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Mobility and transfer of rare earth elements from soil to plants by arbuscular mycorrhizal fungi

Ruoyu Hu

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Docteur de l'Université de Lorraine
en Géosciences

par **Ruoyu HU**

Mobilité et transfert des terres rares du sol aux plantes par les champignons mycorhiziens arbusculaires

Mobility and transfer of rare earth elements from soil to plants by arbuscular mycorrhizal fungi

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Membres du jury :

Rapporteurs :

M. Erik JONER
M. Thierry LEBEAU

Directeur de recherche, Nibio, Ås - Norvège
Professeur, Université de Nantes - France

Examineurs :

M. Alexis DE JUNET
M^{me} Laure GIAMBÉRINI
M^{me} Nathalie SÉJALON DELMAS
M. Ye-Tao TANG

Maître de conférences, Université de Lorraine - France
Professeure, Université de Lorraine - France
Maître de conférences, Université Toulouse III - France
Professeur, Sun Yat-sen University, Guangzhou – Chine

Directeurs de thèse

Mme Corinne LEYVAL

Directrice de recherche CNRS, Université de Lorraine - France

Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC)
UMR 7360 CNRS – Université de Lorraine



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Introduction générale

Cette thèse a été financée par le Laboratoire d'Excellence RESSOURCES21 (LabEx R21) qui réunit des chercheurs de différents centres de recherche et forme une équipe multidisciplinaire visant à comprendre et à gérer les métaux stratégiques au 21ème siècle. Les terres rares (REE en anglais) sont devenues des métaux stratégiques les plus exigeants au niveau international et sont utilisées dans de nombreuses industries, y compris la haute technologie, l'agriculture, l'énergie propre et la médecine, grâce à leurs qualités magnétiques, conductrices, phosphorescentes et thermorésistantes uniques. De nombreuses études ont fait état des quantités croissantes de REE d'origine anthropique dans l'environnement naturel, mais les informations concernant les risques environnementaux et l'écotoxicité des REE anthropiques, en particulier pour l'environnement terrestre, sont rares et contradictoires.

Les champignons mycorhiziens à arbuscules (AM) sont très fréquemment présents dans les sols, établissant une symbiose avec la majorité des plantes vasculaires. Les champignons AM ont été bien documentés en tant que composants importants de la rhizosphère, améliorant la croissance et la nutrition des plantes, et aidant les plantes à faire face aux conditions environnementales stressantes. Les interactions entre ces champignons et les métaux lourds ont été largement étudiées, et de nombreuses études ont montré que la colonisation des AMF favorisait la croissance de manière significative. La mycorhization par ces champignons pourrait améliorer la phytoremédiation de l'environnement contaminé par les métaux lourds. Cependant, le rôle de ces champignons MA sur les plantes dans les sols contaminés par des REE demeurait inconnu.

Les contaminations en REE proviennent principalement de déchets miniers et de déchets industriels (Dev et al. 2020). Les déchets miniers sont produits lors du drainage minier acide et sont rejetés avec les résidus miniers. Les déchets industriels ont été principalement produits lors de la procédure d'extraction des REE. Dans ces déchets et résidus, les REE ont une solubilité assez élevée. Pour d'autres contaminants du sol, la biodisponibilité des REE et leur toxicité pour les organismes vivants a été largement étudiée comme une question clé, avant d'envisager des stratégies de remédiation potentielles, comme par exemple la phytoremédiation.

Les objectifs généraux de la thèse étaient alors de considérer la disponibilité, la mobilité des REE pour les plantes et la contribution des champignons MA à la croissance des plantes et au transfert des REE aux plantes.

Pour 1) simuler la contamination des REE rejetés par les industries ou les résidus miniers dans l'environnement terrestre, 2) connaître la quantité précise de REE, contrôler la mycorhization et fournir des contrôles (sans contamination, et sans MA), 3) contrôler et surveiller les paramètres, des expériences de dopage des REE ont été réalisées en utilisant des plantes modèles cultivées dans des conditions contrôlées en laboratoire.

Des expériences ont été réalisées, allant de simples cultures en pot à des dispositifs à compartiments complexes, pour comprendre le transfert de REE d'origine anthropique du sol aux plantes et préciser comment les champignons MA affectent ce transfert et la croissance des plantes. En complément et pour étudier des sols historiquement contaminés, et grâce au projet ECOLAND et à l'Université de Sun Yat-sen, des sols de résidus miniers à forte concentration en REE ont été utilisés pour préciser la disponibilité et la toxicité des REE pour les champignons MA.

La thèse est composée de 7 chapitres, dont 4 chapitres de résultats et comprend :

1. Etat de l'art et les objectifs
2. Les matériels et méthodes utilisés dans la thèse
3. La biodisponibilité et transfert d'une concentration élevée de Sm vers la luzerne dans des sols enrichis
4. Le transfert de la terre rare samarium du sol enrichi à la luzerne par *Funneliformis mosseae*
5. Le transfert de La, Ce, Sm et Yb à la luzerne et au ray-grass à partir de sols dopés et contribution de l'inoculation mycorhizienne avec *Funneliformis mosseae*
6. La disponibilité et toxicité pour un champignon MA de sols contaminés par des REEs provenant de résidus miniers en Chine
7. Discussion générale, perspectives et conclusion

Les résultats de la thèse ont fait l'objet de deux articles publiés et un troisième est à soumettre:

- Hu R, Beguiristain T, De Junet A, Leyval C (2020a) Bioavailability and transfer of elevated Sm concentration to alfalfa in spiked soils. *Environ Sci Pollut Res* 27:44333–44341. <https://doi.org/10.1007/s11356-020-09223-z>
- Hu R, Beguiristain T, De Junet A, Leyval C (2020b) No significant transfer of the rare earth element samarium from spiked soil to alfalfa by *Funneliformis mosseae*. *Mycorrhiza* 30:761–771. <https://doi.org/10.1007/s00572-020-00991-7>
- Hu R, Beguiristain T, De Junet A, Leyval C Transfer of La, Ce, Sm and Yb to alfalfa and ryegrass from spiked soil and contribution of mycorrhizal inoculation with *Funneliformis mosseae* (to be submitted)

General introduction

This PhD project is funded by the Laboratoire d'Excellence RESSOURCES21 (LabEx R21) project who gathered researchers from different research centers and formed a multidisciplinary team aiming at understanding and managing strategic metals in the 21st century. REEs have become one of the most demanding strategic metals internationally and been applied in many industries including high technology, agriculture, clean energy and medicine thanks to their unique magnetic, conductive, phosphorescent and heat-resistant qualities. Many studies have reported the increasing amount of anthropogenic REEs in the natural environment, however, the information concerning the environmental risks and the ecotoxicity of the anthropogenic REEs especially to the terrestrial environment is scarce and contradictory.

Arbuscular mycorrhizal fungi (AMF) are very frequently present in soils, establishing a symbiosis with the majority of vascular plants. AMF have been well documented as an important component in the rhizosphere, improving plant growth and nutrition, and helping the plants to cope with stressed environmental conditions. The interactions between AMF and heavy metals has been studied extensively, and a significant growth promotion has been found due to AMF colonization in many studies. AMF could enhance phytoremediation of the heavy metal-contaminated environment. However, the role of AMF on plants in REE contaminated environment remained unknown.

The REE wastes mainly come from mining wastes and industrial wastes (Dev et al. 2020). The mining wastes are produced during acid mine drainage and released with mine tailings. The industrial wastes were mainly produced during the REE extraction procedure. In these REE wastes, the REEs are with rather high solubility. For other soil contaminants, the bioavailability of REEs and their toxicity to living organisms is a key question, prior to consider potential remediation strategies, like for example phytoremediation.

The general objectives of the thesis were then to consider the availability, mobility of REEs to plants and the contribution of AMF to plant growth and REE transfer to plants.

To 1) simulate the REE contamination released from industries or mine tailings in the terrestrial environment, 2) know the precise quantity of the REEs and AMF and provide controls (without contamination, and without AMF) 3) control and monitor the parameters, REE spiking experiments using model plants growing in laboratory controlled conditions were performed.

Experiments have been designed from simple pot cultures to complex compartment devices to understand the transfer of anthropogenic origin REEs from soil to plants and how AMF affect this transfer and the plant growth. As a complement and to consider real contaminated soils, and thanks to the ECOLAND project and the University of Sun Yat-sen, mine tailing soils with high REE concentrations were used to precise the availability and toxicity of REEs to AMF.

The PhD thesis is composed of 7 chapters, including four results chapters, presenting

- 1) the state of the art of REEs and AMF
- 2) the materials and methods used in the thesis
- 3) the bioavailability and transfer of elevated Sm concentration to alfalfa in spiked soils
- 4) the transfer of the rare earth element Sm from spiked soil to alfalfa by *Funneliformis mosseae*
- 5) the transfer of La, Ce, Sm and Yb to alfalfa and ryegrass from spiked soil and contribution of mycorrhizal inoculation with *Funneliformis mosseae*
- 6) the availability and toxicity to AMF of REEs contaminated soils from ionic mine tailings in China
- 7) General discussion, perspectives and conclusion

The results of the thesis have been published in two papers and a third one is ready to be submitted:

- Hu R, Beguiristain T, De Junet A, Leyval C (2020a) Bioavailability and transfer of elevated Sm concentration to alfalfa in spiked soils. *Environ Sci Pollut Res* 27:44333–44341. <https://doi.org/10.1007/s11356-020-09223-z>
- Hu R, Beguiristain T, De Junet A, Leyval C (2020b) No significant transfer of the rare earth element samarium from spiked soil to alfalfa by *Funneliformis mosseae*. *Mycorrhiza* 30:761–771. <https://doi.org/10.1007/s00572-020-00991-7>
- Hu R, Beguiristain T, De Junet A, Leyval C Transfer of La, Ce, Sm and Yb to alfalfa and ryegrass from spiked soil and contribution of mycorrhizal inoculation with *Funneliformis mosseae* (to be submitted)

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Chapter 1. State of the art and objectives

Chapitre 1 Etat de l'art et objectifs

1.1 Rare earth elements (REEs)

1.1.1 What are REEs?

Rare earth elements (REEs), also known as rare earths, or rare earth metals are a group of 17 metallic elements that comprise ³⁹yttrium (Y) and 15 Lanthanides: ⁵⁷lanthanum (La), ⁵⁸cerium (Ce), ⁵⁹praseodymium (Pr), ⁶⁰neodymium (Nd), ⁶¹promethium (Pm), ⁶²samarium (Sm), ⁶³europium (Eu), ⁶⁴gadolinium (Gd), ⁶⁵terbium (Tb), ⁶⁶dysprosium (Dy), ⁶⁷holmium (Ho), ⁶⁸erbium (Er), ⁶⁹thulium (Tm), ⁷⁰ytterbium (Yb) and ⁷¹lutetium (Lu) (Figure 1.1). Even though ²¹Scandium (Sc) has been considered as one of the REEs, Voncken (2016) pointed out that the geochemical behaviour of Sc is more similar to the ferromagnesian transition elements such as Iron (Fe), Vanadium (V), Chromium (Cr), Cobalt (Co) and Nickel (Ni) than the other REEs and that Sc should not be considered as an REE.

The 15 lanthanides share very similar physical properties, and the presence of the lanthanides in crystalline compounds are mostly in 3+ oxidation states with the possibility of 2+ or 4+ states for some of lanthanides (Haire and Eyring 1994). The element Y also belongs to REEs due to the similar chemical behaviour and the natural presence of 3+ state (Voncken 2016).

Rare Earth Elements
by Geology.com

H																	He	
Li	Be											B	C	N	O	F	Ne	
Na	Mg											Al	Si	P	S	Cl	Ar	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
Cs	Ba	La-Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	
Fr	Ra	Ac-Lr	Rf	Db	Sg	Bh	Hs	Mt										
Lanthanides																		
La			Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		
Actinides																		
Ac			Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr		

Figure 1.1 The Periodic system of the elements with REEs highlighted in orange. *Reference* <https://geology.com/articles/rare-earth-elements/>

The REEs were firstly discovered in the late 18th century, when a Finnish chemist Johan Gadolin examined a rock containing about 55.5% of an unknown oxide and named the new

oxide as gadolinite and then further changed it to ytterbite. In 1803, the Swedish chemist Jöns Jakob Berzelius and scientist Wilhelm Hisinger have analysed the 'tungsten (heavy stone) of Bastanäs' and have isolated elements similar to gadolinite but also distinct. At the same time, the German chemist Martin Klaproth sampled the 'tungsten from Bastanäs' and made the same conclusion. He also named this new element as ochroite because of the yellow-brown (*ochra* in Greek) colour of the element. And this element was later named as cerium. At the end of the 19th century, except lutetium (Lu) and promethium (Pm), all the other REEs had been discovered and named (Voncken 2016). Lu was identified in the early 20th century, and Pm was discovered in nuclear reactions in 1947 (Marinsky et al. 1947).

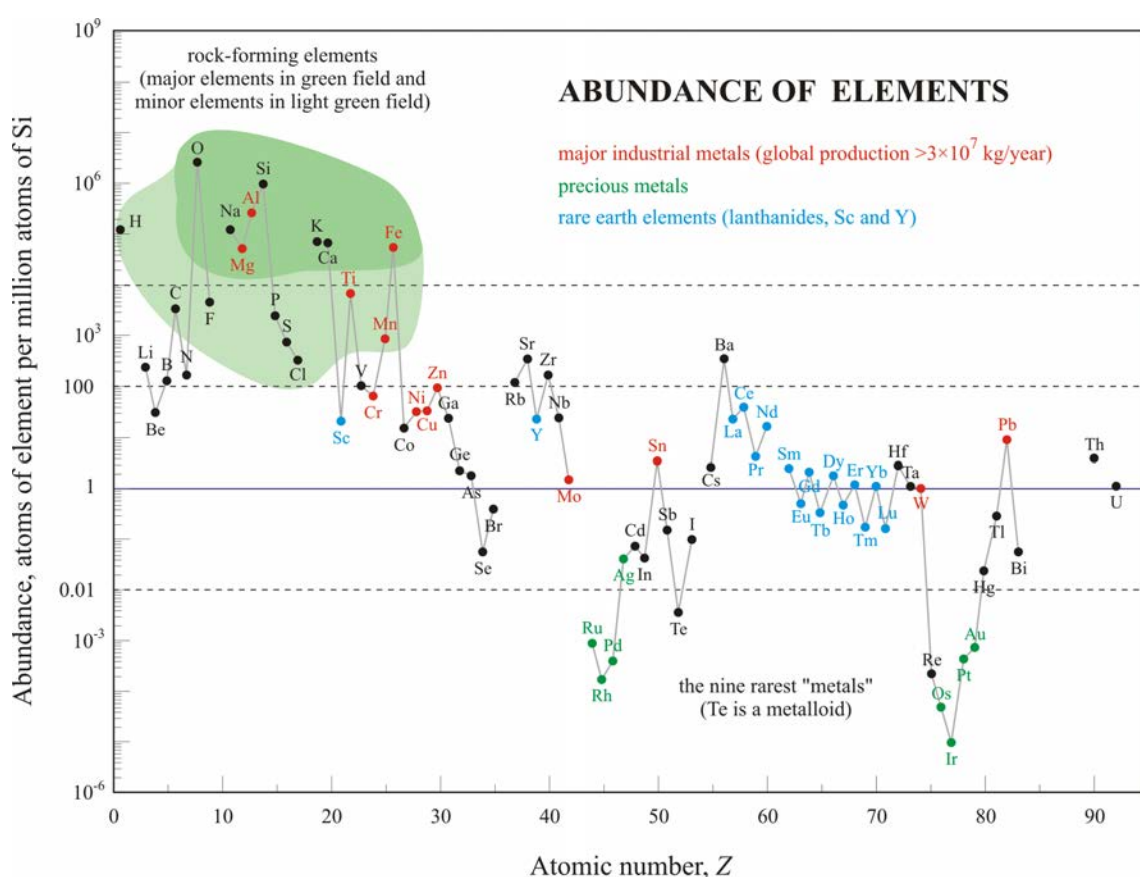


Figure 1.2 Abundance of the elements given in atom fraction as a function of the atomic number. The rare earth elements are indicated in blue. Image from Haxel et al. (2005), courtesy of the U.S. Geological Survey

The term REE was given to Sc, Y and lanthanides due to the discovery of those elements. Most of the REEs were discovered at the end of 19th century and until then the only REE known deposit was in Ytterby in Sweden and therefore, REEs were believed to be rare. Most of the REEs exploited in the early age were oxides and in the major scientific languages in the 19th-century oxide of an element shared the same word with the earth in French, similarly in German written as “Erde” (Voncken 2016). The technology advances and the knowledge on REEs have

increased with time, but even knowing that the REEs are actually not rare in the earth crust, their name has been kept unchanged.

As mentioned before, the REEs are actually not rare concerning their concentrations. The most abundant REEs are Ce, Nd, La and Y. They are comparable to the level of copper (Cu), nickel (Ni) and zinc (Zn). All of the REEs are more abundant in the earth crust than some elements we are familiar with, such as cadmium (Cd) and mercury (Hg) (Figure 1.2). As has been reported in previous studies, the average abundances of La ($30 \text{ mg}\cdot\text{kg}^{-1}$) and Ce ($60 \text{ mg}\cdot\text{kg}^{-1}$) in the crust are comparable to the common metals such as Zn ($70 \text{ mg}\cdot\text{kg}^{-1}$) and Cu ($55 \text{ mg}\cdot\text{kg}^{-1}$) (Tyler 2004a; Kim et al. 2015).

According to the electron configuration (Table 1.1), the REEs are often classified as light rare earth elements (LREEs) and heavy rare earth elements (HREEs). The LREEs include La, Ce, Pr, Nd, Pm, Sm, Eu and Gd. The HREEs comprise Tb, Dy, Ho, Er, Tm, Yb, Lu, and Y (Figure 1.3). Y is classified as HREE due to its similarities in ionic radius and chemical properties with the other HREEs. In general, the LREEs are more abundant in the earth crust and are easier to be exploited in many deposits. Despite the high demand, HREEs are in general less discovered than LREEs and more difficult to be extracted.

Table 1.1 Properties of rare earth elements and their abundance in the continental crust. Summarized from (Taylor 1964; Atwood 2012)

REE	Symbol	Atomic number	Atomic weight	Electron configuration	Abundance ($\text{mg}\cdot\text{kg}^{-1}$)
Yttrium	Y	39	88.91	$[\text{Kr}]4d^15s^2$	33
Lanthanum	La	57	138.91	$[\text{Xe}]5d^16s^2$	30
Cerium	Ce	58	140.12	$[\text{Xe}]4f^15d^1s^2$	60
Praseodymium	Pr	59	140.91	$[\text{Xe}]4f^36s^2$	8.2
Neodymium	Nd	60	144.24	$[\text{Xe}]4f^46s^2$	28
Promethium	Pm	61	Radioactive	$[\text{Xe}]4f^56s^2$	-
Samarium	Sm	62	150.36	$[\text{Xe}]4f^66s^2$	6
Europium	Eu	63	151.96	$[\text{Xe}]4f^76s^2$	1.2
Gadolinium	Gd	64	157.25	$[\text{Xe}]4f^7d^16s^2$	5.4
Terbium	Tb	65	158.93	$[\text{Xe}]4f^96s^2$	0.9
Dysprosium	Dy	66	162.50	$[\text{Xe}]4f^{10}6s^2$	3
Holmium	Ho	67	164.93	$[\text{Xe}]4f^{11}6s^2$	1.2
Erbium	Er	68	167.26	$[\text{Xe}]4f^{12}6s^2$	2.8
Thulium	Tm	69	168.93	$[\text{Xe}]4f^{13}6s^2$	0.48
Ytterbium	Yb	70	173.04	$[\text{Xe}]4f^{14}6s^2$	3
Lutetium	Lu	71	174.97	$[\text{Xe}]4f^{14}5d^16s^2$	0.5

														39 Y 88.906
57 La 138.91	58 Ce 140.12	59 Pr 140.91	60 Nd 144.24	61 Pm (145)	62 Sm 150.36	63 Eu 151.96	64 Gd 157.25	65 Tb 158.93	66 Dy 162.50	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.97
LREE								HREE						

Figure 1.3 Classification of REEs to LREEs and HREEs https://www.periodni.com/rare_earth_elements.html

The REEs are very electropositive, therefore, it is really easy for REEs to form ionic compounds. REEs mainly exist as metallic form, easily forming oxides, halides, carbonates, phosphates and silicates, borates or arsenates. This is one of the reasons why REEs were discovered rather late. Most of REE metals are grey or silver metals with high luster but easily tarnish in the air. They have good electrical conductivity but low solubility (Voncken 2016).

1.1.2 REE applications

During the past decades, REEs have become a hot topic of discussion in different mass media and have been more and more familiar to people. REEs have been used into lots of technological products and are mostly crucial in industries and high technologies. REEs are, for this reason, highly demanded in modern society.

Most of the REEs have good magnetic properties, catalytic abilities and some have fluorescent abilities. Some of the REEs can improve other metal abilities in metal alloys or improve non-metallic substance ability as additives. The major applications of each REEs are summarized in Table 1.2.

Table 1.2 Main applications of REEs

<http://www.rsc.org/Education/EiC/issues/2011June/TheElements.asp> (Voncken 2016; Kim et al. 2018)

REE	Utilizations
Sc	Aluminum-scandium (Al-Sc) alloys are used in sport equipment and aerospace due to the high strength and performance (Dorin et al. 2018). Scandium has also been applied in lasers and ceramics.
Y	Y has wide applications. Y alloys YVO ₄ -Eu and Y ₂ O ₃ -europium phosphorus are the standard red components used in LEDs and TV displays. Yttrium oxide is an additive in lenses fabrication in order to improve the shock and heat resistance of the glass.
La	La is applied in industries as misch metal or metal alloys. The most common application is lanthanum-nickel (NiMH) batteries. La is applied in catalytic cracking or in FCC operations.

	La is also applied as additive to improve the optical properties and alkali resistance of the glass.
	Ce is one of the most abundant REEs and has wide applications.
	Ce is used as a medical treatment and in gas mantles.
Ce	Cerium oxide (CeO_2) is used as polishing compound, as catalyst in catalytic cracking and catalytic converter.
	Ce alloys is used in special lighting source, red pigments and chromium plating.
Pr	Pr is mainly used as alloys. Pr-Mg has high strength and is applied in aircraft industries. Other alloys are applied as pigment for glass and enamel, catalysts and fluoride-glass fibres.
Nd	Nd is known for its strong magnetic properties (neodymium-iron-boron magnets i.e. NdFeB-magnets) and is used as permanent magnets in all kinds of modern technologies like electronic devices, sound devices and sensors.
Pm	Pm is known for its radioactivity and has not been applied in the industries.
Sm	Two Sm magnets (SmCo_5 and $\text{Sm}_2\text{Co}_{17}$) have been applied in industries as high temperature permanent magnets. Sm has also been used as catalyst and chemical reagent.
Eu	Not many industrial applications. Mainly been used as red and blue phosphors.
Gd	Gd is specifically used in shielding of nuclear reactors and neutron imaging.
Tb	Tb is mainly applied as a dope in calcium chlorides in luminescent materials.
Dy	Dy is used as an additive in permanent magnets. Dy oxide-nickel cermet is applied in nuclear-reactor rods as cooling reagent.
Ho	Ho is used as a magnetic flux concentrator due to its high magnetic field strength. Ho is also applied in nuclear reactors as it can absorb neutrons and can keep the chain in reaction.
Er	Er is applied in optical fibers to amplify the signals, and in lasers. Er phosphors is used as green lighting.
Tm	Tm does not have wide application due to its high price.
Yb	Yb has little application due to the low production. Mainly in optical lenses of electronic devices.
Lu	Lu is also a rare metal and is mainly used in advanced computer technologies.

The early application of Sm was used in permanent magnets. It was firstly developed in early 1960s using Sm alloyed with cobalt (SmCo). However, this application was replaced by neodymium iron boron (NdFeB) magnets in the 1980s as Nd magnets are stable up to 300 - 400 °C while Sm-Co magnets are stable even about 700 °C. The Nd magnets are still used now (British Geological Survey report 2011). Even though with less industrial applications, the high

magnetic strength at a higher temperature enables SmCo magnets to be widely applied in the precision-guided system in the military utilisations (Dushyantha et al. 2020). Another important application of Sm is industrial catalysts in plastic decompositions and ethanol productions. Sm triflate is considered as one of the most efficient catalyst promoting Friedel–Crafts alkylation with alkenes (Hajra et al. 2007).

1.1.3 Source of REEs

The principal rare earth ore minerals are monazite, bastnaesite and xenotime. The most known monazite is $CePO_4$. Beside Ce, some light REEs such as La, Pr, Nd and Sm also occur in monazite. Monazite also contains Thorium (Th) and/or Uranium (U) but with a considerably low amount. Monazite is generally present as a minor part in granites, granodiorites, associated pegmatites and some metamorphic rocks (Voncken 2016). Bastnaesite is a major rare earth ore containing mainly LREEs and low proportion of HREEs. Bastnaesite is widely spread all over the world, generally related to carbonatite intrusions. Therefore, bastnaesite was usually found with the presence of carbonatite at the same time (Atwood 2012; Voncken 2016). The general formula of xenotime YPO_4 , different from monazite or bastnaesite, is mainly composed of HREEs. Xenotime contains a large amount of rare earth oxides (up to 67%) (Voncken 2016). The most commonly occurring elements are Dy, Yb, Er and Gd. Similar to monazite, xenotime also contains Th and/or U, depending on the situations of different locations, and Th and U can be a by-product (Voncken 2016).

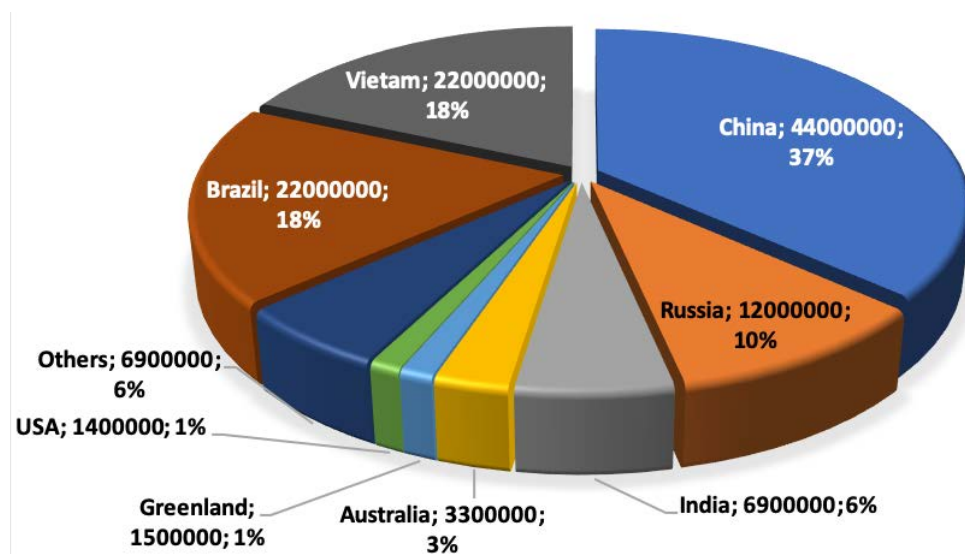


Figure 1.4 REE world reserves sorted countries (tonnes) (data from USGS 2020 report)

1.1.4 REE reserves and major mining deposits

1.1.4.1 REE reserve worldwide

The REE reserve was estimated to more than 100 million tonnes all over the world (Chen 2011). Even though the REEs are widely distributed and rather abundant in the earth crust with the total REE concentration reaching 100-200 mg·kg⁻¹ (Tyler 2004a; Liang et al. 2005), they are not evenly distributed. According to United States Geological Survey (USGS) 2020 report (USGS 2020), Asia holds the largest REE reserve among all the continents. China, being the biggest REE reserve country, holds about 37% of the world REE reserve. Vietnam holds 22 million tonnes of REE reserve, accounting for 18% of the world reserve, then followed by India who holds 6% of the total reservoir. A large number of rare earth reserves was found in South America, mainly in Brazil who holds 18% of the world reserve and has a long rare earth production history. Comparatively, North America holds less REE reserve only about 1% in the USA and less than 1% in Canada. In eastern countries, several countries have REE deposits such as Russia (10% world reserve), Greenland (1% world reserve), Estonia, Sweden and so on. REE deposits have also been found in Australia with 3.3 million tonnes accounting for 3% of world reserve (Figure 1.4 & 1.5). Except for Northern Europe, rare earth occurrences were found with no intensive mining activities. In France, there is little information about REE occurrence. Few REE enrichments were found in east France located in low grade metamorphosed black shales of Dinantian (Châteauneuf-du-Faou, Corlay) and Ordovician age (le Grand-Fougeray, Châteaubriand) and in south France (EuRare.eu) (Figure 1.6).

1.1.4.2 Major REE deposits in the world

REE deposits can be formed through primary or secondary geological processes. Primary processes include magmatic, hydrothermal and/or metamorphic processes, during which apatite, monazite, and synchysite are formed. Secondary REE minerals are Fe-oxyhydroxides, secondary monazite, churchite, and plumbogummite-group which are formed due to the erosion and weathering of primary deposits (Voncken 2016; Dushyantha et al. 2020). In the rare earth exploitation, REE mineralization is classified as REE deposit or REE occurrence depending on its economic interest and possible exploitation (EuRare website).

