and 2.6). This implies that lower germination and poorer root growth as the concentration of REE increased.

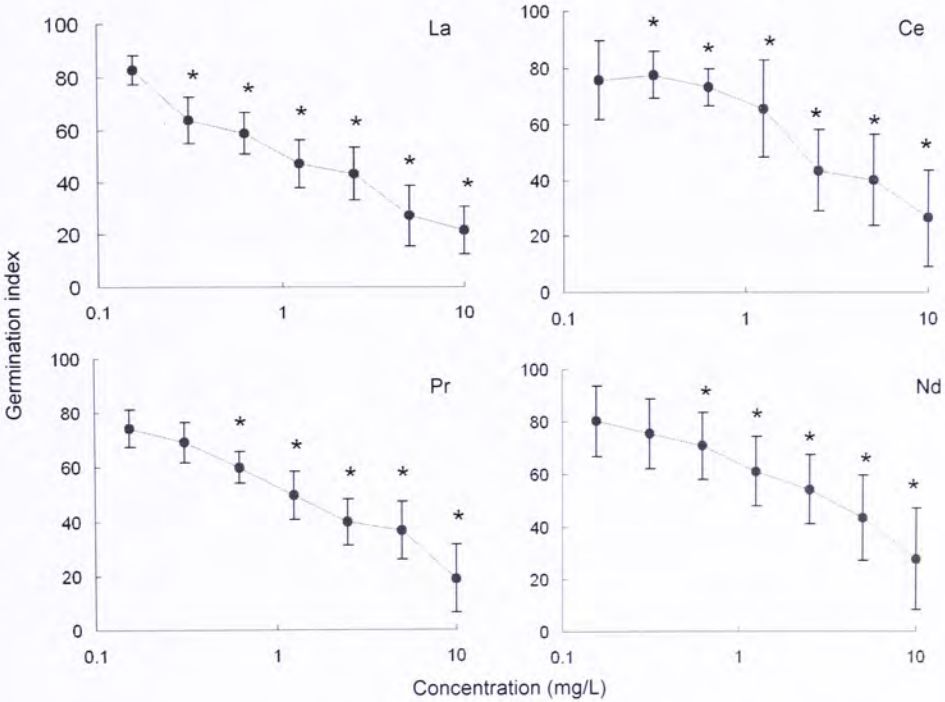


Figure 2.5 Germination index of *B. chinensis* in solutions of different concentration of REEs. Error bar represents standard deviation (n=3). Asterisk indicates significant difference ($p < 0.05$) when compared with the control.

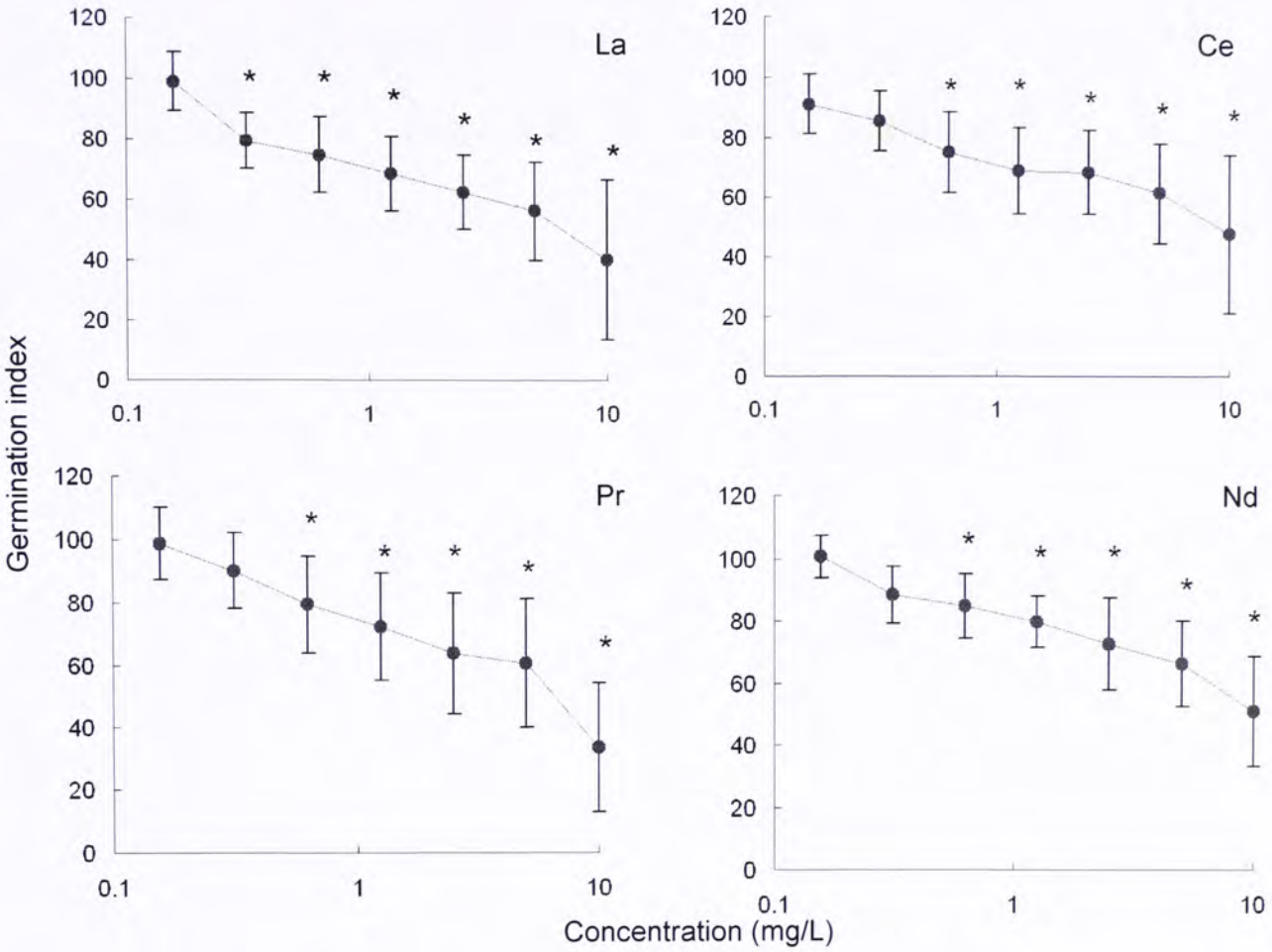


Figure 2.6 Germination index of *L. perenne* in solutions of different concentration of REEs. Error bar represents standard deviation (n=3). Asterisk indicates significant difference ($p < 0.05$) when compared with the control.

La and Ce gave significantly ($p < 0.05$) inhibitory effect on *B. chinensis* at concentration of 0.31 mg/L, where GIs were 63.8 and 77.6 for La and Ce respectively. For Pr and Nd, inhibitory effect was significant only at concentrations greater than 0.63 mg/L. Similarly for *L. perenne*, La had the lowest concentration (0.31 mg/L) that resulted in significant reduction in germination index. For the other three REEs, the germination index was significantly reduced at a higher concentration (0.63 mg/L).

In general, the germination indexes of *B. chinensis* in all treatments were lower than those of *L. perenne*. The germination index of *L. perenne* exposed to 0.31 mg/L was 24.7% significantly higher than that of *B. chinensis*. At a higher concentration (10 mg/L), the germination index of *L. perenne* was almost twice that of *B. chinensis*. This agree with the results obtained from root length and germination rate that *B. chinensis* was more sensitive to REEs than *L. perenne*.

2.3.3 The median effective concentration

The median effective concentrations (EC50s) for the inhibition of the development of seeds of *B. chinensis* and *L. perenne* were calculated and are shown in Table 2.4. The EC50 of La for *B.* chinensis was the lowest (9.52 mg/L). When comparing the 95% confidence interval (CI95) values, the EC50s of La and Ce were not significantly different, but were significantly lower than those of Pr and Nd. The highest EC50 was obtained in the treatment of Pr (20.1 mg/L), but it was statistically similar to the result of Nd. This shows that La and Ce were more toxic to *B. chinensis* than Pr and Nd; La was twice as toxic as Pr. La and Ce had lower atomic mass than Pr and Nd. The adverse effects of REEs on root growth of *B. chinensis* diminished as the atomic mass of REE increased.

For *L. perenne*, the lowest EC50 was obtained from the treatment of Nd, and the highest from Pr. However, the EC50 values for all the REEs were not significantly different from each other when regarding their CI95 values.

No significant difference was evidenced between the two species when treated with the same REE, since the CI95 of the two species overlapped with each other. Both *B. chinensis* and *L. perenne* were equally sensitive to REEs.

Table 2.4 Median effective concentration (EC50) (mg/L) of the various REEs with 95% confidence interval in parentheses.

REE	EC50	
	B. chinensis	*L. perenne*
La	9.52 (6.39-12.6)	16.2 (12.4-20.0)
Ce	10.2 (7.26-13.2)	16.0 (7.22-34.7)
Pr	20.1 (15.3-25.0)	19.9 (19.6-21.3)
Nd	15.1 (14.6-15.7)	14.5 (10.5-18.6)

2.4 Discussion

2.4.1 Dose-response curves of REEs

At low concentration, seed germination of *B. chinensis* and *L. perenne* were not different from the control. Root elongation of *L. perenne* did not differ significantly from the control when La and Ce concentrations were lower than 1.25 mg/L. The results indicate that seeds did not show toxic responses at low concentrations. Hu *et al.* (2002) tested the effect of La and Ce on wheat (*Triticum aestivum*) and found that root length was not decreased by REEs at concentration below 1 mg/L. The shoot of *Vigna radiata* grew similarly to the control until the concentrations of REE fertilizer solution were higher than 0.5% (Diatloff *et al.*, 1996b).

Both seed germination and root elongation were adversely affected when seeds germinated in solution of REEs at concentrations exceeding 10 mg/L. Seeds in all treatments were affected. All seeds did not germinate when concentrations of the REEs reached 100 mg/L, which indicated that the REEs concentration was too high for seed to germinate. High concentration of REEs would inhibit growth of seeds. The results are consistent with other studies. The root length of wheat when treated with La and Ce solution at 10 mg/L decreased 53.6% and 53.1% (Hu *et al.*, 2002). Elongation of onion roots treated with 40 μM La was inhibited by 40% when compared with the control (Clarkson, 1965). A REE solution with the concentration of 0.5% caused leaf damages on cucumber and tomato (Järvan, 2006). Solution containing > 2.0 μM Ce and > 50 μM La inhibited the vegetative growth of *Zea mays* and *Arabidopsis thaliana* (Diatloff *et al.*, 1996b; He and Loh, 2000).

2.4.2 Relative toxicity of the four REEs

In all the three endpoints, results of seeds treated with La solution differed significantly from the other REEs at low concentration. Ce affected the root elongation of *B. chinensis* more severely than Pr and Nd. For *B. chinensis*, the EC50s of La and Ce were significantly different from those of Pr and Nd. La had similar effects as Ce on root elongation of *Triticum aestivum* (Hu *et al.*, 2002). Although all the four REEs are classified as light REEs, there was difference between one and the other. The ionic radii of the four REEs were slightly different. The ionic radius gradually decreases (from 106.1 to 99.5 pm) as atomic number increases. Such difference may affect the affinity of the ion to bind to sites on cell

surface and consequently cause different effects on germination (Laul *et al.*, 1979; Brown *et al.*, 1990). Nevertheless, in the current study, the EC50 of the four REEs were indifferent from each other in the case of *L. perenne*. Since the difference in ionic radii between the four REEs is very small, *L. perenne* may not be sensitive enough to show the difference.

REEs are usually compared with heavy metals due to their similarity in properties. The phytotoxicities of heavy metals are well documented. Shu *et al.* (2002) reported root growth of *Cynodon dactylon* was severely inhibited when exposed to 0.17 mg/L of Cu and there was no root growth when Cu concentration reached 2 mg/L. Leaves of *L. perenne* showed chlorosis when Ni was added to the growth medium (Khalid and Tinsley, 1980). The EC50s of REEs were higher than those for most heavy metals (Table 2.5). Wong and Bradshaw (1982) determined the toxicity of several heavy metals using root elongation of *L. perenne* as endpoint. The EC50s of Cu, Mn and Ni were lower than 1 mg/L. The EC50s of Cu ranged from 0.0015 to 0.5 mg/L for different plant species (Wong and Bradshaw, 1982; Gorsuch *et al.*, 1990; Shu *et al.*, 2002; Charles *et al.*, 2006). The EC50s of heavy metals were much lower than those of REEs. Cu was 725-995 times more toxic than the REEs. Zn is a less toxic heavy metal and is also an essential element for plant growth; its EC50s ranged from 1.6 to 4.2 mg/L for different plant species (Wong and Bradshaw, 1982; Gorsuch *et al.*, 1990; Shu *et al.*, 2002). The EC50s of Zn found from root elongation of *L. perenne* was 9-12 times more toxic than the four REEs. By comparing EC50 with heavy metals, the four tested REEs were in

general much less toxic to plants.

Table 2.5 Summary of literature on phytotoxicity tests with heavy metals.

Test chemicals	Test organisms	Duration	EC50s	References
Cd	*Lolium perenne*	14-day	1.85 mg/L	Wong and Bradshaw, 1982
Cr	*Lolium perenne*	4-week	2.5 mg/L	Dijkshoorn *et al.*, 1979
	Lolium perenne	14-day	2.00 mg/L	Wong and Bradshaw, 1982
Cu	*Chlorella* sp.	72-hour	0.0015 mg/L	Franklin *et al.*, 2000
	Cynodon dactylon	14-day	0.17 mg/L	Shu *et al.*, 2002
	Lactuca sativa	5-day	0.5 mg/L	Gorsuch *et al.*, 1990
	Lemna aequinoctialis	48-hour	0.0162 mg/L	Charles *et al.*, 2006
	Lolium perenne	14-day	0.02 mg/L	Wong and Bradshaw, 1982
	Paspalum distichum	14-day	0.19 mg/L	Shu *et al.*, 2002
	Zea mays	14-day	0.27 mg/L	Craig, 1978
Mn	*Lolium perenne*	14-day	0.45 mg/L	Wong and Bradshaw, 1982
Ni	*Lolium perenne*	14-day	0.18 mg/L	Wong and Bradshaw, 1982
	Zea mays	14-day	0.39 mg/L	Craig, 1978
Pb	*Cynodon dactylon*	14-day	8.2 mg/L	Shu *et al.*, 2002
	Lolium perenne	14-day	1.7 mg/L	Wong and Bradshaw, 1982
	Paspalum distichum	14-day	7.8 mg/L	Shu *et al.*, 2002
Zn	*Cynodon dactylon*	14-day	1.8 mg/L	Shu *et al.*, 2002
	Lactuca sativa	5-day	2.16 mg/L	Gorsuch *et al.*, 1990
	Lolium perenne	14-day	1.6 mg/L	Wong and Bradshaw, 1982
	Paspalum distichum	14-day	4.2 mg/L	Shu *et al.*, 2002

REEs did not cause serious harmful effect on other tested organisms. Biomass of wheat was reduced when REE concentration was > 5.5 g/L (Järvan, 2006). The leaf biomass of wheat treated with Eu was similar to those without Eu (Shtangeeva and Ayrault, 2006). The 48-h LC50 of *Hypophthalmichthys molitrix* and *Carassius auratus* to REEs was 12.2 and 36.0 mg/L respectively (Hu and Luo, 1980). All the *Cyprius carpio* that exposed to 0.5 mg/L Gd and Y solution survived during the exposure period (Tu *et al.*, 1994).

Some studies found that REE gave beneficial effects on seedlings. The root dry weight of *Zea mays* was increased by Ce and La at concentration lower than 0.56 mg/L (Diatloff *et al.*, 1996b). REEs increased the dry weight of seedling of *Beta vulgaris* at a range of 4.0-19.2% (Tian *et al.* 1990). The root biomass of wheat increased by 20% when it was exposed to 0.01 mg/L Eu (Shtangeeva and Ayrault, 2006). The pollen growth of *Nicotiana tabacum* and *Prunus perscia* were also promoted by 14.9-27.7% at low La concentration (Sun *et al.*, 2003). The amaranthin synthesis and the plant growth were promoted after the plant exposed to 0.4 mmol/L Eu (Wahid *et al.*, 2000; Zeng *et al.*, 2003). However, significant growth promotion was not observed in the present studies. The deviation may be attributed to the different growth stages of plants. Seeds were used in the present study while seedlings were used in other studies. Seed is a vulnerable stage in the life of a plant. Once there is damage on the seeds during germination, no remedy can be done to make the seed germinate. The remaining stages in the life cycle will not occur. Therefore, testing the toxic effect of a chemical on plants using

seed germination is highly valuable. Seeds contain virtually all the materials to start off a new plant. No external sources of nutrients are required for seeds to germinate. The promotional effect of REEs may be shown by seedlings but not seeds. After seed grows into seedling, external nutrients are needed by the seedling to grow. REEs may act as nutrient or mimic other nutrients to promote growth at low concentrations.

2.4.3 Mechanism of effect of REEs on seed growth

The mechanism underlying the effects, both positive and negative, of REEs on plants is still not clear. The most widely discussed mechanism was the displacement or replacement of Ca^{2+} by REE on cells. Activities of many enzymes and proteins depend on Ca^{2+}-binding sites in the cell membrane. Change in element on the binding site will affect the function of enzymes, and eventually plant growth. The ionic radii of REE ions were similar to that of Ca^{2+}. REEs would compete for binding sites with Ca^{2+} (Jie *et al.*, 2003). As the valence of REE ion was higher than that of Ca^{2+}, once REEs have bound to the sites, the binding would be less reversible (Lettvin *et al.*, 1964). Displacement of Ca^{2+} from the binding sites would convert the active site structure and the consequent enzyme activities. Effect of REE on pollen grain was reported to be related to calmodulin, which is a Ca^{2+} receptor protein (Sun *et al.*, 2003). In the presence of La, calmodulin is stimulated and started the transduction of signal into the cell which causes germination. Ca^{2+}-dependent protein kinases from silver beet leaves were inhibited by the presence of REE ions. The inhibition ranged from 66-93% for different

REEs (Brown *et al.*, 1990). Another type of enzyme, spinach ferredoxin, was inhibited by the replacement of Ca^{2+} by REE ions. Replacement of Ca^{2+} by REEs in enzyme may not always inhibit its activity. On the other hand, REEs can activate enzymes after the replacement of Ca^{2+} (Chen *et al.*, 2001; Jie *et al.*, 2003; Sun *et al.*, 2003). Such activation may explain the promotion of seed germination and plant growth in the presence of REEs.

Besides influence on the calcium level, REE was reported to block Ca^{2+}-channel (Pickard, 1970). Ca^{2+}-channel is a major path for flux of many ions through the cell membrane. Since the channel was blocked by REEs, uptake of nutrients was disturbed which inhibited seed growth. However, some studies found that such inhibition occurred at high La concentration only (Zeng *et al.*, 2003). When external La concentration was low, the nutrient levels in cells were the same as those in treatment without La (Hu *et al.*, 2002). The blocking may be concentration dependent.

2.4.4 Comparison between different endpoints

Effects of chemicals on the plants may be analyzed by seed germination, shoot or root length change over the duration of the exposure, plant growth measured by fresh weight or qualitative observations such as yellowing of leaves and chlorosis. (Klaine *et al.*, 1995). Among the various endpoints, quantitative measurement would provide an evaluation of toxicity of the chemical tested. Two quantitative endpoints were used to test the phytotoxicity of REE in this experiment.

The sensitivities of the endpoints REEs were different. Percentage of germination remained at relatively high levels at low concentrations. Germination rate was less sensitive to REEs. Although the difference between germination rate and root elongation for *B. chinensis* was not greatly apparent, the sensitivity of root elongation was higher.

Root elongation is generally inhibited at lower toxicant concentrations than seed germination (Gorsuch *et al.*, 1990; Linder *et al.*, 1990; Wang and Keturi, 1990; Klaine *et al.*, 1995; Peralta *et al.*, 2000; Lau *et al.*, 2001; Wong, *et al.*, 2001; Lee *et al.*, 2002; Ali *et al.*, 2004). The lowest concentrations that gave significant inhibition to root elongation for *B. chinensis* were 0.16 mg/L for La and Ce, which had no difference in germination rate. The lowest concentration of La and Ce which reduced germination significantly were 0.63 mg/L and 2.5 mg/L respectively. Root elongation may be a more sensitive indicator for REEs. The germination of seeds of *Vicia faba* was not affected by different solutions of Ca, Co, Ni and Zn chlorides alone and in combination, but effects on root elongation were recorded (Misra *et al.*, 1994). The median inhibitory concentration (IC50) of Cr on lettuce was lower for root growth than seed germination (Cureton *et al.*, 1994). The IC50 estimated from seed germination was 90 μg/g while that from root growth was 3 μg/g. Ali *et al.* (2004) also obtained similar results that the IC50 from root growth was lower than that measured from germination for barley. This applied also to the whole experiment of spent pig litter (Tam and Tiquia, 1994).

Root elongation is more sensitive than germination rate with response to REEs. Therefore, root elongation should be a better indicator about the toxicity of REEs on plants.

2.4.5 Comparison between different species

The concentrations that cause significantly inhibition to *B. chinensis* and *L. perenne* were different. When the seeds were treated in La solution, root lengths of *B. chinensis* and *L. perenne* were significantly shorter than that in the control at concentration of 0.16 mg/L and 1.25 mg/L respectively. *B. chinensis* was affected at lower concentration than *L. perenne*. Similarly, germination index of *B. chinensis* was lower. It may be due to the shorter root elongation of *B. chinensis* when treated with REEs. However, the EC50s of *B. chinensis* and *L. perenne* were not significantly different for each REE, showing that the sensitivity of *B. chinensis* to REE was comparable to that of *L. perenne*.

L. perenne is proposed by the USEPA, OECD and USFDA as a test species for phytotoxicity test using seed germination and root elongation as endpoints. The species is sensitive to many chemicals, including both organic and inorganic chemicals such as acetic acid, herbicides, heavy metals, ammonium, salts and minerals. Gorsuch *et al.* (1990) evaluated 26 commercially important chemicals, including various heavy metals using seed germination as well as root and shoot elongation of *L. perenne* as endpoint. *L. perenne* has been commonly used in phytotoxicity test.