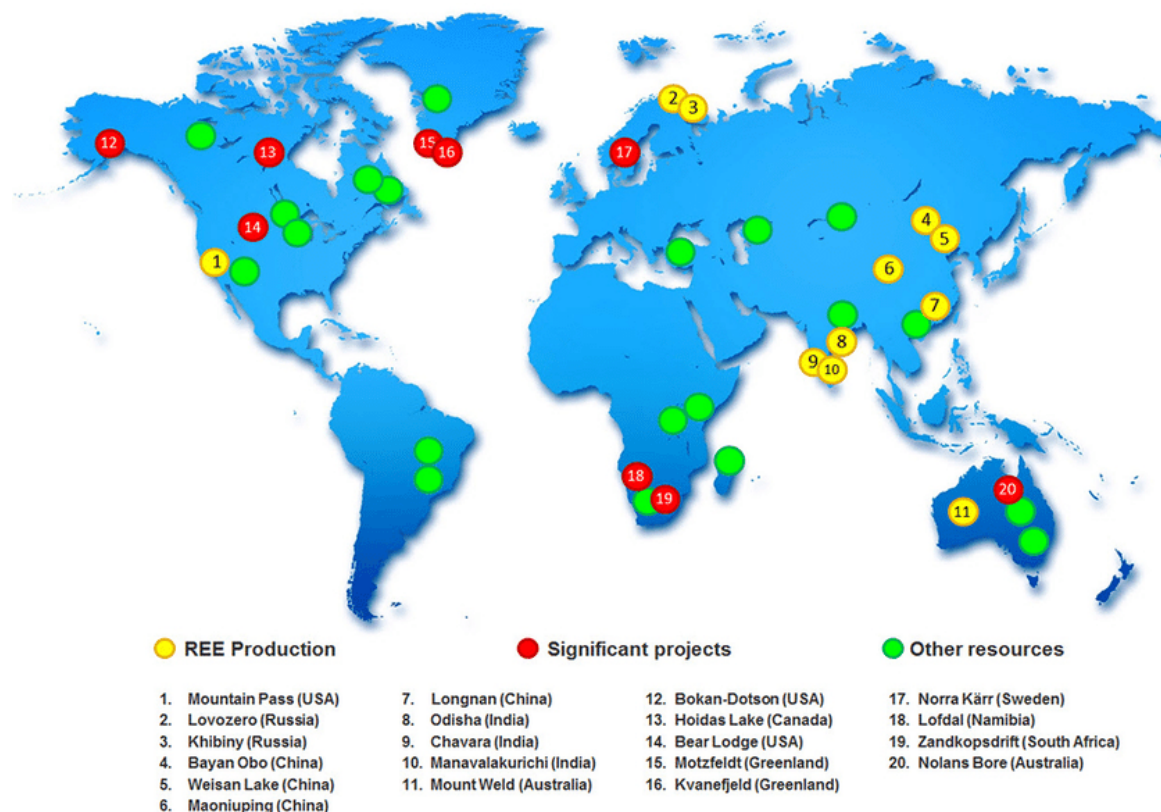


Figure 1.5 The global distribution of REE deposits and mines. (Barakos et al. 2016)

Mountain Pass, the USA

Mountain Pass deposit is an open-pit mine located in California, United States. It has the longest exploitation history of REEs. Mountain Pass deposit was the major rare earth deposit in the USA containing about 8 to 12% rare earth oxides (USGS 2013). From the mid 1960s to the mid 1980s, Mountain Pass was the dominant source of REEs, and the USA were largely self-sufficient in REEs.

Bayan Obo Deposit, China

Since 1985, the REE production in China has increased largely due to the exploitation of the Bayan Obo REE deposit (Figure 1.5). Bayan Obo (白云鄂博 in Chinese, also Baiyun-Obo or Baiyun'ebo) Mine is the largest known rare earth mine worldwide, located in the inner Mongolia region of China, bordering Mongolia and Russia. Bayan Obo deposit is formed by large carbonate and hydrothermal rocks and was discovered in 1927 as an iron deposit but not been exploited as rare earth mine until the REE minerals were discovered in 1936. The ore reserves of Bayan Obo are about 1500 million metric tonnes iron ore with an average grade of 35%, 1 million tonnes of niobium ore with an average grade of 0.13% and more than 48 million

tonnes of REE ore with an average grade of 6% (Voncken 2016). The REE minerals are bastnaesite, parasite, and monazite. Due to the multi-mineral reserves, the Bayan Obo deposit is also called as the Bayan Obo iron-niobium-REE deposit. The intensive REE exploitation of the Bayan Obo deposit area started at the end of 1970s and China began to dominate the rare earth global supply (Figure 1.5).

Mount Weld, Australia

Mount Weld deposit is located 35 km south of Laverton in South-West Australia with laterite and carbonatite rocks (Figure 1.5). This deposit was discovered in 1988 (Voncken 2016). The Mount Weld deposit was not exploited until REE-crisis in the late 2000s. The rare earth ores are mainly apatite, monazite, synchysite, and churchite. According to the Critical Mineral Resources of the United States, Mount Weld holds 23.9 million tonnes of REE ore at an average grade of 7.9 %.

Ilímaussaq Alkaline Complex, South Greenland

Ilímaussaq complex, also called as Ilímaussaq Alkaline Complex, is located in Southwest Greenland (Figure 1.5). The complex is made of alkaline igneous rocks which are defined in terms of the alkali and silica content (Voncken 2016). Ilímaussaq has a large amount of uranium, rare earth elements and zinc resources. The complex is located in southern Greenland with more favourable weather compared to the northern Greenland and more intensive population which made it easier to exploit. A large scale rare earth project, Kvanefjeld project, was launched to produce rare earth oxide as the main goal and uranium, zinc and fluorspar as by-products (Greenland Minerals and Energy 2019).

1.1.4.3 REE deposits and occurrences in France

There are few rare earth occurrences found in France. The relatively intensive REE deposits that have been reported are located in the monazite nodules of detrital origin in shales from Central Bretagne (EUrare project). According to previous studies, some REE deposits were located in north-western France in the Bay of Morlaix, Corlay, Châteauneuf-du-Faou, Craon, Châteaubriant, Le Grand Fougeray. The Le Grand Fougeray deposit is the most studied area with a large number of monazite nodules which contains REE oxides (Figure 1.6). Some deposits were reported in southern France, close to the border with Spain, in Arize, Trimouns, Montagne Noire and Le Vigan. Other known REE occurrences like Massif Central, Armorican

Massif, Estérel, Pyrenées are also reported to have mineralogical interest and even resource potential (EURare project).

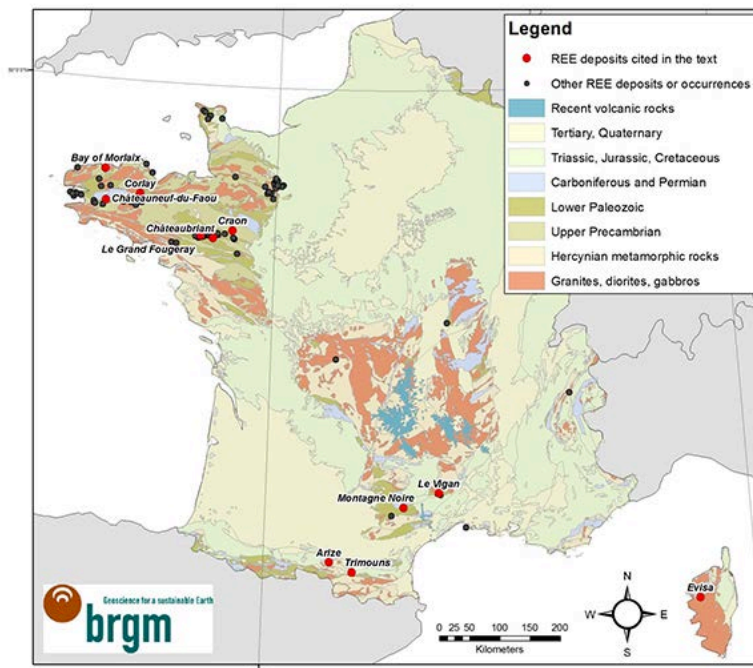


Figure 1.6 Main REE deposits and occurrences in France (reference <http://www.eurare.eu/countries/france.html>)

1.1.5 REE production

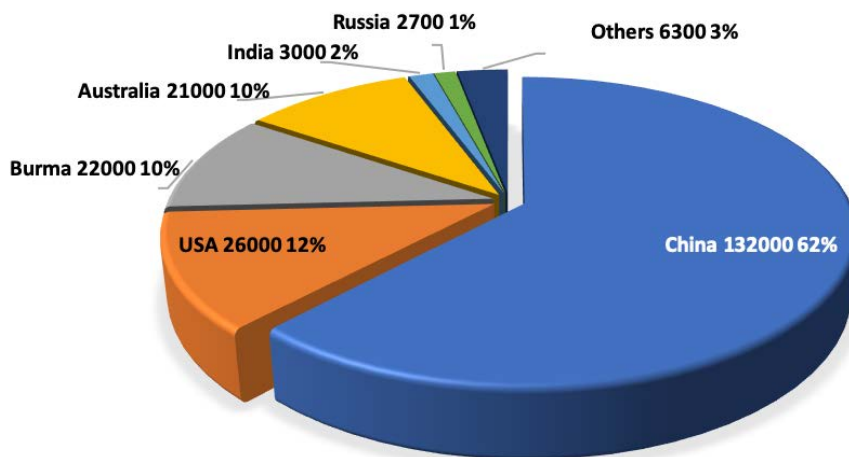


Figure 1.7 REE mine production by countries (data from USGS 2020 report)

Figure 1.8 illustrates the history of REE production since the early 1900s. With a limited utilisation of REEs, rare earth mines have not been exploited much worldwide before the 1960s. From the mid 1960s to the mid 1980s, Mountain Pass was the dominant source of REE (Figure 1.8). Since the 1980s, China provides more than 80% of the worldwide supply of REEs (Kynicky et al. 2012; Chao et al. 2016). For the year 2019, China still dominated the REE

production globally, took up 62% of the world production. Followed by USA, Burma, Australia, accounting for 12%, 10% and 10% respectively (Figure 1.7 & 1.8).

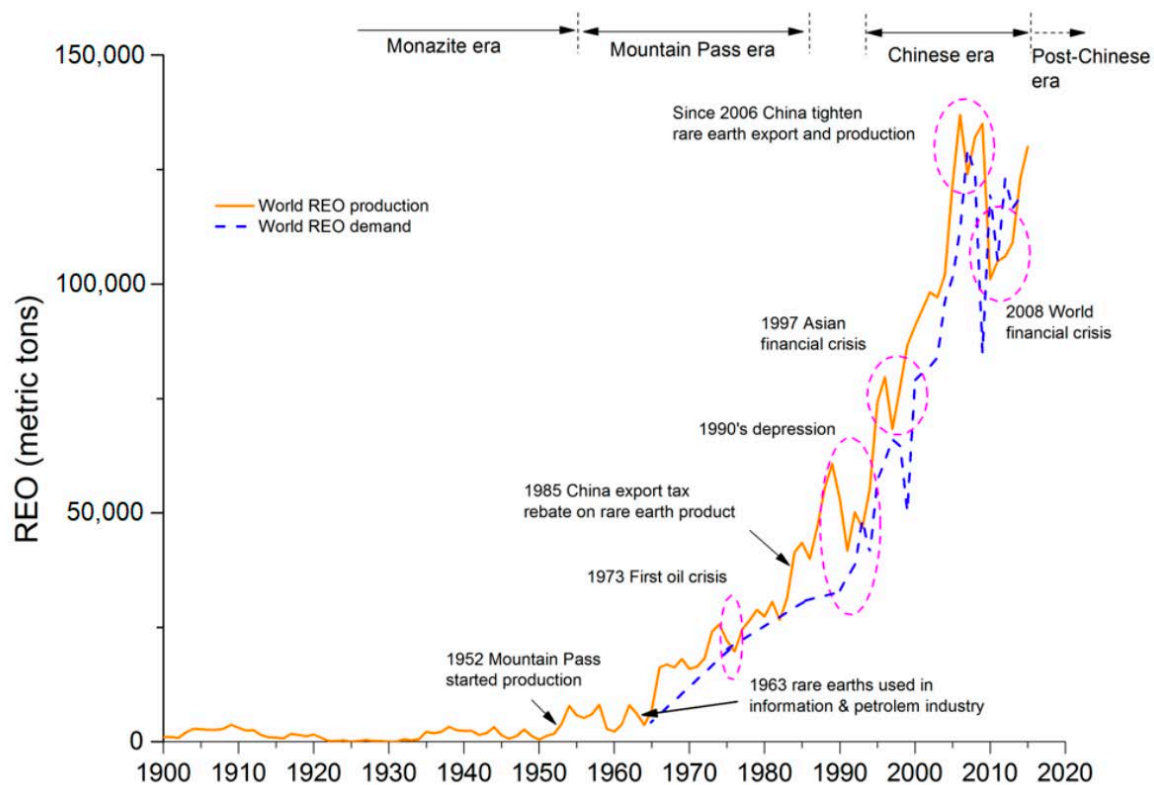


Figure 1.8 Global rare earth production (orange line) and demand (blue line) from 1950 to 2020 (Zhou et al. 2017)

1.2 REEs in the environment

1.2.1 REEs in soil

REEs can be transported in the environment in multiple ways. With the rather abundant amount in the earth crust, REEs can be transferred through hydrological and wind-driven processes (Gwenzi et al. 2018). Besides the transportation, REEs can be mobilized through geological and geochemical processes. With these natural processes, the total REEs concentration in the soil ranges from 100 to 200 mg·kg⁻¹.

Beside natural processes, anthropogenic activity influenced largely the mobilisation and the transfer of REEs in the environment in the last decades. With the large number of mining, industrial and agricultural activities, the total REE concentration can even reach thousands mg·kg⁻¹ in the soil, forming non-vegetated zones (Tyler 2004a; d'Aquino et al. 2009a; Gonzalez et al. 2014; Carpenter et al. 2015; Chao et al. 2016). In the biggest REE producing country China, the average concentration of REEs is 181 mg·kg⁻¹. However, some extreme values have been reported in some industrial and mining areas. For example in Baotou, a city where the biggest rare earth mine Bayan Obo Deposit locates, the soil REE concentration ranged from 156 to 5.65 × 10⁴ mg·kg⁻¹ (average 4.67 × 10³ mg·kg⁻¹) and the average concentration for Sm is 48.16 mg·kg⁻¹ (Wang & Liang, 2015). Harlavan et al. (2009) have analysed 6 soil profiles from Wind River Mountains in the USA, where the REE concentrations ranged from 150 mg·kg⁻¹ up to 500 mg·kg⁻¹ and the Sm concentration between 3.2 and 12.5 mg·kg⁻¹. The average REE concentration in soils collected from 88 sites all over Brazil was 205.18 mg·kg⁻¹ for the topsoil and 224.39 mg·kg⁻¹ for the subsoil, whereas Sm concentration ranged from 0.01 to 22.47 mg·kg⁻¹ (De Sá Paye et al. (2014). However, with the increasing mining activities and the application of REEs in high-tech industries in recent years and the increasing demand in the future decades, the REEs are and will remain under high demand. The exploitation of REE deposits remain an open-pit mining technique and has been mainly carried out in developing countries with intensive mining activities and improper disposal of material containing REE compounds. As a result of this mining method, REEs have caused soil pollution in different countries, especially in China (d'Aquino et al. 2009a; Carpenter et al. 2015; Chao et al. 2016). Except the open-pit mining, industrial waste is another big source of REE emission. The utilisation of REEs in industries has increased exponentially the corresponding emission of rare earth waste. Besides the two ways of emission, the application of REE enriched fertilisers in agriculture is also an emission risk. In this way, the REEs were emitted directly to soil and

water systems. The rare earth enriched fertilisers were commonly used in some countries since the early 1980s (Pagano 2016).

The bioavailability of REEs in the soil has been discussed in my many studies. Most of the studies considered that the bioavailability of REEs in the soil is low. The transfer of REEs from soil to plants is affected not only by the sources and forms of REEs, but also by soil parameters such as soil particle size, soil pH and organic matter content (Tyler 2004a; Babula et al. 2008; MacMillan et al. 2019). In order to predict the bioavailable fraction of REEs, different methods have been developed and applied, including single extractions or sequential chemical extractions (Cao et al. 2000b) (see Chapter 3.4).

1.2.2 REE transfer and toxicity to microorganisms

With more concern about the environmental impact of REE emission, more and more studies have been carried out on REEs and organisms. As an important biological component in the soil, microorganisms represent the largest biodiversity and play a crucial role in the biogeochemical cycle of the REEs. Previous studies showed that microorganisms can affect the mobility and the availability of REEs in the soil (Taunton et al. 2000; Perelomov and Yoshida 2008). However, very little information can be found related to REE effect, uptake and accumulation by microorganisms. Bergsten-Torralba et al. (2020) studied the toxicity of La, Nd and Sm on organisms including the fungi *Penicillium simplicissimum* and *Aspergillus japonicus*. Compared to the algae and crustaceans in the same experiment, the two fungi showed high resistance. Besides the studies on their resistance to REEs, microorganisms were also reported to affect the biogeochemical cycle of REEs. Fathollahzadeh et al. (2018) showed that *Klebsiella aerogenes* enhanced the bioleaching of REEs from monazite ore solutions. A few studies found that some bacteria (e.g. *Pseudomonas aeruginosa*, *Variovorax paradoxus* and *Comamonas acidovorans*) (Texier et al. 1999) and fungi (e.g. *Amanita flavorubescens* and *A. rubescens*, *Russula pectinatoides*,) (Aruguete et al. 1998; Kamijo et al. 1998) can absorb REEs in their cells and excrete REE- containing materials up to 10 mg/g. Yeast, as a model organism to study the metal toxicity and accumulation mechanisms, has been used in many REE studies. Different yeast strains were reported to have high ability to accumulate Nd (Palmieri et al. 2000; Vlachou et al. 2009). High doses of REEs can be toxic to microorganisms, still, a large number of microorganisms can adapt to the REE contaminated environment and even accumulate REEs.

1.2.3 REE transfer and toxicity to aquatic environment

The aquatic environment was considered a sink for contaminations including REEs (Gwenzi et al. 2018). The behaviour of REEs differs from element to element due to their physico-chemical properties. Gd based agents were reported having high stabilities and mainly exist in the dissolved form (Holzbecher et al. 2005; Knappe et al. 2005). On the contrary, anthropogenic La and Sm predominantly exist in the colloidal or nanoparticulate form according to the complexing agents and pH of the environment (Kulaksiz and Bau 2011).

In order to study REE toxicity different aquatic organisms have been tested. Using 3 REEs spiked in the aquatic system with algae, microcrustaceans and fungi, results showed that algae are very sensitive to REEs and can be used as a model organism to indicate the toxicity of REEs in the aquatic environment (Bergsten-Torralba et al. 2020). Spiking experiments were used to study the toxicity of different forms of REEs to algae, and showed that the water soluble REEs are more toxic than the acid extractable and the organic bound species (Hao et al. 1998). Besides algae, *Daphnia*, have also been used in different studies as a model organism to understand the toxicity and the biogeochemical cycle of REEs in the aquatic environment (Barry and Meehan 2000; Ma et al. 2016; Blaise et al. 2018; Galdiero et al. 2019). There is less information about REE toxicology to fish. Some REE risk assessment and bioaccumulation studies have been carried out on fresh water fishes (Yang et al. 2016a; Nørregaard et al. 2019). When it comes to marine fish species, studies mainly concern the analysis of REEs in fish tissues (Picard et al. 2002; Mayfield and Fairbrother 2015).

1.2.4 REE transfer and toxicity to plants

Even though REEs are not nutrient elements to plants, and not considered as essential elements for plants, positive effects of REEs at low concentrations were reported on plant growth (Diatloff et al. 1995c; Xu et al. 2002; Hong et al. 2003; Xiangsheng et al. 2006; Cheng et al. 2015). Since the early 1990s, REEs have even been used to promote plant growth and have been widely applied in agriculture in China. It has been reported that the yield increases from 5% to 15% due to REE addition for native crop species under varying soil and nutrient conditions (Hu et al. 2004).

However, when a high dose of REE fertiliser has been applied, plant growth was limited and even a toxic effect was found (Hong et al. 2003). Many studies have been carried out on the toxicity of REEs to plants. The concentration of REEs in plants growing in natural soil is

rather low, ranging from 0.01 to 2 mg·kg⁻¹ depending on the plants and the soils (Tyler 2004a). Despite the low REE concentrations in unpolluted soils, the phytotoxicity can become a concern in the mining, industrial or other contaminated areas (Carpenter et al. 2015). Spiking experiments have been performed to study the REE acute toxicity to plants. The early REE spiking studies have been carried out by Diatloff et al. (1995a, b) on La and Ce toxicity to corn and mungbean seedlings growing in nutrient solutions. The results showed a strong inhibition of mungbean root elongation and plant growth with Ce (even at micromolar level) while no significance with La. Similar toxicity study has been carried out in artificial soils with different pHs by Thomas et al. (2014). They used La, Ce and Y and all 3 elements showed inhibition effects to the germination and the growth of native and crop plants. The effects of REEs on 6 plant species have been studied by Carpenter et al. (2015). Seed germination rate was not affected by REE polluted soil; however, high levels of growth inhibition were found in the REE polluted soil, especially of native species. All species showed the ability to take up and accumulate REEs from soils in roots and shoots. Comparatively much higher tolerance to REEs was found in crop species than native species (Carpenter et al. 2015). Yuan et al. (2017) also studied the uptake and fractionation of REEs in plants and the influence of REEs on plant biomass. The results showed that the biomass and accumulation were enhanced at low concentration of REEs but inhibited at higher concentration.

Plants growing in polluted soils can absorb heavy metals and accumulate the metals in their cells. The term “hyperaccumulator” was used to describe plants which can grow on metalliferous soils and accumulate extremely high amounts of heavy metals in the aerial organs, far over the levels found in the majority of species, without suffering phytotoxic effects (Rascio and Navari-izzo 2011). Many plants have been studied and found having hyperaccumulating abilities. *Pteris cretica* var. *nervosa* (Zhang et al. 2017) and *Pteris vittata* (Liu et al. 2018; Evandro et al. 2019; Abou-shanab et al. 2020) were reported to accumulate arsenic (As). Many plants were reported to be Cd hyperaccumulators, such as *Solanum nigrum* L. (Sun et al. 2008; Dou et al. 2020), *Lantana camara* L. (Liu et al. 2019), *Phytolacca americana* (Zhao et al. 2011), *Sedum alfredii* (Lu et al. 2010). With the intense REE mining activity and environmental issue caused by REE wastes, more and more studies have been carried on REEs hyperaccumulators. The earliest REE hyperaccumulator has been reported by Robinson et al. (1938) and Robinson (1943) who showed that hickory leaves can accumulate REEs up to 2296 mg·kg⁻¹ dry weight. Till now, many plants have been reported to be REE hyperaccumulator. *Phytolacca americana* was repeatedly reported to be an excellent REE hyperaccumulator (Yuan et al. 2017; Grosjean

et al. 2019) with a high biomass and wide distribution all over the world (van der Ent et al. 2018), which can accumulate up to $4000 \text{ mg}\cdot\text{kg}^{-1}$ REEs in the dry weight (Yuan et al. 2017). *Dicranopteris dichotoma* collected on-site also contained high concentrations of REEs ($675\text{-}3358 \text{ mg}\cdot\text{kg}^{-1}$) in the dry leaves (Wang et al. 1997). Other than these plants, *Carya tomentosa*, *Pronephrium triphyllum* were also reported to be good hyperaccumulators with up to 1350 and $1027 \text{ mg}\cdot\text{kg}^{-1}$ REEs in their dry weight (Thomas 1975; Lai et al. 2005).

Beyond the inhibition of plant growth, REEs were also reported to influence the ionic fluxes into cells in different ways which may affect several plant processes. Previous studies on the effects of REEs on nutrient (N, P, and K) uptake by plants reported different conclusions (Hu et al. 2002a; Wang and Liang 2014; Chang et al. 2018; Grosjean et al. 2019). However, different analytical methods were applied by researchers and complex phenomena are involved in the influence of REEs on nutrient uptake (Hu et al. 2004).

The excessive amounts of REEs can be toxic not only to plants but also enter the food chain and accumulate in herbivorous consumers, higher class predators and even human beings (Loell et al. 2011a; Turra et al. 2013). Studying the transfer of REEs in the terrestrial environment, their effect on plants, and their transfer from soil to plants are important to understand the biogeochemical cycle of REEs, the effect of REEs to organisms and human health and also soil remediation.



Figure 1.9 Workers in Baiyun Obo rare earth mine site in China (Source Pagano 2016)

In order to understand the transfer of an element, the bioavailability is a key parameter. Lots of studies have focused on the bioavailability of heavy metals in soils and on methods to quantify it but not many for REEs (Kim et al. 2015; Menzies et al. 2007). It has been reported that the availability of REEs in soils was affected by REE sources and forms, soil particle size, pH and organic matter content (Tyler 2004a; Babula et al. 2008; MacMillan et al. 2019). For example, Thomas et al. (2014) studied the toxic effect of REEs to plants and showed that it was not only affected by the concentration of REEs in the soil and the plant type, but related to the low soil pH. To estimate and predict the plant-available fraction of REEs, different methods and extractants have been applied, including sequential chemical extractions (Cao et al. 2000b) 0.1 M HCl (Li et al. 2001), CaCl₂ (Li et al. 2018), DTPA (Censi et al., 2017). These authors showed that bioavailability of REEs estimated by these extraction methods differed with the REEs: in some studies, it was correlated with their concentration in wheat roots and shoots in cultivated soils, or to lanthanide toxicity to soil invertebrates. Many of these studies were performed using non-contaminated soils, or agricultural soils with fertilizer amendments. There is still a need to get more knowledge on the bioavailability of REEs in contaminated soils and on extraction methods that would give a good indication of the plant-available REEs. This could be done by combining spiking experiments, with proper non-contaminated controls, and analyses of in situ contaminated soils.

1.2.5 REE transfer and toxicity to humans

The early studies on REEs effects to humans were carried out on occupational toxicology of REEs. As mentioned before, REEs are mainly exploited by open-pit mining with workers directly exposed to the REEs and even some radioactive elements (Figure 1.9). Studies have been carried out on the concentration of REEs in the hair and urine of the on-site workers and mining region residences (Wei et al. 2013; Meryem et al. 2016). Human risk assessment has also been evaluated in different studies (Rim et al. 2013; Borowiak et al. 2018). The toxic effect of REEs, the hazard of REEs in the occupational environment and the results of REE toxicology studies have been summarized in Table 1.4.

Table 1.4 Summary of the toxic effects of REEs to humans (Gong 1996; Rim et al. 2013; Pagano 2016; Shin et al. 2019) (LD₅₀ : median dose that will lead to the death of 50% of the population) (Gad 2014)

Element	Toxicological information	LD ₅₀	
		Oral (mg·kg ⁻¹)	Inhalation (mg·L ⁻¹ , 4h)
<i>Sc</i>	Elemental Sc is considered to be non-toxic. However, Sc can be really dangerous in an occupational environment as it can be inhaled through damps and gasses.	ScCl₃ rats: 755	
<i>Y</i>	Elemental and water-soluble Y have very low toxicity. Y was reported to cause lung and liver damage. Inhaling Y citrate can cause dyspnea and pulmonary edema while inhaling YCl ₃ can cause edema, pleural effusions, and pulmonary hyperemia. Workers may suffer lung problem with the symptom of shortness of breath, coughing, chest pain and even edema	Y rats: >2000 Y₂O₃ rats: >5000 Y(NO₃)₃ rats: 1650	Y₂O₃ rats: >5.09
<i>La</i>	Action of lanthanum can cause hyperglycaemia, low blood pressure and degeneration of the spleen and hepatic alterations	La₂O₃ rats: >8500 La(NO₃)₃ rats: 4500 La(OH)₃ rats: >2000 LaF₃ rats: >2000	LaF₃ rats: >5.1
<i>Ce</i>	Ce is easily igniting above 65 °C and Ce fumes are toxic. Injection of high dose of Ce can cause cardiovascular collapse and death. Workers exposed to Ce have to be careful with the temperature. Meanwhile, symptoms like itching, sensitivity to heat, and skin lesions have been reported from occupational environment with Ce exposure	CeO₃ rats: >5000 Ce(NO₃)₃ rats: 4200 CeCl₃ rats: 2800 Ce₂(CO₃)₃ rats: >5000 CeF₃ rats: ≥2000	CeO₃ rats: >5.05 CeF₃ rats: >5.53
<i>Pr</i>	Lack of study and information about Pr toxicity to animal or human beings.	Pr₂O₃ rats: >2000 Pr(NO₃)₃ rats: 3500 PrCl₃ mice: 4500 Pr₂(CO₃)₃ rats: >2000	Pr₂O₃ rats: >5.21 Pr₂(CO₃)₃ rats: >5.25
<i>Nd</i>	Not much information has been reported on Nd toxicity. Nd dust and salt were reported irritating mucous membranes, eyes and skin. Working in Nd environment, workers need to be careful about Nd dust. Inhale Nd dust may lead to mucous membranes and skin damage, even causing damage to lung embolisms and accumulating in liver and bringing harmful effects	Nd₂O₃ rats: >5000 Nd(NO₃)₃ rats: 2750 NdCl₃ rats: >5000 Nd₂(CO₃)₃ rats: >2000 Nd(OH)₃ rats: >2000 NdF₃ rats: >5000	Nd₂O₃ rats: >4.98
<i>Pm</i>	Lack of study and information about Pm toxicity to animals or human beings even it has been reported having an effect to bone tissue.		
<i>Sm</i>	The total Sm in human body is around 50 µg especially located in kidneys and livers. Non-water-soluble Sm salts are not toxic and the water-soluble ones are slightly toxic to humans.	Sm(NO₃)₃ rats: 2900 SmCl₃ rats: 3200	
<i>Eu</i>	Lack of study and information about Eu toxicity to animals or human beings. Eu dust generated from fire or explosion was reported to be toxic.	Eu(NO₃)₃ rats: >5000 EuCl₃ mice: 5000	
<i>Gd</i>	Gd is highly toxic as free ion and the toxicity elevates on Gd-based contrast agents (also called as MRI contrast media)	Gd rats: >2000 Gd₂O₃ rats: >1000 Gd(NO₃)₃ rats: >5000	
<i>Tb</i>	Lack of study and information about Tb toxicity to animals or humans, the water-soluble Tb has low toxicity.		
<i>Dy</i>	Non-water-soluble Dy compounds are not toxic and the water-soluble ones are slightly toxic to human. Dy dust and powder, however, may be explosive during the mixing procedure with a fire source exists.	Dy₂O₃ rats: >5000 Dy (NO₃)₃ rats: 3100 DyCl₃ mice: 7650	
<i>Ho</i>	Non-water-soluble Ho compounds are not toxic and the water-soluble ones have low toxicity to humans. Ho was even reported as being able to stimulate metabolism		
<i>Er</i>	Er compounds have low toxicity. Er dust can be explosive		
<i>Tm</i>	Non-water-soluble Tm compounds are not toxic and the water-soluble ones are slightly toxic to humans.	TmCl₃ mice: 6250	
<i>Yb</i>		Yb₂O₃ rats: >2000	Yb₂O₃ rats: >1.31

Yb compounds are all considered to be highly toxic, Yb was reported irritating mucous membranes, eyes, skin and even causing teratogenic effects. Yb dust can be explosive.

In the occupational environment, Yb should be preserved in air-tight closed environment from air or moisture.

Yb(NO₃)₃ rats: 3100

YbCl₃ mice: 6700

YbF₃ rats: >2000

Lu

Non-water-soluble Lu compounds are not toxic and the water-soluble ones are slightly toxic to human. However, LuF₃ and Lu₂O₃ are considered toxic as the inhalation of LuF₃ is irritating to skin. Lu(NO₃)₃ is also reported to be dangerous as it can explode and burn once heated.

Lu₂O₃ rats: >2000

Lu(NO₃)₃ rats: >300

LuCl₃ mice: 7100

1.3 Arbuscular mycorrhizae

1.3.1 Introduction

Mycorrhizae are symbiotic associations between the roots of most plant species worldwide and symbiotic fungi. There are 7 kinds of mycorrhizae according to their structural characters: Arbuscular mycorrhiza, Ectomycorrhiza, Ectendomycorrhiza, Arbutoid mycorrhiza, Monotropoid mycorrhiza, Ericoid mycorrhiza and Orchid mycorrhiza (Smith and Read, 2008). Arbuscular mycorrhizae (AM) are the most common mycorrhizal type that can be found in a large variety of host plants by forming a symbiotic relationship (Smith and Read 2008). According to morphological and molecular studies, AM are considered highly likely having an over 460-480 million years' history (Luc et al. 1993; Heckman 2001). Due to the beneficial effect on plant and fungus nutrient uptake, this ancient symbiotic activity remains an important interaction in ecosystems until now and was reported found in 80% of vascular plants (Smith and Read, 2008). AM are formed with bryophytes, pteridophytes, gymnosperms, and angiosperms, and are widely spread in most temperate and tropical ecosystems including agricultural systems (Johnson and Gehring 2007). Only a limited number of families, such as Brassicaceae and Chenopodiaceae, do not form AM.

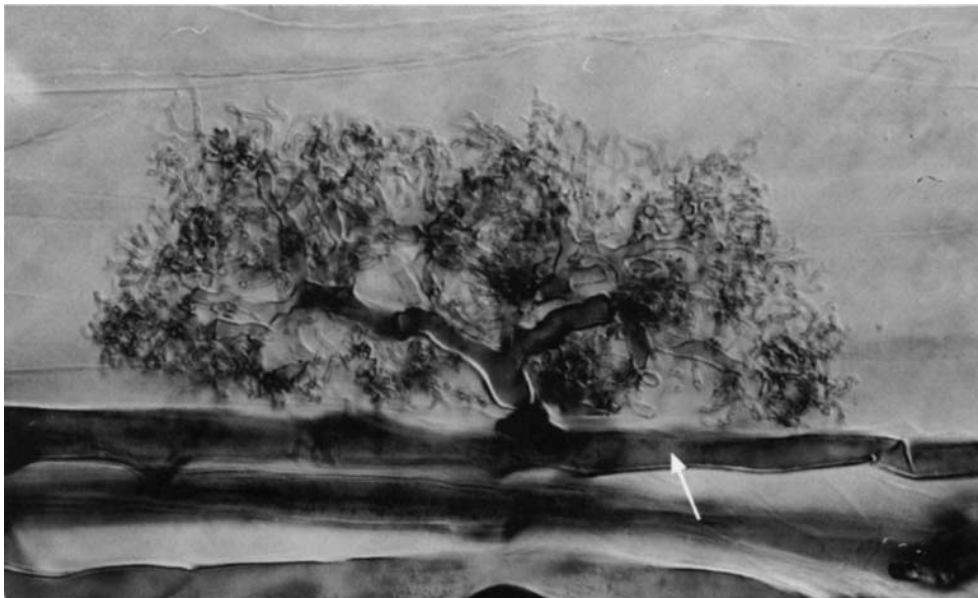


Figure 1.10 A mature arbuscule of *Funneliformis mosseae* within a cortical cell of *Allium porrum* (leek). The arbuscule has grown from a well-developed intercellular hypha (arrow) (Smith and Read 2008).

The tree liked root structures that formed by AM was named arbuscules (means ‘tree-like’ in the Latin language) (Dalpé and Monreal 2004). AM fungi (AMF) have spores containing a huge number of nuclei, a large amount of lipid, some carbohydrate and a thick

layer of spore wall, and the size of AMF spores can reach to 500 µm diameter. A mycorrhiza is formed from a germinated spore in the soil. The germinated spore forms a germ tube mycelium, when a host plant root was found, and the mycelium enters the root by entering the root hair and outer cortical cell. However, mycorrhizal fungi are not able to grow in the absence of a host plant (Smith and Read 2008). After entering into the plant root, the fungus develops more by penetrating other cortical cells and by forming a coil-shaped structure and repeating, finally forming the ‘little brush’ like arbuscules (Walker et al. 2004) (Figure 1.10).

There are two main parts of AMF, the extraradical hyphae or mycelium (EH) and the intraradical hyphae (IH). The EH refers to the part of fungal hyphae that grow in the soil. They originate from spore germination and have unlimited growth in the soil, expanding even beyond the rhizosphere (Souza 2015). However, the EH growth can be limited by the soil physicochemical characteristics (Souza 2015). The EH are formed by coenocytic mycelia, with the thick cell wall, mitochondrion, vacuoles, ergosterol crystals, ribosomes, nuclei, endoplasmic reticulum, lipid bodies, plasma membrane and Golgi apparatus (Souza 2015). Under favourable conditions, EH can form spores and start a new cycle of life. IH are similar to EH but with thinner cell walls. Thicker cell walls allow EH having stronger support capacity in the soil and protecting its structure. Intraradical hyphae are able to transfer nutrients and water taken up by EH to the root cell of the host plant. At the same time, the host plants give energy and sugar to AMF. After entering into the cortex cells, IH can form different structures such as vesicles (e.g., *F. mosseae*), arbuscules (e.g., *F. mosseae*) or spores (e.g., *R. intraradices*) (Figure 1.10). The lifecycle of AMF is illustrated in Figure 1.11.

1.3.2 Effects of AMF on plants

The AMF and their symbiotic relationship with plants were first recognized and described in the 1890s and a large number of studies have reported that AMF can significantly promote plant growth in many ways. These studies showed that AMF can promote plant growth, improve nutrient uptake, help plant to better survive in harsh environment such as polluted or weathered areas, and can even decrease pest infestation and other pathogens (Hildebrandt et al. 2007; Fitter et al. 2011; Baum et al. 2015; Basu et al. 2018). Different inocula of AMF are commercially available and used, since studies showed that AMF inoculation promoted the growth and survival of crops and vegetables (Smith and Smith 2011a; Douds et al. 2012). AMF are not only economically valuable but also environmentally friendly, making it plays a more and more important role in future agriculture (Basu et al. 2018).

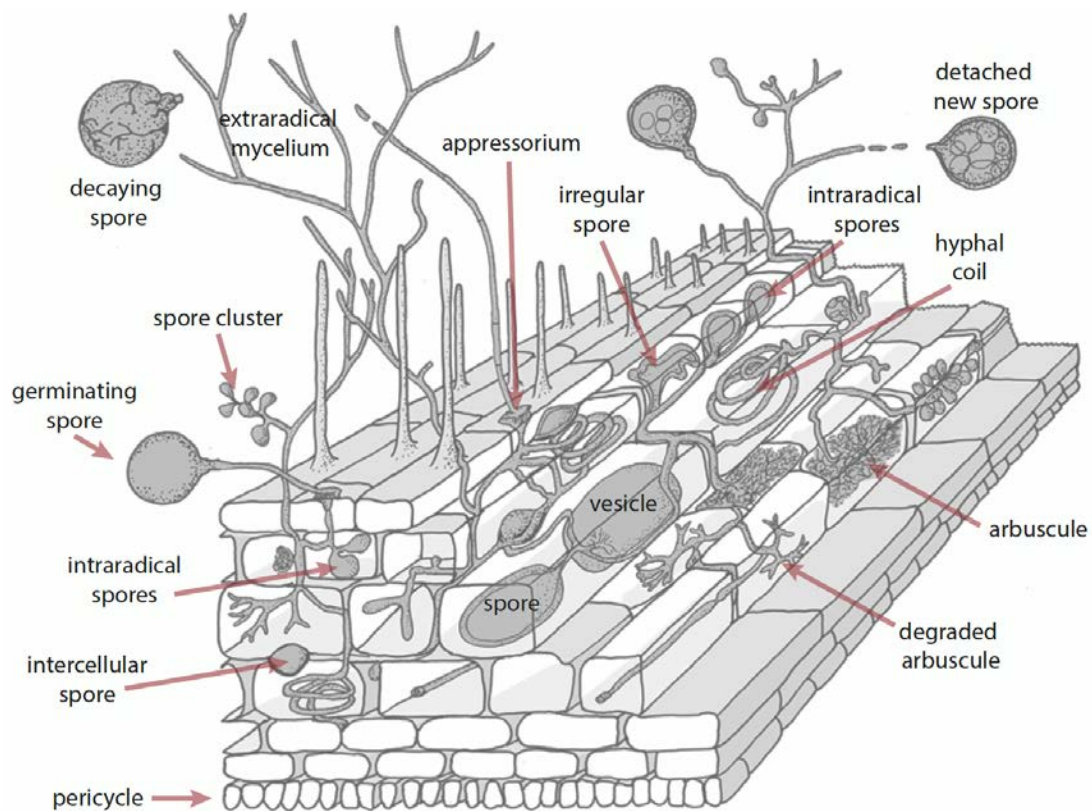


Figure 1.11 Schematic of an arbuscular mycorrhiza, showing the different structures formed by the fungal partner in the outer root cells (Walker 2013).

1.3.2.1 AMF promotion of plant growth and nutrient uptake

Growth promotion and nutrient uptake increase have been reported in many studies on different plants using different AMF species (Smith and Read 2008). In the study of Affokpon et al. (2011), 30 AMF strains have been inoculated to soils to study the growth of tomato and carrot. Among the 30 strains, AMF strain *A. scrobiculata* BEN202 was reported promoted carrot yields by 300% and tomato yields by 26% compared to the non-AMF control. Same growth promotion has been found in barley (*Hordeum vulgare*), cucumber (*Cucumis sativus*), field pea (*Pisum sativum*), tomato (*Solanum lycopersicum*) and wheat (*Triticum aestivum* cv. *Kulin*) (Johansen et al. 1992, 1993; Graham and Abbott 2000; Nagy et al. 2009; Smith and Smith 2011b; Mbodj et al. 2018).

The promotion of host plant growth is strongly correlated with the nutrient supply contributed by AMF. In fact, AMF significantly improve water and nutrient uptake, especially P uptake and transport, which is a diffusion limited nutrient in the soil. Many pieces of evidence showed that the main place of the symbiotic activity where the exchanges of water, nutrient and

photosynthate sugar occurred is the arbuscule/cortical cell interface of AMF (Dalpé and Monreal 2004; Fitter et al. 2011). Several studies focused on the nutrient supply that has been improved by AMF and therefore improved plant growth and fruit yield. Charron et al. (2001a, b) have carried out experiments to study AMF effects on onions and the nutrient transfer. Even though the two AMF (*G. versiforme* and *G. intraradices*) have different abilities on the P and N transfer, both of them showed significant higher P and N concentration in plant tissue. AMF were reported paving the way for nitrogen-fixing bacteria to form intracellular symbioses with plant cells and therefore promoted better growth (Parniske 2008). Other than P and N, AMF also increased significantly the uptake of other mineral nutrients such as K, Ca, Mg, Fe, Mn and Zn. AMF also promoted P and Zn uptake in leeks and therefore improved leek growth (Sorensen et al. 2003). Ortas (2012) pointed out that AMF enhanced the concentration of P and Zn in green pepper, especially in nutrient-deficient conditions. However, Koide and Mosse (2004) mentioned that a growth depression can be found due to AMF inoculation for non-host species or host species when phosphate availability is high, while in most of the natural soils, P availability is a limiting factor for plant growth. Antunes et al. (2012) applied an AMF strain isolated from a nutrient-deficient soil to plants growing in different nutrition level conditions. They found that plant growth was largely promoted in the nutrient depleted soil while the plants showed no growth promotion and even inhibition with fertilization treatments.

1.3.2.2 Alleviating biotic and abiotic stress

Abiotic stress

AMF are also known to alleviate plant stress due to soil weathering, pollution or even pathogens. AMF were reported to enlarge the absorptive surface area of roots in the soil by the extraradical hyphae getting nutrients and water outside the root depletion zone, which is crucial in a poor nutrient and water-deficient soil (Baum et al. 2015). The study by Aroca et al. (2008) showed that AMF inoculated plants grew better in a water-deficient environment by influencing the expression of plant genes and modifying plant physiology. The mycorrhizal plants regulate abscisic acid levels better and faster than non-mycorrhizal plants, balancing between leaf transpiration and root water transfer (Aroca et al. 2008). Beyond water and nutrient deficiency, salinity is another main stress to plants and AMF were shown to alleviate salinity stress successfully to plants (Aroca et al. 2008). Saline soils are toxic to plants because the ions such as sodium and chloride can damage the structure of plant enzymes and some macromolecules, and further disrupt the respiration and photosynthesis processes. Meanwhile, saline soils can a

cause nutrient imbalance in the plants (Juniper and Abbott 1993). Porcel et al. (2012) reviewed that AMF improvement of plant growth in saline conditions was due primarily to improved nutrient uptake and then to gene expression modification. AMF were also reported to reduce cold stress on *Elymus nutans* Griseb (Chu et al. 2016) and eggplants (*Solanum melongena* L.) (Pasbani et al. 2020) and also heat stress on tomato plants (Duc et al. 2018).

However, most of the studies on AMF alleviation of abiotic stress concerned soil pollution, especially with metallic trace elements also called heavy metals, which will be presented in a paragraph below (see in 3.3). A few studies considered other soil pollutants such as polycyclic aromatic hydrocarbons (PAHs) (Binet et al. 2000; Joner and Leyval 2003) and showed potential for using AMF for phytoremediation of PAH-contaminated soils.

Biotic stress

AMF showed a significant beneficial effect against fungal pathogens and disease to plants. Baum et al. (2015) pointed out that AMF colonization can change the root morphology and therefore bring physical and physiological changes to mycorrhizal plants. Matsubara et al. (2001) studied fusarium root rot caused by *Fusarium oxyporum* f. sp. *asparagi* in asparagus, and showed that the sensitivity to fusarium root rot was alleviated due to AMF inoculation which suppressed invasion by Foa in feeder roots. Matsubara et al. (2004) also found that AMF can increase the tolerance to fusarium wilt in strawberry plants. A study carried out by Affokpon et al. (2011) pointed out that AMF significantly reduced nematodes (*Meloidogyne* spp.) and protected crops from root-knot nematode infection.

1.3.3 Effect of AMF on plant in metallic polluted soils

Many studies have been carried out on the effects of AMF on plants growing in metallic contaminated soils during the past decades, and AMF were found to be able to largely improve plant survival and growth in such soils (Haselwandter et al. 1994; Leyval et al. 1997; Hildebrandt et al. 2007). They were also proposed to contribute to the restoration and vegetation of such contaminated soils.

The beneficial effect of AMF has been described for many different plant species, growing in soils contaminated with different metallic pollutants, for example maize (Chen et al. 2004; Chang et al. 2018), alfalfa (Redon et al. 2008), and tobacco (Janoušková et al. 2005). These experiments were mainly performed with Cd (references cited above), Cu (Da Silva et

al. 2005; Chen et al. 2007b), Merlos et al. 2016) and Pb (Hovsepian and Greipsson, 2004) contaminated soils. They showed that AMF inoculated plants grow better than non-inoculated ones in the contaminated soils. These studies showed that besides increased plant growth, the AM plants had lower concentrations of the toxic metallic pollutants (Redon et al. 2008). However, different effects were reported among AMF, including fungi of the same species, but isolated from contaminated or non-contaminated areas (Redon et al, 2009).

The tolerance of AMF to heavy metals was studied and reported in many studies. It has been reported that AMF spores isolated from Cd and Zn contaminated soils were more tolerant to Cd and Zn than isolates from non-contaminated soil (Weissenhorn et al. 1993; Joner et al. 2000; Joner and Leyval 2001a; Rivera-Becerril 2002; Rivera-Becerril et al. 2005). Metal tolerant AMF survive better and perform better symbiotic relationship with plants than the less tolerant ones (Redon et al. 2009). Experiments on Cd sorption by the AM hyphae carried out by Joner et al. (2000) showed the high Cd sorption ability of an AM fungus from a metal-polluted soil. However, the bio-sorption of Cd decreased with the addition of Ca and Zn. This high sorption capacity could explain its higher tolerance to Cd than other isolates from non-contaminated soils and its higher protective effect on Cd transfer to plant.

In order to understand better the role of AMF on the transfer of elements to plants, including pollutants, compartment devices were developed allowing to separate the plant roots and the AMF hyphae, often combined with the use of radioelements to lower analytical detection limits. Joner & Leyval (1997) carried out an experiment using radiolabelling technique to study the Cd transfer from soil to subterranean clover (*Trifolium subterraneum L.*) in symbiosis with the *F. mosseae* fungus. A significantly higher concentration of ^{109}Cd and related ^{109}Cd content was found in the roots of mycorrhizal plants than those of non-mycorrhizal plants, showing that this metal tolerant fungus transported Cd from soil to roots through the hyphae. However, the concentration of ^{109}Cd in the shoots was not significantly higher in the mycorrhizal plant, suggesting retention of Cd at the root/fungus interface. With the same experimental approach, Joner et al. (2004) found no transport of cesium (Cs) from soil to *Medicago truncatula* root and shoot by AMF.

Therefore, AMF isolated from metal contaminated sites may be more tolerant to the metallic pollutants, and maybe more efficient in protecting plants against the toxicity of the metals. However, there may be differences between metallic pollutants, AMF and host-plants, and interactions of the metallic pollutants with the soil characteristics affecting their bioavailability should also be considered.

Even though many studies have focused on the effects of AMF on plants growing in heavy metal contaminated soils, few studies have been carried out on their effect on plant growth and REE transfer to plants in REE-contaminated soils. The only data reported came from Joner et al. (2004) and Chang et al. (2018) concerning Eu or La contaminated environments. Therefore, more work on the effects of AMF on plant growth in REE-contaminated soils should be launched and the specific transfer of these elements from soil to plants by AMF should be investigated.

1.4 Objectives

Rare earth elements (REEs) are a group of elements that are widely used in modern technologies (Chapter 1-1.2). The corresponding industrial emission and the overexploitation of REEs led to environmental pollution, especially of the terrestrial environment and these REEs can be toxic to living organisms (Chapter 1-2.4) as well as human beings (Chapter 1-2.6). Arbuscular mycorrhizal associations are found in more than 70% of plant species (van der Heijden et al. 2015) and have been reported to promote plant growth, nutrient uptake and survival in harsh environments (Smith and Read 2008). Particularly, arbuscular mycorrhizal fungi (AMF) have been repeatedly reported to alleviate the metallic trace element stress to plants (Chapter 1-3.3). However, there is little information on the effect of REEs and their availability on plants. Moreover, scarce data are available on the effects of AMF on plants and REE transfer to plants in REE-contaminated soils and no study has focused on the multiple REE effects on plants by AMF.

This PhD project has been launched to answer four questions to fill the research gap concerning the mobility and transfer of REEs from soil to plants and the contribution of AMF (Figure 1.12). The data of the experimental experiments were arranged in 4 result chapters corresponding to the 4 proposed questions.

Question 1: [What are the bioavailability of REEs in the soil and their effects on plants?](#) In the Chapter 3, a pot experiment was performed with two natural soils spiked or not with a model REE samarium (Sm) and the model plant alfalfa (*Medicago sativa*). Two chemical extractants were tested to estimate the bioavailability of Sm in the soil at different aging times, and the effect of Sm on alfalfa growth and Sm uptake. This chapter has been published in ESPR (Hu et al. 2020a).

Question 2: [What are the effects of an AM fungus on plants growing in an REE contaminated soil and on the transfer of REEs to the plant?](#) Two experiments were launched to answer this question. A pot experiment with alfalfa and a soil spiked with two concentrations of Sm and a non-spiked control soil, inoculated or not with a metal-tolerant AMF *Funneliformis mosseae*. A further compartmented pot experiment was performed with a separated compartment containing Sm-spiked sand which is only accessible by *F. mosseae* fungal hyphae to further study the transport of Sm from the soil to alfalfa (Chapter 4). This chapter has been published in Mycorrhiza (Hu et al. 2020b).

Question 3: [What are the effects of an AM fungus on plants growing in a soil with multiple REE contamination and on the transfer of these REEs to plants?](#) In the Chapter 5, the same compartmented pot experiment as in Chapter 4 was launched with a separated compartment containing 4 REEs: La, Ce, Sm and Yb and two plant species: alfalfa and ryegrass (*Lolium perenne*) inoculated or not with *F. mosseae*.

Question 4: [What are the bioavailability of REEs in historically contaminated mining tail soils and their effects on AMF?](#) In this chapter we analysed the soils collected from a rare earth mining area from China with different dilutions and studied REE availability and effect on AMF germination and colonization, using a spore germination test and a pot experiment with plant (Chapter 6).

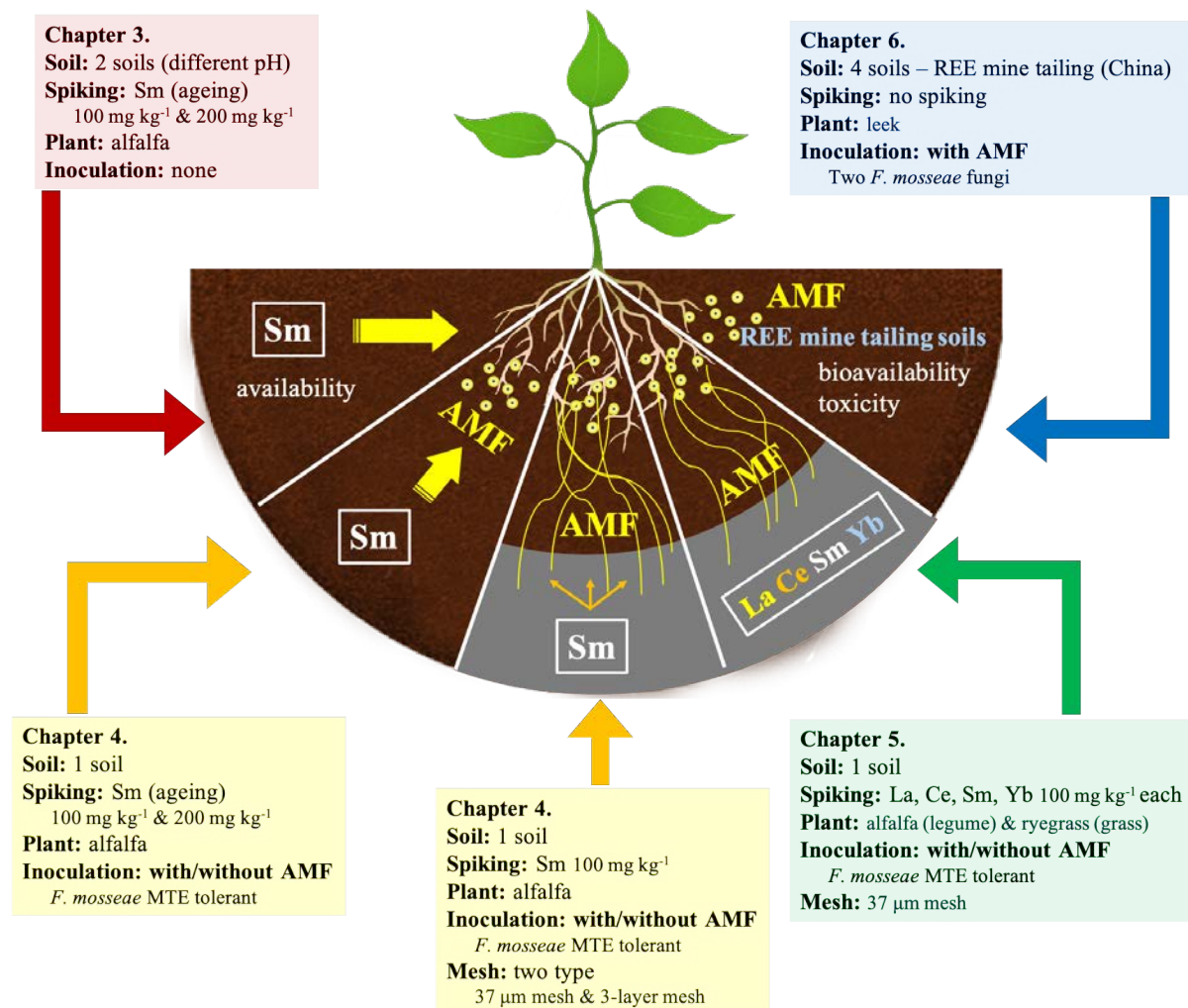
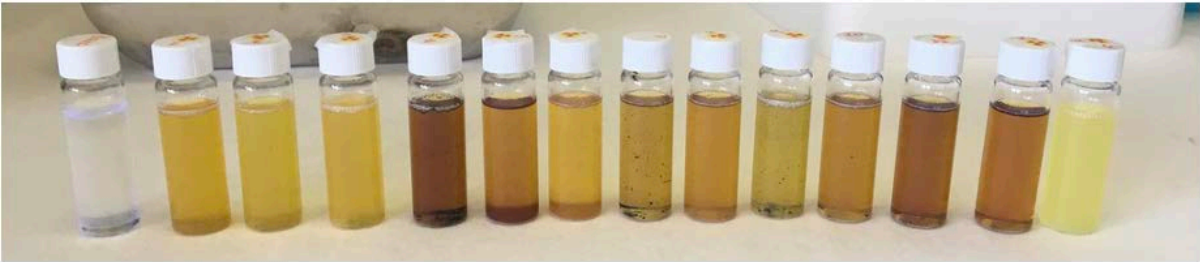


Figure 1.12 General scheme of experimental design of this PhD project according to chapters. The latter experiments had higher complexity and with more parameters considered in the experimental design.

Chapter 2 Materials and methods

Chapitre 2 Matériels et méthodes



2 Materials and methods

2.1 Soil, plant and mycorrhizal fungi

2.1.1 Soil

2.1.1.1 Soil sampling and characteristics

In total 6 soils have been used in this PhD project including two uncontaminated soils and four contaminated soils collected from REE mine tailings sites and the surrounded unmined sites in China (Soil parameters in Table 2.1 & 2.2). In addition, one uncontaminated OCDE/ISO artificial soil has been used in the Chapter 6.

The two uncontaminated soils were sampled (5-25 cm depth) in North-Eastern France: a forest soil (Maron, 48°38'45.75"N, 6°4'48.44"E) and an agricultural soil (Bouzule, 48°44'7.95"N, 6°19'9.64"E). The two soils have different pH values and the physicochemical parameters of the two sampled soils can be found in the Table 2.1. The collected soils were dried in a dark and ventilated room until the weights remained constant. Dried soil samples were ground and sieved to 2 mm. After homogenization, the two soils were packed and stored in a dry and dark room ready for experiments. To avoid the compaction of Bouzule soil, it was partially amended with sand (sand/soil = 30w/70w) which has been pre-sieved (2 mm), washed and dried to allow better seedling growth. The two uncontaminated soils have been spiked with REEs in the Chapter 3, 5 and 6 to understand the toxicity of REEs to plants and the transfer of REEs in the terrestrial environment.

The soil parameters of the two uncontaminated soil were analysed by Laboratoire d'Analyse des Sols d'Arras (Table 2.1).

Table 2.1 Physico-chemical characteristics of the 2 unpolluted natural soils (Maron & Bouzule) used in this PhD project

<u>Soil parameter</u>	<u>Maron soil</u>	<u>Bouzule soils</u>	<u>Methods</u>
pH	5.34	6.68	<u>ISO 10390</u>
sand: silt: clay (%)	81:362:557	167:513:320	<u>NF X 31-107</u>
Organic carbon (g·kg ⁻¹)	40.1	15.8	<u>ISO 14235</u>
Total nitrogen (g·kg ⁻¹)	3.09	1.65	<u>ISO 14235</u>
Organic matter (g·kg ⁻¹)	69.4	27.4	<u>ISO 14235</u>
C/N	13.0	9.6	<u>ISO 14235</u>
Olsen P (mg·kg ⁻¹)	8	91	<u>ISO 11263</u>
Ca (cmol ⁺ ·kg ⁻¹)	18.1	13.1	<u>ISO23470</u>
Mg (cmol ⁺ ·kg ⁻¹)	1.07	1.79	<u>ISO23470</u>
Na (cmol ⁺ ·kg ⁻¹)	0.0739	0.11	<u>ISO23470</u>

K (cmol ⁺ ·kg ⁻¹)	0.504	1.14	<u>ISO23470</u>
Fe (cmol ⁺ ·kg ⁻¹)	0.0266	0.01	<u>ISO23470</u>
Mn (cmol ⁺ ·kg ⁻¹)	0.731	0.3	<u>ISO23470</u>
Al (cmol ⁺ ·kg ⁻¹)	1.45	0.050	<u>ISO23470</u>

The uncontaminated OCDE/ISO 11268-1 artificial soil was prepared in our laboratory and consisted of 10% dried and finely ground (0.2 mm) peat, 20% kaolinite, 0.3 to 1% CaCO₃ to adjust the pH and 69 to 69.7% siliceous sand. This artificial soil was used as a control substrate to test the toxicity of REE polluted soils on the germination of mycorrhizal fungi spores and AM colonization of plants. Further details can be found in Chapter 6.

The four contaminated soils were collected from a chrono-sequence of unreclaimed ionic REE mine tailings abandoned from 2003 to 2011 situated in the Dingnan county of Ganzhou city, Jiangxi province, China. The control sites were collected from the unmined forested areas close to the abandoned REE mine tailings. These contaminated soils collected from China were used to test the toxicity of REEs polluted soils to the germination of mycorrhizal fungi spores and plants.

The physico-chemical characterization of the Chinese REE mine tailings site soil has been carried out by the Department of Environmental Science of Sun Yat-sen University (Pr Ye-Tao Tang, eesty@mail.sysu.edu.cn) (Table 2.2) and further details can be found in Chapter 6.