B. chinensis is not a species proposed by the USEPA or USFDA, but by the OECD. *B. chinensis* is one of the most important vegetables consumed in Hong Kong and England (Wong and Leung, 1989; Mahmud *et al.*, 1999). The species is endemic to the area of concern. They are economically important and constitute major cash crops in Hong Kong and China. The seeds can be easily purchased from local suppliers. Its root was thicker than that of *L. perenne*; it is more easily and efficiently to handle during root length measurement. The germination rate of seeds in aqueous medium was high and uniform, with an average of over 95%, which meets the criteria for standard phototoxicity test protocol. *B. chinensis* is a suitable species for phytotoxicity test. It has been used in many studies, for toxicity testing of spent pig litter, landfill leachate, dredged sediment and sewage sludges (Tam and Tiquia, 1994; Wong *et al.*, 2001; Chen *et al.*, 2002; Cheng and Chu, 2007). From the result of the present study, *B. chinensis* would be a suitable species for testing REEs.

2.4.6 Limitations and improvement

Seeds germinate in a short time, generally 2-4 days. This is an advantage with regard to the experimental duration. However, as the time for seed exposed to chemicals is short, germination test can only determine acute rather than chronic effect of chemicals.

Endpoints of seed germination/root elongation test are germination rate and root length, which provided limited information about the effect of REEs on plants.

Symptoms such as changes in height, foliar biomass, standing leaf number, colour of leaf and flower cannot be assessed by germination test. Seedlings should be tested also to display the effect of REEs on these aspects.

Since most of the germination tests are carried out in laboratory where conditions such as light and humidity are favourable for seeds to germinate; the test may not provide situation similar to the field, which are more variable. In application aspect, REEs are usually applied to plants in cultivated and forestry areas, test using seedlings will better reveal the field conditions.

Seeds were soaked in REE solution in the germination test. However, the usual method for applying REEs to plants is by mixing REEs with soil. After REEs was added to soil, reactions will occur and lead to change in their availability. Based on these limitations, test using seedlings grown in soil will be an alternative to seed germination and root elongation test to determine the effects of REEs on plants.

2.4.7 Methods of measuring root length

Root length was measured manually by a caliper in the present study. This had the advantages that the appearance of seeds and roots can be observed during measurement. However, the method has several limitations. It is labour and time consuming. One root can be measured at the same time. Most of the time would be spent on measurement. It would be worsened if a large scale experiment was

carried out. The length was judged by the naked eyes, which affects the accuracy of measurement. In order to improve the precision, computer-aided analysis is suggested.

Image of root would be captured using either a scanner or camera. Germinated seeds are placed horizontally on top of a flat-bed scanner to get the image of root (Geneve and Kester, 2001). Camera can capture continuous image of root growing vertically. The image are then transferred to a computer and analysed by specialized software to obtain the length of root. The greatest stride over manual measurement is the improvement of precision to less than 0.1 mm. The result should be more reproducible. Tens of roots can be handled at the same time so that measurement time is saved. The image can be saved in a computer to allow review in the future.

2.5 Conclusions

Toxicity of four REEs, viz. La, Ce, Pr and Nd, were tested by seed germination and root elongation. Inhibition to root elongation by REEs was more severe than inhibition to germination. Root elongation was a more sensitive parameter than seed germination. La and Ce were more toxic to *B. chinensis* than Pr and Nd. The effects of REEs on *L. perenne* were statistically indifferent. Although *B. chinensis* was not a highly recommended species in phytotoxicity test, EC50s of REEs measured by the two species were not significantly different. Seeds of both *B. chinensis* and *L. perenne* were sensitive to the four REEs. EC50s

of REEs were much higher than those of heavy metals, implying lower acute toxic effect. Seeds are more sensitive than seedlings. The low toxicity to seed germination implies that the chance for REEs exhibiting toxic effect to seedling would be low. However, the effects of REEs on other aspects of plants are still not well known. This information will allow a better understanding of the value and the application of REEs to plants.

Chapter 3 Growth of tree seedlings in soil treated with rare earth elements

3.1 Introduction

Rare earth elements (REEs) are commonly used in industrial and agricultural applications. Nd and Sm are used to make permanent magnet. Catalysts for the cracking of crude petroleum contain mixture of rare earth oxides. Since 1990s, REE fertilizers have been widely applied, especially in China, and the application rate increased rapidly. The yield and quality of products irrigated with REE solution were claimed to be improved. Yield of corn and wheat grown in soil added with REE were increased by 7-14% and 6-15% respectively (Xiong *et al.*, 2000). Quality benefits to plants included a greater production of roots, a darker green foliage, an enhanced rate of development, and better fruit colour in apples, oranges and watermelons (Brown, *et al.*, 1990).

Experimental studies about application of REEs for plant growth have reported both positive and harmful effects. The length of primary roots, dry weight and number of flowers of *Arabidopsis thaliana* subjected to La and Ce solution increased (He and Loh, 2000). Growth of *Dryopteris erythrosora* in La solution was more than two fold when compared with control (Ozaki *et al.*, 2000). Root biomass of wheat (*Triticum aestivum*) seedling grown in Eu solution was significantly higher against control (Shtangeeva and Ayrault, 2007). In contrast, oat coleoptile growth was restricted by Nd while leaf of corn was burnt when treated in Ce solution (Pickard, 1970). Most of the studies focused on agricultural crops, such as wheat, corn, maize

and bean, which are grown in farms which are isolated from natural environment. It may not extrapolate the situation in the natural environment.

Forestry plants, especially trees, are important to both environment and human. Trees purify air by taking in carbon dioxide and releasing oxygen. They are great energy collectors that convert solar energy to chemical energy. Trees reduce force of rainsplash at soil surface by intercepting and absorbing water, so run off as well as erosion are prevented. They harbour a diversity of wildlife that they are home of many animals. Every part of tree is economically important: timber, oil, rubber, flower and fruit. Studies of the effects of REEs on forestry plants would better reflect the influence on the natural environment, but such studies are lacking. In this study, tree seedlings will be used to investigate the effect of REEs on forestry plants. Results of the phytotoxicity test in the previous chapter shows that the toxicity of REEs to sensitive crop species was not so high. Since the REEs were found to be beneficial to agricultural crops, it would be a contribution to forest ecosystems if such positive effects were also demonstrated in tree seedlings.

Despite similarities in the physical and chemical properties of REEs, some studies reported that there is difference in absorption and effect on plants between REEs. Maize absorbed more La than Lu under the experimental conditions carried out by Xu *et al.* (2002). Wheat tended to absorb light REEs at higher rates when compared with heavy REEs (Liang *et al.*, 2005). Results from seed germination/root elongation test showed that effects of the tested REEs were different from one another.

The major components in REE fertilizers are La, Ce, Pr and Nd which are all light REEs. The four REEs make up of more than 80% of commercial fertilizers that contain REEs. Studies using light REEs would be emphasized.

The present study aims to examine the effect of REEs on tree growth. Plants were exposed to different REE application rates in a greenhouse pot experiment. Biological responses and tissue contents in tree seedlings were monitored and analysed during and at the end of the experiment.

3.2 Materials and methods

3.2.1 Soil

Soil was collected from the campus of The Chinese University of Hong Kong. The soil was sieved through a 5-mm mesh sieve to remove large particles before use. One part of peat moss was added to every three parts of soil as soil conditioner.

3.2.2 Tree seedlings

Seedlings of *Acacia auriculiformis* and *Eucalyptus citriodora* with height of 15-20 cm were used. They were chosen since they were species that commonly planted in Hong Kong and they can grow rapidly. Seedlings of *Acacia auriculiformis* were bought from the Tai Tong Nursery of the Agriculture, Fisheries and Conservation Department, while seedlings of *Eucalyptus citriodora* were provided by Pegasus Greenland Ltd (Hong Kong).

3.2.3 REEs

REEs used in this study were lanthanum (La), cerium (Ce), praseodymium (Pr) and neodymium (Nd). All of them were light REEs and occupied the largest proportion (83%) in the commercial fertilizer. Reagent grade chemicals were used and all chemicals were in their nitrate forms (Fluka, Riedel-de Haën (RdH) and Aldrich) which is the form used in the commercial fertilizer. The purity of the chemicals used was $\geq$ 99.9%.

3.2.4 Greenhouse experiment

Pot experiment was carried out in a greenhouse of The Chinese University of Hong Kong. One seedling of either *A. auriculiformis* or *E. citriodora* was planted to pot (19 cm in diameter and 18 cm in height). They were acclimated for 1 month after received. Known amounts of REEs were added to the soil before seedling transplant and mixed thoroughly to result in soil concentrations of 0, 1, 5, 25 mg REE/kg of the different REEs. There was only one REE per pot which treated with REE. Each treatment had four replicates and the pots were arranged in a randomized block design (Plate 3.1). To each pot, 1.22 g of urea and 1.40 g of potassium dihydrogen phosphate were added to supply 200 kg/ha nitrogen and phosphorus respectively (Bradshaw, 1983).

Plate 3.1 Tree seedlings of *A. auriculiformis* and *E. citriodora* in 19-cm pots arranged in randomized blocks in a greenhouse.

The seedlings were watered with deionized water every day. The growth and health of the plants were monitored. The height, standing leaf number and basal diameter of the seedlings were measured every 15 days. Chlorophyll fluorescence was represented as F_v/F_m which were measured using a plant efficiency analyser (PEA) (Hansatech, England) every 30 days. The plants were harvested after 90 days, and the harvested tissues were chemically examined.

3.2.5 Soil analysis

3.2.5.1 Initial properties

Soil used in the pot experiment was sampled and air-dried for 2 weeks. The soil was then sieved through 2-mm mesh sieve.

Soil texture

Soil texture was determined by the Bouyoucos hydrometer method which measures the decrease in density of a suspension as soil particles settle (Allen, 1989; Grimshaw, 1989). A soil sample of 50 g was stirred with 25 mL 5% sodium hexametaphosphate (Calgon solution) and 400 mL water in an electric blender for 15 minutes. The mixture was poured into a measuring cylinder and stirred with paddle for 1 minute. The hydrometer readings were taken at 4 min 48 seconds for silt and clay contents and 5 hours for clay content. The sand, silt and clay contents were expressed as percentages by weight. Classification of the International Society of Soil Science was used to find out the textural class of the soil.

pH

Water extract of the soil was measured by the glass and Pt electrode using the Jenway 4330 pH and conductivity meter. Milli-Q water was added to 10 g of soil to form aqueous slurry in 1:1 (w/v) ratio. The mixture was shaken at 150 rpm for 15 minutes and allowed to stand for 30 minutes before measurement.

Organic matter

Organic matter in soil was determined by wet combustion method modified by Kandeler (1995). Potassium dichromate solution and concentrated sulphuric acid were added to 2 g of air dried soil. The mixture was allowed to stand for 3 h and then fill up to 100 mL. The samples stood overnight before spectroscopic measurement at 570 nm.

Cation exchange capacity

Cation exchange capacity of soil was determined by ammonium acetate method modified by Kim (1996). Air-dried soil was weighed in a 100-mL centrifuge tube and 25 mL ammonium acetate solution was added. The soil mixture was mechanically shaken for 1 h. The supernatant solution was separated from the soil by centrifugation. The NH_4-saturated soil was washed three times with 20 mL 95% ethanol, by shaking and centrifugation. After removing ethanol, the soil was mixed with 25 mL sodium chloride (pH 2.5) solution and shaken mechanically for 30 minutes. The supernatant was separated from the residue by centrifugation. The ammonium concentration was determined by a Skalar SAN^{Plus} segmented flow auto-analyser (Skalar Analytical BV, Breda, The Netherlands).

3.2.5.2 Post harvest analysis

Soil was sampled from each pot after harvesting. Collection of the soil samples was made after soil in each pot was mixed thoroughly. The soil samples were then air-dried for 2 weeks. Air-dried soil was sieved through 2-mm mesh sieve

before analysis. Sieved soils were stored in plastic bags to prevent contamination until being analysed.

REE contents

A portion of 0.4 g of dried soil samples was digested with mixed acid of concentrated nitric acid and 30% hydrogen peroxide by microwave digestion using a Milestone ETHOS touch control close vessel microwave digester following the method of Milestone (2003). The digested samples were stored at 4°C before analysis. The total contents of REEs in soil were determined by Optima 4300 DV inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer, USA) after mixed acid digestion.

Total Kjeldahl and mineral nitrogen

Soil samples were digested by semi micro-Kjeldahl digestion (Skalar, 1995). Total N content in soil was determined by a Skalar SAN^{Plus} segmented flow auto-analyser.

Extractable NHx-N and NOx-N were measured by Skalar SAN^{Plus} segmented flow auto-analyser after extraction with 1 M potassium chloride at 150 rpm for 1 h (Rowell, 1996).

Total and available phosphorus

Total P content was measured by Skalar SAN^{Plus} segmented flow auto-analyser

using the same method as described for total N.

Available phosphorus was extracted by Troug's reagent (0.001 M $(NH_4)_2SO_4$ buffered at pH 3) at 150 rpm for 30 min (Troug, 1930). The content was then determined by Skalar SAN^{Plus} segmented flow auto-analyser.

Mineral nutrients

The sample used to measure REE content was also used to determine metals using ICP-OES.

3.2.6 Plant analysis

Leaves and roots were harvested at the end of the experiment. Leaves were washed with tap water and Milli-Q water. Roots were separated from soil by washing in tap water until no soil particles were visually detected on the roots, and then with Milli-Q water. The plant samples were dried in an oven at 65°C until constant weight. The dry biomass of the plant samples was weighed. Dried plant samples were ground to pass through 1 mm mesh before further analysis. The methods of digestion and determination were the same as those for soil analysis.

3.2.7 Statistical analysis

The data were processed by SigmaStat 3.1. The differences between treatments were tested by three-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference test at $p = 0.05$ wherever appropriate.

3.3 Results

3.3.1 Growth

All tree seedlings survived until the end of the experiment. No apparent illness appearance such as spots and change in leaf colour was observed throughout the period before harvesting.

3.3.1.1 Height

When comparing the height of tree seedlings, in general, those treated with REEs grew better than those without REEs (Table 3.1). All REEs with application rate of 1 mg/kg resulted in greater heigh in *A. auriculiformis* than the control. Seedlings in the lowest La and Nd concentration were four times and twice as tall as that in the control. There was no significant difference ($p > 0.05$) between the treatment of 25 mg/kg and the control for most of the REEs, whereas *A. auriculiformis* treated with 25 mg/kg Ce was significantly taller than that of the control. Seedlings treated with 1 mg/kg Pr were significantly taller than the control, while those in the other two concentrations were not different from control.

For *E. citriodora*, the concentrations that gave the best height increment were 1 mg/kg for La and Pr, and 5 mg/kg for Ce and Nd. Seedlings in the most effective concentration were almost twice as tall as the control. Except for Ce, all other REEs in the highest concentration did not give significantly higher height than the control.

All the four REEs promoted height growth for both *A. auriculiformis* and *E.*

citriodora. Promotional efficiencies of them were similar. The height increments of the two species were similar except those exposed to Nd.

Table 3.1 Plant growth in height (% change) after 90-day exposure to REEs.

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	30.1 b	128 a	99.5 a	68.2 ab
	Ce	30.1 b	81.0 a	71.1 a	69.9 a
	Pr	30.1 b	104 a	78.7 ab	52.8 ab
	Nd	30.1 b	73.6 a	56.1 a	41.2 b
E. citriodora	La	66.0 b	127 a	101 a	78.3 b
	Ce	66.0 c	91.9 ab	114 a	79.8 b
	Pr	66.0 b	108 a	101 a	77.5 b
	Nd	66.0 b	101 a	132 a	85.1 b

For each REE, the same letter represents no significant difference between concentrations ($p < 0.05$, Tukey's test).

At the initial growth period, the height of *A. auriculiformis* increased slowly in all REE treatments (Figure 3.1). An obvious difference was observed for most REEs after 45 days. The height increment of the control was the slowest when compared with REEs. Height increase in soils treated with Pr was the highest at 1 mg/kg throughout the whole period of study. However, for the other REEs, application rate at 1 mg/kg gave the highest growth only at the end of the experiment. Height of *A. auriculiformis* exposed to Ce at the highest concentration was similar to the second highest concentration. For *E. citriodora*, the growth rate was consistent throughout the experiment (Figure 3.2). Similar to the growth of *A. auriculiformis*, height

increment of eucalyptus without REE application was the lowest. Tree seedlings treated with 5 mg/kg Ce grew the fastest when compared with other concentrations of the same REE. For La and Pr, the growth in various application rates were similar at the beginning of the experiment, differences were apparent at a later period of the experiment. At the end of the experiment, seedlings exposed to 1 and 5 mg/kg doubled their height.

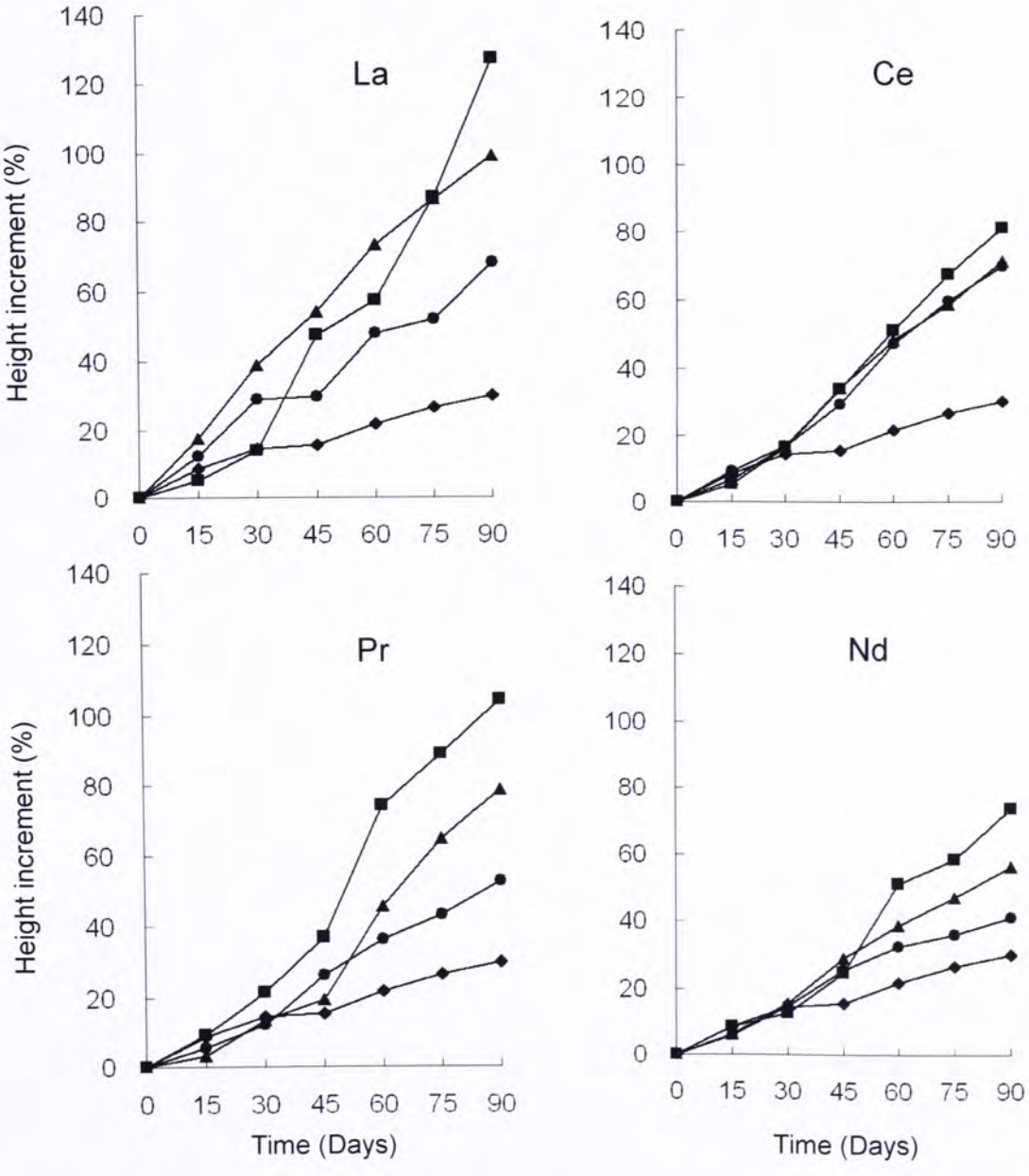


Figure 3.1 Growth of *A. auriculiformis* exposed to REEs at 0 mg/kg (◆), 1 mg/kg (■), 5 mg/kg (▲) and 25 mg/kg (●).

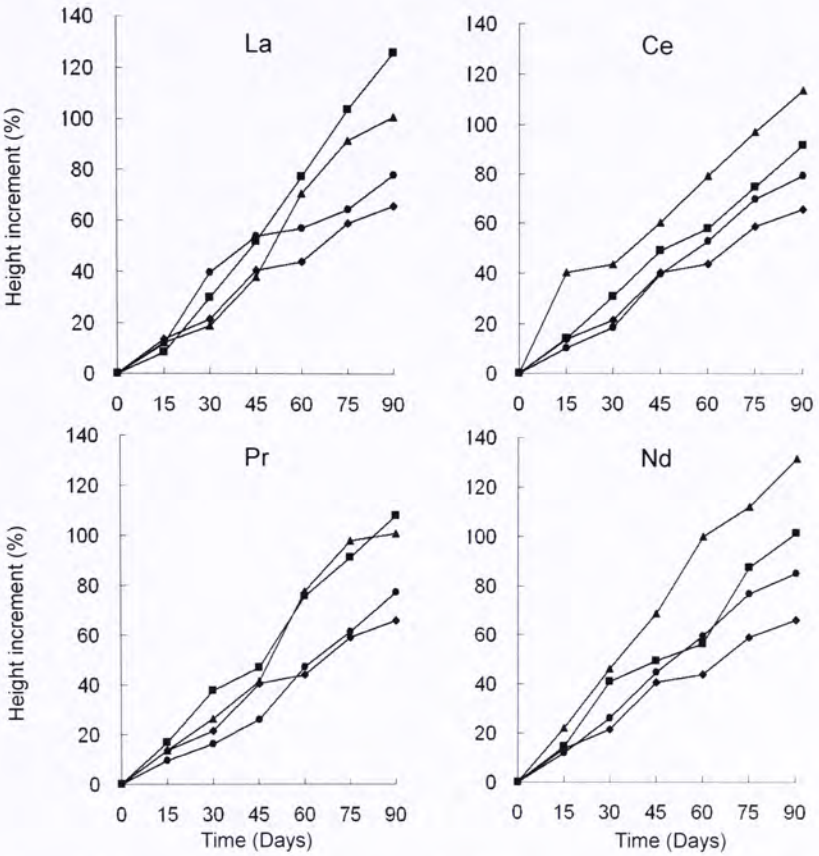


Figure 3.2 Growth of *E. citriodora* exposed to REEs at 0 mg/kg (◆), 1 mg/kg (■), 5 mg/kg (▲) and 25 mg/kg (●).