Table 2.2 Physico-chemical characteristics of the REE mine tailings soils used in this PhD project

	Unit	LOD*	Control_1	Control_3	Aband_2003	Aband_2007
proportion coarse fragments	%		11.2	20.8	31.2	21.1
pH			4.39	4.57	4.88	4.89
temperature	°C		21.1	21.2	21.1	21
TOC	%	0.04	1.89	1.93	0.28	0.18
TN	%	0.015	0.11	0.13	0.02	0.02
C:N	-		17	15	12	10
Al	%	0.005	10.1	11.1	10.8	7.2
Ca	%	0.007	0.01	0.01	0.01	0.01
Fe	%	0.007	3.5	3.3	2.6	2.9
K	%	0.008	3.4	4.6	5.7	4.4
Mg	%	0.006	0.09	0.11	0.12	0.07
Mn	%	0.008	0.02	0.06	0.03	0.05
Na	%	0.007	0.13	0.14	0.22	0.14
P	%	0.004	0.014	0.023	0.018	0.013
Si	%	0.005	30.2	29.6	30.0	34.0
S	%	0.004	0.02	0.02	0.01	0.01
Ti	%	0.006	0.40	0.38	0.34	0.46
As	mg kg ⁻¹	76	< 80	< 80	< 80	< 80

	Ba	mg kg ⁻¹	0.50	625	972	1255	955	
	Co	mg kg ⁻¹	79	< 80	< 80	< 80	< 80	
	Cr	mg kg ⁻¹	10.00	10	10	10	10	
	Cs	mg kg ⁻¹	0.01	4	5	6	5	
	Cu	mg kg ⁻¹	80	< 80	< 80	< 80	< 80	
	Ga	mg kg ⁻¹	0.1	32	35	33	24	
	Hf	mg kg ⁻¹	0.2	20	18	16	28	
	Nb	mg kg ⁻¹	0.2	58	51	46	70	
	Ni	mg kg ⁻¹	79	< 80	< 80	< 80	< 80	
Trace elements	Pb	mg kg ⁻¹	93	< 93	< 93	< 93	94	
	Rb	mg kg ⁻¹	0.2	201	225	259	212	
	Sn	mg kg ⁻¹	1.00	8	7	6	7	
	Sr	mg kg ⁻¹	0.1	35	45	53	43	
	Ta	mg kg ⁻¹	0.01	4	3	3	4	
	Th	mg kg ⁻¹	0.05	39	33	35	27	
	U	mg kg ⁻¹	0.05	7	7	6	8	
	V	mg kg ⁻¹	5.00	30	30	25	22	
	W	mg kg ⁻¹	1.00	3	4	4	8	
	Zn	mg kg ⁻¹	80	83	84	< 80	< 80	
	Zr	mg kg ⁻¹	2.00	745	679	571	1043	
	REEs	La	mg kg ⁻¹	0.5	54.0	186.0	240.9	99.5
		Ce	mg kg ⁻¹	0.5	136.3	254.6	231.8	185.7
		Pr	mg kg ⁻¹	0.03	13.7	39.1	43.9	21.2
Nd		mg kg ⁻¹	0.1	53.5	145.6	153.0	76.7	
Sm		mg kg ⁻¹	0.03	11.0	26.1	32.2	15.5	
Eu		mg kg ⁻¹	0.03	1.9	5.0	6.8	2.9	
Gd		mg kg ⁻¹	0.05	9.2	21.6	35.0	14.1	
Tb		mg kg ⁻¹	0.01	1.4	3.0	5.3	2.1	
Dy		mg kg ⁻¹	0.05	8.4	16.7	29.1	12.5	
Ho		mg kg ⁻¹	0.01	1.7	3.1	5.5	2.4	
Er		mg kg ⁻¹	0.03	4.6	8.0	14.3	6.9	
Tm		mg kg ⁻¹	0.01	0.7	1.1	1.9	1.0	
Yb		mg kg ⁻¹	0.03	4.7	6.6	11.3	6.6	
Lu		mg kg ⁻¹	0.01	0.7	0.9	1.6	1.0	
Y	mg kg ⁻¹	0.5	37.4	74.1	144.8	62.5		
	sum 14 REEs	mg kg ⁻¹		301.9	717.3	812.5	448.1	

*DL: Detection limit

2.1.1.2 Soil spiking

The soil spiking techniques were applied in Chapter 3, 4 and 5. In Chapter 3 and 4, pot experiments were launched with soils spiked with SmCl₃•6H₂O and reaching to 100 and 200 mg·kg⁻¹ Sm. In order to homogenize better the spiked Sm in large amount of soils, a ten-time concentrated solution of SmCl₃•6H₂O in deionized water was added to 1/10 of both soils and dried at 50-60 °C, homogenized, and added to the rest of the non-spiked soils and homogenized to obtain 100 and 200 mg·kg⁻¹ Sm.

In the compartmented pot experiments, the bottom sand compartments were spiked with REE solutions to reach 100 mg·kg⁻¹ Sm (Chapter 4) and 100 mg·kg⁻¹ La, 100 mg·kg⁻¹ Ce, 100 mg·kg⁻¹ Sm, 100 mg·kg⁻¹ Yb (Chapter 5).

2.1.2 Plants

The legume plant alfalfa (*Medicago sativa* L. var. Europe) has a rapid growth, is responsive to AM colonization and has been used in many previous studies on the interaction between plants. Therefore, it has been chosen as a model plant to study the toxic effects of Sm to plants, the transfer of Sm from soil to plants by AMF (Chapter 3 and 4) and further the transfer of four REEs from soil to plants by AMF (Chapter 5).

The grass plant ryegrass (*Lolium perenne* L., cv. Barclay) has been used as a model plant to compare with the legume plant alfalfa to study the transfer of four REEs from soil to plants in Chapter 5.

In addition, leek (*Allium porrum* L.) as a common host plant for AMF cultivation, has been used as a model plant in Chapter 6 to study the colonization of AMF to leek plants under the REEs mining soil conditions.

2.1.3 Mycorrhizal fungi

The metal-tolerant AM fungus *Funneliformis mosseae* (previously *Glomus mosseae*) strain BEG 69 was used in all the experiments concerning REE contamination studies. It was isolated from a soil with a 60-year history of industrial metal pollution (Weissenhorn et al. 1993; Tonin et al. 2001) and permanently cultured in our laboratory in the same contaminated soil using leek (*Allium porrum* L.) as the host plant.

The second *F. mosseae* strain BEG 12 originated from non-polluted soil and has been cultured on non-contaminated expanded clay in our laboratory using leek as the host plant. The *F. mosseae* BEG 12 has been used in the germination and colonization studies in REE mine tailings soils in Chapter 6.

2.2 Experiment design

2.2.1 Seed germination

The plants in all experiments were cultured in the laboratory condition from seeds. The boxes with vermiculite have been prewashed with deionized water and then autoclaved. Plant seeds were prewashed with deionized water before putting on the autoclaved vermiculite. The boxes with seeds and vermiculite were wrapped with aluminium foils to provide dark environment and then placed in 22°C growing chamber until the seeds germinate. The

aluminium foils were then removed to let seedlings grow for 4-5 days in a growth chamber (see 4.4) before the transplantation procedure.

2.2.2 Pot experiments

Pot experiments have been applied in Chapter 3, 4 and 6. One kg pots were used in experiments in the Chapter 3 and 4 with 1 kg of spiked or non-spiked soil inside. Water was added into the soils to reach 70% water holding capacity (WHC) of the corresponding soil. Pots with soil and water were preserved in a cold dark room at 4°C (T0) for 2 weeks for the ageing of the spiked contamination. After soil ageing, 4-5-day-old alfalfa germinated seedlings were transplanted to the pots with or without 200 *F. mosseae* BEG 69 spores extracted under an optical microscope from a pot culture on leek.

250 g small pots were used in the experiment in the Chapter 6, filled with 120 to 190 g (volume of the soil is constant, the weight depends on the density of the soil) of mixed REE mine tailing soil. Water was added into the soils to reach 70% WHC of the corresponding soil. 4-5-day-old leek seedlings were transplanted to the pots with 200 *F. mosseae* BEG 12 or BEG 69 spores.

2.2.3 Compartment experiments

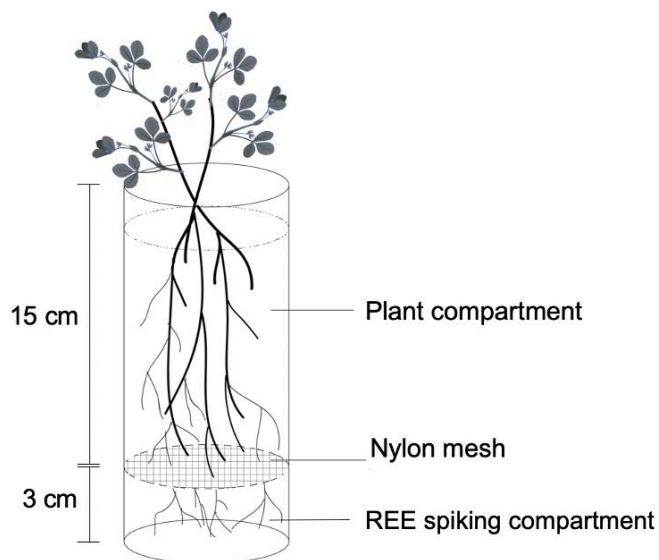


Figure 2.1 Compartmented pots used in the experiments

Compartmented pots were also used for a better understanding of the transfer of the REEs from soil to plant by AMF (Figure 2.1). A compartmented pot was made with two polyvinyl chloride (PVC) tubes with a 150-mm tall main compartment on the top (also called

plant compartment) and a 30-mm bottom compartment. Both compartments have 50 mm outer diameter and 45 mm inner diameter. The bottom compartment was filled with Fontainebleau sand spiked with REEs and the main compartment was filled with soils. The two compartments were separated with nylon meshes. Two size of nylon meshes were used: a one-layer 37 μm mesh (Chapter 4 and 5) and a 3-layers (37 μm , 1000 μm and 37 μm) mesh, called “air-mesh” below (Chapter 4). Both meshes separated the main compartment and the bottom compartment and only allowed AMF hyphae to pass through to get access to the REE spiked sand.

2.2.4 Growth conditions and watering

According to the growing situation of the plants, the plants were cultured 28 to 49 days in the growth chamber. The growth chamber has 16 h photoperiod with 22°C/18°C day/night, 250 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$, and 80% relative humidity.

During the growing period, plants were watered twice per week to maintain 70% WHC of the soils. In Chapter 5, plants were watered with deionized water for the first two weeks and the rest four weeks were watered once with deionized water and once with P-deficient nutrient solution. The composition of the P-deficient nutrient solution is as follows (Table 2.3):

Table 2.3 The composition of the P-deficient nutrient solution

Component	Dose/L
NH_4NO_3	1 mM
$\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$	1 mM
$\text{Na}_2\text{HPO}_4\cdot 2\text{H}_2\text{O}$	0.1 mM
K_2SO_4	1 mM
$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$	0.75 mM
H_3BO_3	12.5 μM
$\text{MnSO}_4\cdot 4\text{H}_2\text{O}$	2.5 μM
$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$	0.3 μM
$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$	1 μM
$\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$	0.05 μM
$\text{CoCl}_2\cdot 6\text{H}_2\text{O}$	0.2 μM
$\text{CoSO}_4\cdot 7\text{H}_2\text{O}$	0.2 μM
$\text{FeEDTA}(\text{C}_{10}\text{H}_{14}\text{FeN}_2\text{O}_8)$	20 μM

2.2.5 Spore germination tests

A spore germination test following ISO/TS 10832 was carried out using different dilutions of mine tailing soils collected from China (Chapter 6). 30 spores were carefully put on a nitrocellulose membrane filter and covered with another layer of filter, forming a

“sandwich” structured device. The sandwiches containing 30 spores were fixed by slide frames and put in the test soil (figure 2.2). Water was added to reach 90% WHC of the soils and then the Petri dishes were sealed with parafilm and stored in a dark chamber for 14 days at 24 °C.

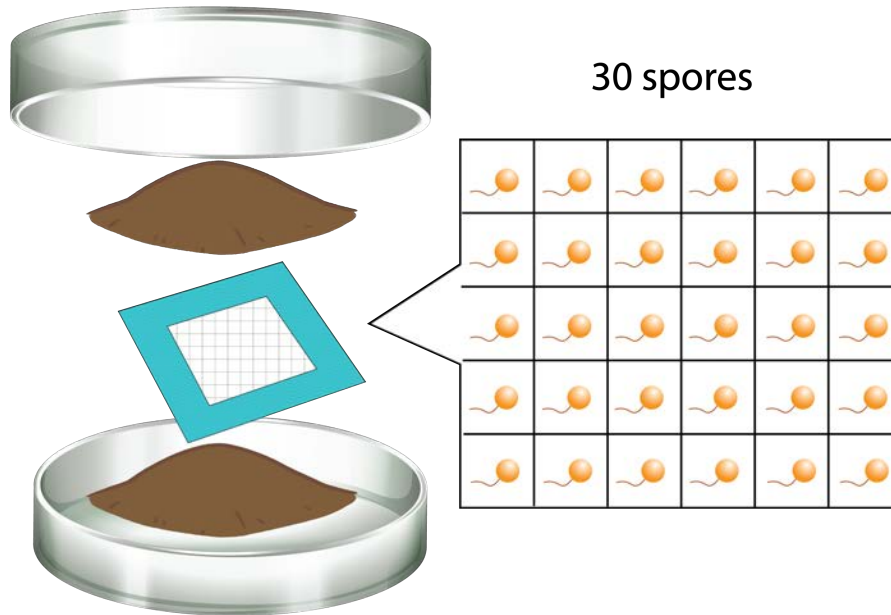


Figure 2.2 Illustration of the experimental design of spore germination test

After 14 days' culturing, the sandwiches were gently withdrawn from the Petri dishes, cleaned, stained with 0,05 % trypan blue solution for 15 minutes and rinsed before the observation under the microscope. The total number of spores recovered and germinated were counted (Chapter 6).

2.3 Analysis

2.3.1 Bioavailability of REEs in soil

The bioavailable concentrations of REEs in soils were estimated using two different extractants: diethylenetriaminepentaacetic acid (DTPA) and CaCl_2 (Miles and Parker 1979; Feng et al. 2005). DTPA extracting solution consists of diethylenetriaminepentaacetic acid (DTPA) (0.005M), CaCl_2 (0.01M) and triethanolamine (0.1M), and the extraction procedure is described in Chapter 3.

2.3.2 Total REE and major elements analysis in soil and plant

Soil. To analyze the REEs and other elements in soil, 200 mg of soil sample was transferred into a cleaned Teflon tube designed for CEM Mars 5 Digestion Microwave System® and 10 mL of *Aqua regia*. Digestion was processed under the adapted programme (15 min digestion at 25°C and 10 min digestion at 170°C). After the digestion and cooling down procedure, the digested samples were filtered (DigiFILTER, SCP Science® 010-500-070) and the solutions were adjusted into 10 mL in centrifuge tubes before the analysis.

Plant. Dry vegetal samples were digested in nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) solution (6 mL of 69% HNO₃ and 3 mL of 30 % H₂O₂) (CAS: 7722-84-1). 200 mg of plant sample was transferred into a DigiTUBEs followed by a DigiPREP MS 48 & TS (SCP Science® 010-500-205 & 010-500-275) hot plate digestion. After cooling down, the solution was filtered, adjusted to 10 ml and kept at 4 °C until the analysis. REEs, nutrient element P and other major elements (K, Ca, Na, Mg) were analysed in the Chapter 4,5 and 6. For the two pot experiments, the chemical analysis was performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) analytical technique using a Varian Inc.® (Part A) - 720/730-ES ICP-OES. For the compartmented pot experiments, inductively coupled plasma mass spectrometry (ICP-MS) technique was used, with the certified reference material BCR®-667 estuarine sediment (rare elements, Th, U) as a reference. The detection limit for Sm concentration in the plant samples analyzed by ICP-MS was 0.0001 mg·kg⁻¹.

2.3.3 Root mycorrhizal colonization

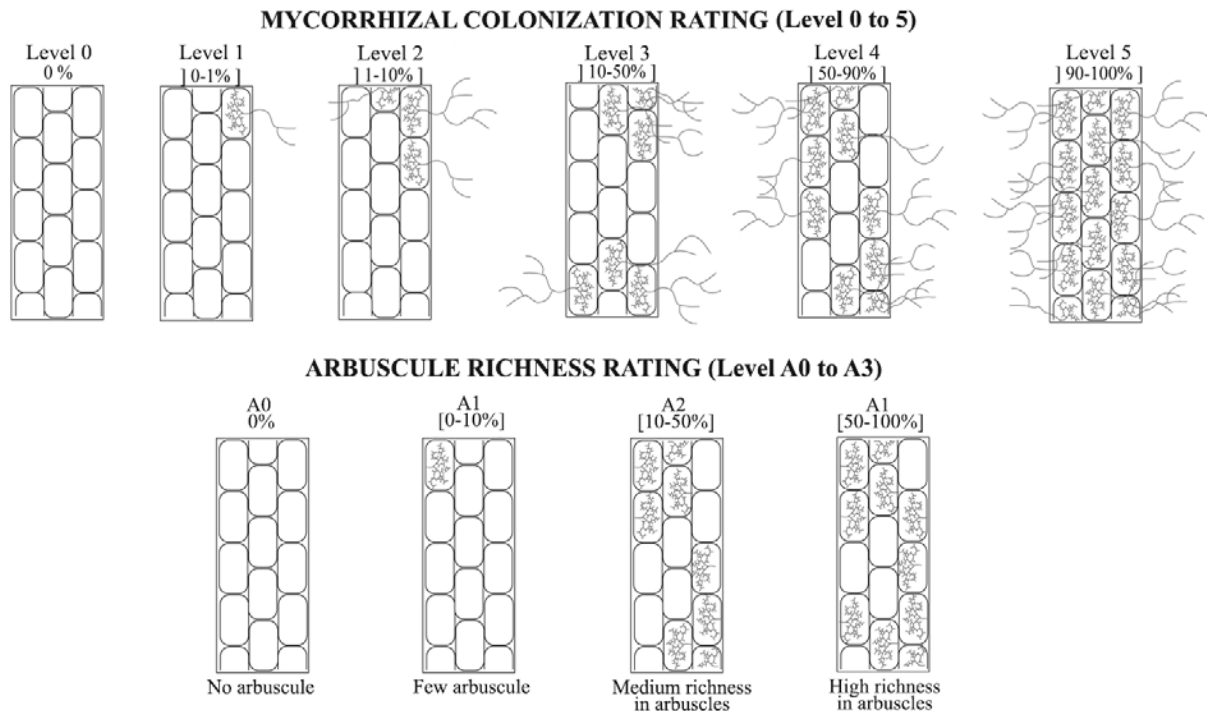


Figure 2.3 Illustration of the analysis of the mycorrhizal colonization rate of the root cortex and arbuscular richness rate (Trouvelot et al., 1985).

The root samples were stained with the trypan blue method to determine mycorrhizal colonization (Koske and Gemma 1989). Thirty 1-cm pieces of stained root segments were sealed on slides, and the arbuscular mycorrhizal colonization was observed and determined according to the protocol of Trouvelot et al. (1986) (Figure 2.3), with the following formula :

■ *Mycorrhizal frequency, F*

$$F\% = 100 \times \left(\frac{N - n_0}{N} \right)$$

N = number of fragments observed

n_0 = number of non mycorrhizal fragments

■ *Intensity of mycorrhization, M*

$$M\% = \frac{95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1}{N}$$

N = number of fragments observed

n_5-n_1 = number of fragments quoted 5 to 1

■ *Content of arbuscules in the mycorrhizal part, a*

$$a\% = \frac{100mA_3 + 50mA_2 + 10mA_1}{100} \qquad mA = (95n_5A + 70n_4A + 30n_3A + 5n_2A + n_1A) \times \frac{F}{M \times (N - n_0)}$$

mA_3 to mA_1 = % of mycorrhizal fragments, m, with quotation A3 to A1

■ *Content of arbuscules in the root system, A*

$$A\% = a \times \frac{M}{100}$$

M = intensity of mycorrhization of the cortex

a = content of arbuscules in the mycorrhizal part

2.3.4 Data analysis

The statistical analysis of the first pot experiment was performed using RStudio software (Version 1.1.463© 2009–2018 RStudio, Inc.). After checking the normality of the data, one-way and two-way analysis of variance (ANOVA 1 and 2) and the Tukey honestly-significant-difference (HSD) post-hoc test were performed. The data obtained in the other experiments were analyzed using XLSTAT version 2020.3. Statistical analyses were performed using two-way analysis of variance (ANOVA) and Tukey's (HSD) post-hoc test. For the non-normally distributed data parameters, we used rank-transformed data. Mycorrhizal colonization data were arcsine transformed before statistical analyses. The data presented in the figures are the means \pm standard deviations (SDs). *P*-values are given in the figure legends.

Chapter 3. Bioavailability and transfer of elevated Sm concentration to alfalfa in spiked soils

Chapitre 3. Biodisponibilité et transfert d'une concentration élevée de Sm vers la luzerne dans des sols enrichis



Résumé du Chapitre 3

La très forte augmentation de l'utilisation des terres rares (REE en anglais) dans les nouvelles technologies au cours des dernières décennies, a induit des émissions industrielles et des déchets d'exploitation avec des teneurs élevées de ces éléments dans l'environnement naturel. L'augmentation de la contamination des REE dans l'environnement suscite de fortes inquiétudes sur leur impact et notamment sur leur mobilité dans les sols et leur toxicité pour les plantes. Dans ce chapitre, l'objectif était d'étudier la biodisponibilité et le transfert des REE du sol vers les plantes. Une expérience en pot a été réalisée en utilisant deux sols avec des caractéristiques chimiques différentes et notamment le pH, un sol forestier avec un pH acide et un sol agricole avec un pH neutre, plantés avec de la luzerne et contaminés en laboratoire avec 0, 100 et 200 mg.kg⁻¹ de samarium (Sm), utilisé comme REE modèle. Le sol a été placé pendant 15 jours en chambre froide pour induire un vieillissement de la contamination par le Sm, avant de planter les plantules de luzerne. Les teneurs totales de Sm ont été analysées dans le sol au début et à la fin de l'expérience et dans les parties aériennes de la luzerne. Deux extractants chimiques, le DTPA et le CaCl₂, ont été utilisés pour estimer la biodisponibilité du Sm dans le sol. Le transfert du Sm a été analysé en utilisant le rapport entre la teneur en Sm dans la luzerne cultivée et dans les sols. Les effets du Sm sur les plantes ont été analysés en fonction du poids sec des parties aériennes et des racines.

Les résultats ont montré un effet significatif du vieillissement puisque le Sm extractible par DTPA a diminué de manière significative après deux semaines. La disponibilité du Sm était nettement plus faible dans le sol à pH neutre par rapport au sol acide. Le Sm extractible par DTPA était néanmoins bien corrélé avec l'absorption de Sm par la luzerne, ce qui indique que l'extraction au DTPA donne une bonne indication du Sm biodisponible pour la luzerne dans les sols. L'absorption du Sm du sol par la luzerne dépendait du pH du sol et de la concentration de Sm. Le facteur de transfert sol-plante était faible (< 0,08), mais une réduction de 30 % de la biomasse de luzerne a été observée lorsque les sols étaient dopés avec 100 à 200 mg.kg⁻¹ de Sm. Une inhibition significative de la croissance de la luzerne a été constatée dans le sol contaminé avec 200 mg.kg⁻¹ de Sm dans le sol acide et pour les deux teneurs de Sm dans le sol neutre.

Bioavailability and transfer of elevated Sm concentration to alfalfa in spiked soils

Ruoyu HU, Thierry BEGUIRISTAIN, Alexis De JUNET, Corinne LEYVAL*

Université de Lorraine, CNRS, LIEC, F-54000 Nancy, France

* Corresponding author: corinne.leyval@univ-lorraine.fr, tel. 33(0)372745204

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Abstract Rare earth elements (REEs) have been widely used in recent decades and their exploitation has led to industrial REE emission and to contaminated soils especially in former mining areas. This raised people's concerns on the accumulation and toxicity of REEs in soils and plants, and consequences on plant health. Although many studies dealt with REE in soils and plants, there is still a need to precise their toxicity, bioavailability and transfer to plants in contaminated sites in order to restore such ecosystems. We studied the bioavailability and transfer of an REE to *Medicago sativa* grown on two contaminated soils differing in their chemical characteristics. A pot experiment was set up in a growth chamber where two natural soils were spiked or not with samarium (Sm) as a model REE. Two chemical extractants were tested to estimate the bioavailability of Sm in the soil, its decrease with time and its transfer to the plants. Results showed that DTPA extractable Sm was well correlated with Sm uptake in alfalfa shoots. The experiment pointed out a significant ageing effect since DTPA extractable Sm significantly decreased within two weeks in the soils and was significantly lower in the less acidic soil than in the other. The uptake of Sm from soil to alfalfa shoots depended on the soil pH and on the spiking concentration. The soil to plant transfer factor was low (<0.08) but a 30% reduction of alfalfa biomass was observed when the soils were spiked with 100 to 200 mg kg⁻¹ of Sm.

Keywords:

Rare earth element, Transfer, Soil, Bioavailability, Plant, Ageing

1. Introduction

Rare earth elements (REEs) are a group of elements in the periodic table consisting of 15 lanthanide group elements from lanthanum (La) to lutetium (Lu), along with the pseudo lanthanide (Tyler 2004a). According mainly to the geochemical behaviour of the REEs, they were divided into light (LREEs; La to Sm) and heavy REEs (HREEs; europium to Lu) (Brioschi et al. 2013). Because

of their magnetic, fluorescent and catalytic properties, REEs, especially LREEs are nowadays widely used in many industries especially high-technology industries like electronics and medicine (Loell et al. 2011a; Pagano 2016). In natural soils, REE concentrations range from 40 to 700 mg·kg⁻¹ all over the world and are not considered as toxic for the environment. However, the higher demand and mining of REEs in the past decades have led to a notable increase in REEs in the environment (MacMillan et al. 2019) and high soil REE concentrations in former mining areas (Chao et al. 2016; Khan et al. 2017). For example, REE concentration in soil of Bayan Obo rare earth mining area in China is higher than 2.7 × 10⁴ mg·kg⁻¹ due to the mining activity (Liang et al. 2014). Landfills with improperly discarded materials containing REEs might also be a source of contamination.

REE concentrations in plants are very low in natural soils (Tyler 2004a), but their phytotoxicity may be a concern in contaminated areas (Carpenter et al. 2015). REEs are not considered as essential elements for plants, but positive effects of REEs at low concentrations were reported on plant growth (Diatloff et al. 1995a; Xu et al. 2002; Hong et al. 2003; Xiangsheng et al. 2006; Cheng et al. 2015). In China, REEs have even been used to promote plant growth and have been widely applied in agriculture since the 1990s. However, when a high dose of REE fertiliser has been applied, plant growth was limited and even a toxic effect was found (Hong et al. 2003). Diatloff et al. (1995a, b) studied the toxicity of lanthanum (La) and cerium (Ce) on corn and mungbean seedlings in nutrient solutions and showed strong inhibition of mungbean root elongation and plant growth with Ce (even at 0.2 μM). Thomas et al. (2014) studied the phytotoxicity of La, Ce and Y (yttrium) on germination and growth of native plants and crops in two artificial soils with different pH. However, there is little information on the phytotoxicity of REEs in contaminated soils. The excessive amounts of REEs are not only harmful to plants but are also able to be taken up by plants and enter the food chain, resulting in their accumulation in organisms and finally in human beings (Loell et al. 2011a; Turra et al. 2013). Therefore, studies regarding the effects of REEs in contaminated soils on plants, and their transfer from soil to plants should be carried out (Gonzalez et al. 2014). The LREE uptake by plants involves calcium (Ca²⁺ ion) channels and HREE uptake uses aluminium (Al³⁺ ion) channels (Gonzalez et al. 2014; Yuan et al. 2017). The analysis of REEs in different plants on mining and control areas was performed for health risk assessment and showed an enrichment of LREEs in the former (Zhuang et al. 2017) or to identify potential hyperaccumulating plants (Khan et al. 2017).

The bioavailability of REEs has been discussed in many studies but many of them considered natural soils where their concentrations are low, or agricultural soils with fertilizer inputs. The mobility of REEs in soils is affected by REE sources and forms, soil particle size, pH and organic matter content (Tyler 2004a; Babula et al. 2008; MacMillan et al. 2019). The bioavailability of REEs and transfer to plants in soils contaminated due to industrial or mining activities has been less considered. Different methods or development of methods have been studied to estimate/predict the uptake of metals in different plants. Among these methods, simple extractants have not been widely investigated for REEs.

Using sequential chemical extractions, Cao et al. (2000b) showed that bioavailability of REEs differed with the REEs. Li et al. (2001) compared different extractants and found that 0.1 M HCl extractable fraction of REEs was well correlated with their concentration in wheat roots and shoots in cultivated soils in China. Li et al. (2018) used CaCl_2 to correlate lanthanide toxicity to soil invertebrates with bioavailable fraction in soil. None of these studies considered the ageing of REEs in soil and the consequence on their bioavailability.

This work aimed to study the bioavailability and transfer of an REE to a crop plant *Medicago sativa* grown on two contaminated soils differing in their chemical characteristics. A pot experiment was set up in a growth chamber where two natural soils were spiked or not with elevated concentrations of Samarium (Sm) as a model REE. The bioavailability of Sm was estimated using two chemical extractants in the spiked and control soils just after spiking, after two weeks of ageing and at plant harvest. Sm phytoavailability was also estimated by plant accumulation at harvest. Plant growth, P and Ca plant uptake were also estimated.

2. Material and methods

2.1. Experiment design

The soils used for the experiments were collected from the surface horizon (5-25 cm) of two sampling sites in north-eastern France: Maron (48°38'45.75"N, 6°4'48.44"E) and Bouzule (48°44'7.95"N, 6°19'9.64"E). They both belong to Luvisol with different pH values. The physicochemical parameters of the two sampled soils are shown in Table 3.1. Soil samples were air-dried, ground, sieved to 2 mm, homogenised and stored in a dry and dark room ready to be used in the experiments. The Bouzule soil was partially amended with pre-sieved (2 mm), washed and dried sand (30w/70w) to avoid compaction and allow seedling growth. A ten times concentrated solution of $\text{SmCl}_3 \cdot 6\text{H}_2\text{O}$ in deionized water was added to 1/10 of both soils. The concentrated spiked soils were dried at 50 °C, homogenised, and added to the rest of the unspiked soils and homogenised to obtain 100 and 200 $\text{mg} \cdot \text{kg}^{-1}$ Sm. 1 kg of soils were placed in 1kg pots with eight replicates and three Sm conditions (0, 100, 200 $\text{mg} \cdot \text{kg}^{-1}$) according to LREE concentrations in abandoned mining sites (Chao et al. 2016). Deionized water was added to reach 70% of water holding capacity (WHC) and pots were placed in a dark room at 4°C (T0) for 2 weeks for the ageing of the Sm contamination.