3.3.1.2 Basal diameter

Basal diameters of *A. auriculiformis* seedlings treated with REEs were generally significantly larger than those without REEs (Table 3.2). Except Ce, other REEs gave higher basal diameter to seedlings at all concentrations. At 5 mg/kg Ce, seedling basal diameter was not significantly different from the control. The

increment of basal diameter ranged from 16.7 to 47.6% under treatment of REEs, which was smaller than that of height. There was no significant difference ($p > 0.05$) between all REE concentrations for each REE. When comparing the REEs at the same concentration level, there was no significant difference between the four REEs studied.

Table 3.2 Plant growth in basal diameter (% change) after 90-day exposure to REEs.

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	9.56 b	30.0 a	23.4 a	47.6 a
	Ce	9.56 b	40.6 a	16.7 ab	24.9 a
	Pr	9.56 b	28.2 a	21.4 a	33.9 a
	Nd	9.56 b	29.6 a	23.9 a	30.4 a
E. citriodora	La	30.1 c	59.6 a	42.6 b	35.6 bc
	Ce	30.1 b	34.3 b	65.0 a	35.5 b
	Pr	30.1 c	78.4 a	40.8 b	26.9 c
	Nd	30.1 b	72.0 a	64.0 a	64.5 a

For each REE, the same letter represents no significant difference between concentrations ($p < 0.05$, Tukey's test).

Most of the four REEs at concentrations of 1 and 5 mg/kg significantly promoted basal stem growth of *E. citriodora* seedlings (Table 3.2). Seedlings treated with the lowest concentration (1 mg/kg) of La and Pr had significantly greater basal diameter than other application rates, while this happened at 5 mg/kg Ce. The greatest basal diameter was 2-2.5 times of the seedlings that did not expose to REEs. The greatest increment occurred at 1 mg/kg Pr. Basal diameter of *E. citriodora*

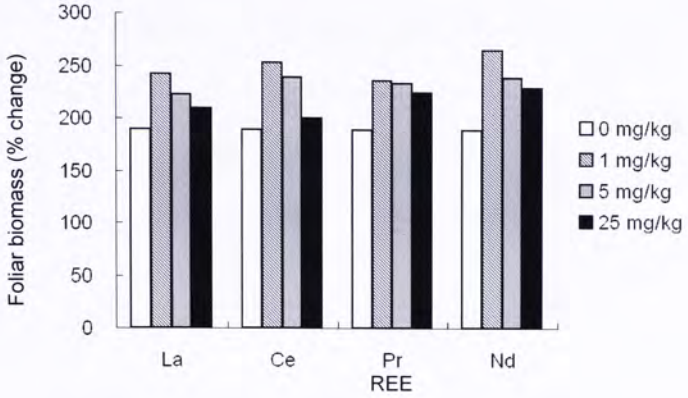
treated with Nd was similar at all the three application rates. The increase of basal diameter ranged from 64.0 to 78.4% when exposed to Nd, while the control was 30.1%. Change in basal diameter in treatments of La, Ce and Pr at 25 mg/kg were similar to the control; only Nd at 25 mg/kg resulted in significantly higher than the control. At the lowest concentration, the basal diameter of seedlings treated with Ce was smaller than the other REEs, while at 5 mg/kg REEs, Ce gave the highest growth in stem diameter.

3.3.1.3 Biomass

After the 90-day experiment, the control foliar biomass was almost double of the initial biomass (Figure 3.3). The increase of foliar biomass of *A. auriculiformis* ranged from 201 to 266% when treated with different application rates of REEs. There are no significant differences ($p > 0.05$) among all treatments. For *E. citriodora*, seedlings treated with 1 and 5 mg/kg REEs had significantly higher foliar biomass than those without REE. The promotional effect of 1 mg/kg REEs was the greatest, whereas those treated with 25 mg/kg REEs did not grow better than the control.

In general, the four tested REE performed similarly regarding foliar biomass production in various concentrations.

(a)



(b)

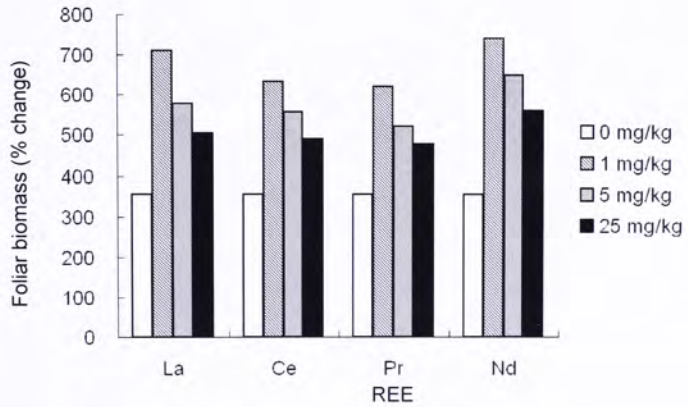


Figure 3.3 Foliar biomass (% change) after 90-day exposure to REEs (a) *A. auriculiformis* and (b) *E. citriodora*.

The effect of REEs on root biomass was more obvious than that on foliar biomass. For *A. auriculiformis*, there was a significant difference ($p < 0.05$) between the applied concentrations (Figure 3.4). Root biomass treated with REEs at the two

lowest concentrations was greater than control. However, all REEs added at the highest concentration did not significantly promote root growth of *A. auriculiformis* seedlings. Nd at the highest application rate had similar effect as the other two concentrations, though it was not significantly different from control.

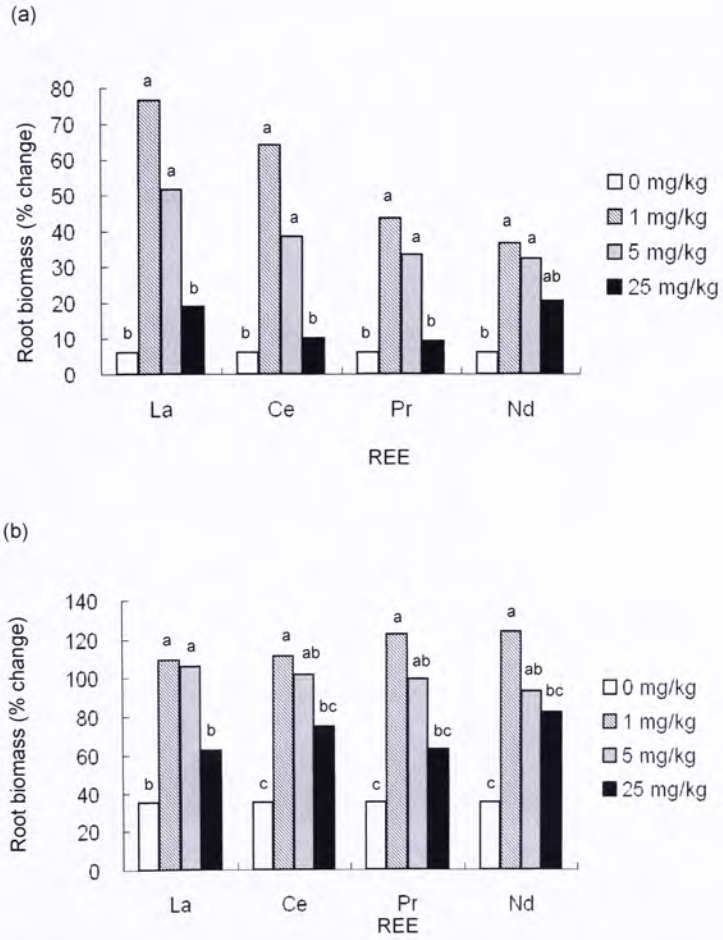


Figure 3.4 Root biomass (% change) after 90-day exposure to REEs (a) *A. auriculiformis* and (b) *E. citriodora*. When compared within REE in the same species, bars with the same letters are not significantly different at $p > 0.05$ by Tukey's test.

E. citriodora had root growth similar to that of *A. auriculiformis*. The concentration that brought about the highest root biomass was 1 mg/kg for all REEs. With the addition of 5 mg/kg REEs, they were significantly different from the control. With the exception of La, root biomass at 5 mg/kg was not significantly different from the root biomass at 25 mg/kg. The difference in root biomass of the control and the highest REE concentration were not statistically significant.

When comparing the performance between the REEs, there were no significant differences in all levels for both species.

3.3.1.4 Standing leaf number

There was no massive leaf fall during the experiment period. Both *A. auriculiformis* and *E. citriodora* treated with REEs had more standing leaf number than the control seedlings. In general, the effects of the four REEs were not different with statistic significance ($p > 0.05$).

All REEs resulted in greater standing leaf number than the control. With the addition of REEs, the standing leaf number at the end of the experiment increased by 60-256% (Table 3.3). The four tested REEs at the lower two concentrations gave significant beneficial effect to *A. auriculiformis* seedlings. At 1 mg/kg Ce and 5 mg/kg La and Nd, the leaf numbers were over 300% of the initial numbers. In the other treatments, the standing leaves on REE treated seedlings were almost twice of the control. The standing leaf number of tree seedlings treated with Ce and Pr at the

highest application rates were significantly higher than the control, while seedlings treated with La and Nd were not.

Table 3.3 Standing leaf number (% change) after 90-day exposure to REEs.

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	129 B	271 a	314 a	193 ab
	Ce	129 B	325 a	286 a	261 a
	Pr	129 B	296 a	272 a	253 a
	Nd	129 C	266 ab	356 a	160 bc
E. citriodora	La	190 C	549 a	284 b	229 bc
	Ce	190 C	280 b	423 a	257 b
	Pr	190 C	545 a	352 b	240 c
	Nd	190 C	406 a	395 a	324 b

For each REE, the same letter represents no significant difference between concentrations ($p < 0.05$, Tukey's test).

The concentration which brought about the highest standing leaf number of *E. citriodora* for La and Pr was 1 mg/kg, while that for Ce was 5 mg/kg. The effects of two lower Nd concentrations (1 and 5 mg/kg) were similar to one another. The leaf numbers under treatments of REEs were twice to five times more than the initial number. The leaf numbers from 1 mg/kg La and Pr were 3 times of the control, which had the highest standing leaf number among all treatments. Seedlings treated with the highest concentration of Ce and Nd had significantly more leaves than those from the control ($p < 0.05$).

The changes in standing leaf number are shown in Figures 3.5 and 3.6. The increase in the leaf number of *A. auriculiformis* was rapid initially that the growth was more than 100%. The increment in most treatments then slowed down during 15th to 45th day. The growth rate rose again before harvest. The leaf number of seedlings treated with REEs was obviously higher than that in the control after 15 days. The trend for 5 mg/kg Nd was unlike the others that growth rate was even over the experimental period. The trends of Ce and Pr treatments were similar to one another, while those La and Nd were more varied. Increase in leaf number at 1 and 5 mg/kg La were faster than the other treatments.

The highest increment rate in standing leaf number of *E. citriodora* appeared after 45 days. Seedlings in the absence of REEs produced the least number of leaves. A higher increase was found in seedlings treated with 25 mg/kg La and Ce. However, the growth rate was superior by others after 45 days. For Pr and Nd, 1 mg/kg gave the best leaf growth of *E. citriodora* throughout the 90 days. The cumulative increment was more than twice after 45 days, and more than 400-500% of the beginning. There was no adverse effect in leaf number throughout the whole period.

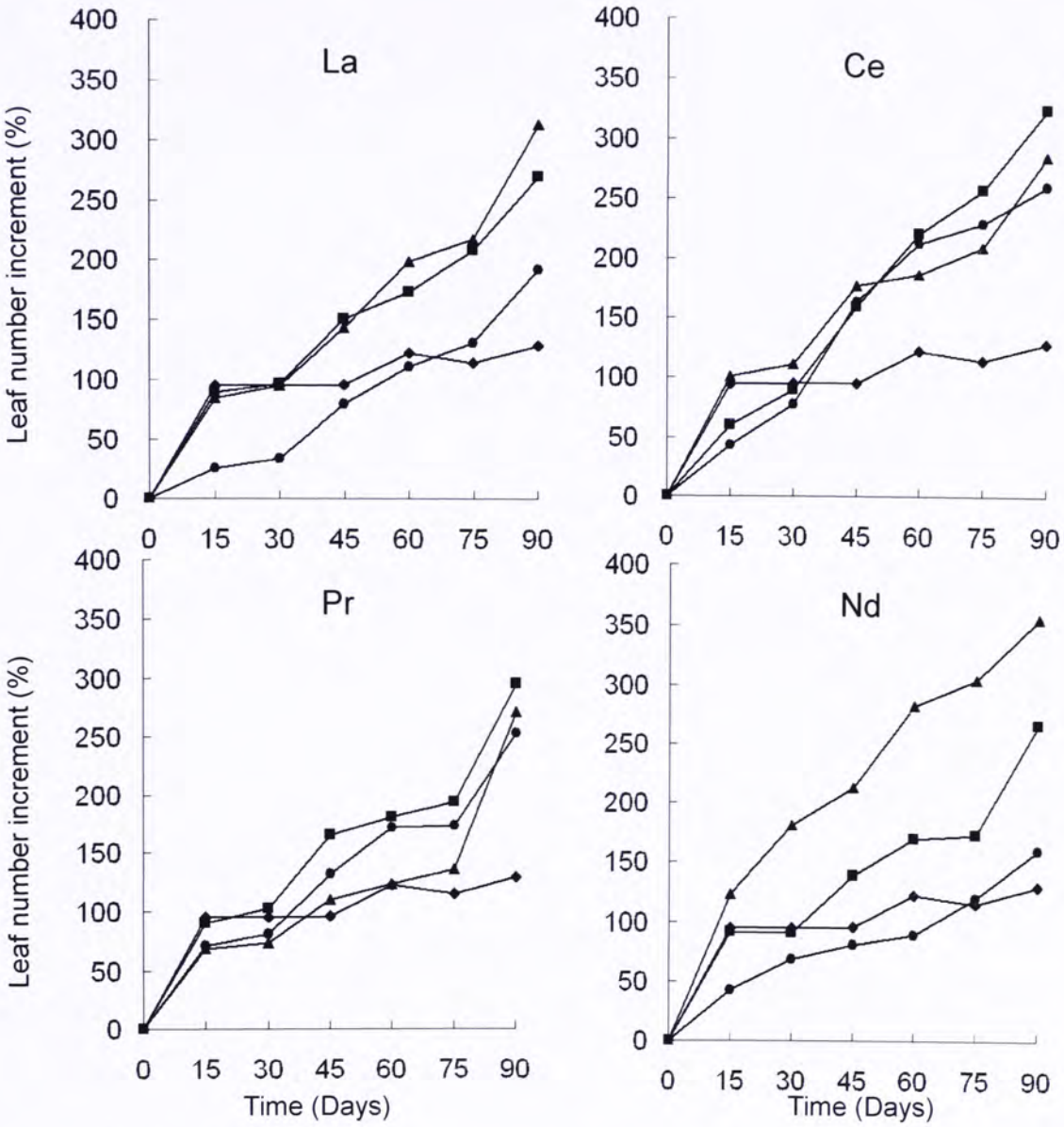


Figure 3.5 Standing leaf number of *A. auriculiformis* exposed to REEs at 0 mg/kg (◆), 1 mg/kg (■), 5 mg/kg (▲) and 25 mg/kg (●).

3.3.1.5 Chlorophyll fluorescence

In general, there were no significant differences between all the treatments as well as control for the F_v/F_m for both species (Table 3.4). The photosynthetic system was not significantly affected by the application of REEs.

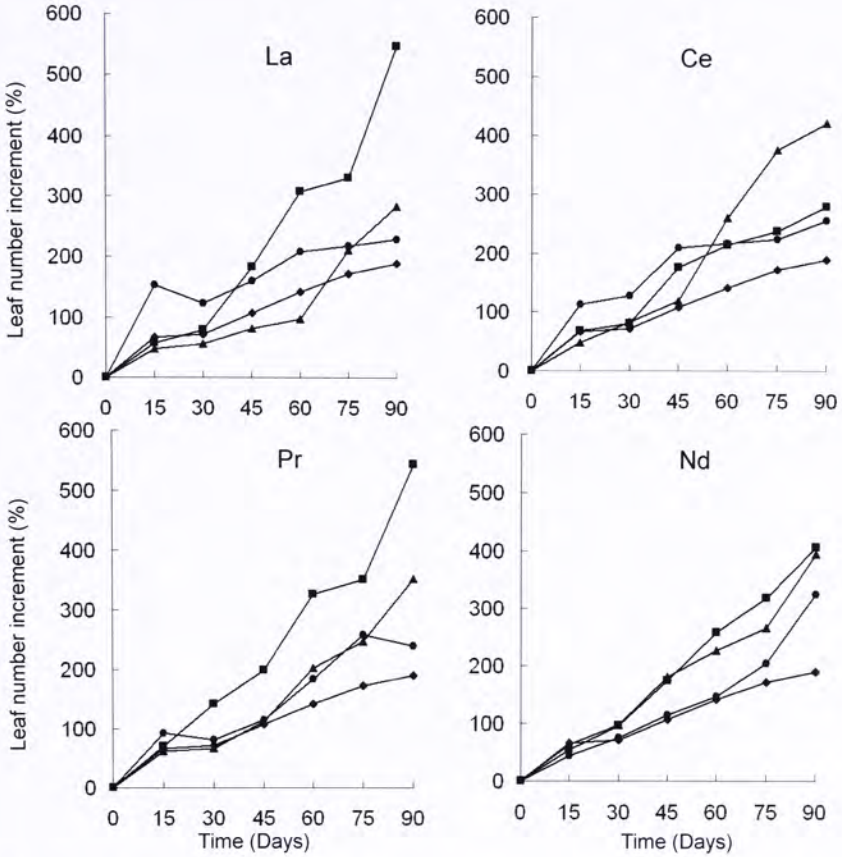


Figure 3.6 Standing leaf number of *E. citriodora* exposed to REEs at 0 mg/kg (◆), 1 mg/kg (■), 5 mg/kg (▲) and 25 mg/kg (●).

Table 3.4 Chlorophyll fluorescence (F_v/F_m) after 90-day exposure to REEs (n=4).

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	0.80	0.82	0.82	0.81
	Ce	0.80	0.82	0.83	0.81
	Pr	0.80	0.83	0.81	8.82
	Nd	0.80	0.82	0.82	0.78
E. citriodora	La	0.83	0.83	0.83	0.83
	Ce	0.83	0.83	0.84	0.83
	Pr	0.83	0.84	0.82	0.83
	Nd	0.83	0.83	0.83	0.83

3.3.2 Tissue contents

3.3.2.1 REEs concentrations

Foliar REE contents in *A. auriculiformis* were significantly higher ($p < 0.05$) at 25 mg/kg than those in the control without REE addition to the soil (Table 3.5). The accumulation of Ce and Pr in leaves from treatments of 1 and 5 mg/kg REE were not significantly different from the control. The La content in leaf exposed to 1 mg/kg REE was significantly higher than the content in control leaf. All Nd treatments gave significantly higher Nd contents in leaf. The Ce concentration in leaf was relatively lower than those of the other three REEs.

Table 3.5 Foliar REE content (mg/kg) after 90-day exposure to REEs.

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	0.18 c	1.14 b	3.10 b	8.30 a
	Ce	0.007 b	0.054 b	0.18 b	3.94 a
	Pr	0.69 b	0.17 b	1.58 b	7.05 a
	Nd	0.36 b	0.98 a	2.68 a	4.41 a
E. citriodora	La	0.28 c	1.27 bc	2.85 b	6.55 a
	Ce	0.21 b	3.95 a	3.75 a	3.11 a
	Pr	0.13 c	2.15 bc	2.38 b	5.21 a
	Nd	0.32 c	1.90 b	3.63 ab	5.86 a

For each REE, the same letter represents no significant difference between concentrations ($p < 0.05$, Tukey's test).

With the exception of Ce, the REE concentrations in leaves of *E. citriodora* gradually increased as the applied level increased (Table 3.5). The foliar concentrations of La and Pr from 1 mg/kg application rate were similar to those of the

control, while those from 25 mg/kg were significantly higher than other application rates. Foliar Ce contents increased with the presence of the element but irrespective of its application rate. The concentrations of the four tested REEs in leaves of *E. citriodora* were similar.

Similarly, the REE concentrations in roots of *A. auriculiformis* increased with the application rates (Table 3.6). There were significant differences in the root La contents between the control and all the application rates. The La contents were the highest among the four REEs. Concentrations of 1 mg/kg Ce, Pr or Nd from 1 mg/kg did not differ significantly from the control.

Table 3.6 Root REE content (mg/kg) after 90-day exposure to REEs.

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	0.19 d	4.31 c	7.28 b	12.4 a
	Ce	0.16 b	2.86 b	2.78 b	6.70 a
	Pr	0.11 b	0.99 b	1.28 b	4.39 a
	Nd	0.18 c	1.66 bc	4.54 ab	5.68 a
E. citriodora	La	0.18 c	5.60 b	5.86 b	10.3 a
	Ce	0.16 b	2.05 b	2.46 b	7.64 a
	Pr	0.18 c	1.65 bc	3.00 b	8.08 a
	Nd	0.19 c	1.87 bc	4.36 b	7.41 a

For each REE, the same letter represents no significant difference between concentrations ($p < 0.05$, Tukey's test).

With respect to root of *E. citriodora*, the treatment of the highest application level always gave significantly more REE content than the other application levels (Table 3.6). The root REE concentrations of seedlings treated with 1 mg/kg REE were similar to the control except in the treatment of La. There was significant difference among the root contents of various REEs at the same application rate.

When compared within a application rate, the results of La were always significantly higher than that of Pr, whereas results of Ce were always similar to other REEs.

The concentrations of REEs in leaves of *A. auriculiformis* were statistically indifferent ($p > 0.05$) with those in leaves of *E. citriodora* respectively. There was also no statistically difference between their roots.

3.3.2.2 Nitrogen concentration

The nitrogen content in leaves of *A. auriculiformis* did not differ significantly ($p > 0.05$) with respect to different REEs and different application rates of REEs (Table 3.7). The same applies to both species, though *A. auriculiformis* had higher foliar N than *E. citriodora*. The foliar N concentration of *A. auriculiformis* ranged from 4.30 to 4.72 mg/g, while those of *E. citriodora* ranged from 3.40 to 3.99 mg/g (Table 3.7).

Table 3.7 Foliar N content (mg/g) after 90-day exposure to REEs (n=4).

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	4.40	4.30	4.59	4.48
	Ce	4.40	4.45	4.72	4.60
	Pr	4.40	4.65	4.73	4.32
	Nd	4.40	4.49	4.58	4.56
E. citriodora	La	3.75	3.55	3.49	3.92
	Ce	3.75	3.77	3.76	3.99
	Pr	3.75	3.40	3.83	3.48
	Nd	3.75	3.58	3.57	3.44

Similarly, for the N content in roots, there was no significant difference ($p > 0.05$) among all treatments (Table 3.8). The average N concentration in roots of *A. auriculiformis* and *E. citriodora* were 2.30 mg/g and 1.78 mg/g respectively. The average N content in roots of *A. auriculiformis* was significantly higher than those in roots of *E. citriodora*.

Table 3.8 Root N content (mg/g) after 90-day exposure to REEs (n=4).

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	2.32	2.40	2.38	2.34
	Ce	2.32	2.14	2.31	2.07
	Pr	2.32	2.57	2.06	2.48
	Nd	2.32	2.31	2.19	2.27
E. citriodora	La	1.74	1.80	1.88	1.85
	Ce	1.74	1.90	1.74	2.19
	Pr	1.74	1.63	1.79	1.85
	Nd	1.74	1.50	1.81	1.63

3.3.2.3 Phosphorus concentration

The foliar P of *A. auriculiformis* from the control was significantly higher than those treated with REEs ($p < 0.05$) (Table 3.9). However, the P contents in treatments of REEs were similar to each other. Comparing among various application rates in individual REE, significant difference occurred within Ce only. The P concentration was 16.4 mg/kg in 25 mg/kg Ce, which was significant lower than that in control, which was 138 mg/kg. For *E. citriodora*, there was no significant difference between REEs and control. There was no significant difference among the P content in all REE treatments.

Table 3.9 Foliar P content (mg/kg) after 90-day exposure to REEs.

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	138 a	58.3 a	70.0 a	115 a
	Ce	138 a	70.6 ab	84.9 ab	16.4 b
	Pr	138 a	83.6 a	67.4 a	39.6 a
	Nd	138 a	27.6 a	23.5 a	43.4 a
E. citriodora	La	77.9 a	112 a	87.9 a	105 a
	Ce	77.9 a	103 a	59.0 a	123 a
	Pr	77.9 a	70.6 a	93.0 a	66.0 a
	Nd	77.9 a	71.1 a	68.3 a	65.5 a

For each REE, the same letter represents no significant difference between concentrations ($p < 0.05$, Tukey's test).

The P content in root was lower than the detection limit (< 5 mg/kg) in some treatments, such as in the control, 25 mg/kg Ce, Nd and Pr of *A. auriculiformis* and 5

mg/kg of Ce and Pr of *E. citriodora* (data not shown). The P concentrations in roots of both *A. auriculiformis* and *E. citriodora* are not significantly different between control and various REE treatments.

The P contents in leaf and root of *E. citriodora* were generally higher than those in *A. auriculiformis*. The P content in *E. citriodora* leaf from treatment 1 mg/kg La, 5 mg/kg La, 1 mg/kg Ce and 5 mg/kg Pr were 92.5%, 25.5%, 45.1% and 38.0% higher than those in *A. auriculiformis* leaf respectively. The P contents in *E. citriodora* root exposed to 5 mg/kg and 25 mg/kg La were 86.4% and 80.7% higher than the contents in the root of *A. auriculiformis*.

3.3.2.4 Mineral concentrations

Most of the foliar mineral nutrient contents did not significantly change after application of REEs for both species (Table 3.10). The concentrations of metals in leaf were significantly different ($p < 0.05$) between *A. auriculiformis* and *E. citriodora*. *E. citriodora* had significantly higher K, Mg and Zn contents but less Fe and Na contents than *A. auriculiformis*.

The concentrations of Ca in leaves under the influence of REEs were in general significantly ($p < 0.05$) higher than those seedlings in soil without REEs. Effect of REE application rate on Ca content was not apparent. In the treatment of 5 and 25 mg/kg REE, seedlings treated by La had the lowest Ca in their leaves among four REEs. Seedlings exposed to 1 mg/kg Nd and Pr had the highest Ca content.

Leaves of control had significantly more Mn than leaves affected by 5 and 25 mg/kg REE. Among various REEs, Mn concentration in leaves of *E. citriodora* exposed to La was significantly higher than the values in Nd and Ce.

Table 3.10 Foliar metal contents (mg/kg) after 90-day exposure to REEs (n=4).

Species	REE	Application rate of REE (mg/kg)	Concentration (mg/kg)						
			Ca	Fe	K	Mg	Mn	Na	Zn
A. auriculiformis	Control		2.36	0.042	4.42	0.79	0.16	0.94	0.009
	La	1	3.36	0.042	5.24	0.74	0.18	0.97	0.007
		5	2.72	0.052	5.47	0.83	0.16	0.90	0.007
		25	2.24	0.044	5.27	0.78	0.16	0.92	0.008
	Ce	1	2.27	0.044	4.62	0.80	0.18	0.97	0.009
		5	2.97	0.043	5.29	0.79	0.13	0.95	0.013
		25	5.82	0.047	4.57	0.78	0.092	1.02	0.006
	Pr	1	3.69	0.046	5.74	0.93	0.17	0.97	0.009
		5	3.25	0.045	5.64	0.81	0.15	0.82	0.005
		25	3.77	0.045	6.96	0.89	0.17	0.91	0.010
	Nd	1	3.66	0.047	4.35	0.79	0.13	0.89	0.007
		5	4.34	0.045	5.66	0.84	0.14	0.94	0.010
		25	3.78	0.036	4.80	0.86	0.15	0.81	0.008
E. citriodora	Control		3.92	0.042	6.78	1.10	1.15	0.37	0.033
	La	1	4.13	0.042	7.03	1.18	1.00	0.35	0.044
		5	4.12	0.037	5.77	1.20	1.21	0.35	0.042
		25	3.52	0.037	7.37	1.14	1.03	0.36	0.035
	Ce	1	3.30	0.033	6.81	1.02	1.10	0.35	0.029
		5	5.81	0.037	6.15	1.15	0.405	0.43	0.033
		25	5.67	0.036	7.27	1.13	0.89	0.46	0.030
	Pr	1	4.95	0.032	6.85	1.21	0.92	0.34	0.037
		5	5.28	0.036	7.24	1.14	0.91	0.39	0.037
		25	6.50	0.044	7.61	1.31	0.75	0.44	0.043
	Nd	1	6.16	0.035	6.79	1.10	0.74	0.40	0.031
		5	5.53	0.041	6.74	1.20	0.87	0.35	0.033
		25	4.68	0.036	6.89	1.03	0.75	0.36	0.029

In general, the metal concentrations in roots of *A. auriculiformis* were significantly different from those in roots of *E. citriodora* (Table 3.11). Metals like Fe and Zn were not different among various REE treatments. Some metals, like Ca, Na and Mg, in roots of seedlings exposed to REEs were more than that of the control. K contents in the roots exposed to 25 mg/kg La were significantly higher than those in the control and 1 mg/kg La. The concentrations of Mg and Mn were similar when comparing among the four REEs. REEs at different concentrations gave similar effect on the Mn content in roots of *E. citriodora*. REEs did not affect the Na concentration in roots of *A. auriculiformis*, whereas REEs at all levels increased significantly the Na concentration in roots of *E. citriodora*.

3.3.3 Soil

3.3.3.1 Initial properties

The soil for this study was a sandy loam with 74.0% sand, 15.5% silt and 10.5% clay (Table 3.12). The soil was slightly acidic with pH value of 6.59. The cation exchange capacity and organic matter were 13.1 cmol/kg and 4.21% respectively. The concentrations of total La, Ce, Pr and Nd before the experiment were 0.494, 0.376, 0.209 and 0.488 mg/kg respectively.

Table 3.11 Root metal contents (mg/kg) after 90-day exposure to REEs (n=4).

Species	REE	Application rate of REE (mg/kg)	Concentration (mg/kg)						
			Ca	Fe	K	Mg	Mn	Na	Zn
A. auriculiformis	Control		0.95	1.74	1.50	0.20	0.16	0.43	0.012
	La	1	1.31	1.08	1.60	0.22	0.15	0.45	0.011
		5	1.08	1.93	1.82	0.24	0.20	0.48	0.014
		25	0.82	1.82	1.89	0.22	0.19	0.45	0.011
	Ce	1	1.02	1.95	1.60	0.24	0.19	0.50	0.010
		5	1.09	1.48	1.55	0.21	0.15	0.46	0.011
		25	2.81	1.48	1.69	0.31	0.13	0.51	0.011
	Pr	1	1.44	1.73	1.74	0.24	0.17	0.49	0.016
		5	1.19	1.72	1.46	0.22	0.18	0.45	0.013
		25	1.20	1.76	1.59	0.22	0.15	0.42	0.010
	Nd	1	1.45	1.74	1.76	0.25	0.17	0.48	0.011
		5	2.18	1.91	1.44	0.25	0.15	0.52	0.013
		25	1.27	2.35	1.50	0.23	0.24	0.45	0.018
E. citriodora	Control		2.62	1.17	1.68	0.23	0.28	0.57	0.016
	La	1	2.83	1.16	1.71	0.22	0.23	0.61	0.015
		5	2.67	1.10	1.87	0.25	0.25	0.55	0.019
		25	3.06	0.75	2.13	0.25	0.28	0.62	0.014
	Ce	1	2.90	0.92	1.76	0.23	0.26	0.63	0.014
		5	5.16	1.04	1.56	0.32	0.13	0.90	0.022
		25	4.58	1.23	1.68	0.32	0.19	0.75	0.021
	Pr	1	3.46	0.69	1.62	0.26	0.21	0.71	0.014
		5	4.00	0.61	1.59	0.28	0.20	0.65	0.013
		25	4.43	1.00	1.79	0.30	0.17	0.78	0.018
	Nd	1	3.87	0.75	1.76	0.27	0.16	0.77	0.013
		5	4.20	1.06	1.57	0.28	0.21	0.74	0.018
		25	3.54	1.05	1.61	0.27	0.19	0.69	0.018

Table 3.12 Initial properties of the soil.

	Soil
Sand (%)	74.0
Silt (%)	15.5
Clay (%)	10.5
Texture (class)	Sandy loam
pH	6.59
Cation exchange capacity (cmol/kg)	13.1
Organic matter (%)	4.21
Total La concentration (mg/kg)	0.494
Total Ce concentration (mg/kg)	0.376
Total Pr concentration (mg/kg)	0.209
Total Nd concentration (mg/kg)	0.488

3.3.3.2 REEs concentrations

The total REE contents in soil are shown in Table 3.13. For soil planted with *A. auriculiformis*, soil REEs in the control and treatment added with 1 mg/kg REE were similar, irrespective to the REE examined. When comparing among treatments of different application levels, significant differences were observed. The final soil concentrations were consistent with the application rates. At the highest application level, soil concentrations of Ce and Pr were lower than those of La and Nd.

In soil grown with *E. citriodora*, the total REE concentration in the control was not significantly different from that in treatment with 1 mg/kg REE added (Table 3.13). The total contents of Pr in soil added with 1 and 5 mg/kg Pr were similar, while contents of other REEs in the 5 mg/kg treatment were significantly higher than those in the 1 mg/kg treatment. When combining the data from the two species, the concentrations of La and Nd were generally higher than those of Ce and Pr.

Table 3.13 Soil REE content (mg/kg) after 90-day exposure to REEs.

Species	REE	Application rate of REE (mg/kg)			
		0	1	5	25
A. auriculiformis	La	0.45 c	0.49 c	4.87 b	12.7 a
	Ce	0.36 c	0.24 c	3.89 b	7.67 a
	Pr	0.20 c	0.20 c	2.83 b	6.60 a
	Nd	0.46 c	0.44 c	4.42 b	11.3 a
E. citriodora	La	0.45 c	0.49 c	4.15 b	11.8 a
	Ce	0.25 c	0.23 c	3.86 b	7.00 a
	Pr	0.14 b	0.24 b	2.16 b	6.89 a
	Nd	0.46 c	0.43 c	4.54 b	11.0 a

For each REE, the same letter represents no significant difference between concentrations ($p < 0.05$, Tukey's test).

3.3.3.3 Nitrogen and phosphorus concentrations

The total and extractable N contents of soil after harvest are shown in Table 3.14. Extractable N should include NH_4^+ and NO_3^-. However, the NH_4^+ content in soil was below the detection limit of 1 mg/kg. The extractable N therefore consisted of NO_3^- only. The soil grown with *A. auriculiformis* had significantly ($p < 0.001$) lower extractable N than the soil of *E. citriodora* at harvest. The extractable N levels in soil with REEs added were slightly higher than the control. The levels in the control were 16.7 and 26.4 mg/kg for *A. auriculiformis* and *E. citriodora* respectively, while the averaged extractable N in REE treatments was 17.2 and 27.5 mg/kg respectively.

Table 3.14 Amount of total and extractable N contents in soil after 90-day exposure to REEs (n=4).

Species	REE	Application rate of REE (mg/kg)	Extractable N (mg/kg)	Total N (mg/g)
A. auriculiformis	Control		16.7	1.59
	La	1	18.2	1.88
		5	17.0	1.99
		25	17.3	2.16
	Ce	1	16.3	1.41
		5	17.4	1.46
		25	16.9	2.09
	Pr	1	15.9	1.33
		5	17.5	1.53
		25	18.4	1.74
	Nd	1	17.6	2.00
		5	16.9	2.00
		25	16.8	1.26
E. citriodora	Control		26.4	2.31
	La	1	20.4	2.16
		5	23.7	1.15
		25	49.3	2.02
	Ce	1	23.2	1.98
		5	26.3	1.99
		25	26.2	1.46
	Pr	1	21.7	1.96
		5	31.6	1.82
		25	36.7	1.16
	Nd	1	31.8	1.25
		5	22.0	0.97
		25	17.4	1.48

In soil planted with *A. auriculiformis*, the levels of total N (after the addition of 25 mg/kg La and Ce) were higher than the control. However, soils in some other treatments, such as 1 mg/kg Ce and Pr, had less total N than the control. There was overall no significant difference ($p > 0.05$) among REE treatments. For *E. citriodora*, there was significantly ($p < 0.01$) lower total N content in soil added with 5 mg/kg Nd than control. Significant difference was not observed between other treatments. The total N contents in soil added with 5 and 25 mg/kg REEs were significantly lower than the control.

Total and available P concentrations in soil with and without REEs were below the detection limit (5 mg/kg).

3.3.3.4 Mineral concentrations

Soils added with REEs had more Fe, Mn and Zn than the control (Table 3.15). The Zn content in soil added with 25 mg/kg Ce was significantly higher than those in treatments of La and Pr at the same application rate. The treatment with the highest REE application rate gave a significant difference from the control ($p < 0.05$) with respect to soil Zn content, while the treatments with lower application rates did not cause significantly difference. The Ca concentrations in soil added with Pr and Nd were the lowest among soil added with REEs. Soils planted with *A. auriculiformis* had similar metal concentrations when compared with those of *E. citriodora*.

Table 3.15 Total metal contents (mg/kg) in soil after 90-day exposure to REEs (n=4).

Species	REE	Application rate of REE (mg/kg)	Concentration (mg/kg)						
			Ca	Fe	K	Mg	Mn	Na	Zn
A. auriculiformis	Control		0.14	3.00	1.01	0.095	0.20	0.33	0.003
	La	1	0.42	3.59	1.08	0.119	0.52	0.35	0.008
		5	0.20	3.40	0.80	0.092	0.19	0.29	0.007
		25	0.41	3.13	0.91	0.099	0.17	0.32	0.005
	Ce	1	0.46	3.38	0.99	0.10	0.15	0.28	0.005
		5	1.16	3.38	1.01	0.11	0.47	0.27	0.008
		25	0.53	3.56	1.00	0.12	0.44	0.27	0.017
	Pr	1	0.46	3.22	1.39	0.13	0.40	0.29	0.012
		5	0.41	3.40	1.03	0.11	0.19	0.35	0.007
		25	0.31	3.22	1.02	0.12	0.22	0.29	0.010
	Nd	1	0.16	3.26	0.96	0.11	0.28	0.26	0.010
		5	0.32	3.11	0.98	0.11	0.35	0.26	0.015
		25	0.23	3.52	1.10	0.13	0.27	0.31	0.012
E. citriodora	Control		0.40	2.98	0.93	0.097	0.16	0.28	0.005
	La	1	0.24	3.34	1.24	0.11	0.18	0.30	0.005
		5	0.24	3.22	1.02	0.11	0.26	0.26	0.005
		25	0.46	3.43	1.02	0.10	0.16	0.26	0.008
	Ce	1	0.28	2.99	0.93	0.095	0.16	0.30	0.005
		5	0.50	3.48	0.87	0.11	0.39	0.28	0.015
		25	0.37	3.10	0.79	0.099	0.25	0.27	0.021
	Pr	1	0.22	3.53	0.90	0.12	0.32	0.27	0.009
		5	0.37	3.27	0.94	0.11	0.45	0.25	0.010
		25	0.38	3.27	0.86	0.11	0.25	0.27	0.010
	Nd	1	0.25	3.17	1.00	0.12	0.30	0.27	0.014
		5	0.43	3.61	0.81	0.12	0.28	0.35	0.013
		25	0.18	3.36	1.02	0.12	0.23	0.29	0.012

3.4 Discussion

3.4.1 Effects of REEs on growth

There was no evidence to support that REEs are essential for plant growth. The results obtained in the early investigation showed that REEs brought about

adverse effects to plant growth (Brown *et al.*, 1990). However, more recent studies showed that plants applied with REEs grew better than those without REEs. The contradictory results may be explained by the levels of REEs applied and the species being used. In the present experiment, addition of the four REEs significantly improved the growth of *A. auriculiformis* and *E. citriodora* in terms of height, basal diameter, biomass and standing leaf number. However, the positive effects were dose-dependent that seedlings grew better only at application rates of 1 and 5 mg/kg. Zhang and Shan (2001) obtained similar results that the dry shoot weight was significantly higher than the control when the concentration was below 20 mg/kg. Growth stimulations at low REE concentration were also obtained in other studies. Applying 0.05-6 mg/L La or Ce promoted the yield of rice in terms of weight and the number of grain per plant (Xie *et al.*, 2002). When sprayed with fertilizer containing less than 0.5% REE, the shoots of corn and mungbean grew better than the control (Diatloff *et al.*, 1996b); the dry root weight of corn increased after applying < 2 μM of Ce and La. Root growth of coconut was stimulated when 1 g/pot of REE was added to the soil (Wahid *et al.*, 2000). The optimum level for the growth of cucumber was 0.02 mM La (Zeng *et al.*, 2000). Pollen tube growth of *Nicotiana tabacum* was promoted by 44.1% when 10 μM of La was added (Sun *et al.*, 2003). There were earlier and more flowering of *Arabidppsis thaliana* after being exposed to low levels of La or Ce (He and Loh, 2000).

Beneficial response was found at REE concentration lower than 10 mg/kg (Shtangeeva and Ayrault, 2007). Higher concentration diminished the beneficial

effect and may become harmful to plant. The dry weight of shoot decreased gradually when the application levels of REEs were higher than 40 mg/kg (Zhang and Shan, 2001). Symptoms of leaf burn and small necrotic spots were observed, and shoot dry weight was reduced when received 0.5-1.0% La and Ce (Diatloff *et al.*, 1996b). In the present experiment, the growth stimulation was not significant for addition of REEs at concentration of 25 mg/kg.

The differences in growth performance at various REE concentrations may be related to the tissue concentration in plant. The foliar and root REE concentrations increased with the application rate which agreed with other studies (Chang *et al.*, 1991; Chua *et al.*, 1998; Xu *et al.*, 2003). The abundance of REEs in root and shoot of corn increased sharply when soil was applied with 1-5 mg/kg REE (Wang *et al.*, 2001). When applied with 2.6 kg/ha of REEs mixture to soil, REEs in root of winter wheat increased by 10% (Liang *et al.*, 2005). When the application rate increased to 7.7 kg/ha, REE abundance in both shoot and root increased 2 times compared with the control. Increasing level of REEs in the plant tissues outweighed the beneficial effects and symptoms of intoxication became apparent.

3.4.2 Mechanisms of the effect of REEs

REEs significantly promoted the growth of tree seedlings in many aspects, including height, basal diameter, standing leaf number and biomass. As the concentrations of REEs in roots of treatments were significantly higher than control, REEs may enhance the uptake of nutrients, such as P and Ca, which consequently

promoted plant growth. Ozaki *et al.* (2000) found that the absorbed REEs were mainly accumulated in the active growing points of *Dryopteris erythrosora*.