After soil ageing, 4-5-day-old alfalfa (*Medicago sativa*) seedlings germinated on sterilised vermiculite were transplanted to the pots (T1) with 15 plants in each pot. Pots with plants were transferred to a growth chamber with 16 h photoperiod, 22°C/18°C day/night, 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 80% relative humidity for 6 weeks. During the 6-week growing period, the pots were watered twice a week and a nutrient solution (Redon et al. 2009) was added once per week to maintain 70% WHC.

Table 3.1 Physico-chemical characteristics of the soils used in the experiment

	Maron	Bouzule
Soil pH	5.34	6.68
Sand: silt: clay (%)	81:362:557	167:513:320
CaCO ₃ (g·kg ⁻¹)	0	0
Organic carbon (g·kg ⁻¹)	40.1	15.8
Total Nitrogen (g·kg ⁻¹)	3.1	1.7
Organic matter (g·kg ⁻¹)	69.4	27.4
C/N	13.0	9.6
P (mg·kg ⁻¹)	8	91
CEC cobalthexamine (cmol ⁺ ·kg ⁻¹)	23.2	16.1
Saturation rate (%)	85	100
Ca (cmol ⁺ ·kg ⁻¹)	18.1	13.1
Mg (cmol ⁺ ·kg ⁻¹)	1.07	1.79
Na (cmol ⁺ ·kg ⁻¹)	0.07	0.11
K (cmol ⁺ ·kg ⁻¹)	0.50	1.14
Fe (cmol ⁺ ·kg ⁻¹)	0.03	0.01
Mn (cmol ⁺ ·kg ⁻¹)	0.73	0.30
Al (cmol ⁺ ·kg ⁻¹)	1.45	0.05

2.2. Sampling and analysis

Soil samples were collected at T0 and T1 in centrifuge tubes and dried at 80 °C for 72h. Alfalfa plants and soil samples were harvested (T2) 28 days (Maron) or 42 days (Bouzule) after transplantation. Pots with Maron soil were harvested earlier due to the poor growth of plants on this soil. Shoots and roots were harvested, gently rinsed with deionized water then dried at 60 °C for 72 h, and the dry weight of shoots and roots of each pot was recorded. Soil samples were collected at T2 in centrifuge tubes and dried at 80 °C for 72 h. All soil and shoot samples were ground in a mortar with a pestle before the analysis.

To analyse the total concentration of Sm in soil, 200 mg of ground soil sample was transferred into an acid-cleaned Teflon tube designed for CEM Mars 5 Digestion Microwave System® and 10 mL of *Aqua regia* (nitric acid: hydrochloric acid =1:3 v/v) was added. Digestion was processed under the adapted programme (15 min digestion at 25°C and 10 min digestion at 170°C). After the digestion and cooling down procedure, the digested samples were filtered (DigiFILTER, SCP Science® 010-500-070), and the filtered solutions were transferred into 15 mL centrifuge tubes, adjusted to 10 mL with ultra-pure deionized water 18 MΩ and preserved at 4 °C till the analysis.

The bioavailable concentrations of Sm in soils were extracted by two different extractants. The DTPA extracting solution consists of diethylenetriaminepentaacetic acid (DTPA) (0.005M), CaCl₂ (0.01M) and triethanolamine (0.1M) (Carter and Gregorich 2007; Kim et al. 2015). One gram of ground soil sample was transferred into a 15 mL centrifuge tube, with 10 mL of DTPA solution and homogenised on a rotary shaker with 99 rpm for 2 hours. Samples were centrifuged at 3227 g for 15 min (20 °C) and the supernatant was filtered in 15 mL centrifuge tubes, adjusted to 10 mL with ultra-pure deionized water 18 MΩ and preserved at 4 °C till the analysis. The second extractant is CaCl₂. One gram of ground sample was transferred into a 15 mL centrifuge tube with 10 mL of 0.01 M CaCl₂ solution with the same homogenization and centrifugation method as above. The supernatant was filtered in 15 mL centrifuge tubes, adjusted to 10 mL with ultra-pure deionized water 18 MΩ and preserved at 4 °C till the analysis.

Dried shoot samples were digested in nitric acid (HNO₃) and hydrogen peroxide (H₂O₂) solution. Six mL of 69% HNO₃ (CAS: 7697-37-2) and 3 mL of 30 % H₂O₂ (w/w H₂O) (CAS: 7722-84-1) were added into DigiTUBEs containing 200 mg of ground shoot sample at 20 °C for a night. Then the DigiTUBEs with the sample solution was placed on DigiPREP MS 48 & TS (SCP Science® 010-500-205 & 010-500-275) hot plate system covered with DigiPROBE Watch Glass (SCP Science® 010-500-081) processing the digestion programme adapted to plant tissue with 5 min solubilization at 45 °C, 10 min pre-digestion at 65 °C and 60 min digestion at 95 °C. After cooling down, the solution was filtered as previously described and kept at 4 °C until the analysis.

The total and the bioavailable Sm concentrations in soil samples and the concentration of Sm in alfalfa shoots were analysed using coupled plasma-optical emission spectroscopy (ICP-OES) analytical technique using a Varian Inc.® (Part A) - 720/730-ES ICP-OES. Calcium (Ca), sodium (Na), potassium (K) and magnesium (Mg) concentrations in alfalfa shoots were also analysed using the same technique and are presented as Supplementary figure 3.1 (Online Resource 1). All the analysis used NCS Certified Reference Material NCS DC 73030 soil (Sm recovery rate = 85.1%) and Labmix24 oriental basma tabacco leaves (track elements) - IC-INCT-OBTL-5 (Sm recovery rate = 70%) as references. The soil-plant transfer factor (SPTF) (Papastefanou et al. 1999; Cao et al. 2000a; Chyi 2003; Šmuc et al. 2012) was calculated as the ratio between Sm concentration in alfalfa shoots and the total concentration of Sm in soils.

Statistical analysis was performed with RStudio software (Version 1.1.463 – © 2009-2018 RStudio, Inc.). One-way and two-way analysis of variance (ANOVA 1 and 2) and the Tukey honestly-significant-difference (HSD) post-hoc test were performed for the significance of the data with a 95% significant level (p-value ≤ 0.05).

3. Results

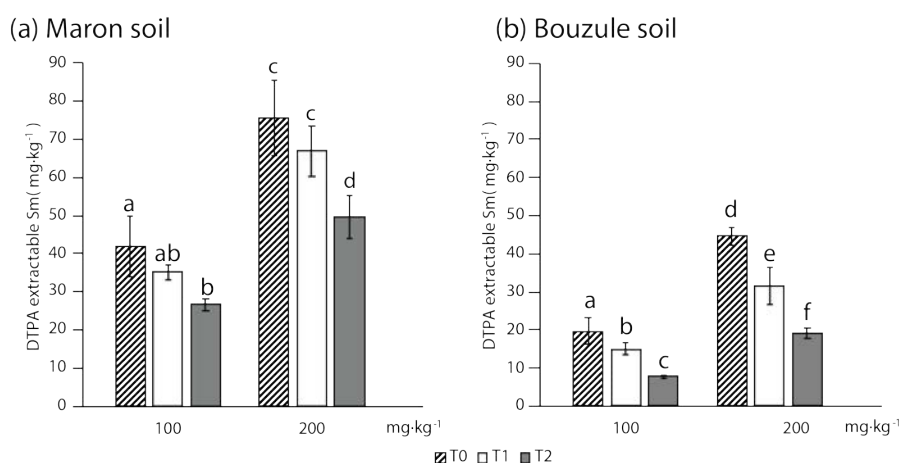
3.1. Total and extractable Sm in both soils

Sm concentration in unspiked Maron and Bouzule soils was $14.83 \pm 0.37 \text{ mg}\cdot\text{kg}^{-1}$ and $4.99 \pm 0.46 \text{ mg}\cdot\text{kg}^{-1}$, respectively. After spiking with 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$ Sm, the concentrations were $82.76 \pm 12.25 \text{ mg}\cdot\text{kg}^{-1}$ and $169.48 \pm 47.11 \text{ mg}\cdot\text{kg}^{-1}$ for Maron soil, and $86.44 \pm 32.62 \text{ mg}\cdot\text{kg}^{-1}$ and $151.49 \pm 63.09 \text{ mg}\cdot\text{kg}^{-1}$ for Bouzule soil, respectively.

Both CaCl_2 and DTPA extractants were used for Maron and Bouzule soils at 3 sampling dates: at spiking (T0), after 2 ageing weeks (T1) and at plant harvest (T2), and in unspiked soils. For Maron soil CaCl_2 extractable Sm was below the detection limit ($0.1 \text{ mg}\cdot\text{kg}^{-1}$) in unspiked soil. In spiked soils, CaCl_2 extractable Sm was $0.2 \pm 0.02 \text{ mg}\cdot\text{kg}^{-1}$ at T0, $0.1 \pm 0.01 \text{ mg}\cdot\text{kg}^{-1}$ at T2 in 100 $\text{mg}\cdot\text{kg}^{-1}$ spiked soil, and $0.39 \pm 0.03 \text{ mg}\cdot\text{kg}^{-1}$ at T0 and $0.18 \pm 0.02 \text{ mg}\cdot\text{kg}^{-1}$ at T2 in 200 $\text{mg}\cdot\text{kg}^{-1}$ spiked soil. For Bouzule soil, CaCl_2 extractable Sm was below the detection limit in all samples.

The DTPA extractable Sm in unspiked Maron and Bouzule soils was also low: $4.16 \pm 0.58 \text{ mg}\cdot\text{kg}^{-1}$ at T0, $4.13 \pm 0.35 \text{ mg}\cdot\text{kg}^{-1}$ at T1 and $3.27 \pm 0.45 \text{ mg}\cdot\text{kg}^{-1}$ at T2 in Maron soil, without significant difference between the 3 sampling dates. Much lower DTPA extractable Sm was found in Bouzule soil with $0.36 \pm 0.05 \text{ mg}\cdot\text{kg}^{-1}$ at T0, $0.30 \pm 0.03 \text{ mg}\cdot\text{kg}^{-1}$ at T1 and $0.20 \pm 0.005 \text{ mg}\cdot\text{kg}^{-1}$ at T2, with a significant difference between T0 and T2.

Figure 3.1 DTPA extractable Sm concentration (mg kg^{-1}) in (a) Maron and (b) Bouzule spiked soils as a function of time (T0=0, T1=14 days, T2 = 42 days (Maron) and 56 days (Bouzule)) and spiked Sm concentration (100 and 200 mg kg^{-1}). Different letters within a soil indicate significant differences (two-way ANOVA, $p < 0.05$).



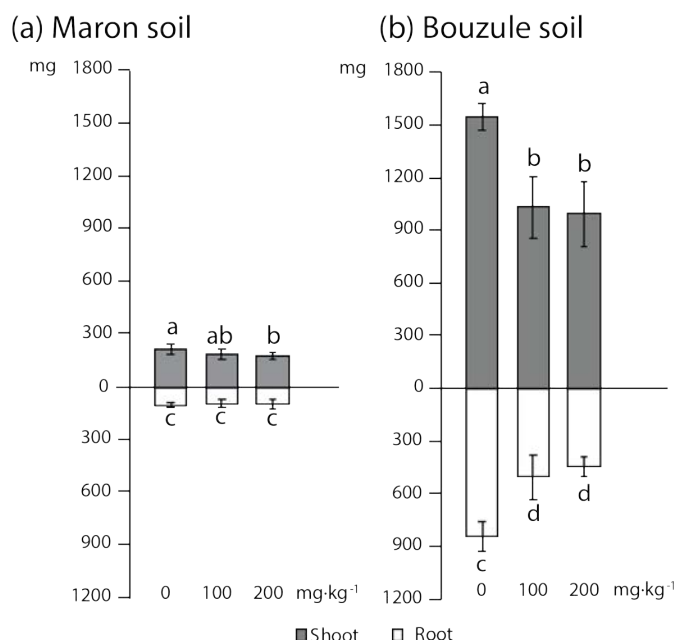
The DTPA extractable concentration of Sm in the spiked soils decreased as a function of time (Figure 3.1). It decreased by 16% and 11% respectively in 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$ spiked Maron soil during the 2-week ageing period from T0 to T1. For Bouzule soil, there was a significant 24% and 29% decrease of DTPA extractable Sm in 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$ spiked soil respectively during the 2-week ageing (T0 to T1). A significant decrease was also observed between T1 and T2, during the 4 weeks' alfalfa growing time, with 24% and 26% decrease respectively in 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$ spiked Maron soil. During the 6 weeks' alfalfa growing time (between T1 and T2), the available Sm concentration in

Bouzule spiked soil also significantly decreased with a 48% and 40% decrease at 100 and 200 mg·kg⁻¹ Sm spiked concentration.

3.2. Alfalfa growth in Sm spiked and unspiked soils

In Maron soil, the shoot biomass showed a significant decrease in the 200 mg kg⁻¹ Sm spiked soil compared to the unspiked control but the root biomass showed no significant difference between control and spiked soils. Shoot and root biomass in Bouzule soil was significantly lower in Sm spiked than in the unspiked soil (Figure 3.2). It was significant already with 100 mg·kg⁻¹ Sm spiked concentration (p-value ≤ 0.05) and no further decrease was observed at 200 mg kg⁻¹. For Maron soil, the shoot biomass showed a significant decrease in the 200 mg kg⁻¹ Sm spiked soil compared to the unspiked control but the root biomass showed no significant difference between control and spiked soils.

Figure 3.2 Shoot and root dry weight (mg) of alfalfa plants in (a) Maron and (b) Bouzule soil as a function of spiked Sm concentration (0, 100 and 200 mg kg⁻¹). Mean ± SD (n= 8). Different letters within a soil indicate significant differences (two-way ANOVA, p<0.05).



3.3. Sm transfer to plants

The concentration of Sm in alfalfa shoots was significantly higher in the spiked soils than in the controls and was twice higher with 200 than with 100 mg kg⁻¹ Sm spiked soils (p-value = 0.05) (Figure 3.3). The soil-plant transfer factor (SPTF) was around 0.05-0.08 in Maron soil, and it was not significantly different between spiked and unspiked soils. However, in Bouzule soil, the SPTF was around 0.02 to 0.035 and was significantly lower in spiked than in unspiked soil (Figure 3.3). The Sm uptake by plants as a function of DTPA-extractable concentration of Sm in soil was also calculated (Figure 3.4) and showed a positive linear correlation (R² = 0.9334, linear regression model).

We analysed P and Ca concentrations in plants. There were no significant differences in P and Ca concentrations in alfalfa shoots between unspiked and spiked soils (data not shown). P and Ca content in shoots was also not significantly different between spiked and unspiked Maron soil. However, in Bouzule spiked soil, the content of P and Ca in shoots was significantly lower than in unspiked soil (Figure 3.5).

Figure 3.3 Sm concentration (mg kg^{-1}) in alfalfa shoots growing in (a) Maron and (b) Bouzule soil as a function of Sm spiked concentration ($0, 100 \text{ mg kg}^{-1}$ and 200 mg kg^{-1}) and soil-plant transfer factor. Mean \pm SD ($n= 8$). Different letters within a soil indicate significant differences (two-way ANOVA, $p<0.05$).

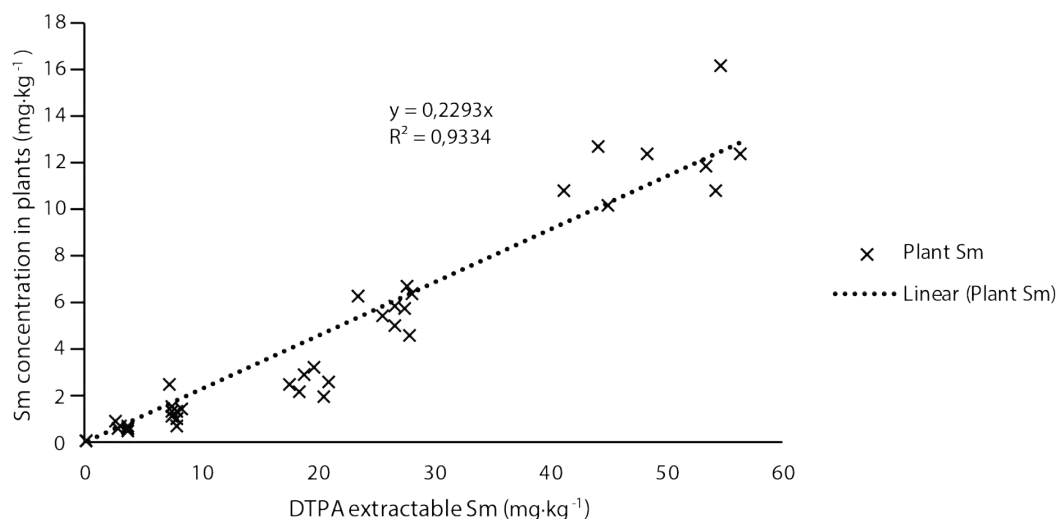
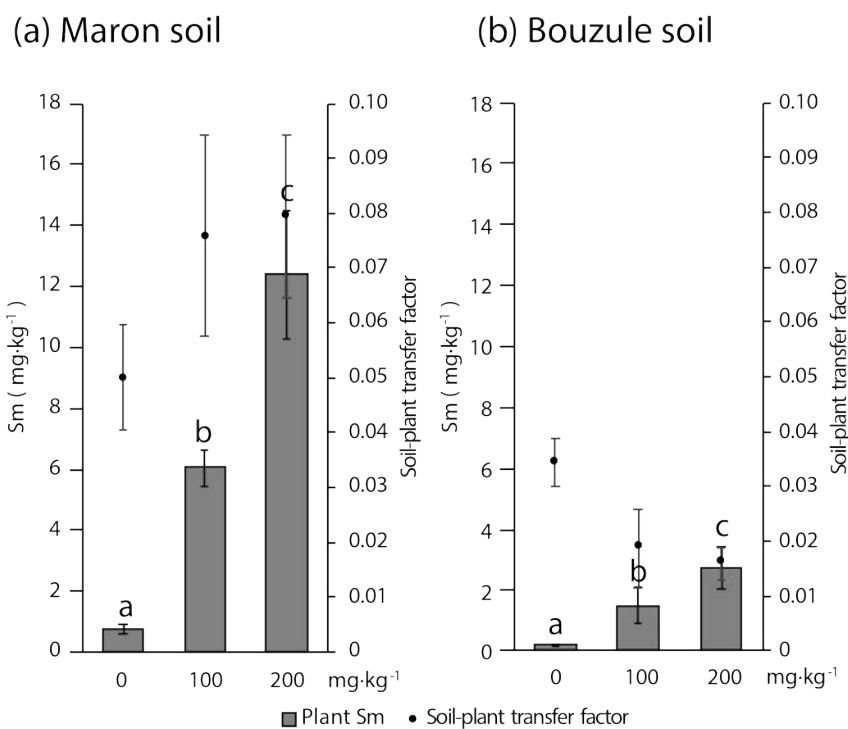


Figure 3.4 Sm concentration (mg kg^{-1}) in plants as a function of the DTPA extractable Sm in spiked and unspiked soils.

4. Discussion

4.1. Assessment of bioavailability of Sm using different extractants

Different extractants and extraction methods have been applied to study the bioavailability of REEs in the soil or their phytoavailability and potential uptake by plants. In our study, we performed a single extraction method using two neutral extractants: DTPA and CaCl₂. Our results showed that CaCl₂ extractant had a low extracting ability, while the phytoavailable Sm in the soil was well predicted by DTPA extraction as the Sm plant uptake accounted for 22.9% of DTPA extractable Sm (with R²=0.93). According to Rao et al. (2010), the bioavailability of REEs can be overestimated by strong acid reagents such as CH₃COOH and HCl. Meanwhile, the complexing extractants such as DTPA and EDTA showed moderate extraction ability and have been applied in different studies. EDTA was used to indicate that 10% to 16% of REEs were phytoavailable in natural soils in central Germany (Loell et al. 2011a, b). EDTA showed a stronger extracting ability than DTPA as it can extract both carbonate and organically bound fractions of metals in soils with low carbonate content (Sahuquillo et al. 2003). For Censi et al. (2017), DTPA gave a good indication of the plant-bioavailable fraction of REEs and other trace elements in rural, urban and industrial soils in Sicily. The neutral salt 0.01M CaCl₂ was recommended as a good extractant of phytoavailability (Menzies et al. 2007; Kim et al. 2015). However, in our study, CaCl₂ could not be used to represent alfalfa available Sm in both soils due to its weak extraction ability, while DTPA showed a good indication of alfalfa available Sm in the soil.

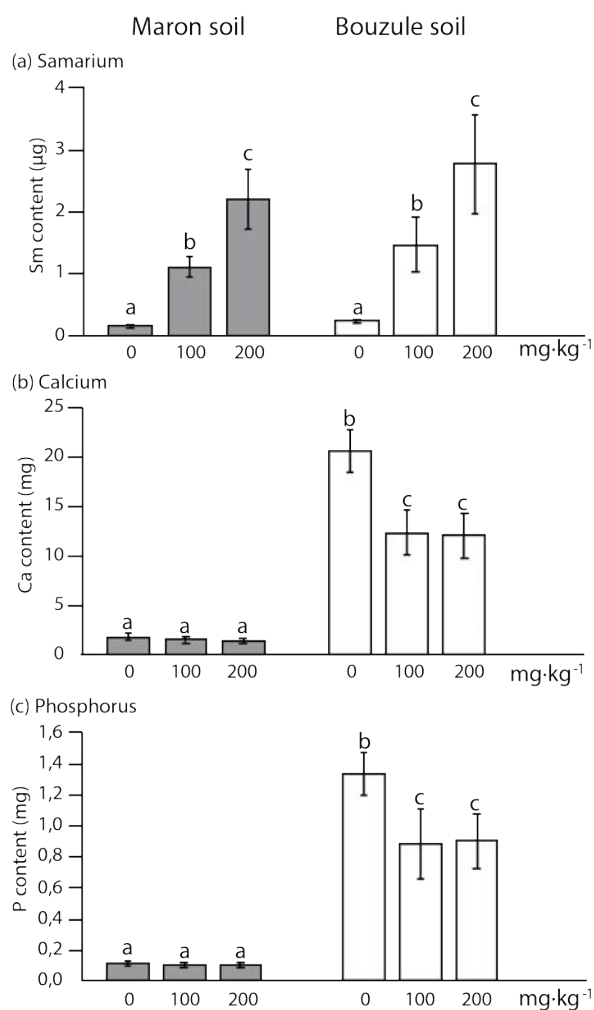


Figure 3.5 Content of (a) Sm (µg), (b) Ca (mg) and (c) P (mg) in alfalfa shoots growing in two soils as a function of Sm concentration (0, 100 ppm and 200 ppm). Mean ± SD (n=8). Different letters within a soil indicate significant differences (two-way ANOVA, p<0.05).

4.2. Bioavailability of high concentration of Sm in soils and influence of ageing

In our study, the bioavailable Sm, estimated by DTPA extraction, accounted for 40% of the total Sm concentration (extracted by *aqua regia*) in freshly spiked Maron soil and 25% in freshly spiked Bouzule soil. Despite the same spiking concentrations in both soils, it was significantly higher in the acidic Maron soil than the more neutral Bouzule soil. In a study of four types of Chinese soils, with both control and spiked ($200 \text{ mg}\cdot\text{kg}^{-1}$ REEs) soils, the red earth with lowest pH had the highest exchangeable REEs (Liang et al. 2005). A higher REE availability in lower pH soil was also reported in a study comparing total REE contents and the potentially bioavailable fraction using EDTA in three different soils in Germany (Diatloff et al. 1995c). Diatloff et al. (1996) found that the $\text{Ca}(\text{NO}_3)_2$ extractable REEs decreased with the soil pH increase when adding CaCO_3 and increased when soil pH decreased by adding $\text{CaSO}_4\cdot 2\text{H}_2\text{O}$. Yufeng et al. (2003) applied artificial acid rainwater to Chinese red soil and showed an increase in the REE concentrations in the soil leaching solution after the acid rainwater application. Our result of higher bioavailable Sm in Maron soil further confirmed pH as an important parameter influencing the bioavailability of REEs.

Despite soil pH and soil composition, the organic matter in soil with a large amount of negatively charged groups is able to adsorb or chelate cations as REEs and thereby decrease their availability and transfer (Tyler 2004a; Loell et al. 2011b; Fiket et al. 2017). In our study, the bioavailability of Sm is higher in Maron soil even though the organic matter was higher than in Bouzule soil, indicating that the pH had a major influence on REE bioavailability. Ca^{2+} was reported to have an influence on the bioavailability of LREE to plant, as LREE are reported to use the same ion channels as Ca^{2+} when entering plant root (Yuan et al. 2017). In our study, there was no indication that Ca concentration influenced the bioavailability of the LREE Sm, but calcium concentration in both soils was similar.

Other than the above-mentioned factors, the ageing significantly affected the bioavailability of Sm in the spiked soils. In our study, the bioavailable fraction of Sm significantly decreased within the two weeks of ageing. Cao et al. (2000b) studied the bioavailability of REEs in natural soils using the same model plant alfalfa (*Medicago sativa*) and sequential extractions. They showed that exchangeable REE fraction contributed to the bioavailable REEs, which explained the decreased bioavailability of REEs, including Sm, with time as REEs were adsorbed to soil particles. In the unspiked soils, the bioavailable fraction of Sm was much lower than in the spiked soils (26 and 6 % for Maron and Bouzule, respectively), which was also observed for natural and spiked soils by Liang et al. (2005). The bioavailable fraction of REEs accounted for only 10.1 % of total REEs and 13.8% of Sm in natural soil (Loell et al. 2011b). We also observed a further decrease of the bioavailable Sm from T1 to T2 down to 25 % in Maron and 9 % in Bouzule soil which could be due to the ageing effect and the presence of alfalfa plants as well. Therefore, REE toxicity studies with spiked soils (Thomas et al., 2014) should include an ageing period of the soil before to introduce the plants.

4.3. Impact of high concentration of Sm on plant growth

Although some studies have mentioned a positive influence of REEs on plant growth at low concentration in soil (Hong et al. 2003; Cheng et al. 2015), other studies reported the inhibition of plant growth and toxic effects of high concentrations REEs to plants (Carpenter et al. 2015). However, most of the studies on the effects of REEs on plant growth were performed in liquid media (Diatloff et al. 1995a, b, c; d'Aquino et al. 2009a) on two or three LREEs (yttrium, lanthanum and cerium) and not in soil. Few data are focusing on the effect of single elements (Pagano 2016). In our study, we focused on the effect of a single REE on alfalfa. REE toxicity and transfer to plants may differ with plant species and REEs. This was observed with elevated La, Y and Ce concentrations in artificial spiked soil where lower IC_{25s} values for Ce and some plants were observed (Thomas et al. 2014). However, we showed a strong decrease, around 30%, of alfalfa shoot biomass when the Sm concentration in soil reached 100 to 200 mg·kg⁻¹. Carpenter et al. (2015) showed that the IC_{25s} of native plants in artificial soils spiked with 6 REEs was between 100 and 300 mg·kg⁻¹ REEs, but the ageing of the contamination was not considered neither in the experiment of Liang et al. (2005) where ryegrass weight was inhibited by spiking REEs on natural soils. Since the spiking was performed with SmCl₃, a potential effect of Cl addition might be considered. Salinity, for example addition of NaCl, can be harmful to alfalfa growth and lead to decreased concentration of shoot K, Ca and P (Li et al. 2010b; Ashrafi et al. 2018). However, compared to these experiments, the added Cl concentration in our study was much lower. Further, the concentration of Ca, and P, and Na and K in plants was not significantly different between SmCl₃ spiked and non-spiked soil (Online Resource 1), suggesting no side effect of the addition of Cl.

4.4. Transfer of Sm to plants

The SPTF of Sm was rather low: 0.05 and about 0.08 in the control and spiked Maron soil, respectively, and 0.035 and about 0.02 in the control and spiked Bouzule soil. The difference between control and spiked soils can be explained by the source and ageing time of Sm. In studies focussing on natural soils, the transfer of REEs to plants, especially to wheat seeds (Liang et al. 2005) or fruits (Cheng et al. 2015) was also low (around 0.03). According to Tyler (2004), the concentrations of REEs in the plant are generally low due to the low SPTF, ranging between 0.04 to 0.09 and even lower in grass (Tyler and Olsson 2001) and vegetables (Bibak et al. 1999). In an experiment using REE-containing coal fly ash amendment He et al. (2019) showed a transfer factor from soil to alfalfa shoots of the light REEs La and Ce, and of the heavy REE Y ranging from 0.01 to 0.10. The transfer factor depended on the element and was higher for Ce than the two other REEs. Our results for Maron soil fit with this range of data and the SPTF is lower in Bouzule soil which was mainly influenced by the availability of Sm in the soil. Moreover, the preferable neutral soil for alfalfa lead to better growth in Bouzule soil and a dilution effect occurred. Other plants reported to be hyperaccumulators, such as *Phytolacca Americana* that can accumulate up to 623 mg·kg⁻¹ REEs or the Chinese fern *Dicranopteris dichotoma*, showed higher SPTF values, up to 75, depending on the soil properties and REE concentrations (Chen et al.