The most widely accepted mechanism of the effect of REEs on plant growth is through their binding of sites which are originally occupied by Ca^{2+}. The ionic radii of ions of REEs and Ca are similar. For the first REE, La^{3+}, the radius of ion is 3.1 Å, while the radius of Ca^{2+} is 2.8 Å (Lettvin *et al.*, 1964). Owing to the similarity in ionic radii, ions of REEs and Ca compete for the binding sites (Chang, 1991; Xie *et al.*, 2002; Jie *et al.*, 2003). The higher valence of REE ions allows them to bind superficially to Ca^{2+} sites in a less reversible manner than Ca^{2+} (Nagahashi *et al.*, 1974; Hu *et al.*, 1998; Tyler, 2004). The structure of Ca-binding sites and ion channels are altered. The ion distribution and thus the complex formation are changed. Such modification may interfere with hormones responsible for controlling the physiological and biochemical activities of plants.

Ca^{2+} is one of the messengers in plant responses to stimuli, which affects plant growth (Zeng *et al.*, 2000). REE was reported to mimic or interfere with signal system involving Ca^{2+}. Calmodulin (CaM) was originally combined with Ca^{2+} to form Ca^{2+}-CaM. When La^{3+} and $Ca^{2+}$was present simultaneously, La^{3+} had higher affinity to site than Ca^{2+} for binding with CaM (Sun *et al.*, 2003). The La^{3+}-CaM signal passed across the plasma membrane through the pathway of Ca^{2+}-CaM. By modifying the signal transduction system, the levels of proteins and enzymes were increased. Eu^{3+} promoted the synthesis of amaranthin while La^{3+}, Ce^{3+} and Nd^{3+} had

positive effect on the synthesis of phenylethanoid glycosides (PeG) at low concentration (Jie *et al.*, 2003; Zeng *et al.*, 2003). The extent of stimulation by Eu^{3+} was higher than that by Ca^{2+}.

The level and metabolism of Ca^{2+} were also reported to be adjusted in the presence of intracellular REEs. REEs were illustrated to increase the transport of Ca^{2+} across plasma membrane (Xie *et al.*, 2002; Zeng *et al.*, 2003). The level of Ca^{2+} was important in regulating ion absorption and the activities of other substances. The activities of Ca^{2+}-ATPase was affected by the presence of REEs, which may be the consequence of regulating Ca^{2+} level (Zeng *et al.*, 2000). Synergistic stimulation was reported when solution of Ca^{2+} and La^{3+} was applied by foliar spray (Brown *et al.*, 1990). One of the ways that REEs affect plant growth may be related to the concentration of Ca^{2+}. The absorption and use of nutrients may be improved and gave better growth. Nevertheless, beneficial effects brought about by La may be attributed to the intrinsic nature of La rather than replacing the function of Ca^{2+} (Ozaki *et al.*, 2000). Further studies are needed to get more information about the mechanism.

3.4.3 Nutrient uptake

Measuring the amount of nutrients in plant tissue reflects the nutrient taken up by plant. The nutrient contents in seedlings were influenced by the presence of REEs, which implied the nutrient uptake was affected when soil was added with REEs. There was no significant enhancement in N uptake after application of REEs.

This contrasted with previous studies that N uptake by coconut increased after addition of REEs at low concentration (Wahid *et al.*, 2000). The deviation may be attributed to the N content in soil. Improvement in nutrient uptake may be obvious under deficiency conditions. In the present experiment, sufficient N was provided such that the plants had optimal rate of N uptake, and therefore, addition of REEs could not further increase the rate of N uptake.

The foliar P of *A. auriculiformis* treated with REEs was significantly lower than that of the control, which may be attributed to the interference in translocation of P in seedlings (Wahid *et al.*, 2000; Huang *et al.*, 2003). However, the previous studies only examined the foliar P content. REE application did not cause accumulation of P in root. It may show that the absorption of P from soil was influenced by REEs.

In most cases, the foliar mineral contents in REE-treated plants and the control were similar. However, concentration of foliar Ca under exposure to REEs was significantly higher than the control, whereas foliar Mn in treated plants was significantly lower. The level of K significantly increased whereas the level of Mn was similar to the control. Diverse results were also observed in other studies. Absorption of K, Mg and Zn was suppressed, but uptake of Ca, Fe, Mn and Na was promoted (Chang, 1991; Wahid *et al.*, 2000; Zeng *et al.*, 2000; Zeng *et al.*, 2003; Järvan, 2006). Shtangeeva and Ayrault (2007) obtained different results that Eu did not affect the uptake of K, but increased the tissue contents of Ca, Zn and Au. Zn

and Au concentrations in *Triticum aestivum* were 2.5 and 1.7 times higher than those in the control. The uptakes of Cu, Fe, Mg, Ca, Mn and Zn in rice were also elevated at La concentration of 0.75 mg/L (Xie *et al.*, 2002). Besides major nutrients, the uptakes of some other minerals such as Se, Co and V were also affected by exposure to REEs (Huang *et al.*, 2003). The mechanism for the alteration in mineral uptake was still not clear. Chang (1991) suggested that change in the permeability of plasma membrane led to leakage of metals ions. Equilibrium of ions thus shifted, and this triggered a change in uptake of metal ions. Huang *et al.* (2003) argued that La was involved in the ion transport in plants. Change in the concentration of La would affect the mechanism and lead to alteration in ion uptake.

Among the metal studied, Ca received the most concern owing to the similarity in the ionic radii and chemical properties between REE ions and Ca^{2+} (Aruguete *et al.*, 1998). Uptake of Ca^{2+} by seedlings was increased in the treatment with REEs applied in low concentration in the present study. However, contradictory results were obtained in the previous studies as both stimulation and inhibition in uptake were observed (Chang, 1991; Zeng *et al.*, 2000; Zeng *et al.*, 2003). More investigations are needed to study the effect of REEs on nutrient uptake.

3.4.4 Soil nutrient contents

Some studies revealed that soil reactions involving N and P were affected by REEs. N mineralization in soil with < 45 mg La/kg or < 23 mg mixed REE/kg did not significantly differ from the control (Xu and Wang, 2001). The effect of La on

ammonia oxidation was similar to that of N mineralization. Nitrification was another important process of N transformation of NH_4^+ to NO_3^- state (Xu and Wang, 2001; Chu *et al.*, 2002). In the present study, NH_4^+-N was not detectable, probably because of the increased nitrification that converts NH_4^+ to NO_3^-. The rate of nitrification was increased by 20% and 14% in red soil and fluvo-aquic soil respectively after the addition of La at low doses (Zhu *et al.*, 2002).

The extractable N in soil grown with acacia was not significantly higher than that grown with eucalyptus. This may be attributed to the increased nitrification in soil grown with eucalyptus after addition of REEs. Besides, it may be due to the addition of urea as N source and high N level could be harmful to nodule activity and N fixation (Roberts and Bradshaw, 1985a, b)

The improvement in nitrification may be due to the increase of microbial activity in soil. Chu *et al.* (2002) showed that the biomass carbon and nitrogen increased 4 weeks after addition of La. Pot culture of *Oryza sativa* demonstrated that the biomass increased in the treatment with La addition which may be related to the elevated nitrate in soil.

Most P compounds in soil are highly insoluble, making them not available to plant uptake. Even though P in available form was applied to soil in the form of fertilizer, they rapidly changed to insoluble complexes. As P is essential to plant growth, the transformation of unavailable P to available form is beneficial to plant.

Chu *et al.* (2002) found that the P transformation process was stimulated by low La concentration. However, P transformation would be inhibited when the concentration of La was over 30 mg/kg, implying that less P would be available to plant. In the present study, the P contents in soil in all treatments were below detection limit. This may be attributed to leaching and uptake by the seedlings. Moreover, the tissue P content in seedlings exposed to the highest REE concentration in this experiment was significantly lower than the control, implying less P was taken up. As the soil P content is closely related to the efficiency of nitrogen fixation of legumes, the improvement in P availability not only increased the amount of available P, but also improved the content of NH_x-N, as well as NO_x-N (Xu and Dell, 2003). Addition of low dose of REEs would be beneficial to the conversion of unavailable forms of N and P to available ones.

3.4.5 Comparison between REEs

Owing to the similarity in ionic radii and outermost electronic configuration, REEs rarely exist in compounds of single elements. REEs were absorbed together from soil, and thus there should be no preference in the assimilation of individual REE by plants. Fungi absorbed REEs as a group and there were similar pattern of REE accumulation in fungi with the nearby soil (Aruguete *et al.*, 1998). The patterns of REE composition in rice and wheat were similar to the soil profile, which implied that there was no obvious selective uptake of REEs (Liu *et al.*, 1997; Wang *et al.*, 2001). There was no fractionation in REE abundance in matured wheat (Ding *et al.*, 2006). The concentrations of La, Ce, Pr and Nd in corn exposed to various

application levels were not statistically different (Wang *et al.*, 2001). Contrasting, results of the present study shows a significant difference among the various REEs. A significantly higher La content was observed in *A. auriculiformis* and *E. citriodora*. The results were not consistent with the above studies, whereas in line with other studies which found that plant did not absorb each REE at the same extent.

Plants preferred light REEs to heavy REEs in soil-plant systems. The ratio of light to heavy REEs was much higher in plants than in soil (Liang *et al.*, 2005). Enrichment of La and Ce was found when a mixture of REEs was applied to rice and wheat (Sun *et al.*, 1994). There were more light REEs than heavy REEs in the tissues of *Dicranopteris pedata, Elaeocarpus sylvestis* and *Pinus massoniannai* (Gao *et al.*, 1999). There was also a light REE enrichment in the environment of estuary (Borrego *et al.*, 2004). Norway spruces in Switzerland and Southern Germany preferred La to Sc (Wyttenbach *et al.*, 1994). In wheat, the preference was more obvious at the early stage of life cycle, including the sprouting and jointing stages (Ding *et al.*, 2006). The ratio of chondrite-normalized light REEs to heavy REEs (LREE/HREE) represents the difference in the abundance of light REEs and heavy REEs and ratio greater than 1 implies fractionation. The concentration ratios of La/Sm in stem and leaf of *Triticum aestivum* were statistically higher than 1 (Ding *et al.*, 2006). The La/Sm values and Gd/Yb values of stem and lamina of *Dicranopteris linearis* were greater than 1 (Wei *et al.*, 2001). La/Lu ratios were high in the region of northern Ethiopia, ranging from 8.49 to 10.42 (Worash and Valera, 2002). This showed that REEs did not behave as a completely homogeneous group

in the uptake process.

When compared within members of light REEs, there were usually differences in tissue concentration. Plants preferred taking up La to Ce and Nd (Franca *et al.*, 2002). The concentration ratio of La/Ce was greater than 3.0, which showed that there was an apparent preference to La in maize (Xu *et al.*, 2002). The results were consistent with the present study that La concentration was higher than the other REEs studied. Besides discrimination of light REEs, it was commonly reported that Ce did not show the same abundance pattern as other REEs. The theoretical level of Ce was expected to be in intermediate position between concentrations of La and Pr. However, the Ce level measured in the environment was lower than the theoretical level. In the environment of transition beds, there was a significant negative Ce anomaly (Worash and Valera, 2002). It was an outcome of the formation of Ce^{4+}. The other REEs as ions only have the highest oxidation state of +3 but not +4. The chemistry of Ce^{4+} were different from those trivalent REEs (Wyttenbach *et al.*, 1998). Ce^{4+} was less bioavailable than Ce^{3+} because it was retained in oxygenated subsoil in the form of CeO_2, which has very low solubility and penetrability and thus being less taken up by plants (Laul *et al.*, 1979; Fu *et al.*, 2001; Xu *et al.*, 2003). In the present study, negative Ce anomaly was not observed. The content of Ce was not significantly lower than other REEs, probably because Ce was added to soil in form of $Ce(NO_3)_3$ which is relatively soluble. Szefer *et al.* (1999) demonstrated an absence of Ce anomaly in Vistular Lagoon. Sun *et al.* (1994) even found that there was an enrichment of Ce in roots of wheat and rice sprayed with Ce-containing fertilizer.

Although there was variation in the absorption of REEs, owing to their similarity in many aspects, fractionation may not always be apparent. Furthermore, there were great differences between individual plants in natural environment which may mask the fractionation in plants (Wyttenbach *et al.*, 1998).

3.4.6 Comparison between species

A. auriculiformis and *E. citriodora* are commonly planted in Southeast Asia, including Hong Kong and southern China. Forests of *A. auriculiformis* have covered 30000 ha in China since 1960s, while there were 1547000 ha of eucalyptus plantations (Zhang *et al.*, 1998; Qi, 2003). They were widely planted since they are well adapted to the southern China environment, grow fast and mature precociously (Zhang *et al.*, 1998; Chen *et al.*, 1999; Cheung *et al.*, 2000; Qi, 2003; Simpson *et al.*, 2003). Some of the eucalypts are planted for economic purposes due to their excellent fibre and relatively high wood density (Martin, 2003; Midgley *et al.*, 2003; Xu and Dell, 2003). *A. auriculiformis* has been commonly used as a pioneer species to grow on the infertile soil due to its symbiotic N-fixing capability. Both species are important to the economy and ecological restoration in Southern China.

The effects of the four REEs on the growth of *A. auriculiformis* and *E. citriodora* were similar in some of the growth parameters, such as height and biomass increment. The amounts of REEs retained in the plants were also similar when compared within the same application rate. The results were consistent with Laul *et al.* (1979) that peas and corns had similar concentrations of REEs. Nevertheless, the

results did not agree with some other studies that showed inter-species differences. Various species growing under the same condition generally took up different amounts of REEs (Markert and Li, 1991; Wyttenbach *et al.*, 1996; Wyttenbach *et al.*, 1998; Fu *et al.*, 2001; Franca *et al.*, 2002; Merten *et al.*, 2005). Fern and maple growing in a forest in Swiss midlands had ten times more REEs than spruce, whereas ivy had similar REE contents as spruce (Wyttenbach *et al.*, 1998). *Pachystroma longifolium* had higher REE contents than *Esenbeckia leiocarpa* growing in the same tropical forest (Franca *et al.*, 2002). *Populus* species had the highest REE abundance whereas *Robinia* species grown on the same site had the lowest REE contents (Merten *et al.*, 2005). In the forest of Grasmoor, levels of REEs in *Sphagnum* species and *Polytrichum* species were higher by a factor of three and two respectively than those in other plants (Markert and Li, 1991). The abundance of REEs in *Populus sieboldii* was several times higher than that in *Sasa nipponica* (Fu *et al.*, 2001). The variation in REE contents may reflect differences in the uptake of REE by various species. Not only there were inter-species differences in the concentration of REEs in plants, intra-species variation was also observed (Wyttenbach *et al.*, 1998). Such variation may be due to the variation between individuals and difference in environmental factors. Since the previous studies were carried out in natural forests, where growth conditions were not well controlled, it may reflect the possible effect of environmental factors to REE uptake.

A. auriculiformis generally grows better than other species in infertile soil due to its ability to fix atmospheric N by the symbiotic rhizobia. However, after adding

REEs, the superiority from symbiotic N-fixing capability was less apparent. Addition of small amount of REE increased the rate of nitrification and ammonification in soil (Zhu *et al.*, 2002). The amount of available N in soil could be increased even in the absence of N-fixing legume providing that there was adequate capital of N. Therefore, non N-fixing could take up more N from soil, resulting to a better growth after being exposed to REEs. Therefore, even there was more N in soil growing with acacia, the growth of *A. auriculiformis* may not be better than another species.

3.5 Conclusions

Application of REEs to soil is favourable to the growth of *A. auriculiformis* and *E. citriodora*. Growth in the terms of height, standing leaf and biomass production were enhanced after treatment with REEs. The beneficial effects of REEs were dose dependent that small amount of REEs promoted the growth but the effects diminished at high dose, causing negative effects at further higher application rates. The uptake of nutrient was also influenced by the presence of REEs. The amount of REEs available to plants would affect the amount being taken up. Further research on factors influencing the bioavailability of REEs would help the understanding of the effects of REEs on plant growth.

Chapter 4 Bioavailability and accumulation of rare earth elements

4.1 Introduction

There is extensive literature on the abundance and distribution of REEs in the natural environment, but information on the biogeochemistry including transport, bioaccumulation and bioavailability of REEs in the soil plant system is scanty. There have been increasing quantities of proceeded REEs entering into the environment from various sources and pathways because of the rapid growth in the usage of REEs in human life. The information about the bioavailability of REEs in soil is important to study on the uptake of REEs by plants. After being absorbed, the REEs would be stored in the different parts of the plant. Their distribution inside the plant body determines the effect of REEs on plants.

Plants do not take up all forms of REEs in soil. Various fractions of metals in soil have different availability to be taken up by plants. Total content in soil may not accurately reflect the concentration of actual assimilation. Information on the physicochemical forms of elements is thus essential for assessing their behaviour. One-step extraction of REEs from soil using strong chelating agents can examine the bioavailability of REEs (Cao *et al.*, 2000). However, this method suffered from the difficulty of finding a single reagent that only extracts out the non-residue fractions but not attacks the detrital forms (Tessier *et al.*, 1979). Sequential extraction developed in recent decades solves the drawback. REEs from different fractions are extracted from soil in individual step using corresponding extractants. Concentration of REEs from a variety of fractions can be examined separately so that their

availability can be determined. The most commonly applied sequential extraction methods nowadays are that proposed by Tessier and his colleagues (1979), and another proposed by Standard Measurements and Testing Programme of the European Community (SMT) formerly the Community Bureau of Reference (BCR) (Quevauviller *et al.*, 1993). The SMT method was widely used in experiments on soils and sediments. Although these methods also have the disadvantage of nonselectivity of the extractants used during the extraction processes, they are still the most popular methods.

Mobility, transformation and bioavailability of REEs in soil are driven by various mechanisms such as precipitation-dissolution and sorption-desorption processes. These mechanisms are governed by the soil properties, such as pH, redox potential, cation exchange capacity and organic matter content. Changing the soil properties would alter the biogeochemistry and bioavailability of REE in soil, and consequently affect the plant growth. Since bioavailability affects the movement of REEs along the tropic level, controlling their bioavailability is essential to management of REE application. Therefore, information on soil conditions that influences the fractionation and hence bioavailability of REEs is valuable.

Among a variety of edaphic properties, pH and organic matter are the most important factors which govern metal bioavailability (Masscheleyn *et al.*, 1990; Cao *et al.*, 2001; Gu *et al.*, 2001; Shan *et al.*, 2002). Metals are more available in conditions with lower pH. Organic matter was reported to be a great pool for REEs

to be deposited. However, the uptake and accumulation of REEs under the influence of pH and organic matter have attracted less attention. This study investigated the uptake of REEs by plants under different pH and organic matter conditions. The aims of the present study are to examine the chemical speciation of REEs in soils after being applied to soil, to examine the bioavailability and distribution of REEs under different soil conditions, and to determine the relationship between plant uptake and chemical speciation of REEs.

4.2 Materials & Methods

4.2.1 Soil

The soil used was collected from the campus of The Chinese University of Hong Kong. The soil was sieved through 5-mm mesh sieve to remove large particles before use.

4.2.2 Tree seedlings

Seedlings of *Eucalyptus citriodora* with height of 15-20 cm were used. *E. citriodora* is successfully introduced and well adapted to the southern China (Zhang *et al.*, 1998; Chen *et al.*, 1999; Cheung *et al.*, 2000; Qi, 2003; Simpson *et al.*, 2003). Also, seedling growth was promoted after being treated with REEs (Chapter 3). Seedlings were bought from the Tai Tong Nursery of the Agriculture, Fisheries and Conservation Department (Hong Kong).

4.2.3 Pot experiment

Soil was modified to obtain variation in pH and organic matter content. The pH of soil was adjusted to 4, 6 and 8 by adding 1 M hydrochloric acid (HCl) or 1 M sodium hydroxide (NaOH) (Masscheleyn *et al.*, 1990; Chuan *et al.*, 1996; Cao *et al.*, 2002; Xie *et al.*, 2002). Peat moss was mixed with soil in three different ratios, viz 3:1, 1:1 and 1:3 soil to peat (v/v). Totally there were nine soil treatments after combining various pH and peat moss ratio (Table 4.1). Lanthanum (La) was added to the treated soils at a concentration of 5 mg/kg soil. A set of control was included without the addition of La. The soil received urea and potassium dihydrogen phosphate to provide 200 kg/ha of N and P respectively (Bradshaw, 1983).

Seedlings of *E. citriodora* were planted in pots of 19 cm in diameter and 18 cm in height. One seedling was planted in each pot. Each treatment was replicated four times and the pots were arranged in a randomized block design in a greenhouse (Plate 4.1). During the 90-day experiment, the growth and health of seedlings were monitored. The methodology was the same as that described in Chapter 3.

The seedlings were harvested at the end of the experiment (after 90 days). Leaves and stems were washed with tap water and then with Milli-Q water. Roots were collected by washing with tap water and Milli-Q water until visibly clean. Plant tissues were oven-dried at 65°C until constant weight for the determination of biomass. The dried materials were ground to powder by an electric grinder. Soil was collected after harvesting and then air-dried for 2 weeks.

Table 4.1 Abbreviations for various combinations of soil conditions.

Organic matter (%)	pH		
	4	6	8
25	4L	6L	8L
50	4M	6M	8M
75	4H	6H	8H

Plate 4.1 Tree seedlings in 19-cm pots arranged in randomized blocks in a greenhouse.

Both soil and plant samples were analysed using the method described in Chapter 3.

4.2.4 Chemical speciation of soil

In soil, REEs present in various forms and associated with different chemicals. Chemical fractionation helps to find out the form of REEs in soils and thus bridges the relationship between the contents of REEs in soil and in plant (Wen *et al.*, 2006). Sequential extraction was a commonly used method to fractionate the chemical types of REEs in soils. REEs were divided into fractions based on the three-stage sequential extraction scheme developed by the Standard Measurements and Testing Programme of the European Community (SMT). The procedure resulted in three chemically distinct fractions, exchangeable and carbonated bound fraction (B1 fraction), Fe-Mn oxide bound fraction (B2 fraction) and organic and sulphide bound fraction (B3 fraction). The extraction was performed with 0.5 g dried soil samples which was placed into a 50 mL centrifuge tube and then added with 0.1 M acetic acid. The centrifuge tubes were shaken mechanically at 25°C for 16 h. The mixture was centrifuged to separate the supernatant (B1 fraction) and the residues. The remaining soil was washed with Milli-Q water and further extracted using hydroxylamine hydrochloride (0.1 M), the pH of which was adjusted to pH 2 by concentrated nitric acid. The tubes were mechanically shaken again at room temperature for 16 h, and centrifuged to give the B2 fraction. After the second extraction, 5 mL of 30% (w/v) hydrogen peroxide was added to the soil. The tubes were placed at room temperature for 1 h and then at 85°C for another 1 h. After the solution was nearly dried, a further 5 mL hydrogen peroxide was added and incubated in water bath for 1 h. After the tube was cooled, 25 mL of ammonium acetate was added. The shaking, centrifugation and washing processes were repeated to obtain

the B3 fraction. Extracts were analysed by an Optima 4300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (PerkinElmer, USA).

4.2.5 Statistical analysis

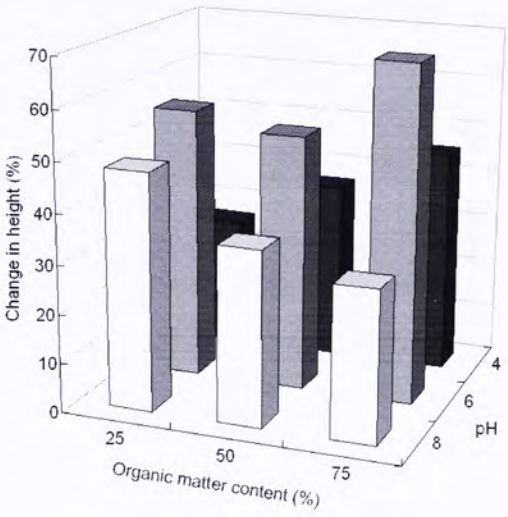
The data were processed by SigmaStat 3.1. The differences between treatments were tested by three-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference test at $p = 0.05$ wherever appropriate.

4.3 Results

4.3.1 Plant performance

The height of *E. citriodora* seedlings exposed to La was in general higher than that of the control (Figure 4.1). The height increment of the control seedlings ranged from 26.4 to 68.7%, while those in the La treatment ranged from 42.0 to 80.5%. The growth differed when compared among groups with different soil properties. In the control, the seedlings grew best in soil with pH 6, while in La treatment, the height was the highest in soil with pH 4. However, in the soil with pH 8, the height was significantly lower than in the above pH conditions. The effect of organic matter was not as apparent as that of pH (Table 4.2). No significant difference could be observed among difference soil organic mater contents.

(a)



(b)

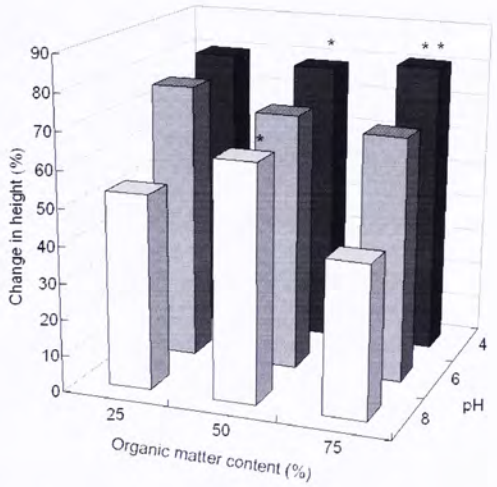


Figure 4.1 Percentage change in height in *E. citriodora* after being exposed to La under different conditions in (a) control soil and (b) La treated soil. When compared within the same level of pH and organic matter, treatments indicated with asterisk and 2 asterisks were significantly different from the controls at $p < 0.05$ and $p < 0.01$ respectively by Tukey's test.

Table 4.2 ANOVA table for height of *E. citriodora* under various soil conditions and treatment.

Source of variation	df	p
Treatment	1	<0.001
pH	2	0.009
Organic matter	2	0.937
Treatment × pH	2	0.005
Treatment × Organic matter	2	0.615
pH × Organic matter	4	0.340
Treatment × pH × Organic matter	4	0.826

The height increased with time throughout the experiment period (Figure 4.2). The growth at the beginning of the experiment was relatively slow, but became faster afterwards. For each condition, the slope of growth curve of the control was smaller than that in the La treatments. Growth stimulation by La was the most apparent in 4L. Since the growth rate of seedlings exposed to La was faster than those in the control, the difference between the control and treatment became larger. In the control group with soil pH of 6, plant grew faster after Day 60 and reduced the deviation between the control and the La treatment. Their growth rates were diminished towards the end of the experiment, regardless of the treatment.

The effect of La on the growth of basal diameter was less obvious than that of height (Table 4.3). Thus, there was no significant difference between the treatment and the control. When compared within treatment groups, change in soil pH and organic matter did not lead to significant change in stem growth.

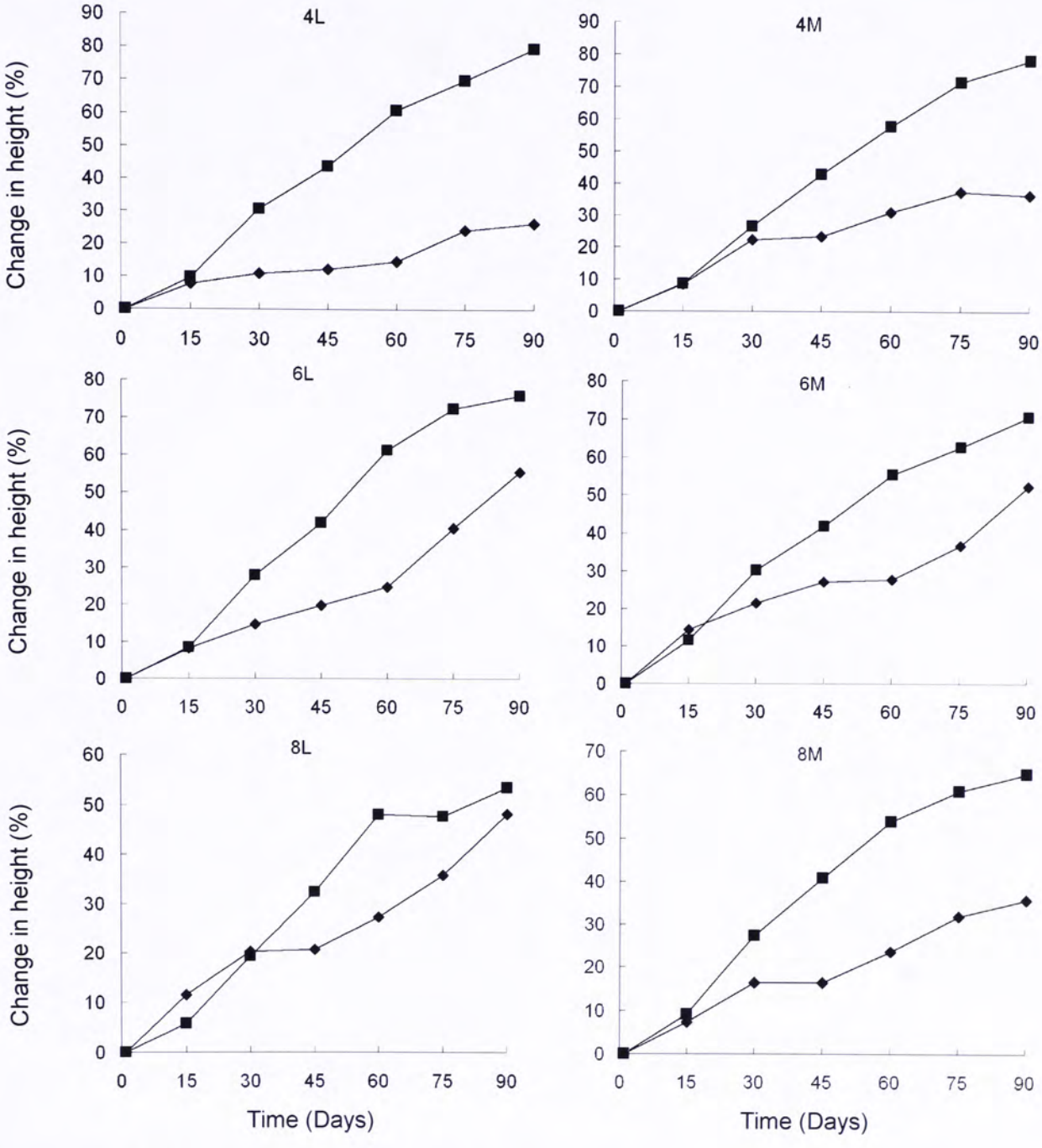


Figure 4.2 Height of *E. citriodora* grown in soil with La application (■) and without La application (◆) in a 90-day experiment.

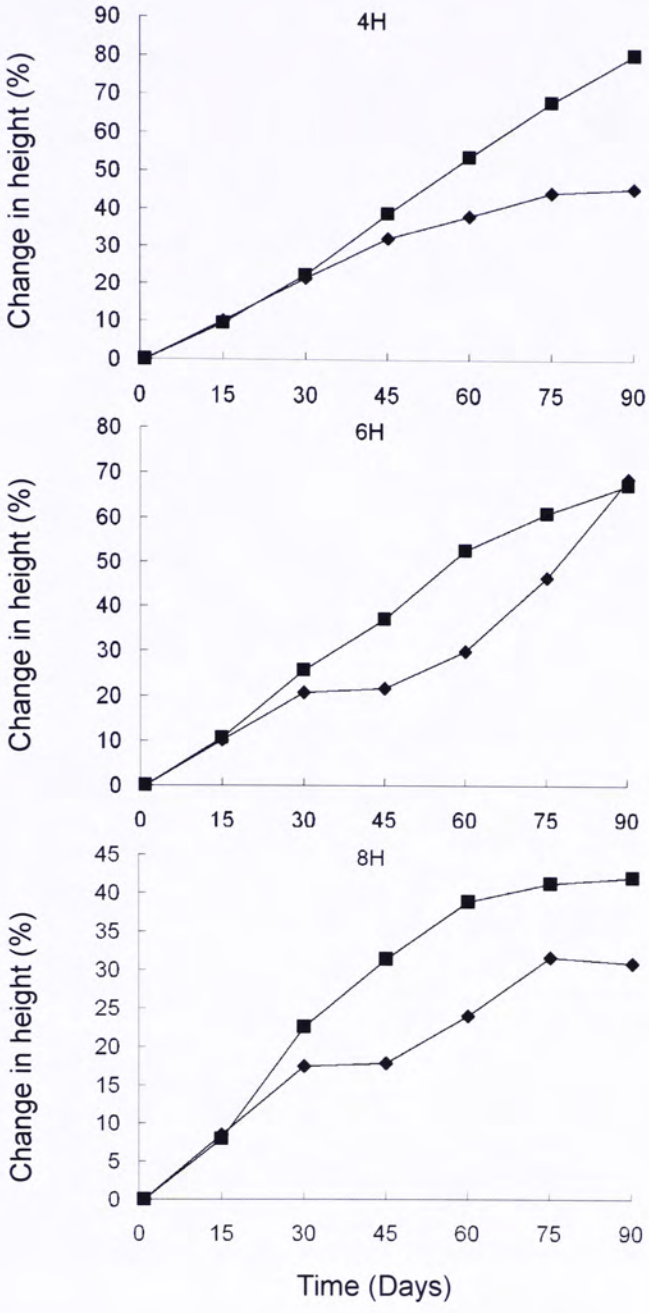


Figure 4.2 Height of *E. citriodora* grown in soil with La application (■) and without La application (◆) in a 90-day experiment (continued).

Table 4.3 Percentage change in stem diameter in *E. citriodora* after being exposed to La under various soil conditions (n=4).

Soil condition	Treatment	Increase in stem diameter (%)
4L	Control	70.2
	La	61.2
4M	Control	38.7
	La	30.4
4H	Control	46.0
	La	29.8
6L	Control	52.7
	La	56.4
6M	Control	38.1
	La	22.3
6H	Control	35.6
	La	33.6
8L	Control	50.0
	La	50.3
8M	Control	28.1
	La	46.5
8H	Control	45.8
	La	55.5

There were great increments in the biomass for the foliages and roots (Table 4.4). At the end of the experiment, the foliar biomass in the control was increased by four times, while foliar biomass in the La exposed plants was increased by eight times. An improvement effect can be observed for the La treated trees. The increment in root biomass was less than that of the leaf. The final root biomass for the control was 91-279% of the initial biomass, while the root biomass was 248-576% of the initial one for La treated plants. An acidic condition with lower organic matter content resulted in a significantly higher biomass of the seedlings. However, for the leaf biomass of seedlings exposed to La, there was no statistical difference among different soil conditions.

Table 4.4 Percentage change in biomass in *E. citriodora* after being exposed to La under various soil conditions.

Soil condition	Treatment	Leaf biomass (%)	Root biomass (%)
4L	Control	721	171
	La	1330 *	576 **
4M	Control	756	224
	La	1140 **	409 *
4H	Control	744	279
	La	933	304
6L	Control	585	195
	La	1074	290
6M	Control	608	235
	La	1120 **	296
6H	Control	512	206
	La	1010 **	237
8L	Control	722	239
	La	1060	265
8M	Control	717	179
	La	1093 *	248
8H	Control	567	91
	La	1190 **	322 **

When compared within the same level of pH and organic matter, treatments indicated with asterisk and 2 asterisks were significantly different from the controls at $p < 0.05$ and $p < 0.01$ respectively by Tukey's test.

When exposed to La, there were greater variations with respect to the root biomass among different soil conditions. Roots of seedlings grew at pH 4 had a significant higher biomass than those grown at pH 6 and 8. At a lower level of organic matter, the effect of pH on plant growth was more apparent. When comparing different pH levels within the group of 25% organic matter, the root biomass at pH 4 was significantly larger than those at pH 6 and 8. At a higher level of organic matter, the root biomass at pH 4 was statistically different from that at pH 8,

but not at pH 6. At the highest organic matter content level, there was no difference among all pH levels. Nevertheless, the effect of changing organic matter content on root biomass was significant only in acidic solution. With soil pH of 4 and organic matter of 25%, the root biomass of seedlings was significantly higher than those grew in other two organic matter content levels.

A three-way ANOVA reveals that the addition of La gave a significantly higher root biomass to *E. citriodora*. The root biomass of *E. citriodora* was significantly different ($p < 0.05$) under various soil pH conditions (Table 4.5). However, there was no significant difference ($p \geq 0.05$) among various organic matter levels tested.

Table 4.5 ANOVA table for root biomass of *E. citriodora* under various soil conditions and treatment.

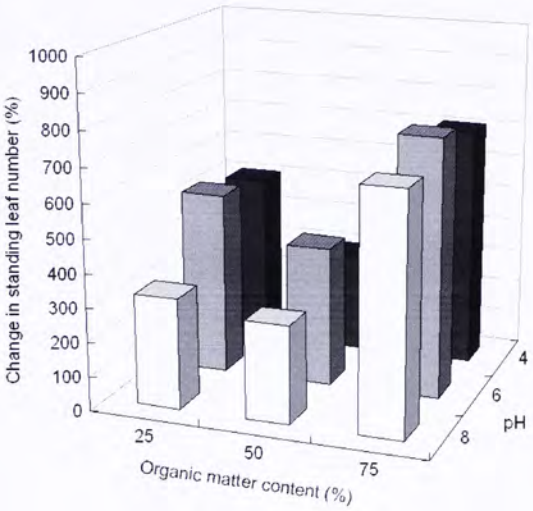
Source of variation	df	p
Treatment	1	<0.001
pH	2	<0.001
Organic matter	2	0.183
Treatment × pH	2	0.010
Treatment × Organic matter	2	0.157
pH × Organic matter	4	0.776
Treatment × pH × Organic matter	4	<0.001

In the treatments, the seedlings gave large change in standing leaf number throughout the experiment period (Table 4.6). At the end of the experiment, the average standing leaf numbers of the control seedlings and seedlings exposed to La were 504% and 557% of the initial leaf number (Figure 4.3). Although the average standing leaf number in the control and the treatment was similar, exception was observed in a condition (4L) where the standing leaf number was significantly higher than the control. The condition with the highest standing leaf number for treatment was 4L, the seedlings exposed to La had nine times more leaves counted at the end of the experiment. Under the same condition, the final leaf number of the control seedling was five times of the initial one, which was almost half of the standing leaf number of treatment. Seedlings grew in soil with lower pH conditions (pH 4 and 6) had more standing leaves than seedlings grew in alkaline soil (pH 8) (Figure 4.3). However, there was no trend between growth and the level of soil organic matter (Table 4.6).

Table 4.6 ANOVA table for standing leaf number of *E. citriodora* under various soil conditions and treatment.

Source of variation	df	p
Treatment	1	0.389
pH	2	0.010
Organic matter	2	0.120
Treatment × pH	2	0.057
Treatment × Organic matter	2	0.066
pH × Organic matter	4	0.352
Treatment × pH × Organic matter	4	0.816

(a)



(b)

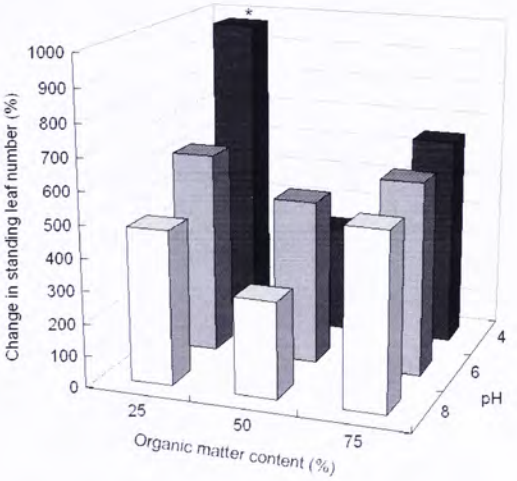


Figure 4.3 Percentage change in standing leaf number in *E. citriodora* after being exposed to La under different conditions in (a) control soil and (b) La treated soil. When compared within the same level of pH and organic matter, treatments indicated with asterisk were significantly different from the controls at $p < 0.05$ by Tukey's test.

The leaf number increased in most of the time, with only minor defoliation between the 30^{th} and 60^{th} day (Figure 4.4). However, the foliage soon recovered and even became denser after then. The standing leaf number increased at a rapid rate at the beginning of the experiment. The curve patterns for various experimental conditions were not the same. Under the conditions of 6M, 8M and 8H, the leaf numbers of the control plants were similar to that in the treatment group, whereas under other conditions, greater differences were observed between the control and the treatment. In general, the increase in foliage number was faster when grew in acidic soils compared with the alkaline soils.

The chlorophyll fluorescence parameter F_v/F_m was used to evaluate the maximum quantum efficiency of photosystem II (Figure 4.5). The F_v/F_m of seedlings exposed to La was significantly higher than the values in the control (Table 4.7). When considering the individual condition, only results in 4L had significant difference between the treatment and the control. The effect of La application on chlorophyll fluorescence was not obvious for other soil conditions.

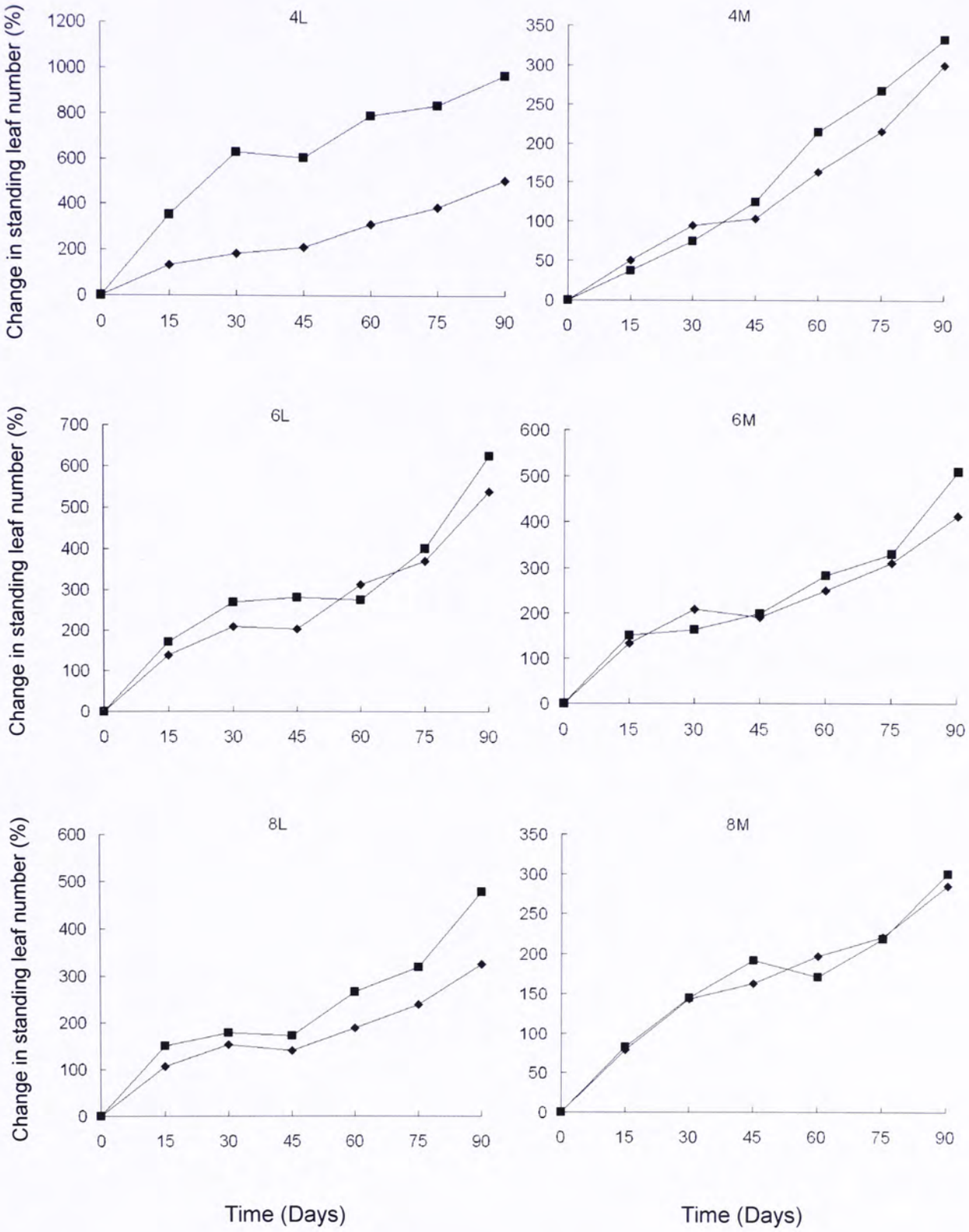


Figure 4.4 Standing leaf number of *E. citriodora* grown in soil with La application (■) and without La application (◆) in a 90-day experiment.