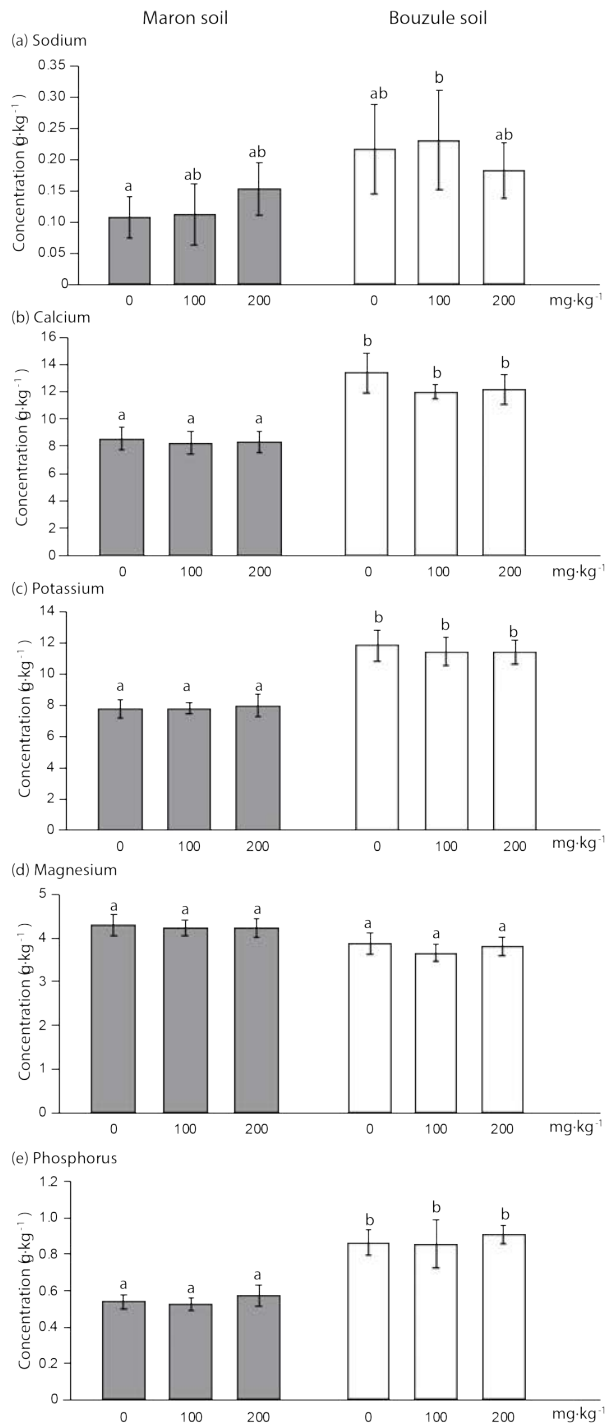
2016; van der Ent et al. 2018). Our study focused on REE transfer from a contaminated soil to a widespread crop plant with rapid growth, where, although the SPTF value is lower, the plant can be strongly impacted by the presence of REEs.

5. Conclusions

Although the SPTF of Sm to alfalfa represented a low percentage, the presence of Sm at high concentration inhibited the growth of alfalfa. In areas with a high natural abundance of REEs, in post-mining sites, or with the increasing risk of uncontrolled landfills, non-adapted plants might then be strongly impacted. pH was a key parameter of the bioavailability of Sm since higher Sm availability and higher SPTF were found in the acid soil. The bioavailable Sm decreased during the ageing process in the soils, suggesting interactions between Sm and soil constituents. A decrease with time of the bio- and phytoavailability of REE in contaminated soils can be expected and help native or crop plants to cope with the presence of REEs. However, in order to further reduce the bioavailability and the transfer to plants of REEs in contaminated soils, and restore such ecosystems, other soil parameters could be considered such as Ca content and organic matter. Rhizospheric processes, such as root exudation or the contribution of root-associated microorganisms, which are known to affect the mobility and availability of metallic trace elements, should also be investigated for REEs.

Acknowledgements

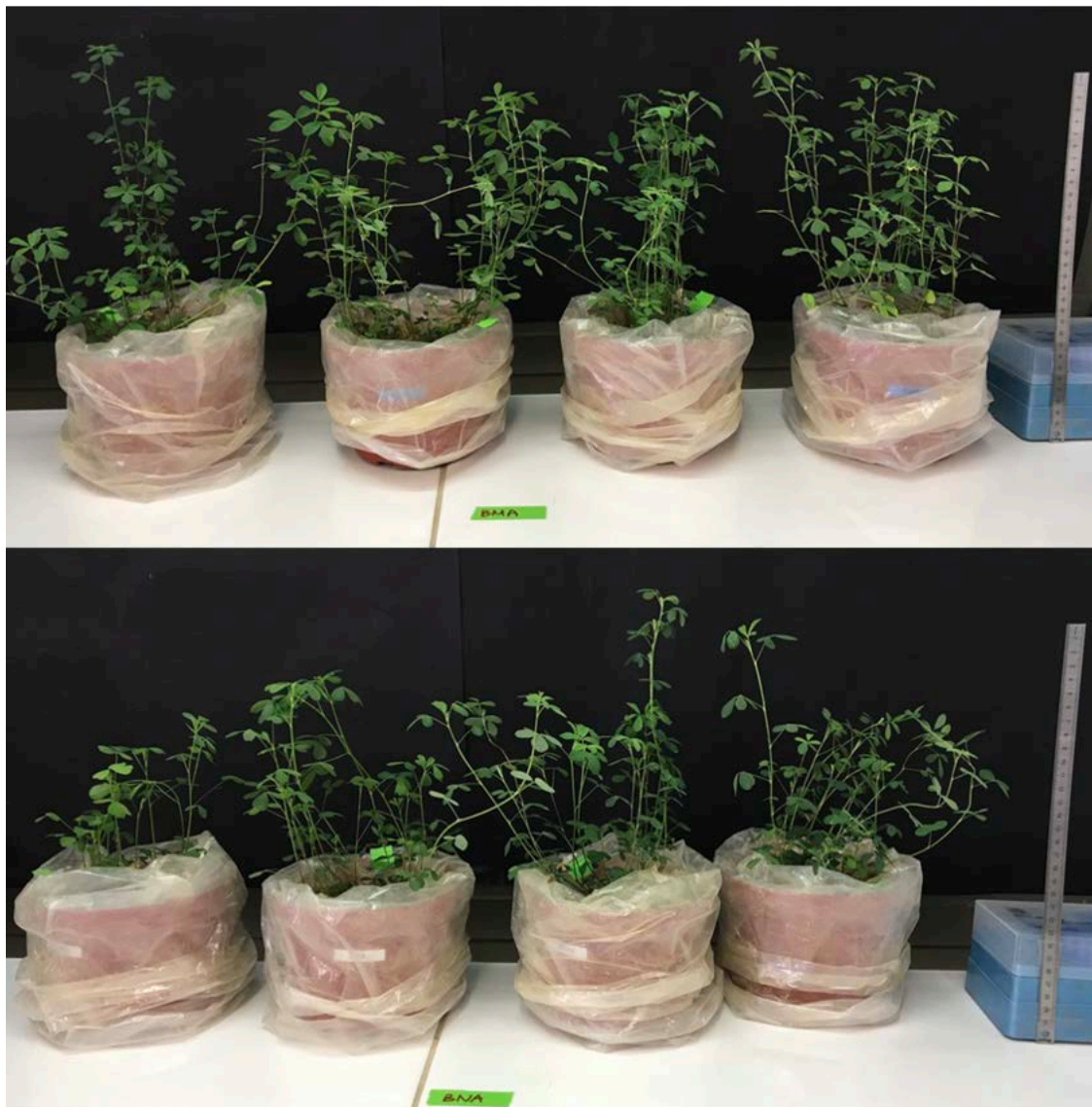
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Supplementary figure 3.1: Concentration of (a) Na, (b) Ca, (c) K, (d) Mg and (e) P (mg kg⁻¹) in alfalfa shoots growing in two soils as a function of Sm concentration (0, 100 ppm and 200 ppm). Mean ± SD (n=8). Different letters within a soil indicate significant differences (two-way ANOVA, p<0.05).

**Chapter 4. No significant transfer of the rare earth element samarium
from spiked soil to alfalfa by *Funneliformis mosseae***

**Chapitre 4. Pas de transfert significatif de la terre rare samarium du sol
enrichi à la luzerne par *Funneliformis mosseae***



Résumé du Chapitre 4

Les champignons mycorhiziens à arbuscules (AM) forment les symbioses mycorhiziennes les plus répandues avec les plantes et sont associés à une grande variété de plantes-hôtes. De nombreuses recherches menées dans des sols contaminés par des métaux ont montré que les champignons AM améliorent largement la croissance des plantes et diminuent les effets toxiques des métaux pour les plantes dans ces sols. Cependant, aucune information n'était disponible concernant le rôle des champignons AM dans les sols contaminés par des terres rares (REE), un groupe d'éléments qui sont de plus en plus largement utilisés dans les nouvelles technologies.

Lors du chapitre précédent, le comportement et la biodisponibilité du Sm dans deux sols ont été étudiés, et un transfert significatif de Sm du sol vers la luzerne a été montré. L'objectif de l'étude, présentée dans ce Chapitre 4, était d'étudier le rôle des champignons AM sur la croissance des plantes et le transfert sol-plante du Sm dans un sol contaminé. Deux expériences en mésocosmes ont été réalisées avec de la luzerne plantée dans un sol artificiellement contaminé avec du Sm :

- une expérience en pot avec la luzerne inoculée ou non avec *Funneliformis mosseae* BEG 69, un champignon tolérant aux métaux, et un sol contaminé avec 100 et 200 mg.kg⁻¹ de Sm ou non contaminé.
- une expérience en pot compartimenté, afin d'étudier plus précisément le transport du Sm du sol vers la luzerne via les hyphes de *F.mosseae*. Les plantes et le champignon AM ont été cultivés dans le compartiment supérieur, avec un compartiment inférieur séparé par une membrane permettant uniquement le passage des hyphes fongiques et contenant du sable contaminé par du Sm (100 mg kg⁻¹).

La biomasse de la luzerne était plus faible dans les sols artificiellement contaminés par du Sm par rapport aux témoins. Mais cette biomasse était plus élevée pour les plantes inoculées par *F.mosseae* que les non mycorhizées, que le sol soit contaminé par du Sm ou non. Aucune différence significative de teneurs en Sm n'a été constatée dans les parties aériennes de la luzerne, mycorhizées ou non, contrairement aux teneurs en P qui étaient nettement plus élevées dans les plantes mycorhizées. L'expérience en pot compartimenté a confirmé l'absence de transfert significatif de Sm dans la luzerne induit par *F. mosseae*.

No significant transfer of the rare earth element samarium from spiked soil to alfalfa by *Funneliformis mosseae*

Ruoyu HU, Thierry BEGUIRISTAIN, Alexis DE JUNET, Corinne LEYVAL*

Université de Lorraine, CNRS, LIEC, F-54000 Nancy, France

* Corresponding author: corinne.leyval@univ-lorraine.fr, tel. 33(0)372745204

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Abstract

Rare earth elements including samarium have been widely used in modern technologies in recent decades. Following over-exploitation and soil contamination, they can accumulate in plants and be toxic at high concentrations. Arbuscular mycorrhizae benefit plants in metal-contaminated soils by improving their survival and growth and alleviating metal toxicity, but little information is available about soil contaminated by rare earth elements. We performed two experiments using samarium to study the role of arbuscular mycorrhizal fungi on plant growth and samarium transfer to alfalfa in a samarium-spiked soil. A pot experiment was conducted in a soil spiked with two concentrations of samarium and a non-spiked control, inoculated or not with a metal-tolerant *Funneliformis mosseae*. A compartmented pot experiment was then performed with a separated compartment containing samarium-spiked sand only accessible by *F.mosseae* fungal hyphae to further study the transport of samarium from the soil to alfalfa. The biomass of alfalfa grown on samarium-spiked soil was reduced, while it was significantly higher following arbuscular mycorrhiza inoculation in the pot experiment, both in the control and samarium-spiked soil. Although mycorrhizal plants had a higher phosphorus content than non-mycorrhizal ones, there was no significant difference in samarium concentrations between mycorrhizal and non-mycorrhizal plants. The compartment experiment confirmed that there was no significant samarium transfer to the plant by *F.mosseae*. Other fungi and plants should be tested, and field experiments performed, but our results suggest that arbuscular mycorrhizal plants might be considered in phytoremediation of rare-earth-contaminated soils.

Keywords

Rare earth element, *Funneliformis mosseae*, Compartment experiment, Metal tolerance

Introduction

Rare earth elements (REEs) are a group of elements that comprise 15 lanthanides and yttrium (Y). Despite their name, they are rather abundant in the earth crust, as abundant as some metallic trace elements (MTEs). Due to exceptional conductivity, magnetic and other properties, REEs have been increasingly used in advanced technologies and industries. The potential anthropogenic input of REEs to natural ecosystems and to the food chain will increase correspondingly in the near future (Jiang et al. 2012; Falandysz et al. 2017). In parallel, the over-exploitation of REE ores can cause a dramatic issue to local environment, with large amounts of REE deposits in soil (Li et al. 2010a; Liang et al. 2014).

Samarium (Sm) is a lanthanide found half-way among the 15 lanthanides in the periodic table. It is the fifth most abundant REE with about 700 tons of Sm oxide production every year (Zhang et al. 2014). It has been used in lasers, magnets, screens, catalysts, and fertilizers (Zhang et al. 2014; Pagano 2016). This wide use has made it one of the REEs with the highest commercial interest (Massari and Ruberti 2013). REEs can accumulate in crops and wild plants and be toxic at high concentrations (Carpenter et al. 2015; Pagano 2016). Sm is not only phytotoxic at high concentrations, but has also been reported to be toxic to mammals (Zhang et al. 2014). The toxicity of REEs depends on their bioavailability, which can be affected by their sources and forms and also by soil parameters like the pH, particle size and organic matter (Babula et al. 2008; MacMillan et al. 2019). Other elements such as calcium can affect the bioavailability of light REEs because the uptake of light REEs by plant roots involves Ca channels (Lansman 1990; Yuan et al. 2017).

Arbuscular mycorrhizal (AM) symbiosis represents the most common symbiotic association between fungi and plants and can be found in the roots of 90% of plants on earth. AM fungi bring remarkable benefits to plant growth, especially in stressful conditions like polluted soils. Many studies have been carried out on the effects of AM fungi on plants growing in MTE-polluted soils: AM fungi decrease MTE concentrations in plants and increase plant biomass (Haselwandter et al. 1994; Leyval et al. 1997; Hildebrandt et al. 2007). This beneficial effect of AM fungi on plant growth and a lower metal concentration in AM plants have been found for cadmium (Cd) in maize (Chen et al. 2004; Chang et al. 2018), alfalfa (Redon et al. 2008), and tobacco (Janoušková et al. 2005). They have also been shown for copper (Cu) in both Cu-mine-tailing soil (Da Silva et al. 2005; Chen et al. 2007b) and spiked soil (Merlos et al. 2016); for zinc (Zn) in maize grown in Zn-spiked soil (Zhang et al. 2010), and in plants growing in Zn waste soil (Turnau et al. 2001); and finally for lead (Pb) (Hovsepian and Greipsson, 2004). AM hyphae transported Cd from the soil to the roots in a compartmented pot and radioactive Cd experiment (Joner and Leyval 1997; Chen et al. 2018). However, no AM transport of caesium (Cs) or europium (Eu) to *Medicago truncatula* occurred using the same experimental approach (Joner et al., 2004). Despite the large number of studies carried out on the effects of AM fungi on plants in MTE-polluted soils, few data are available on the effects of AM fungi on plant growth and REE transfer to

plants in REE-contaminated soils, and such studies concern only Eu and lanthanum (La) (Joner et al. 2004; Chang et al. 2018).

Many studies have compared different AM fungi for their tolerance to MTE and showed that AM fungi isolated from MTE-contaminated soils were more tolerant to Cd and Zn than isolates from non-contaminated soils (Weissenhorn et al. 1993; Joner et al. 2000; Joner and Leyval 2001a; Rivera-Becerril 2002; Rivera-Becerril et al. 2005). Metal-tolerant AM fungi are more efficient to improve plant growth and reduce metal uptake by plants than less tolerant ones (Redon et al. 2009). To date, information on whether AM fungi can also be tolerant to other trace elements such as REEs is lacking.

The objective of our study was to investigate the effect of an AM fungus on plant growth and on REE transfer to plants in an REE-contaminated soil, using Sm as a model REE. We performed two experiments using the model legume alfalfa, inoculated or not with a metal-tolerant *Funneliformis mosseae*. Mycorrhizal and non-mycorrhizal alfalfa plants were first grown in pots with Sm-spiked soil. Then a compartment experiment was performed to determine whether hyphae could transfer Sm to plants. Alfalfa was grown in a top compartment without added Sm, separated from a bottom compartment spiked with Sm by two kinds of mesh to allow hyphal crossing and limit diffusion.

Materials and methods

a. Soil, plant and arbuscular mycorrhizal fungus

The soil used in both experiments was a Luvisol collected (5-25cm deep) in north-eastern France (Bouzule, 48°44'7.95"N, 6°19'9.64"E). The sampled soil was air-dried, ground, sieved to 2 mm and then homogenised and stored in a cool, dry and dark room prior to the experiments. Its physico-chemical characteristics are presented in Table 4.1. The soil was amended and homogenized with 2-mm, pre-sieved and washed sand (30% sand/70% soil) to prevent compaction. Both experiments were carried out using the model legume alfalfa (*Medicago sativa L. var. Europe*), which grows rapidly. The AM fungus used in the experiments was metal-tolerant *F. mosseae* strain BEG 69. It was isolated

Table 4.1 Physico-chemical characteristics of the soil used in the experiment

Soil parameter	Value	Methods
pH	6.68	ISO 10390
sand: silt: clay (%)	167:513:320	NF X 31-107
Organic carbon (g·kg ⁻¹)	15.8	ISO 14235
Total nitrogen (g·kg ⁻¹)	1.65	ISO 14235
Organic matter (g·kg ⁻¹)	27.4	ISO 14235
C/N	9.6	ISO 14235
Olsen P (mg·kg ⁻¹)	91	ISO 11263
Ca (cmol ⁺ ·kg ⁻¹)	13.1	ISO23470
Mg (cmol ⁺ ·kg ⁻¹)	1.79	ISO23470
Na (cmol ⁺ ·kg ⁻¹)	0.11	ISO23470
K (cmol ⁺ ·kg ⁻¹)	1.14	ISO23470
Fe (cmol ⁺ ·kg ⁻¹)	0.01	ISO23470
Mn (cmol ⁺ ·kg ⁻¹)	0.3	ISO23470
Al (cmol ⁺ ·kg ⁻¹)	0.050	ISO23470

from a soil with a 60-year history of industrial metal pollution (Weissenhorn et al. 1993; Tonin et al. 2001), and is kept as a permanent culture on the contaminated soil using leek as host plant in our laboratory.

Experimental design

Pot experiment

Alfalfa was grown in 1 kg pots, with (M) or without (NM) AM inoculation, filled with soil spiked with 2 concentrations of Sm (100 and 200 mg·kg⁻¹, using SmCl₃·6H₂O solution) and a control without Sm addition. Four replicates were prepared for each treatment. The spiking concentrations were chosen according to the results of preliminary experiments (Hu et al. 2020a). After spiking, the soil was dried at 60 °C and thoroughly homogenized. Spiked or non-spiked soil was used to fill the pots evenly, and deionized water was added to reach 70% of water holding capacity (WHC). The pots were stored at 4°C in a dark room (T0) for 21 days of ageing. Soil samples were collected at T0, dried at 80 °C and ground for Sm analysis. After the ageing period, 15 pre-germinated alfalfa (3-4-day-old) seedlings were transplanted into each pot. Prior to transplantation, mycorrhizal pots were inoculated with approximately 200 spores of *F. mosseae* BEG 69 extracted from a pot culture on leek and added to the soil at 3 cm depth. The non-inoculated pots received an equal amount of deionized water at the same depth. The planted pots were transferred to a growth chamber with a 16 h photoperiod, 22°C/18°C day/night, 250 μmol photons m⁻²·s⁻¹, and 80% relative humidity. Plants were watered twice a week to maintain 70% WHC.

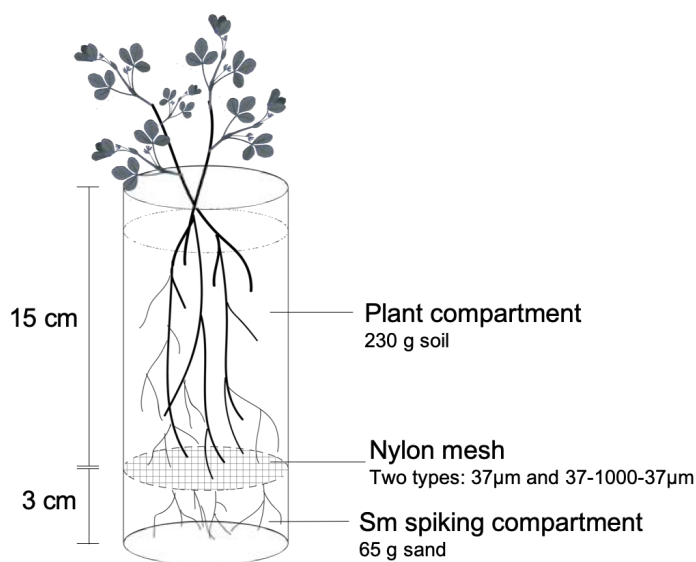
Alfalfa shoots and roots were harvested 49 days after transplantation. The shoot samples were rinsed carefully 4 times with deionized water, then dried, weighed and finely ground for Sm, P, calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) analysis. The root samples were cleaned in the same way and were stained with the trypan blue method to determine mycorrhizal colonization (Koske and Gemma 1989). Thirty 1-cm pieces of stained root segments were sealed on slides, and arbuscular mycorrhizal colonization was observed and determined according to the protocol of Trouvelot et al. (1986).

Compartment experiment

Alfalfa plants inoculated or not with *F. mosseae* were grown in compartmented pots where only AM hyphae could access the bottom compartment containing 100 mg kg⁻¹ spiked Sm. The compartmented pots consisted of a plant compartment made of a 150-mm tall polyvinyl chloride (PVC) tube (50 mm outer diameter, 45 mm inner diameter), and a Sm-spiking bottom compartment made of a 30-mm tall PVC tube with the same diameter separated by a nylon mesh (Supplementary Figure 4.1). Two kinds of nylon mesh were used: a one-layer 37 μm mesh and a 3-layer (37μm, 1000 μm and 37

μm) mesh, called air-mesh below, forming a 0.5 mm air gap between the two compartments (Joner et al. 2004).

The bottom compartment was filled with 65 g of Fontainebleau sand (EINECS: 238-878-4) spiked with $100 \text{ mg}\cdot\text{kg}^{-1}$ Sm ($365 \text{ mg}\cdot\text{kg}^{-1}$ $\text{SmCl}_3\cdot 6\text{H}_2\text{O}$) and deionized water to reach 70% WHC. The main compartment contained 250 g of soil watered with deionized water to reach 70% WHC. Half of the pots were inoculated with approximately 200 spores of *F. mosseae* BEG 69 in the main compartment. Five pre-germinated alfalfa (3-4-day-old) seedlings were transplanted into each pot in the main compartment. Each of the four treatments was carried out in three replicates. All the pots were transferred to the growth chamber with the same conditions as the pot experiment. Plants were watered twice a week to maintain 70% WHC.



Supplementary Figure 4.1 Compartmented pots used in the second experiment

Alfalfa shoots and roots were sampled separately 42 days after transplantation. The shoot samples were rinsed 3 times with deionized water. The root samples were rinsed 3 times with deionized water, then transferred to 5-mM ice-cold CaCl_2 solution for 15 min to remove the soil particles and adsorbed mineral elements from the root surface, and washed with running deionized water (Han et al. 2004). The samples were dried at 60°C and weighed before being ground for Sm, P and Ca analysis. Root samples were blotted dry with paper towels and weighed. One root sub-sample was preserved in 70% ethanol solution to measure arbuscular mycorrhizal colonization as described above. Another root sub-sample was weighed before drying at 60°C , weighed and ground for Sm, P and Ca analysis. Total dry weight was calculated using the total fresh weight along with the fresh and dry weights of the sub-samples.

Sm and nutrient element analysis

The total Sm concentration in the soil samples from both experiments was assessed by *Aqua Regia* digestion. Two hundred mg of ground soil were digested with 10 mL of *Aqua Regia* (nitric acid: hydrochloric acid 1:3 v/v) in an acid-cleaned Teflon tube of the CEM Mars 5 Digestion Microwave System®, and then processed with the digestion program: 15 min digestion at 25°C and 10 min digestion at 170°C. Samples were then filtered (DigiFILTER, SCP Science® 010-500-070), and the volume was calibrated to 10 mL with ultra-pure deionized water 18 MΩ and preserved at 4 °C until analysis.

The soil available Sm concentration was determined using the DTPA extraction method (Carter and Gregorich 2007; Kim et al. 2015) based on preliminary experiments (Hu et al. 2020). One g of ground soil sample was added to 10 mL of DTPA reagent consisting of diethylenetriaminepentaacetic acid (DTPA) (0.005M), CaCl₂ (0.01M) and triethanolamine (0.1M). Samples were homogenized and centrifuged. The supernatants were filtered and calibrated to 10 mL with ultra-pure deionized water 18 MΩ and preserved at 4 °C until analysis.

The ground shoot and root samples (200 mg) were transferred into DigiTUBEs with 6 mL of 69% HNO₃ (CAS: 7697-37-2) and 3 mL of 30 % H₂O₂ (w/w H₂O) (CAS: 7722-84-1) at room temperature overnight. Then, the samples were processed in a DigiPREP MS 48 & TS (SCP Science® 010-500-205 & 010-500-275) hot plate system with a digestion program adapted to plant tissues with 5 min solubilization at 45 °C, 10 min pre-digestion at 65 °C and 60 min digestion at 95 °C. Then, the solutions were filtered and preserved at 4 °C until analysis.

For the pot experiment, shoot Sm, P, Na, Mg, K and Ca concentrations were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES), using certified soil NCS® Reference Material DDC 73030 Soil as a reference. The detection limits for Sm concentrations in the plant and soil samples by ICP-OES were 0.02 and 0.05 mg·kg⁻¹, respectively. For the compartment experiment, Sm, P and Ca were analyzed in the root and shoot samples using inductively coupled plasma mass spectrometry (ICP-MS), using the certified reference material BCR®-667 estuarine sediment (rare elements, Th, U) as a reference. The detection limit for Sm concentration in the plant samples analyzed by ICP-MS was 0.0001 mg·kg⁻¹.

Data analysis

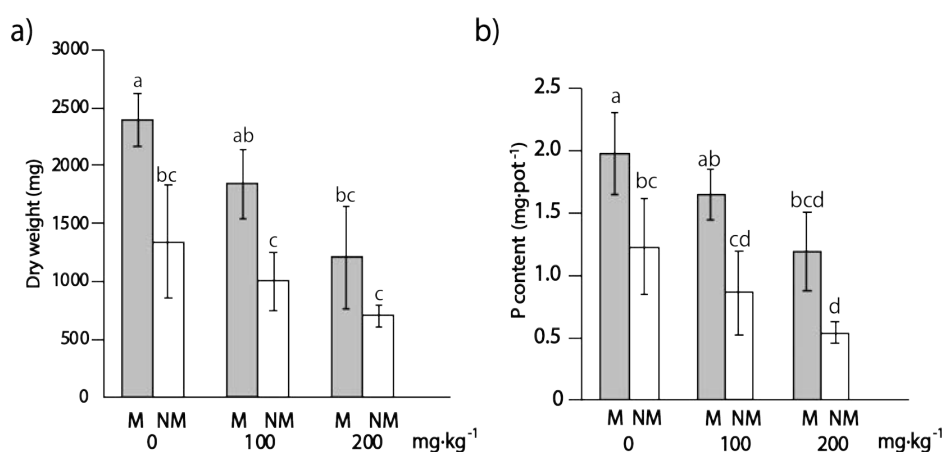
The data obtained in both experiments were analyzed using XLSTAT version 2020.3. Statistical analyses were performed using two-way analysis of variance (ANOVA) and Tukey's honestly-significant-difference (HSD) post-hoc test. For the non-normally distributed data parameters, we used rank-transformed data. Mycorrhizal colonization data were arcsine transformed before statistical analyses. The data presented in the figures are the means ± standard deviations (SDs). *P*-values are given in the figure legends.

Results

Pot experiment

The Sm concentration was analyzed in the soil just after spiking (T0). It was $83.07 \pm 14.88 \text{ mg}\cdot\text{kg}^{-1}$ and $158.48 \pm 24.65 \text{ mg}\cdot\text{kg}^{-1}$ in the 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$ Sm spiked treatments, respectively. In the non-spiked soil, it was $4.47 \pm 0.49 \text{ mg}\cdot\text{kg}^{-1}$. DTPA-extractable Sm in the spiked soil was $27.31 \pm 0.83 \text{ mg}\cdot\text{kg}^{-1}$ and $66.12 \pm 5.61 \text{ mg}\cdot\text{kg}^{-1}$ in the 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$ spiked soils, respectively. In the non-spiked soil, it was $0.53 \pm 0.29 \text{ mg}\cdot\text{kg}^{-1}$.

Figure 4.1 a) Alfalfa shoot dry weight (total per pot) and b) P content in alfalfa shoots as a function of spiked Sm concentration (0, 100 and 200 $\text{mg}\cdot\text{kg}^{-1}$) and with (M) and without (NM) arbuscular mycorrhizal inoculation in pot experiment. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc test ($P < 0.05$)



Alfalfa shoot biomass was significantly higher ($\sim 80\%$) for the mycorrhizal plants grown on the non-spiked and 100 $\text{mg}\cdot\text{kg}^{-1}$ spiked soils than for the non-mycorrhizal ones ($P < 0.0001$) (Figure 4.1a). However, it was significantly lower ($\sim 50\%$, $P < 0.0002$) for the mycorrhizal plants grown on the 200 $\text{mg}\cdot\text{kg}^{-1}$ spiked soil (Figure 4.1a). No mycorrhizal colonization was observed in the non-inoculated plants. Nodules were observed on alfalfa roots in most of the pots, without any clear differences among treatments.