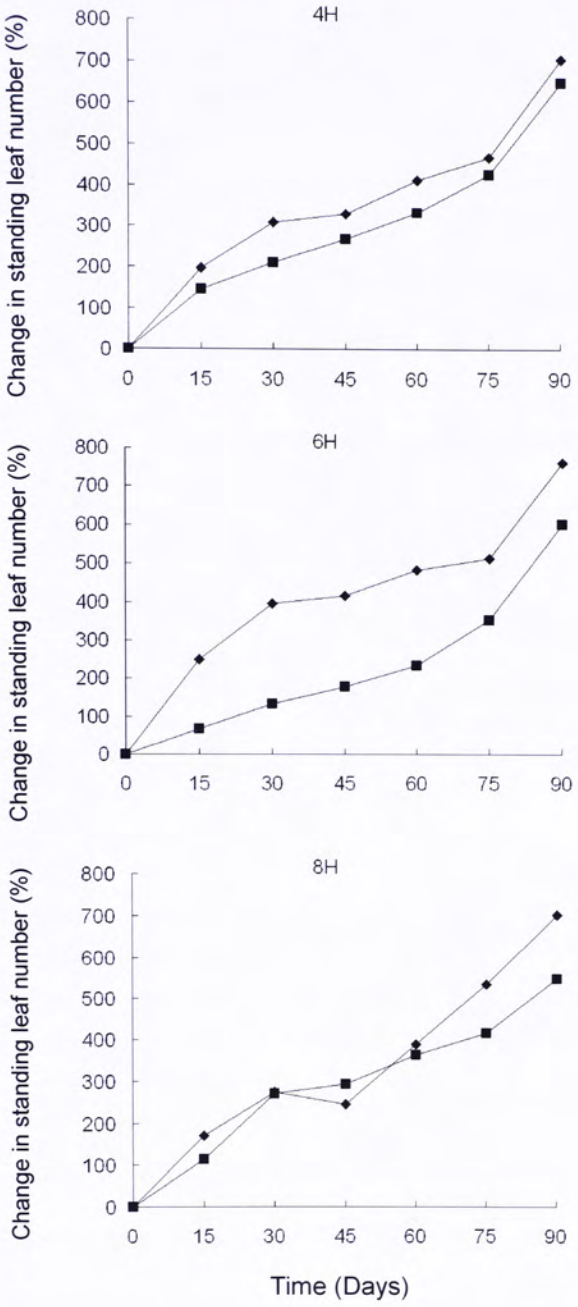
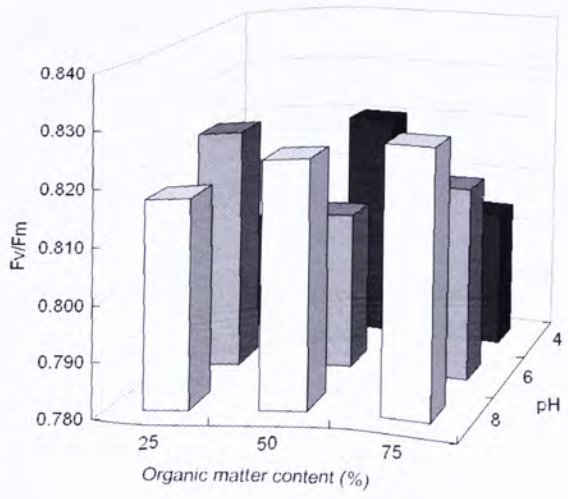


Figure 4.4 Standing leaf number of *E. citriodora* grown in soil with La (■) and without La (◆) in a 90-day experiment (continued).

(a)



(b)

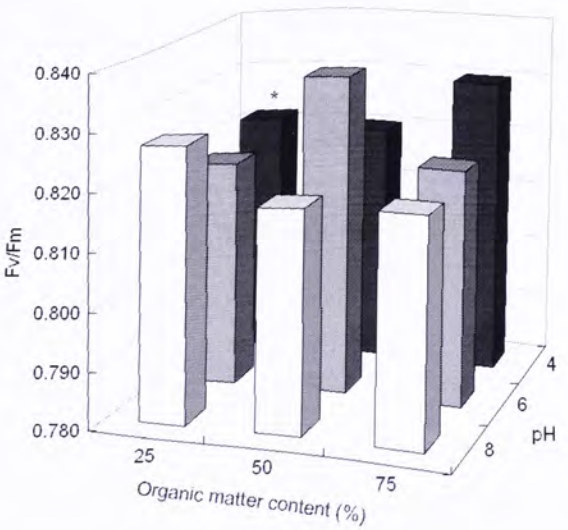


Figure 4.5 The maximal quantium efficiency expressed as F_v/F_m of *E. citriodora* grown in different conditions in (a) control soil and (b) La treated soil. When compared within the same level of pH and organic matter, treatments indicated with asterisk were significantly different from the controls at $p < 0.05$ by Tukey's test.

Table 4.7 ANOVA table for chlorophyll fluorescence parameter F_v/F_m of *E. citriodora* under various soil conditions and treatment.

Source of variation	df	p
Treatment	1	0.028
pH	2	0.490
Organic matter	2	0.835
Treatment × pH	2	0.117
Treatment × Organic matter	2	0.947
pH × Organic matter	4	0.739
Treatment × pH × Organic matter	4	0.051

4.3.2 Tissue contents of La

The foliar La concentrations of seedlings exposed to La ranged from 1.60 to 2.40 mg/kg (Table 4.8). It was on average four times of the control and the difference was significant. Seedlings grown in acidic soil had more La in their leaves.

The contents of La in root ranged from 1.15 to 2.35 mg/kg and 3.02 to 4.86 mg/kg for the control and the treatment respectively (Table 4.8). The root La content was obviously the lowest under alkaline condition. Root REE at pH 4 was significantly different from that at pH 8 at all organic matter content levels. The influence of soil organic matter was not apparent.

4.3.3 Soil

Before manipulation, the soil had a pH of 4.82, and the cation exchange capacity and organic matter content were 16.9 cmol/kg and 0.22% respectively (Table 4.9). The soil was a sandy loam with 74.8% sand, 13.6% silt and 11.6% clay. The

concentrations of La in the B1, B2 and B3 fractions were 0.033, 0.039 and 0.15 mg/kg respectively.

Table 4.8 Concentration of La in leaf and root of *E. citriodora* at the end of experiment.

Soil condition	Treatment	La concentration (mg/kg)			
		Leaf		Root	
4L	Control	0.502		2.32	
	La	2.40	**	4.86	*
4M	Control	0.618		1.86	
	La	2.35	**	3.83	**
4H	Control	0.441		2.11	
	La	1.92		4.24	*
6L	Control	0.647		1.96	
	La	1.94	**	3.02	
6M	Control	0.500		1.97	
	La	2.25	**	2.09	
6H	Control	0.607		2.44	
	La	1.80	*	4.61	
8L	Control	0.298		1.17	
	La	2.20	***	3.04	**
8M	Control	0.755		1.15	
	La	1.93		2.44	*
8H	Control	0.744		2.35	
	La	1.60	*	3.47	*

When compared within the same level of pH and organic matter, treatments indicated with asterisk, 2 asterisks and 3 asterisks were significantly different from the controls at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively by Tukey's test.

Table 4.9 Initial properties of the soil.

	Soil
Sand (%)	74.8
Silt (%)	13.6
Clay (%)	11.6
Texture (class)	Sandy loam
pH	4.82
Cation exchange capacity (cmol/kg)	16.90
Organic matter (%)	0.22
La concentration in B1 fraction (mg/kg)	0.033
La concentration in B2 fraction (mg/kg)	0.039
La concentration in B3 fraction (mg/kg)	0.15

4.3.3.1 Soil final pH

From the results of the ANOVA, the pH was significantly different between various soil conditions, but the values were similar between treatment and control (Tables 4.10 and 4.11). Under the soil condition with pH 4, the final pH was similar to the pre-set pH. For the other two pH conditions, the final pH was lower than the initial pH, but no significant difference was observed.

4.3.3.2 Soil La contents

The soil La concentration was significantly higher for soil added with La than the control in some conditions (Figure 4.6). In the condition with organic matter content of 75%, the average soil La content in the La treatment groups was significant higher when compared with the corresponding controls.

Table 4.10 pH of soil after harvest.

Soil condition	Treatment	pH
4L	Control	4.24
	La	4.18
4M	Control	3.99
	La	4.00
4H	Control	4.63
	La	4.60
6L	Control	6.06
	La	5.95
6M	Control	5.26
	La	5.19
6H	Control	5.63
	La	5.53
8L	Control	7.24
	La	7.20
8M	Control	6.40
	La	6.36
8H	Control	6.56
	La	6.50

Table 4.11 ANOVA table for pH of soil sampled after harvest under various soil conditions and treatment.

Source of variation	df	p
Treatment	1	0.287
pH	2	<0.001
OM	2	<0.001
Treatment x pH	2	0.881
Treatment x OM	2	0.951
pH x OM	4	<0.001
Treatment x pH x OM	4	1.000

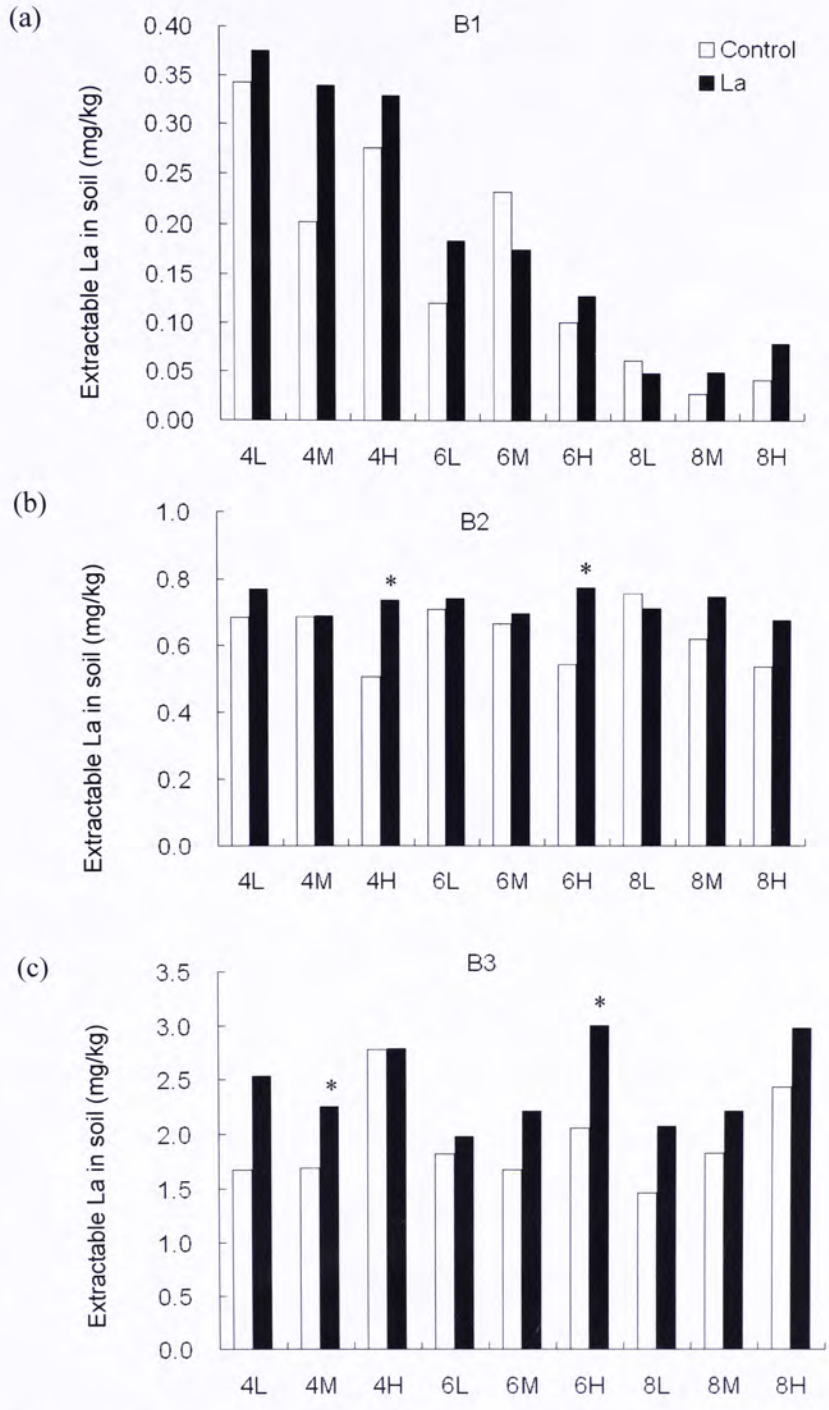


Figure 4.6 Content of La in (a) B1 fraction, (b) B2 fraction and (c) B3 fraction for soil under various conditions. When compared within the same level of pH and organic matter, treatments indicated with asterisk were significantly different from the controls at $p < 0.05$ by Tukey's test.

Regarding the three extractable fractions, concentration of La in the organic and sulphide bound fraction (B3 fraction) was the highest, it contributed 36.6%-83.7% of the total REE in soil (Figure 4.6). The concentration in the exchangeable and carbonated bound fraction (B1 fraction) was the lowest that this fraction occupied 0.994%-12.4% of the total soil REE.

Within the same organic matter content level, B1 fraction in soil with pH 4 was significantly higher than the soil with higher pH ($p < 0.05$). While within treatment of pH 4, lower organic matter content significantly increased the B1 fraction. The trends of Fe-Mn oxide bound fraction (B2 fraction) upon change in pH and organic matter content were less obvious as B1 fraction. Increase in the organic matter gave a higher La concentration in B3 fraction, and the effect was more remarkable at higher pH.

4.3.4 Association between pH, organic matter and La contents in soil and plant

Table 4.12 presents the multiple regression equations between pH, organic matter content and REE concentrations in different tissues. In general, there was a significant association between the parameters.

The La content in fraction B1 had a significant negative regression with pH as well as organic matter content ($p < 0.001$) (Equation 1). For B2 fraction, the concentration of La was positively related with pH but inversely related with organic matter content (Equation 2). The La content in fraction B3 was negatively related

with pH, but positively related with organic matter content (Equation 3).

Table 4.12 Regression equations between pH, organic matter and La concentrations.

	Regression equation	R^2	P
1.	B1 = 0.649-0.0725pH-0.0475OM	0.627	<0.001
2.	B2 = 0.802+0.0322pH-0.327OM	0.587	<0.001
3.	B3 = 1.38-0.0363pH+1.545OM	0.364	<0.001
4.	Leaf La = 2.92-0.0776pH-0.815OM	0.133	0.095
5.	Root La = 5.02-0.331pH+0.943OM	0.176	0.041
6.	Leaf La = 2.32+0.530B1+0.856B2+0.635B3-0.309Total La	0.150	0.060
7.	Root La = 6.195+4.19B1-3.44B2+0.148B3+0.223Total La	0.530	0.016

The reverse associations between foliar tissue contents and pH and organic matter were not significant (Equation 4). The regression equation had a lower coefficient of determination (R^2), revealed that variation in foliar La content was not attributable to pH and organic matter.

A significantly reverse relationship was existed between pH and La content in root of *E. citriodora* ($p < 0.05$) (Equation 5). Root La content changed positively with organic matter content, however, it was not statistically significant. The model can significantly predict 42.0% of the variability of the root La content.

The association between the La content in plant tissue and different soil fractions was analysed separately for foliar content and root content. The foliar La content was not predictable neither by the total content nor the levels in various soil fractions ($p > 0.05$) (Equation 6). However, the association between root La content and contents in soil fractions was significant ($p < 0.05$) (Equation 7). The root La content was predictable by the La in fraction B1 ($p < 0.05$). However, the total La content in soil had a poor predictability for the root content. The regressions between tissue La content and B2 and B3 fractions were not significant, implying that B1 fraction was a better predictor for the La content in root.

4.4 Discussion

4.4.1 Growth performance of tree seedling on different soil conditions

There was generally a better growth performance for those seedlings grown in soil added with La. This may imply that La can promote tree seedling growth at the level applied. Significant differences were observed only in some of the conditions. Growth stimulation was more often observed in acidic soil, which may be attributed to a higher bioavailability at lower soil pH (Zhu *et al.*, 1998a; Zhang and Shan, 2001). More La was taken up by plant to extent its effect and there was a strong regression between tissue La concentration and the pH. They had a significant reverse relationship implying that the trees absorbed more La at lower pH.

Changing the organic matter content in soil seems did not lead to any difference of the La treated plants and the control in the current study. Organic

matter was not a significant factor governing REE bioavailability. Since the uptake of La was not significantly influenced by organic matter content, the effect of La on growth would be less dependent on organic matter. Regression analysis showed a poor association between tissue La content and organic matter level. pH was a stronger determinant for the bioavailability of REEs (Masscheleyn *et al.*, 1990).

4.4.2 Comparison between growth parameters

Tree seedlings exposed to REEs in general had better growth than those without REEs. Significant stimulating effects brought by REEs were exhibited in terms of height, biomass, standing leaf number and chlorophyll fluorescence. Height was the most sensitive parameter (Hemery and Savill, 2001; Brown and Newman, 2003). The application concentration that can affect height increment may not be high enough to affect other parameters such as basal diameter. On the other hand, the duration of experiment may be too short for basal diameter to show a significant effect of REEs. The growth in height was faster than that of basal diameter so that effects could only be demonstrated by height increment.

Shtangeeva and Ayrault (2007) reported that small amounts of REEs could promote biomass production. Biomass of coconut root was the most sensitive growth parameter to the effect of REEs (Wahid *et al.*, 2000). The foliar and root biomasses of seedlings exposed to La were significantly higher than those of the control seedlings. It implied that biomass was also a sensitive parameter in the current study. However, the effect of REEs on tree seedling was not only expressed

in terms of biomass, but also height and standing leaf number. Most of the studies concerning effect of REEs on growth were assessed in terms of yield (Chang, 1991). They mainly concentrated on agricultural crops such as rice and wheat. Leaf is the main site for photosynthesis, which produces food and energy for maintaining tree life. More leaf number indicates a better growth of tree. Leaf number therefore was an important indicator for trees. It was suggested that more non-destructive benchmarks should be assessed for the determination of the effect of REEs on tree seedling.

REEs were claimed to able to improve the rate of photosynthesis and increase chlorophyll concentration (Xiong *et al.*, 2000). The effect was also observed in this study. Measurement of chlorophyll fluorescence was noninvasive, simple, rapid and without disrupting the integrity of the plant (Maxwell and Johnson, 2000; Brown and Newman, 2003; Mallick and Mohn, 2003). It is a useful tool to monitor the photosynthetic performance of plants under effects of chemicals. The optimal value of F_v/F_m was around 0.83 which reflect the highest photosynthetic efficiency. The optimal value of F_v/F_m was not achieved in the control but reached after REE exposure in the present study. It means that the condition in the control might be suboptimal for plant growth, but photosynthetic efficiency was improved by addition of La. The enhanced photosynthesis after application of REEs may be attributed to the increased synthesis of chlorophyll and deposit of REEs in chloroplast and thylakoid (Xu and Wang, 2001). Chlorophyll fluorescence can also be used to measure the suppression of photosynthetic system by chemicals (Maxwell and

Johnson, 2000). It indicates the extent to which PSII is using energy absorbed by chlorophyll and the extent to which it is being damaged. Decrease in F_v/F_m or increase in F_0 indicates the presence of environmental stresses on plants and the extent to which these stresses have damaged the photosynthetic system. The F_v/F_m of *Gracilariopsis longissma* was significantly reduced by copper at applied concentration greater than 0.25 mg/L (Brown and Newman, 2003). However, no decrease in F_v/F_m was observed in REE treatment. This may indicate that REE had little adverse impact on the photochemical system.

4.4.3 Speciation in soils

The proportion of each fraction in soil was not always the same. The proportion of B1 fraction was always the lowest. In a natural environment, the B1 fraction was lower than 4% of the total REE content (Wen *et al.*, 2002). Content of water soluble REEs was the lowest in the topsoil sample collected from China (Zhu *et al.*, 1998a). In the present study, the B1 fraction also occupied the lowest percentage, because REEs in this fraction was in form of simple or complexed ions in the soil solution, so that it was easily absorbed by plants or leached out rapidly after application (Han *et al.*, 2001).

In the present study, the major fraction was B3 fraction, which in agreed with other studies. The soil collected from a corn fields in China had as high as 30.3% of REEs distributed in B3 fraction (Li *et al.*, 1998). Zhang and Shan (2001) also found that there were 28.4% of the total La contents existed as B3 fraction, which was the

greatest percentage among the three fractions. Other than B3 fraction, B2 fraction was often reported to occupy a great portion. Wen *et al.* (2002) found that B2 occupied more than half of the total REEs. In the study carried out by Cao *et al.* (2002), majority of REEs existed in B2 fraction of soil collected from a wheat field.