Table 4.2 Mycorrhizal colonization of roots in the two experiments (Trouvelot et al. 1986). Means followed by the same letter do not differ significantly using one-way ANOVA on arcsine transformed data ($p < 0.05$). M: *Funneliformis mosseae* inoculated alfalfa plants, NM: non inoculated alfalfa plant.

	treatment	Mycorrhizal frequency (F%, % of colonized roots)	Mycorrhizal intensity (M%, % of root length colonized)	Arbuscule frequency (a%)	Arbuscule intensity (A%)
Pot experiment	0 M	42.5±29.7 a	16.1±10.2 ab	10.1±7.1 a	2.1±1.8 a
	0 NM	0	0	0	0
	100 M	60.0±20.0 a	30.4±10.9 a	28.7±13.2 b	8.0±2.9 b
	100 NM	0	0	0	0
	200 M	15.0±16.9 a	5.7±7.0 b	2.4±3.2 a	0.1±0.2 a
	200 NM	0	0	0	0
Compartment device experiment	37-M	28.9±8.4	17.3±2.7	32.7±14.8	5.4±1.9
	37-NM	0	0	0	0
	Air-M	25.6±3.8	16.4±5.4	22.8±16.1	3.7±2.4
	Air-NM	0	0	0	0

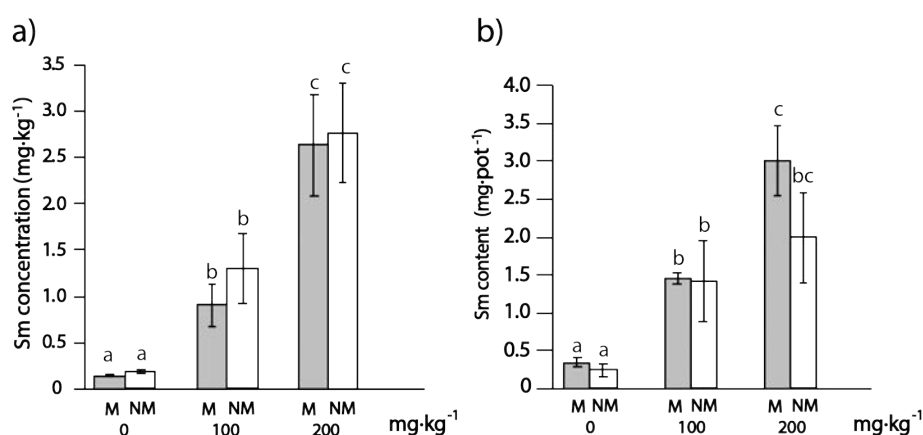
There was a high frequency of mycorrhiza-colonized roots and arbuscules in the inoculated plants, indicating that *F.mosseae* colonized the roots of alfalfa and was active, despite variations among

replicates (Table 4.2). Significantly higher arbuscular frequency and intensity were found in the plants grown on 100 mg·kg⁻¹ Sm spiked soil than in those grown on non-spiked soil. Mycorrhizal intensity, arbuscule frequency and intensity in roots were significantly lower in the 200 mg·kg⁻¹ than in the 100 mg·kg⁻¹ Sm spiked soil, but not significantly different from the non-spiked treatment.

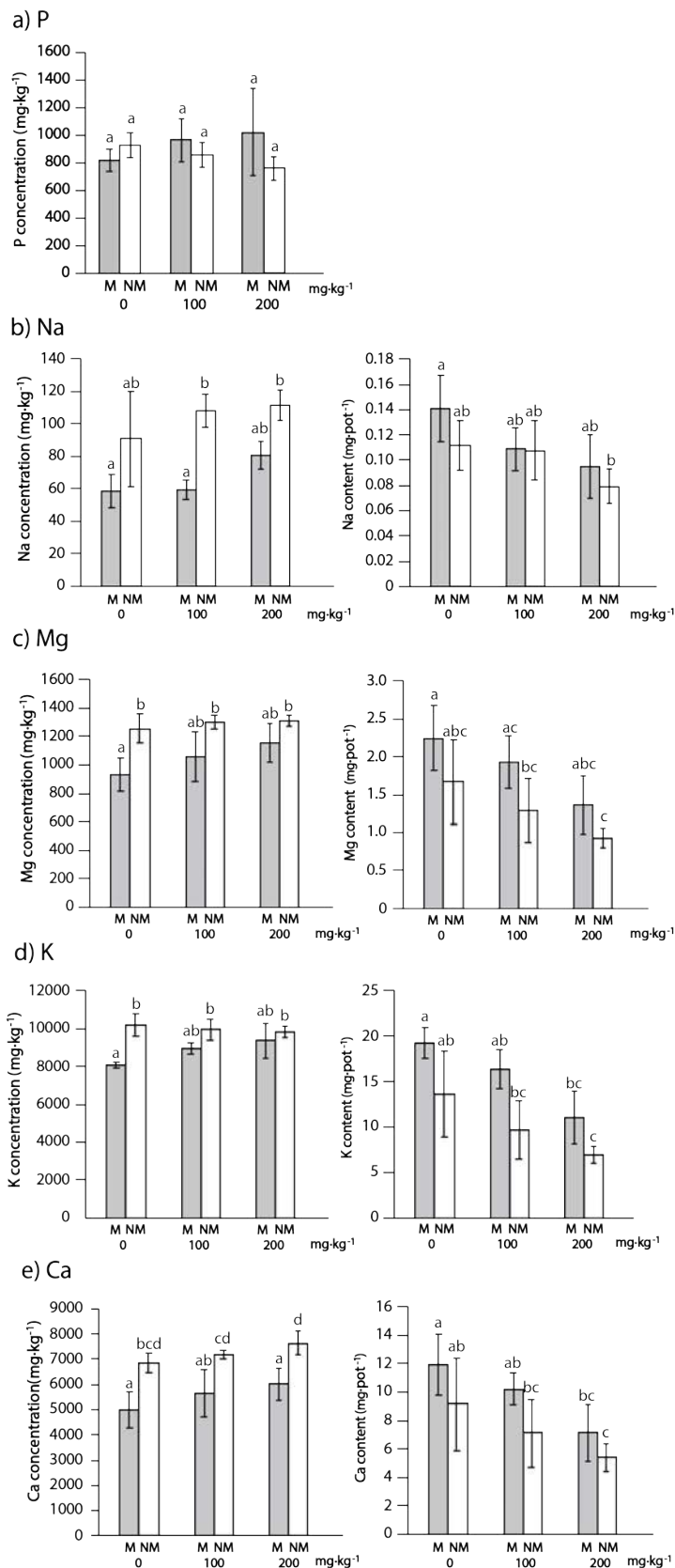
The shoot Sm concentration increased with Sm addition and with the spiking concentration. Sm was detected in alfalfa shoots (0.16 ± 0.02 mg·kg⁻¹) in the non-spiked soil, but it increased to 1.08 ± 0.38 mg·kg⁻¹ in the 100 mg·kg⁻¹ spiked soil and up to 2.70 ± 0.51 mg·kg⁻¹ in the 200 mg·kg⁻¹ spiked soil. There was no significant difference between mycorrhizal and non-mycorrhizal plants (Figure 4.2a and b).

The alfalfa shoot P concentration was not significantly affected by Sm spiking or AMF inoculation, and no significant difference was found among the treatments (Supplementary Figure 4.2). However, the P content was significantly affected by both Sm spiking and AMF inoculation (Figure 4.1b, $P < 0.001$). The shoot P content was significantly higher in mycorrhizal than in non-mycorrhizal plants in the non-spiked and 100 mg·kg⁻¹ spiked soil and not significantly higher in the 200 mg·kg⁻¹ spiked soil. The shoot P content was significantly lower in the plants grown on 200 mg·kg⁻¹ spiked soil than in those grown on non-spiked soil.

Figure 4.2 Sm a) concentration and b) content in alfalfa shoots as a function of Sm spiked concentration (0, 100 and 200 mg kg⁻¹) with (M) and without (NM) arbuscular mycorrhizal inoculation in pot experiment. Means followed by the same letter do not differ significantly using two-way ANOVA on rank-transformed data and Tukey (HSD) post-hoc test ($P < 0.05$)



Shoot Na, Mg, K and Ca concentrations were not affected by Sm spiking, while AMF inoculation significantly decreased shoot Ca concentrations in all Sm treatments, shoot Na concentration in the 100 mg·kg⁻¹ spiked soil, and K and Mg concentrations in the non-spiked soil (Supplementary Figure 4.2). The shoot contents in these elements decreased with increased Sm spiking. However, significant differences in Mg and Na contents were only observed between mycorrhizal plants grown on non-spiked soil and non-mycorrhizal plants grown on 200 mg·kg⁻¹ Sm spiked soil. Shoot K and Ca contents were significantly lower in the plants grown on 200 mg·kg⁻¹ spiked soil than in those grown on non-spiked soil. The shoot contents of these elements tended to increase with AMF inoculation, but differences among treatments were not significant.

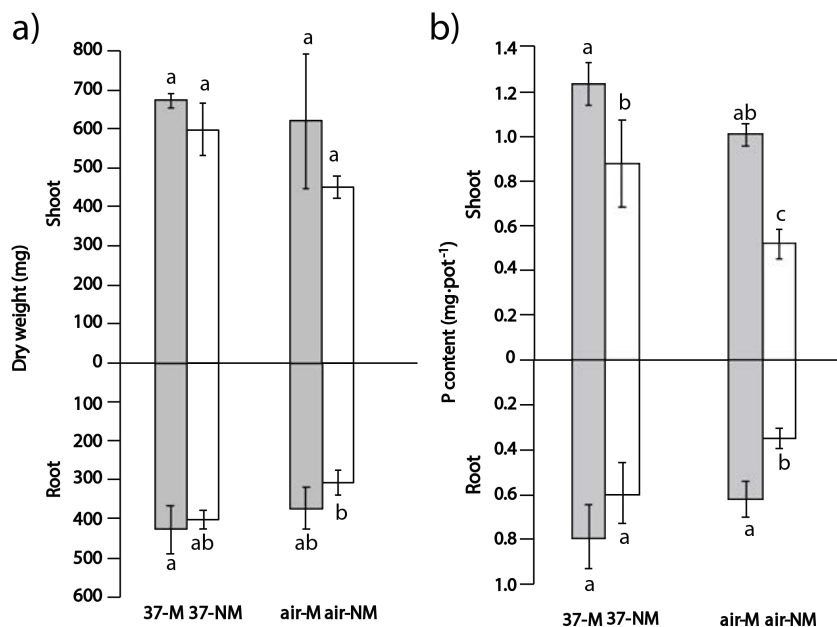


Supplementary Figure 4.2 Concentrations of **a) P b) Na c) Mg d) K e) Ca** and content of **b) Na c) Mg d) K e) Ca** of alfalfa shoots as a function of Sm spiked concentration (0, 100 and 200 mg kg⁻¹) with (M) and without (NM) arbuscular mycorrhizal inoculation in pot experiment. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc tests ($P < 0.05$). Rank transformed data were used for K, Mg and P concentrations.

Compartment experiment

Shoot biomass did not significantly differ among the four treatments in the compartment experiment (Figure 4.3a). Root biomass was significantly affected by mesh size ($P < 0.05$), but it was only significantly different between mycorrhizal plants grown in 37- μm mesh pots and non-mycorrhizal ones grown in air-mesh pots ($P < 0.05$, Figure 4.3a). As in the first experiment, a good level of mycorrhizal root colonization by *F. mosseae* BEG 69 was observed, with the presence of arbuscules in pots with the two kinds of mesh (both above 25% mycorrhizal frequency) (Table 4.2).

Figure 4.3 a) Alfalfa shoot dry weight (total per pot) and b) P content in alfalfa shoots in compartment devices with 37 μm (37) or 37-1000-37 μm (air) mesh with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc tests ($P < 0.05$).

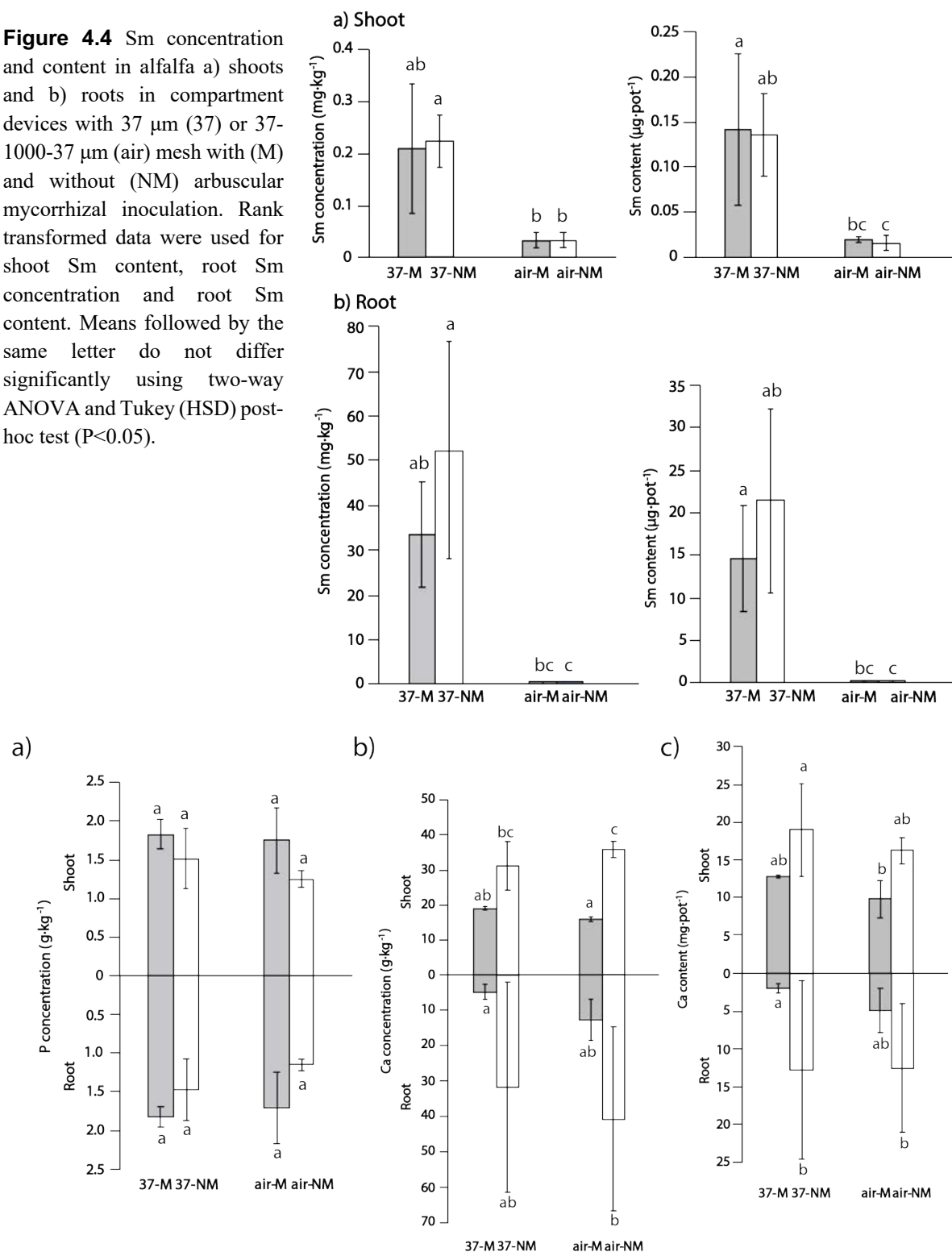


The alfalfa root Sm concentrations and the Sm contents in both alfalfa shoots and roots were not significantly different among the treatments (Figure 4.4), although higher values were observed in the plants grown in the 37- μm mesh pots than in those grown in the air-mesh pots. However, the Sm concentration in alfalfa shoots was significantly higher in the plants grown in the 37- μm mesh pots than in those grown in the air-mesh pots ($F = 28.174$, $P = 0.001$), especially for non-mycorrhizal plants. But no significant difference in Sm shoot and root concentration or content was observed between mycorrhizal and non-mycorrhizal plants.

The P concentration and content in alfalfa shoots and roots were both affected by mycorrhizal inoculation (two-way ANOVA, $P < 0.05$ for shoot concentration and $P \leq 0.006$ for shoot content). However, significant differences were observed only for the shoot content with both kinds of mesh and for the root content in air-mesh pots, with higher values in mycorrhizal plants (Figure 4.3b). The shoot and root P contents were also significantly affected by the mesh type, and significantly higher with the 37- μm mesh than the air mesh for non-mycorrhizal plants (Figure 4.3b). When it comes to the root Ca concentration and content, no significant difference was observed among treatments because of high variance among replicates (Supplementary Figure 4.3). However, the shoot Ca concentration was

significantly lower in mycorrhizal than non-mycorrhizal plants in air-mesh pots (Supplementary Figure 4.3).

Figure 4.4 Sm concentration and content in alfalfa a) shoots and b) roots in compartment devices with 37 μm (37) or 37-1000-37 μm (air) mesh with (M) and without (NM) arbuscular mycorrhizal inoculation. Rank transformed data were used for shoot Sm content, root Sm concentration and root Sm content. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc test ($P < 0.05$).



Supplementary Figure 4.3 a) P concentration b) Ca concentration and c) Ca content of alfalfa shoots and roots in compartmented pots with 37 μm (37) or 37-1000-37 μm (air) mesh with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc tests ($P < 0.05$). Rank transformed data were used for shoot Ca concentration, root Ca concentration and root Ca content.

Discussion

In the pot experiment, the alfalfa shoot dry weight was significantly higher due to AM inoculation, indicating a beneficial effect of *F.mosseae* on alfalfa growth with or without Sm contamination. When the plants were grown on the Sm-spiked soil, although their biomass was reduced at the highest Sm concentration, there was an 80-100% increase of the shoot biomass of *F. mosseae*-inoculated plants as compared to the non-inoculated ones. There was no significant effect of *F.mosseae* on alfalfa biomass in the compartment experiment, but the rootable soil volume was more limited than in the pot experiment and the plants did not grow on a Sm-contaminated soil (only a limited amount of Sm may have been transferred to the main compartments by diffusion). AM fungi had a beneficial effect on plant growth in REE-contaminated soil in a pot experiment with maize and sorghum grown on mine tailing soil with multiple metal contamination including REEs (Guo et al. 2013), where *Glomus versiforme* substantially promoted plant biomass (over 200%). To our knowledge, no other publication has reported such an effect of AM fungi on plant growth in REE-contaminated soils. Large numbers of studies have reported this beneficial effect on plants grown on soils historically polluted by metals. However, results differed depending on plants and AM fungi. Redon et al. (2009) tested the effect of AM fungi on barrel medics (*Medicago trunculata*) grown on heavy metal (Pb, Zn, Cd) polluted agricultural soil; 4 out of the 5 fungi used in the experiment significantly increased shoot weight. Janoušková et al. (2005) studied the effects of two isolates of *G.intraradices* on a variety of tobacco in Cd-spiked soils and found a beneficial effect of AM fungi on tobacco plant growth, with differences among isolates. *Rhizophagus irregularis* helped maize to survive in soil spiked with a high concentration of Cu ($500 \text{ mg}\cdot\text{kg}^{-1}$) by increasing shoot and root fresh weight by 15-90% (Merlos et al., 2016), but results differed depending on the Cu concentration and maize cultivar. For REE-contaminated soils, this beneficial effect of AM fungi on plant growth also may be fungus and host-plant dependent, because another AM fungus – *G.mosseae* – did not increase maize and sorghum growth (Guo et al. 2013).

The improved growth of AM plants is often attributed to nutritional effects, especially P nutrition. Together with the promotion of plant growth, *F.mosseae* increased the P content of alfalfa shoots in our pot experiment. The shoot P content almost doubled in AM-inoculated plants compared to the non-inoculated ones. This increased P content was found even in the highly Sm-contaminated mycorrhizal pots. The same increase in P content was also found in the compartment experiment with the two kinds of mesh, and in alfalfa roots in the air-mesh pots, although plant growth was not increased. In an experiment with REE mine tailings (Guo et al. 2013), *G. versiforme* increased P content by more than 2 fold in maize and 6 fold in sorghum shoots and roots, whereas the other AM species investigated, *G.mosseae*, had no effect.

The beneficial effect of AM fungi on plant nutrition is mainly documented for P, which is a diffusion-limited element in the soil, while the effect on other nutrients is not usually as great. The promotion of nutrient uptake due to AM fungal colonization has been found in mining soil contaminated by Zn, Cd and Pb (Vogel-Mikuš et al. 2005): shoot and root P and S concentrations almost doubled, and the concentrations of mineral nutrients such as K, Ca, Ni and Cu also were significantly higher in AM-inoculated plants. The Ca concentration was analyzed in our experiments because it may interact with REEs. It was not significantly affected in AM-inoculated alfalfa in either experiment. However, the same results were obtained for the concentrations of other mineral nutrients such as K, Mg and Na, suggesting that there was no specific interaction between Ca and Sm uptake. The contents of these elements decreased with increased Sm concentration, probably due to the decreased biomass. In the study of Vogel-Mikuš et al. (2005), the soil was contaminated by several metals, while in our experiment only Sm was present. Chang et al. (2018) showed that maize plants grown in pots in La- and Cd-spiked soil had higher N, P and K concentrations than when grown on single metal-spiked soil and than the controls grown on non-spiked soil. However, there were no increased N, P, K concentrations in plants due to AM symbiosis and even a decrease in some of the treatments. Therefore, further experiments on multi-REE contamination could be considered to clarify the effect of AM fungi on plant nutrient uptake in REE-contaminated soils.

The influence of REE contamination on nutrient element uptake from the soil rarely has been studied, but a few reports indicate that application of REEs decreases nutrient concentrations in plants. La application to maize seedlings decreased root K concentration (Chang et al. 2018), while at a high concentration (>1g REEs/plant, including La, Ce, Pr and Nd) P and Zn uptake by coconut palm was significantly reduced (Wahid et al. 2000). The concentrations of a few plant nutrients (K, Ca, Mg, Zn, Cu, Mn) decreased in wheat seedlings grown on liquid media following exposure to La and Ce (Hu et al. 2002a). In our study, Sm addition had no significant effect on the P concentration in alfalfa shoots, or on the shoot K, Ca, Na, and Mg concentrations. However, compared to experiments with plants grown on nutrient solution (Hu et al. 2002), our study was performed with Sm-spiked soil without any nutrient added. The shoot P, K and Ca contents were significantly lower in the alfalfa plants grown on Sm-spiked soil at the highest Sm concentration, but this could be due to the lower shoot biomass. Wang and Liang (2014) studied the effect of an REE mixture on P adsorption and desorption in 5 different soils and showed that it depended on their respective concentrations and on competitive adsorption between REE and phosphate ions on soil colloids. Grosjean et al. (2019) studied the effects of REEs on the growth and micro- and macro-nutrients of 5 hyperaccumulator *Phytolacca* species; they showed that the leaf P concentration was positively correlated with Sm concentrations, although it was more strongly correlated with heavy REEs. There was no evidence of interactions between Sm and nutrient uptake in plants in our study, but other REEs and soil types should be considered.

Besides promoting plant survival and growth in contaminated soils, AM fungi alleviate metal toxicity by decreasing plant uptake, although conclusions may differ (Pagano 2016). As far as REEs are concerned, no contribution of *G. intraradices* hyphae to Eu uptake by plants has been found (Joner et al., 2004). *Claroideoglossum etunicatum* had no significant effect on root and shoot La concentrations in a pot experiment with spiked soil (Chang et al., 2018). Results differed in a pot experiment with mine tailing soil contaminated with a mixture of REEs and heavy metals, depending on the plant and the fungal species (Guo et al. 2013). *G. versiforme* decreased La, cerium, praseodymium and neodymium in maize roots and shoots, but increased them in the roots of sorghum. However, no data were available for Sm. We observed no significant effect of *F. mosseae* on Sm uptake in either alfalfa shoots from the pot experiment or alfalfa shoots and roots from the compartment experiment. A higher Sm concentration was observed in the plants grown in the 37- μ m mesh pots than in those grown in the air-mesh pots, regardless of the presence of AM fungi. However, this higher concentration was likely induced by Sm diffusion through the single-layer mesh, which was prevented in the air mesh pots by a 0.5-mm air gap. A lot of studies have been carried out on the effect of metal contaminant uptake by AM fungi, but produced different results. Vogel-Mikuš et al. (2006) studied AM fungal colonization of the metal hyperaccumulator *Thlaspi praecox* grown on soil long contaminated by Cd, Zn and Pb. They compared AM-inoculated and non-inoculated plants in a pot experiment: AM colonization significantly decreased Cd, Zn and Pb shoot and root concentrations. The question of the direct uptake of metal pollutants by AM fungi has been a challenging issue because these fungi cannot to be cultured in the absence of host plants and it is difficult to distinguish fungal uptake from root uptake (Leyval et al. 1997). Therefore, some researchers used compartmented pots to only allow fungal hyphae access to the contaminant, and radiolabeled elements to have a strong signal and a low detection limit. A radioelement labeling experiment was carried out with a lateral compartment using ^{109}Cd to study Cd transfer through AM hyphae to plants (Joner and Leyval 1997). The shoot Cd concentration was not significantly influenced by ^{109}Cd spiking, but the root Cd concentration was significantly higher in AM-inoculated roots than in non-inoculated ones. This experiment showed that AM hyphae can transport Cd from the soil to the roots, but Cd translocation from the roots to the shoots was not increased by AM colonization. The hypothesis was that AM fungi can decrease MTE uptake by plants by immobilizing metals in the soil through sorption and by binding metals to intraradical fungal structures (Joner and Leyval 1997; Joner et al. 2000; Chen et al. 2018) or to extracellular polymeric substances possibly including glomalin-like proteins (Wu et al. 2016) reducing their root-to-shoot translocation. Joner et al. (2004) used radiocesium and bottom compartmented pots to study cesium (Cs) transfer by AM fungi to plants. They found no significant difference in Cs radioactivity between mycorrhizal and non-mycorrhizal roots and concluded that there was no significant transfer of Cs from fungal hyphae to plants. On the contrary, radiocesium was present in the roots and shoots of mycorrhizal *Medicago truncatula* plants in *in vitro* culture pots with mycorrhizal plants (Dupré De Boulois et al., 2006). The results of these *in vitro* and *in vivo* studies imply that the sorption capacity of soil clay, K bioavailability

in the soil solution and the plant K nutritional status could explain the different results (Dupré de Boulois et al. 2008). We did not find evidence of any Sm transfer *via* AM hyphae to alfalfa, and the mechanisms involved are to be further investigated, but interactions with soil characteristics may have to be considered.

Among the studies on AM fungi in metal-polluted soils, Leyval et al. (1997) and Hildebrandt et al. (2007) reviewed the tolerance of AM fungi to metals and illustrated that certain AM fungi can adapt to high metal concentrations. *F. mosseae* BEG 69 was isolated from a site polluted with MTEs, and has been cultured since then on that metal-contaminated soil. This *F. mosseae* isolate has been found tolerant to Cu (Weissenhorn et al. 1995), Cd (Weissenhorn and Leyval 1995; Joner and Leyval 1997), and multiple heavy metals (Cd, Zn, Pb) (Redon et al. 2009). The metal-binding capacity of AM hyphae has been shown, especially for metal-tolerant isolates (Joner et al. 2000). This could contribute to explain the beneficial effect of these fungi on plant growth in metal-contaminated soils, as they retain toxic metals in mycorrhizal root structures and reduce metal uptake by the plants. However, the beneficial effect of *G. intraradices* on plant growth in a metal-contaminated soil was instead due to the elevated frequency of N-fixing nodules (Redon et al., 2009). In the work of Janoušková et al. (2005), one isolate was more efficient to promote plant growth in the absence of Cd, while another decreased the negative effect of Cd on plant growth. Therefore, different mechanisms may explain the improved growth of mycorrhizal plants in metal-contaminated soils. In our study, *F. mosseae* BEG 69 was tolerant to elevated soil Sm concentrations. The high colonization rate did not significantly decrease at 200 mg.kg⁻¹. *F. mosseae* BEG 69 even showed higher arbuscule frequency and intensity in 100 mg.kg⁻¹ Sm spiked soil than in the control without Sm added. No information is available on the ability of AM fungal structures to bind REEs, but the compartment experiment showed that there was no significant transfer of Sm through *F. mosseae* BEG69 hyphae.

Conclusion

The AM fungus *F. mosseae* BEG 69 colonized alfalfa roots even at the highest spiked concentration of Sm and significantly promoted alfalfa growth in Sm-spiked and non-spiked soil. This fungus, formerly isolated from MTE-contaminated soil, can tolerate high concentrations of Sm. Other AM fungi from different soils could be tested for their tolerance to REEs, including light and heavy REEs, and their ability to promote plant growth in REE-contaminated soils. This AM fungus increased the P content in both our pot and compartment experiments. The compartment experiment showed that Sm was not transferred from the soil to alfalfa roots by *F. mosseae* extraradical hyphae, in accordance with results previously reported on Eu. In order to have a better understanding of REE transfer in soil-AM fungi-plant systems, further studies should consider a combination of REEs and the interactions between REEs and soil constituents. Other fungi and plants should be tested, and field experiments

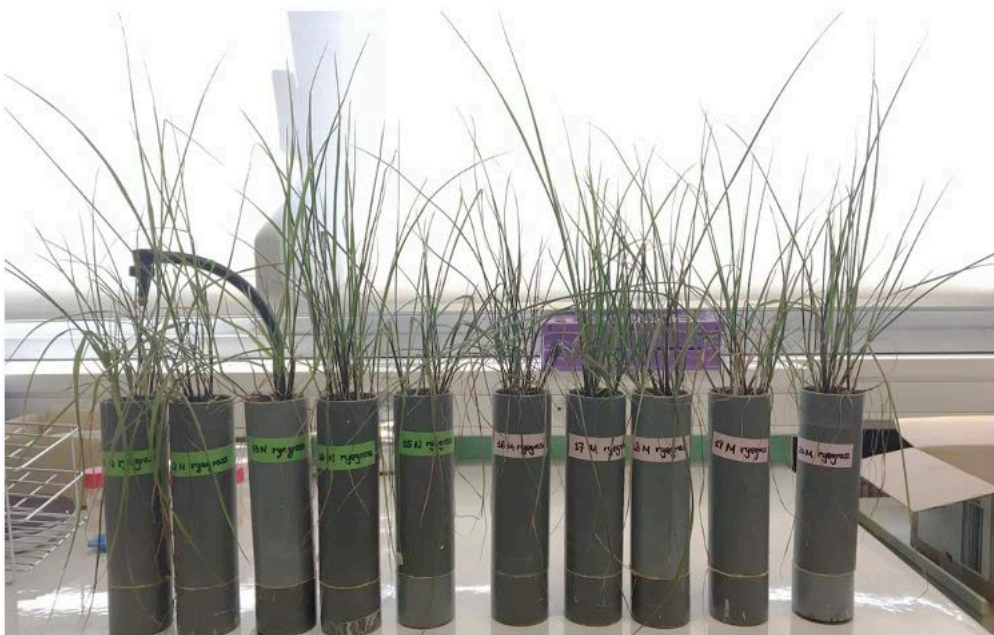
performed, but our results suggest that the beneficial effect of AM fungi on plants in REE-contaminated soils could be considered for applications in phytoremediation or restoration strategies.