The difference in the proportion of REE forms may be attributed to the variation in soil composition. Those soil rich in B2 fraction had a high Fe and Mn content. Concentration of B2 fraction has strong association with Fe and Mn contents (Chuan *et al.*, 1996). REEs adsorbed on the Fe-Mn oxides by coordination with OH^- on surface of Fe-Mn oxides (Cao *et al.*, 2001). For soil with lower Fe and Mn concentration, B2 would no longer become the major fraction, as the elements preferred to bind with organic matter (Cao *et al.*, 2000). There are numerous negatively charged binding sites on the organic matters of soil which had high affinity to metal cations. The reaction between REEs and organic matter was fast. REE would be easily attracted to the organic matter and formed the B3 fraction. Such association is reversible and the distribution among fraction can be shifted in response to the changed soil properties.

4.4.4 Bioavailability of REEs in soil

The contents of La in *E. citriodora* planted in La-treated soil were significantly higher than those grown in soil without addition of La. This implies that the plant could take up La. However, the La contents in tree seedling had poor association with their total contents in soil. The results were consistent with other

studies, in which the total content of La, Pr, Nd, Yb and Y was poorly correlated with the contents in corn and rice and other plant species (Li *et al.*, 1998; Wang *et al.*, 2001; Tyler, 2004; Chojnacka *et al.*, 2005). The REEs in soil are distributed in different fractions, in which not all of them are available to plant. The total REE concentration not only included the portion available to plants, but also those strongly bound within crystal structure of soil. The latter fraction was not expected to be released for plant uptake over a reasonable time span and regarded as fraction of residue (Tessier *et al.*, 1979; Han *et al.*, 2001). In nature, a great portion of metal contents in soil were in this fraction. Yuen *et al.* (2001) reported that there were more than 80% of total REE remained in the form of residue. Since a portion of total REE was not available to plant, total amount was a poor indicator for predicting their contents in plants.

The concentration of La in plants has stronger relationship with defined soil fractions of La. In the present study, the La content in roots was better correlated with B1 fraction whereas there was no significant regression between contents in leaf and all soil fractions. Other studies reported significant positive relationship between B1 fraction and the contents in various parts of plant. Lu *et al.* (2003a) found that REEs in B1 fraction was positively correlated with REEs in root and shoot of rice. Wang *et al.* (2001) demonstrated that concentrations of La, Ce, Pr and Nd in B1 fraction were significantly correlated with their concentration in shoot of wheat. A good correlation between tissue contents and the B1 fraction suggested that REEs in B1 fraction was liable to plant uptake. It was commonly agreed that B1 fraction

represented the most bioavailable form (Laul *et al.*, 1979; Cao *et al.*, 2000; Han *et al.*, 2001; Wang *et al.*, 2001; Tyler, 2004; Liang *et al.*, 2005). Metals were taken up by plants from the soil solution. B1 fraction represented the water soluble, exchangeable and carbonate bound portion, which is the most mobile and easily absorbed form.

The results concerning the bioavailabilities of B2 and B3 fractions were still controversial. In the present study, their correlations with the tissue contents were not significant, implying that the plants would not take up more La even though La in these fractions increased. The REEs in these two fractions were poorly bioavailable to plants. REEs contents in *Vaccinium vitis-idaea* (lingonberry) were poorly correlated with abundance of REEs in the fractions (Markert, 1987), while concentrations of several REEs in six woody plants were independent to these fractions (Wyttenbach *et al.*, 1998). However, other studies reported good correlations between REE contents in B2 or B3 fractions and contents of REEs in plant tissue. Organically bound REEs were available to corn and rice (Li *et al.*, 1998), and a significant correlation existed between shoot uptake and REE contents in B2 fraction (Wang *et al.*, 2001). The inconsistency between results may be attributed to the difference in soil properties. REEs in B3 fraction could be taken up after they were released to the soil solution. However, the REEs associated with organic matter were not easily desorbed owing to slow mineralization of peat moss. For those studies with high bioavailability of B3 fraction, REEs may be bound to soluble organic matter which were easily dissolved into soil solution and assimilated

by plant (Han *et al.*, 2001; Wen *et al.*, 2006).

As the forms of REEs existed in soil highly depend on soil properties. Maintaing a soil condition that favoured the formation of the bioavailable form of REEs can make the application of REEs to plants more efficiently. Chemical fractionation was a useful approach to determine the bioavailability of REEs in soil and suggest the needs for modifying soil properties.

4.4.5 Factors affecting bioavailability of REEs

The total abundance of REEs in soil had a poor correlation with the tissue REEs contents since not all REE forms can be assimilated. The bioavailability of REEs was affected by the soil conditions.

Among the soil properties that would affect the bioavailability of REEs, pH was the most important. In the current study, a significant reverse association was obtained between soil pH and La content in B1 fraction, implying that the REEs would be more available at low pH. This was further proved by the significant association between pH and the tissue content of La. Other than those REEs existed in exchangeable and carbonated bound form, which were readily absorbed, most of them were bound to soil particles. At a higher pH level, the immobilization by soil was more rapid, and insoluble compounds would be formed when REEs reacted with hydroxides or oxides (Obrador *et al.*, 1997). However, at low pH, they dissolved and so released the bound REEs. REEs also formed complexes with Fe-Mn oxides.

The Fe and Mn dissolved at low pH, which released REEs to soil solution. (Masscheleyn *et al.*, 1990).

A lower pH also favoured the uptake of REEs by plants. Rhizosphere has a lower pH than the soil far away from plant roots as root secretes acidic substances such as organic acids to facilitate the metal uptake (Li *et al.*, 1998). The result of present study shows that the final pH was lower than the pre-set pH which may be explained by the secretion. This would help the dissolution of metals so more REEs appeared as free ions for plant assimilation.

Under acidic condition, competition between cations and hydrogen ions leads to release of REEs (Cao *et al.*, 2002; Wen *et al.*, 2002). REEs are soluble in low soil pH. Chuan *et al.* (1996) found that solubilities of REEs at pH 5 were higher than those at alkaline pH and further increased at pH 3. REEs were converted from precipitable forms to soluble ions under acidic condition (Yang *et al.*, 1999a; Rimmer *et al.*, 2001; Shiowatana *et al.*, 2001). The adsorption process diminished and the elements preferred to exist as ions, and remained in soil solution (Jansen *et al.*, 2002; Shan *et al.*, 2002; Borrego *et al.*, 2004; Olías *et al.*, 2005). Those REEs originally bound to Fe-Mn oxides (B2 fraction) and organic matters (B3 fraction) shifted to the soluble and exchangeable fraction (B1 fraction) (Cao *et al.*, 2001). As a result, the REEs contents in B1 fraction increased and so improved the bioavailability of REEs.

Organic matter in soil plays an important role in providing REEs to plants. There have been studies recorded that organic matter could improve the bioavailability of REEs. Åström and Corin (2003) reported an increase in REEs when the amount of colloidal-sized organic matter increased. However, in the present study, altering the amount of organic matter content in soil did not change the La content in tree seedling significantly. The improvement effect of organic matter may also depend on their forms which have different capability in releasing REEs, making the reaction between organic matter and REEs complicated (Olías *et al.*, 2005). Dissolved organic matter had the highest affinity to REEs and was easily assimilated by plants (Nierop *et al.*, 2002). The amount of metals in soil solution was obviously related to the quantity of dissolved organic matter (Zhu *et al.*, 1998b).

The presence of ligand would also change the bioavailability of REEs. There were more REEs in plant tissue when low concentration of fluvic acid was present in soil (Gu *et al.*, 2001). In the absence of ligands, REEs were merely bound to soil particles. Soluble organic ligands in soil compete for REEs with the soil adsorption sites. Organic ligands, such as EDTA and low molecular weight organic acids, reacted with the metals bound to soil particles to form organo-metallic complexes, which are more soluble in soil solution. This helps the release of metals from the insoluble components. Fe and Mn were released from their oxides which were originally insoluble (Wang *et al.*, 2001). Since the products were more soluble, there was reduction in adsorption on soil and, at the same time, there was increase in desorption (Yang *et al.*, 1999a; Shan *et al.*, 2002). Furthermore, the organic acids

can promote REE adsorption onto root surface and thus increase the uptake of REEs (Wang *et al.*, 2004; Wen *et al.*, 2006).

Besides, the influence of organic matter on bioavailability was pH-dependent; alteration in pH would change the forms of organic compounds (Shan *et al.*, 2002). Since various compounds have different affinity to REEs, the desorption from organic matter becomes complicated under the influence of pH.

The dominant forms of metal ion shifted under influence of soil redox potential. Chuan *et al.* (1996) found that solubility of all the tested heavy metals (Pb, Cd and Zn) was higher under reducing condition. It may be because these metals were adsorbed on Fe-Mn oxyhydroxides and dissolution of the solid occurred under reducing condition which resulted in the release of the adsorbed ions. Similar results were also obtained for REEs. More La, Ce, Gd and Y were released into soil solution at decreasing redox potential (Cao *et al.*, 2001). Such REEs ions were proposed to be adsorbed on Fe-Mn oxides or precipitated as hydroxide. Under reducing condition, dissolution of Fe-Mn oxides subsequently releases REEs. Contrastingly, the release of metals was also observed under highly oxidizing condition. The amount of heavy metals released from solid phase of sediment increased under oxidizing condition which might come from the oxidation of metal sulphides (such as iron sulphide) (Masscheleyn *et al.*, 1990; Chuan *et al.*, 1996). Besides, the ionic form changes under various redox conditions. Most of Se in sediment was converted to soluble SeO_4^{2-} under highly oxidizing condition

(Masscheleyn *et al.*, 1990).

4.4.6 Distribution of REEs in plants

The REEs concentrations in roots and shoots were similar in the control. However, the REE concentrations in different parts of the plant varied when seedlings were exposed to REEs. More REEs were accumulated in roots than in leaves. Wheat and rice from soil with REE dressing also had similar distribution pattern (Liu *et al.*, 1997; Yang *et al.*, 1999a; Wang *et al.*, 2001; Nakamaru *et al.*, 2006). REEs were restricted to roots of agricultural crops rather than transported to shoots. The concentrations of La in roots of corn and mungbean were 20-150 times higher than the concentrations in shoots (Diatloff *et al.*, 1995b). Contents of La, Ce, Pr and Nd in roots of *Troticum aestivum* ranged from 1.03 to 4.30 mg/kg, while their contents in shoots were from 0.02 to 0.11 mg/kg (Wang *et al.*, 2001). Roots of *Taxodium japonicum*, *Vivia villosa* and *Thea sinensis* had the highest REE contents while the trunk had the lowest (Fu *et al.*, 2001). Trees mainly accumulated REEs in roots, which could accumulate 20-150 times REEs as much as the shoot (Tyler, 2004). The results were consistent with the present study.

However, leaves had more REEs than other parts when the REEs were sprayed on the leaves (Sun *et al.*, 1994; Chua *et al.*, 1998). A significant increased of La, Ce, Pr, Nd and Gd concentration in shoot was observed when application rate was 2 kg/ha, while it required more than 10 kg/ha to raise the contents in grain (Tyler, 2004). The translocation was relatively slow and REEs was confined at the site of exposure.

However, some studies demonstrated that such pattern will become less significant some time after REE application to either soil or leaves (Chua *et al.*, 1998; Ding *et al.*, 2006). The absorbed REEs will be transported to other parts, which is a kind of homeostatic regulation mechanism. Such even distribution was not obtained in the present experiment probably due to the relatively short duration of the experiment.

REEs were absorbed into roots as ions (Xu *et al.*, 2003). The ions were adsorbed on the surface of roots, passed into the vascular tissues and were transferred to the aerial parts (Yuen *et al.*, 2001; Zhang and Shan, 2001). The transport mechanism of REEs throughout the plant is still not clear. For the REEs applied to soil, the most widely reported pathway would be via mass flow in xylem (Fu *et al.*, 2001; Shtangeeva and Ayrault, 2007), while for REEs applied through foliar spray, the transport route may be via phloem (Ding *et al.*, 2006).

Casparian strip has been claimed to be a barrier to movement of REEs (Brown *et al.*, 1990; Yuen *et al.*, 2001; Xu *et al.*, 2003). Diffusion of REEs in the apoplast was completely avoided and REEs cannot pass through the plasma membrane (Nagahashi *et al.*, 1974; Chang, 1991; Diatloff *et al.*, 1995b; Xie *et al.*, 2002). Deposit of REEs was reported at and along cell wall and cortical side of endodermal cells. There were approximately 10% of REEs deposited in the cell membrane of *Dicropteris dichotoma* (Shan *et al.*, 2003). REEs were also located at the outer membranes and bound tightly with the membrane sites of Ca^{2+} on the cell surface (Xu *et al.*, 2003). La^{3+} was recorded to accumulate on the surface of palsmalemma of

Avena sativa (Brown *et al.*, 1990). The stelar side of Casparian strip and cells in stele were totally free of REEs. It was therefore believed that REE ions could not pass through Casparian strip until recent discovery of REE passage through Casparian strip and enter to cell organelles (Lai *et al.*, 2006). Although most of the REEs (68%) were found to deposit on the cell wall of *Pronephrium simplex*, about 30% REEs passed the cell membrane. Casparian strip was partially permeable to REEs which depended on the development of Casparian strip (Brown *et al.*, 1990). La^{3+} could reach the phloem cells by passing through the Casparian strip in *Yucca flaccida* (Adam's needle). There were some chemicals which could help the passage of REE ions across the membrane, such as pectin acid on the cell wall (Lai *et al.*, 2006). In normal cases, pectin acid bound with Ca^{2+}, but REE ions replaced Ca^{2+} and reacted with pectin acid, and transported across membrane since REE ions have higher ability to bind with the acid.

There were 8% of REEs accumulated in the chloroplast in the leaves of *Dicranopteris dichotoma*. Half of the REEs were present on the chloroplast membrane and another half were in the thylakoid (Wang *et al.*, 2003). In the chloroplast of *Pronephrium simplex*, there were 46.6% and 53.4% of the absorbed REEs deposited on chloroplast membrane and thylakoid respectively (Lai *et al.*, 2006). The REEs ions interacted with thylakoid membrane surface and were associated with photosystem II (PS II) in thylakoid while a small portion was associated with PS I (Yuen *et al.*, 2001). PS II is an important site for photosynthesis, which affects the plant's total productivity directly (Lu *et al.*, 2003b). Deposit of REEs can improve

photosynthesis and thus facilitate plant growth. Other than chloroplasts, REEs were also detected in tannin vacuoles, endoplasmic reticulum and vacuoles (Brown *et al.*, 1990).

4.5 Conclusions

Seedling of *E. citriodora* took up significant amount of the La applied to soil. When REEs in soil were fractionated by sequential extraction, the La content in plant tissue was poorly related with the total La content in soil but positively related to the La concentration in B1 fraction. B1 fraction was the most bioavailable fraction for plants. The magnitude of the B1 fraction can give a rough prediction on the amount of REE assimilated by plants. The speciation of REEs was governed by soil properties. pH was a more important factor than organic matter in affecting the REE fractionation and hence bioavailability. A significantly negative correlation was obtained between La concentration in B1 fraction and pH. More REEs were converted to available form under acidic condition. Since plants take up metals in form of free ions, the results suggest that REEs would be more bioavailable at low pH. However, the bioavailability was also influenced by other factors such as the competition for ion exchange sites, obtaining more information about the soil properties would improve the prediction of bioavailability of REEs.

Most of the absorbed La was stored in roots of seedlings and only a small portion was transported to the aerial parts. As the translocation of REEs was a slow process, a longer experimental period may provide more valuable information about

the translocation of REEs in plants.

Chapter 5 General conclusions

5.1 Summary of major findings

The present research investigated the effects of REEs on plants and the influence of various soil factors on the bioavailability of REEs. The information obtained can fill the knowledge gap.

Seed germination and root elongation test demonstrated reduced germination rate and root growth when seeds of *B. chinensis* and *L. perenne* were immersed in solutions of various REEs including La, Ce, Pr and Nd. The EC50s of REEs ranged from 9.52 to 20.1 mg/L, while the EC50s of the heavy metals such as Cr, Cu, Ni and Zn were not higher than 2.5 mg/L for *L. perenne* (Dijkshoorn *et al.*, 1979). These heavy metals were much more toxic than the REEs. The adverse effect of REEs on seed growth was not as serious as heavy metals. The threat of REEs to environment seems to be smaller. There was difference between the four REEs tested for the results obtained from *B. chinensis*. The EC50s of La and Ce were significantly lower than those of Pr and Nd. It was commonly believed that REEs were highly similar in electronic configuration and chemical properties with each others. However, from the results of the present study, it was found that the difference among each REE would be exhibited when they were examined in a test with high sensitivity assay in a well controlled condition. The germination rate and root elongation were less affected by environmental factors. The results can preciously reflect the toxicity of REEs. Germination and root growth of *B. chinensis* were affected at a lower REE levels than those of *L. perenne*. Nevertheless, when comparing the EC50s, there was

no statistically difference among the values obtained from these two species. *L. perenne* was a species suggested by many standard protocols since it was sensitive towards many chemicals. However, *B. chinensis* has been appeared in the protocol prepared by the OECD only. In the present study, *B. chinensis* was found to be as sensitive as *L. perenne*. *B. chinensis* is one of the most important vegetables consumed in Hong Kong. This species is also common in England because of the influx of immigrants from Hong Kong and mainland China (Mahmud *et al.*, 1999). *B. chinensis* has high economic values. It was proposed that *B. chinensis* would be a useful species when determining the phytotoxicity of REEs.

Germination and root elongation test involving seeds exposed to REE solution was a sensitive method to investigate the phytotoxicity of REE. Nevertheless, it cannot reflect the plant performance in the rest of the life cycle. Directly evaluating the growth of plant in REE-amended soils would provide more completed information about the effects of REEs on plant growth so as to help the application of REEs to soil.

The increments in height, leaf number and biomass of seedlings of *A. auriculiformis* or *E. citriodora* were significantly increased when low application rate of REEs were applied to soil. Plant grew at a higher rate when REEs were added to soil, which may be attributed to the better growth observed at the end of the experiment. The stimulating effect was dose-dependent, which diminished when the concentration was 25 mg/kg. The increment in growth contributed by La, Ce, Pr and

Nd varied. This implies that there was difference among these REEs about their interaction with the plants. It is proven by the higher La concentration in the plant tissue compared with the others. It is reported that REEs increased the nutrient uptake by plants (Wahid *et al.*, 2000). However, no significant improvement in nutrient uptake could be observed in the present study. Further investigation should be carried out to clarify the contradictory results.

In most cases, information about the effect of REEs is derived from crops, especially wheat and rice. However, data should include forestry plants which are also recipients of REEs and are important to human life and ecosystem. REEs were reported to be able to increase yield and improve crop quality. Once these beneficial effects are also exhibited in forestry plants, a great contribution could be given to the ecology and forestry industry. The present study indicates that the influence experienced by tree seedlings was similar to economic crops, thus REEs may be useful in promoting tree growth. Since the effect of REEs could be dose-dependent, the amount of REEs applied should be carefully managed.

The growth of seedlings *E. citriodora* was significantly improved by REEs under acidic condition, under which REE availability and uptake were elevated. The absorbed REEs were mainly stored in roots. The translocation of REEs inside plant may be slow, causing the absorbed REEs to accumulate in the assimilated part. REEs exist in soil in various forms. Some of them are more easily taken up than the other forms. Among the fractions extracted, the water soluble, exchangeable and

carbonated bound fraction (B1 fraction) was the most bioavailable form, while Fe-Mn oxide bound fraction (B2 fraction) and organic and sulphide bound fraction (B3 fraction) was less bioavailable. It was proposed that concentration of REEs in B1 fraction in soil would be a better prediction of the abundance of REEs in plant. The effects of change in soil pH and organic matter content on converting REEs fractions were investigated. There was a significant relationship between B1 fraction and soil pH. The concentration of B1 fraction increased under acidic condition, but decrease under alkaline condition. Therefore, the REE contents in plant tissue were higher under low pH condition. Change in soil organic matter did not cause impact as significant as pH. It was proposed that regulating the soil pH would be the most critical for managing REE bioavailability.

In the field, environmental conditions could vary greatly and change continuously. Plant dose not assimilate all forms of REEs in soil. Those can be easily absorbed by plant are regarded as readily bioavailable. The change in soil properties can lead to alteration of chemical speciation of REEs, and thus converting the proportion of REEs which is bioavailable to plants. The present study provided more information about the bioavailability of REEs under different soil conditions. Such information would help in regulating the amount of REEs being absorbed by plants from soil.

REEs were still not reported to be essential to plant growth. From the present study, REEs were found to have low toxicity and stimulate plant growth at certain

concentrations. Plants with better growth are benefits to human life and ecosystem. It would be a contribution to plant when REEs were applied in a proper condition and application rate. The present study investigated information on the effect of REEs on plant, which is valuable in managing application of REEs to plants.

5.2 Suggestions for further investigation

The present study investigated the effect of REEs on plant growth for three months. It is a relatively short period with respect to the life of trees. Once the REEs were absorbed by plants, they would be stored in the plant body rather than being excreted. The possibility for REEs affecting plant after a long time was least addressed in the current literature. Thus, the information about chronic effect would be highly valuable in the view of environmental concern.

Moreover, one REE was added to soil in each treatment in the present study to investigate the growth performance alternation of plant contributed by the REEs. As an ingredient of fertilizer, mixture of REEs exists. However, the growth of plant under the condition that REEs simultaneously exist was not well studied. Thus, effect of applying more than one REE to plants is an important issue that further research is needed.

There were various forms of REEs existed in soil. Not all of them can be assimilated by plant. The conversion from the unavailable form to the available form is controlled by many factors. Soil properties, including pH and organic matter

were tested to be factors influencing the bioavailability of REEs. Other than the chemical and physical properties of soil, biological aspect is also an important component of soil. Bacteria and soil fauna help to improve the soil conditions that favour plant growth. Nevertheless, their contribution on the bioavailability of REEs has received less concern. Earthworm was reported to increase the concentration of the most available form of REEs in soil (Wen *et al.*, 2006). The mechanism behind the observation was not well known. Further investigation on this aspect is also highly recommended.

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