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Chapter 5. Transfer of La, Ce, Sm and Yb to alfalfa and ryegrass from spiked soil and contribution of mycorrhizal inoculation with *Funneliformis mosseae*

Chapitre 5. Transfert de La, Ce, Sm et Yb à la luzerne et au ray-grass à partir de sols dopés et contribution de l'inoculation mycorrhizienne avec *Funneliformis mosseae*



Résumé du Chapitre 5

Dans le chapitre 3, la disponibilité du Sm dans le sol et son transfert à la luzerne, en fonction de sa concentration et du sol, ont été montrés. Et les 2 expériences du chapitre 4 ont montré que l'inoculation de la luzerne par le champignon mycorhizien *F.mosseae*, dans un sol artificiellement contaminé par du Sm, permettait d'améliorer la croissance de la plante en favorisant l'absorption de P sans augmenter l'absorption de Sm. Cependant, dans les milieux naturels ou contaminés, les REE sont multiples. Les REE possèdent des caractéristiques proches, mais elles sont souvent divisées en deux groupes : les REE dites légères (LREE, du lanthane au gadolinium) et les REE lourdes (HREE). L'objectif de ce Chapitre 5 était d'étudier (i) le transfert de plusieurs REE, dont des LREE et HREE, du sol vers la plante, et (ii) l'effet du champignon *F.mosseae* sur la croissance et l'absorption de ces REE par les plantes dans un sol contaminé. Nous avons utilisé deux plantes modèles, la luzerne et le ray-grass, et réalisé une expérience en utilisant des pots compartimentés comme au chapitre 4 avec ou sans inoculation avec *F. mosseae* BEG 69, et quatre REE : La, Ce, Sm et Yb. Chaque plante et le champignon MA ont été cultivés dans le compartiment supérieur, tandis que les 4 REE à 100 m.kg⁻¹ ont été ajoutés dans le compartiment inférieur. L'utilisation des pots compartimentés devait permettre de déterminer si les hyphes pouvaient transférer les différents REE aux plantes.

La concentration des 4 REE dans les plantes était plus élevée dans les racines que dans les parties aériennes, avec un facteur de transfert sol-racine plus élevé que le facteur de transfert racines-parties aériennes. Le transfert des deux LREE La et Ce vers les parties aériennes était plus élevé que celui du Sm et du HREE Yb pour le ray-gras, mais ceci n'a pas été observé pour la luzerne. La matière sèche de la luzerne était plus élevée pour les plantes mycorhizées, alors que celle du ray-grass n'a pas été augmentée par la mycorhization. Cependant, pour les deux plantes, une augmentation significative de l'absorption de P due à la mycorhization par *F.mosseae* a été observée. Mais aucun effet significatif de l'inoculation par ce champignon n'a été observé sur l'absorption de La, Ce, Sm et Yb dans les racines et les parties aériennes, confirmant les résultats obtenus dans le chapitre 4. Les champignons AM pourraient donc avoir un effet bénéfique pour les plantes dans des sols contaminés par des REE, en permettant une meilleure croissance et nutrition des plantes, sans contribuer au transfert de LREE ou HREE.

Transfer of La, Ce, Sm and Yb to alfalfa and ryegrass from spiked soil and contribution of mycorrhizal inoculation with *Funneliformis mosseae*

Ruoyu HU, Thierry BEGUIRISTAIN, Alexis DE JUNET, Corinne LEYVAL*

Université de Lorraine, CNRS, LIEC, F-54000 Nancy, France

* Corresponding author: corinne.leyval@univ-lorraine.fr, tel. 33(0)372745204

Abstract

Rare earth elements (REEs) are widely used in high-tech industries and REE waste emission to the terrestrial environment has become a concern to the ecosystem, food quality and human beings. Arbuscular mycorrhizal fungi (AMF) have repeatedly been reported to alleviate the stress of plants in metallic contaminated soils. So far, little information is available concerning the role of AMF on the transfer of REEs in the soil-plant system. We recently showed that there was no transfer of Sm from soil to alfalfa by mycorrhizal hyphae, but a single REE was used, while light and heavy REEs are present in contaminated soils. To understand the role of AMF to the transfer of REEs to plants, an experiment was carried out using a legume alfalfa (*Medicago sativa* L. var. *Europe*) and a grass ryegrass (*Lolium perenne* L., cv. *Barclay*) in compartmented pots with separated bottom compartments containing 4 equal concentration REEs (La, Ce, Sm, Y) that was only accessible by *Funneliformis mosseae* fungal hyphae. The concentration of REEs in plants was higher in roots than in shoots with higher REE soil-root transfer factor than root-shoot transfer factor. Meanwhile, significantly higher LREEs La and Ce were transferred to ryegrass shoots than Sm and than the HREE Yb, but this was not observed for alfalfa. The dry weight of alfalfa was significantly increased by *F. mosseae* inoculation. For both plants, there was significantly higher P uptake in the mycorrhizal than the non-mycorrhizal plants but no significant transfer of La, Ce, Sm or Yb to alfalfa and ryegrass roots or shoots due to *F. mosseae* inoculation. Therefore AMF might be beneficial to plants in REE contaminated soils.

Keywords

Rare earth elements, arbuscular mycorrhizal fungi, transfer factor, LREEs, HREEs

1. Introduction

There is a high and increasing demand for rare earth elements (REEs) that are key components in modern technologies. The REE mining activities and potential REE waste production from industrial activities have increased correspondingly. In addition, REE enriched fertilizers have been widely used

in China and contributed to the REE contamination of the terrestrial environment (d'Aquino et al. 2009b; Carpenter et al. 2015). REEs have been considered as emerging contaminants and many studies were carried out on environmental toxicology and risk assessment of REEs (Barry and Meehan 2000; Thomas et al. 2014; Gonzalez et al. 2014; Carpenter et al. 2015; Li et al. 2018; Gong et al. 2019; Bergsten-Torralba et al. 2020). Toxic effects of high concentrations of REEs to microorganisms (Texier et al. 1999; Bergsten-Torralba et al. 2020) and plants (Diatloff et al. 1995a, c; Carpenter et al. 2015; Hu et al. 2020a) were reported. Meanwhile, it has also been reported that REEs can enter the food chain and accumulate in herbivorous consumers, higher class predators and even human beings (Loell et al. 2011a; Turra et al. 2013). The bioavailability of the REEs is poorly reported (Galhardi et al. 2020), but in general, the bioavailability of REEs is strongly affected by soil properties (Galhardi et al. 2020) and plant species (Brioschi et al. 2013). Even though the toxicity of REEs has been studied, little is known on the transfer of the REEs in the terrestrial environment from soil to microorganisms and plants.

The 15 lanthanides of REEs are often classified as light (LREEs) and heavy (HREEs) rare earth elements according to their electron configuration. The LREEs include lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd); while HREEs comprise terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb) and lutetium (Lu) (Miao et al. 2011). The risk assessment of REEs was mainly evaluated based on the total concentration of the REEs and did not consider a possible difference between LREEs and HREEs. Some studies considered the bioavailability of LREEs and HREEs and their concentration in plants growing in solutions and soils (Brioschi et al. 2013; Grosjean et al. 2019; Galhardi et al. 2020). Grosjean et al. (2019) exposed 5 *Phytolacca* species seedlings grown hydroponically to an equimolar REE solution. They found that LREEs were more preferentially transferred to roots and HREEs were more preferentially transferred to leaves. Brioschi et al. (2013) compared REE transfer to local trees from different non contaminated soils. The soils were enriched in HREEs but there was a LREE enrichment except for Ce anomalies in roots and leaves, although differences were observed between plants and plant parts even on the same site. Galhardi et al. (2020) studied the bioavailability of REEs from soils collected from a tropical coal mining area to soya plants. The LREE concentrations in plant leaves and roots were three-fold higher than HREE ones, but the LREE/HREE ratio differed between the grains, leaves and roots.

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with plant roots of most plant species and are well known to enhance plant growth and nutrient uptake (Smith and Read 2008). Especially in contaminated environments, AMF have been reported to improve plant growth and tolerance to metallic contamination. Many studies were performed using *Funneliformis mosseae* (previously *Glomus mosseae*), a worldwide spread vesicular-arbuscular mycorrhizal fungus, and on different isolates, some of them isolated from contaminated soils (Joner and Leyval 2001b; Tonin et al. 2001; Corgié et al. 2006; Colombo et al. 2019; Hu et al. 2020b). Many studies showed that plants such

as clover and maize growing in zinc (Zn), cadmium (Cd), copper (Cu) contaminated soils were bigger when inoculated by *F. mosseae* (Joner and Leyval 1997, 2001a; Joner et al. 2004; Vogel-Mikuš et al. 2006; Dupré de Boulois et al. 2008). AMF colonized plants contained higher concentrations of Zn, Cd, Cu and P concentration than the nonmycorrhizal plants (Joner and Leyval 2001a). In a study focussing on the colonization of *F. mosseae* of the hyperaccumulator *Thlaspi praecox* in Zn, Cd and lead (Pb) contaminated substrate, *F. mosseae* significantly increased nutrient uptake and decreased Cd and Zn uptake (Vogel-Mikuš et al. 2006). Using a radioactive Cd labelled experiment, it was shown that *F. mosseae* transported Cd from soil to plant roots through the fungal hyphae, but reduced the translocation from root to shoots (Joner and Leyval 1997). Mycorrhizal fungi also enhanced plant Cr tolerance through Cr immobilization in mycorrhizal roots (Wu et al. 2016). For Cs, it was shown that there was a transfer or no transfer from the soil to the plant by AMF depending on the addition of a K containing nutrient solution (Joner et al. 2004; Dupré de Boulois et al. 2008). AMF have been shown repeatedly to bring remarkable benefits to plants in metal contaminated soils and their potential application in soil remediation has been considered (Leyval et al. 1997; Khan et al. 2000; Chibuike 2013). Although many studies reported the AMF effects on plant growth and transfer to plants of metallic trace elements, little information is available on their contribution to REE transfer to plants. No significant transfer of Eu to *Medicago truncatula* was observed due to mycorrhizal inoculation (Joner et al. 2004). Chang et al. (2018) studied the effect of AMF to maize growing in soils spiked with La and Cd and found that maize growing in pots with both La and Cd spiked soil had higher N, P and K concentrations than those in single metal-spiked soil and even higher than the controls grown on non-spiked soil. Using a compartment pot experiment with Sm spiked soil, we found no transfer of Sm from soil to alfalfa by *F. mosseae* (Hu et al. 2020b). However, such studies focussed only on single REE spiking experiments, whereas in contaminated soils all REEs including LREEs and HREEs are present (Mihajlovic and Rinklebe 2018; Galhardi et al. 2020).

The objective of this study was to investigate (i) the transfer of multiple REEs, including LREEs and HREEs, from soil to plants and (ii) the effect of the AM fungus *F. mosseae* on plant growth and REE uptake in an REE contaminated soil. We used two model plants, alfalfa and ryegrass, and performed an experiment using compartmented pots (Hu et al. 2020b) with or without *F. mosseae* inoculation, and four REEs : La, Ce, Sm and Yb. The compartment experiment allowed to determine whether hyphae could transfer the different REEs to plants. Each plant and the AM fungus were grown in the top compartment, while REEs were spiked in the bottom compartment. The two compartments were separated by a nylon mesh that only AM fungal hyphae can cross.

2. Materials and methods

2.1. Soil, plants and fungi preparation

The soil used in this experiment was a Luvisol collected from an agricultural site located in north-eastern France (Bouzule, 48°44'7.95"N, 6°19'9.64"E). The soil was sampled from the top layer (5-25 cm deep), air-dried, ground, sieved (2mm), homogenized and stored in a dark, cold and dry room before the experiment. The physicochemical parameters have been presented in Hu et al. (2020b). Due to the compaction of the soil, prior to the experiment, the soil was mixed with sand (30% sand/70% soil) that has been sieved (2 mm) washed 3 times and dried.

Two model plants, the legume alfalfa (*Medicago sativa* L. var. *Europe*), and the grass ryegrass (*Lolium perenne* L., cv. *Barclay*), have been used in this study. Both plants seeds were washed with deionized water and pre-germinated on sterilized vermiculite.

The AMF used in our experiment *F. mosseae* BEG 69 was isolated from a soil with a 60-year history of industrial metal pollution (atmospheric deposition from smelter). *F. mosseae* BEG 69 strain was kept permanently cultured using leek (*Allium porrum* L.) as host plant in the same contaminated soil in the laboratory growth chamber.

2.2. Experimental setup and sampling

The compartmented pots used in this experiment consisted of 2 compartments made of polyvinyl chloride (PVC) tube with 50 mm outer diameter and 45 mm inner diameter (Hu et al. 2020b). The top compartment was 150-mm high and the bottom compartment 30-mm high. The two compartments were separated by a nylon mesh (37 μm) that only allowed AMF hyphae passing through.

Every bottom compartment was filled with 65 g Fontainebleau sand (EINECS: 238-878-4), then spiked with 100 $\text{mg}\cdot\text{kg}^{-1}$ of La (245.26 $\text{mg}\cdot\text{kg}^{-1}$ LaCl_3), Ce (372.58 $\text{mg}\cdot\text{kg}^{-1}$ $\text{CeCl}_3\cdot 7\text{H}_2\text{O}$), Sm (364.81 $\text{mg}\cdot\text{kg}^{-1}$ $\text{SmCl}_3\cdot 6\text{H}_2\text{O}$), and Yb (387.49 $\text{mg}\cdot\text{kg}^{-1}$ $\text{YbCl}_3\cdot 6\text{H}_2\text{O}$) dissolved in a P-deficient nutrient solution (Redon et al. 2009) to reach 70% water holding capacity (WHC) of the sand. The top compartment was filled with 250 g of Bouzule soil and watered to reach also 70% WHC. In total, 20 compartmented pots were prepared with 4 treatments and 5 replicates for each treatment: non-mycorrhizal and mycorrhizal alfalfa and ryegrass. The 10 mycorrhizal pots were inoculated with around 200 *F. mosseae* BEG 69 spores at 3 cm soil depth and the other 10 pots received the same quantity of deionized water at the same depth. Five pre-germinated alfalfa or ryegrass (3-4-day-old) seedlings were then transplanted into the top compartment. All the pots were transferred into a growth chamber with a 16 h photoperiod, 22°C/18°C day/night, 250 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$, and 80% relative humidity. Plants were watered twice a week with deionized water to maintain 70% WHC. After two weeks, the same P-deficient nutrient solution as described above was added once a week.

The shoot and root samples were harvested 6 weeks after the transplantation. Alfalfa and ryegrass shoots were cut and rinsed 3 times with deionized water then dried at 60 °C for 72h, weighed and then ground for chemical analysis. Alfalfa or ryegrass root biomass was harvested, rinsed 3 times with deionized water and then transferred to 5-mM ice-cold CaCl_2 solution for 15 min in order to

remove the soil particles and mineral elements from the root surface (Hu et al. 2020b). The roots were then rinsed with deionized water, dried and weighed. One subsample was weighed and dried at 60 °C for 72h and then ground for chemical analysis. Total dry weight was calculated using the total fresh weight, fresh subsample weight and dry subsample weight. The other subsample was cut into 1-cm root pieces and preserved in 70% ethanol solution to measure arbuscular mycorrhizal colonization.

2.3. Chemical and mycorrhizal analysis

200 mg ground shoot and root samples were transferred into DigiTUBEs containing 6 mL of 69% HNO₃ (CAS: 7697-37-2) and 3 mL of 30 % H₂O₂ (w/w H₂O) (CAS: 7722-84-1) overnight at room temperature and then digested as described in Hu et al. (2020b). After the digestion, the biological samples were filtered, adjusted to 10 mL with ultra-pure deionized water and preserved at 4 °C until analysis.

The REE (La, Ce, Sm and Yb) and major elements K, Ca, Na, Mg and P concentrations (Supplementary Figure 5.1) in the root and shoot samples were analysed using inductively coupled plasma mass spectrometry (ICP-MS) technique with certified reference material BCR®-667 estuarine sediment (rare elements, Th, U) analysed as a reference. The detection limits for REEs La, Ce, Sm and Yb in shoots and roots are 0.0116, 0.0167, 0.0109, 0.0102 µg kg⁻¹ respectively, and the detection limit for P in shoots and roots is 0.5696 µg kg⁻¹.

The mycorrhizal colonization was determined using cut root samples and the trypan blue method (Koske and Gemma 1989). For each root sample, thirty 1-cm stained root pieces were fixed on slides and observed under microscope. The arbuscular mycorrhizal colonization was determined according to Trouvelot et al. (1986).

2.4. Data analysis

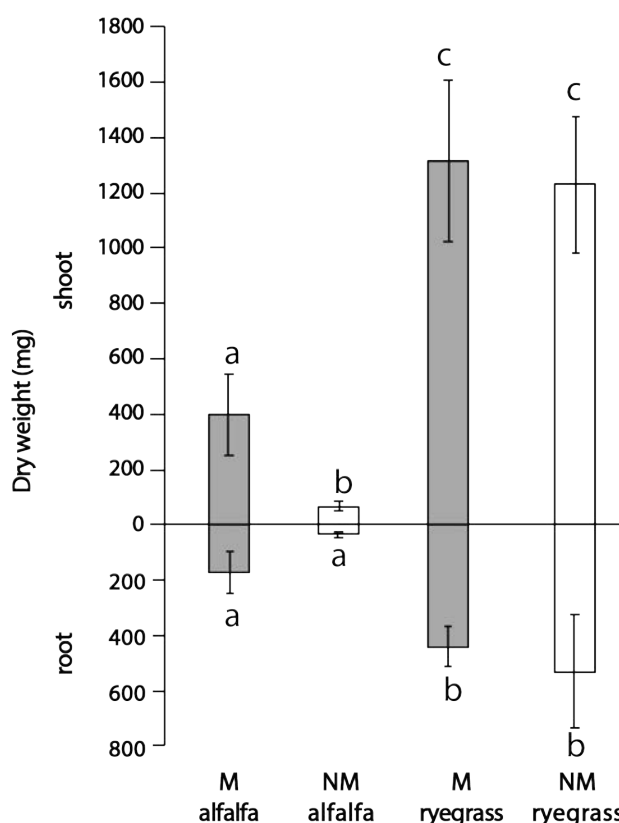
Three transfer factors have been calculated for the four REEs and both plants: soil-root transfer factor (concentration in the plant roots/concentration in the sand) (Tyler 2004b; Brioschi et al. 2013), root-shoot transfer factor (concentration in the shoots/concentration in the roots) (Page et al. 2006; Grosjean et al. 2019) and soil-shoot transfer factor (concentration in the shoot/concentration in the sand) (Adamo et al. 2014; Li et al. 2019; Hu et al. 2020a).

The data were analysed using XLSTAT version 2020.3.1 (Microsoft® Excel version 16.16.22). All tests were used with a 95% significant level. Shapiro-Wilk's tests for normality were performed. When the normality conditions were not met, a rank-transformation was performed. Data were analysed using one-way or two-way analysis of variance (ANOVA 2) and Tukey's honestly-significant-difference (HSD) post-hoc test as precised in the table and figure legends. Prior to the data analysis, all the percentage data (transfer factors, arbuscular mycorrhizal colonization) were arcsine (value<1) or logarithmic log(x+1) (value >1) transformed.

3. Results

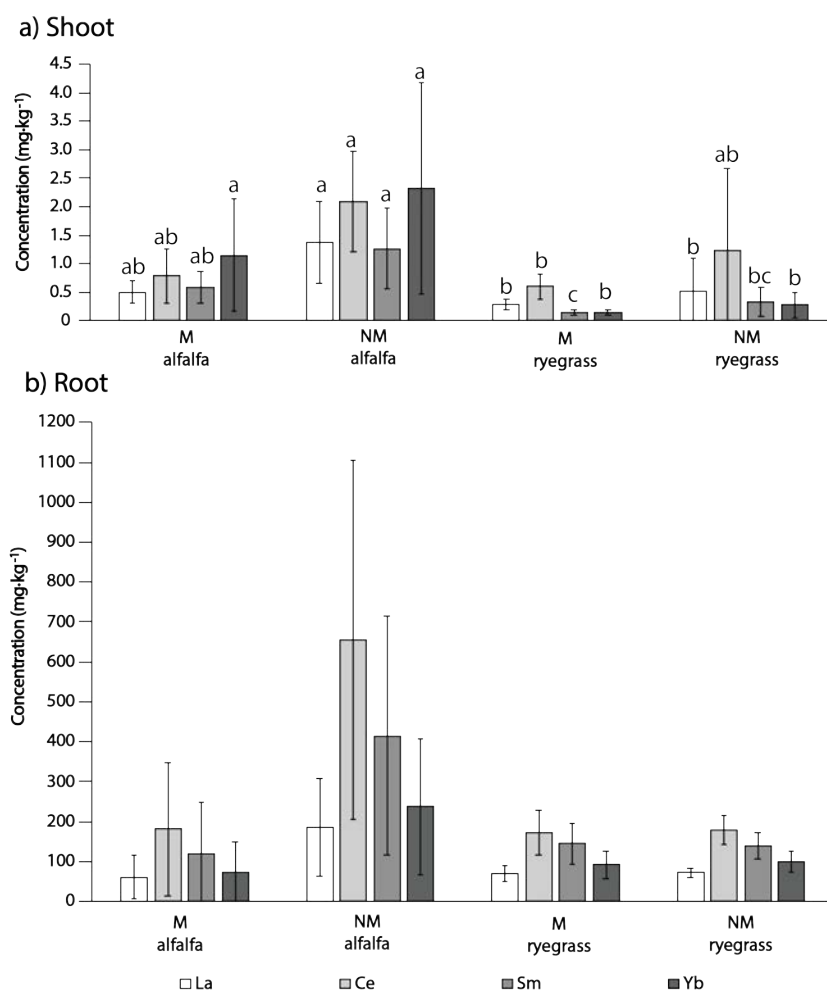
The ryegrass dry weight was significantly higher than that of alfalfa for both shoots and roots, for both mycorrhizal and non-mycorrhizal treatments (Figure 5.1). The mycorrhizal inoculated alfalfa had a significantly higher shoot dry weight than non-mycorrhizal alfalfa. However, there was no significant difference in shoot and root dry weights between mycorrhizal and non-mycorrhizal ryegrass. There was a high frequency of AM-colonized roots for both plants, with a high frequency and intensity of arbuscules.

Figure 5.1. Dry weight of alfalfa and ryegrass shoots and roots (total per pot) with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc test ($P < 0.05$). Rank transformed data were used for shoot weights.



The average concentrations of La, Ce, Sm and Yb in alfalfa and ryegrass roots were 100-fold higher than in their shoots (Figure 5.2). There was no significant difference in the root REE concentrations among the four treatments. For the shoots, Sm and Yb concentrations were significantly higher in mycorrhizal alfalfa than in mycorrhizal ryegrass. Similarly, the shoot concentrations of La, Sm and Yb were significantly higher for non-mycorrhizal alfalfa than non-mycorrhizal ryegrass. However, there was no significant difference in La, Ce, Sm and Yb concentrations between mycorrhizal and non-mycorrhizal plants (Figure 5.2).

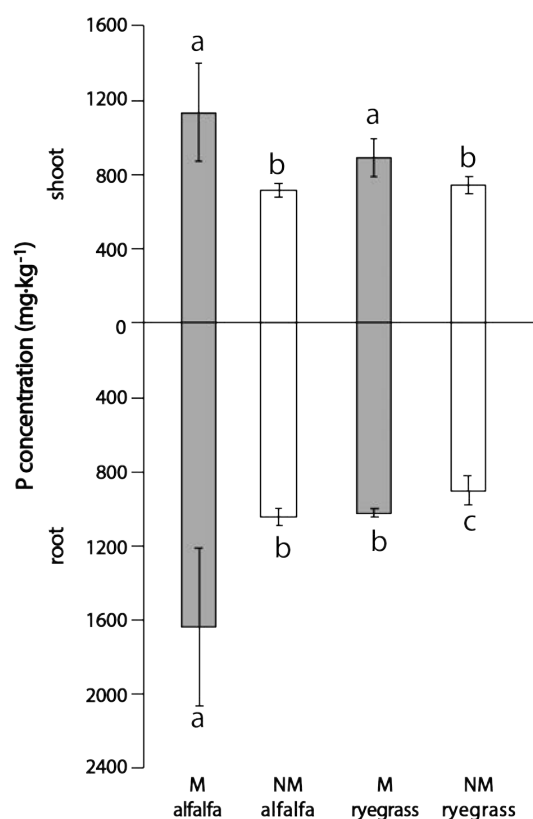
Figure 5.2. La, Ce, Sm and Yb concentrations in alfalfa and ryegrass a) shoots and b) roots with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly among treatments using two-way ANOVA on rank transformed data and Tukey (HSD) post-hoc test ($P < 0.05$).



P concentrations were significantly higher in mycorrhizal than non-mycorrhizal plants, for both shoots and roots and both plant species (Figure 5.3). Shoot and root P concentrations in mycorrhizal alfalfa were about 30 % and 40 % higher than in non-mycorrhizal alfalfa, respectively. For ryegrass, root and shoot P concentrations were about 20% higher in mycorrhizal than in non-mycorrhizal plants.

The average root-shoot transfer factors of La, Ce, Sm and Yb ranged between 0.6% and 2.9% in alfalfa and between 0.1% and 0.7% in ryegrass (Table 5.2). There was a significant difference between plants for Sm and Yb root-shoot transfer factors with higher values for alfalfa than ryegrass. But there was no significant difference in the root-shoot transfer factors between the mycorrhizal and non-mycorrhizal plants. Comparing all the REEs showed that the root-shoot transfer factor of La was higher than the Sm one for ryegrass. For mycorrhizal inoculated ryegrass, there was a significantly lower transfer of Sm than La and Ce, and a significantly lower transfer of Yb than La (Table 5.2).

Figure 5.3. P concentration in alfalfa and ryegrass shoots and roots with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly using two-way ANOVA on rank transformed data and Tukey (HSD) post-hoc test ($P < 0.05$)



The average soil-shoot transfer factors of the four REEs ranged between 0.5% and 2.3% in alfalfa and between 0.1% and 1.2% in ryegrass (Table 5.3). The soil-shoot transfer of Sm and Yb differed between plants and was significantly higher in alfalfa than ryegrass, with no significant difference between the mycorrhizal and non-mycorrhizal plants (Table 5.3). For mycorrhizal inoculated ryegrass La and Ce soil-shoot transfer factors were significantly higher than that of Sm and Yb.

The average soil-root transfer factors of the four REEs ranged between 61% and 656% in alfalfa and between 69% and 178% in ryegrass (Supplementary Table 5.1). There was no significant difference between both plants and between non-mycorrhizal and mycorrhizal plants. However, comparing the soil-root transfer factors of the four REEs showed that Ce transfer factor was significantly higher than that of La and Yb for both mycorrhizal and non-mycorrhizal ryegrass (Supplementary Table 5.1).

Table 5.1 Mycorrhizal colonization of alfalfa and ryegrass roots according to Trouvelot et al (1986). Means \pm SD followed by the same letter do not differ significantly using one-way ANOVA on arcsine transformed data ($P < 0.05$). M, *Funneliformis mosseae* inoculated alfalfa plants; NM, non-inoculated alfalfa plants

	Mycorrhizal frequency (F%, % of colonized roots)	Mycorrhizal intensity (M%, % of root length colonized)	Arbuscule frequency (a%, content of arbuscules in colonized part of roots)	Arbuscule intensity (A%, content of arbuscules in total root system)
M-alfalfa	56.0 \pm 19.1 a	47.3 \pm 15.1 a	99.3 \pm 1.5 b	46.9 \pm 14.6 a
NM-alfalfa	0	0	0	0
M-ryegrass	73.3 \pm 10.3 a	38.8 \pm 5.7 b	90.3 \pm 4.7 c	35.1 \pm 6.5 a
NM-ryegrass	0	0	0	0

Table 5.2 Root-shoot transfer factors (%) of La, Ce, Sm Yb for alfalfa and ryegrass. Means \pm SD followed by the same letter do not differ significantly among treatments (lowercase letters) using two-way ANOVA and among REEs using one-way ANOVA (capital letters) on arcsine transformed data ($P < 0.05$). M, *Funneliformis mosseae* inoculated alfalfa plants; NM, non-inoculated alfalfa plants

Transfer coefficient (%)	La	Ce	Sm	Yb
M-alfalfa	1.53 \pm 1.12	0.78 \pm 0.50	1.23 \pm 1.06a	2.89 \pm 2.16a
NM-alfalfa	1.34 \pm 1.25	0.56 \pm 0.49	0.98 \pm 1.46a	2.08 \pm 2.52a
M-ryegrass	0.43 \pm 0.16A	0.37 \pm 0.18AB	0.11 \pm 0.05 b C	0.18 \pm 0.10 b BC
NM-ryegrass	0.67 \pm 0.58	0.62 \pm 0.56	0.25 \pm 0.25ab	0.31 \pm 0.33b

Table 5.3 Soil-shoot transfer factors (%) of La, Ce, Sm Yb for alfalfa and ryegrass. Means \pm SD followed by the same letter do not differ significantly among treatments (lowercase letters) using two-way ANOVA and among REEs using one-way ANOVA (capital letters) on arcsine transformed data ($P < 0.05$). M, *Funneliformis mosseae* inoculated alfalfa plants; NM, non-inoculated alfalfa plants

Transfer coefficient (%)	La	Ce	Sm	Yb
M-alfalfa	0.50 \pm 0.20	0.79 \pm 0.47	0.59 \pm 0.29ab	1.15 \pm 0.99a
NM-alfalfa	1.38 \pm 0.72	2.10 \pm 0.88	1.26 \pm 0.71a	2.32 \pm 1.85a
M-ryegrass	0.29 \pm 0.09 A	0.60 \pm 0.23 A	0.15 \pm 0.05c B	0.14 \pm 0.05b B
NM-ryegrass	0.53 \pm 0.56	1.23 \pm 1.43	0.32 \pm 0.26bc	0.27 \pm 0.22b

Supplementary Table 5.1 Soil-root transfer factors (%) of La, Ce, Sm Yb for alfalfa and ryegrass. Means \pm SD followed by the same letter do not differ significantly among REEs using one-way ANOVA (capital letters) on arcsine transformed data ($P < 0.05$). M, *Funneliformis mosseae* inoculated alfalfa plants; NM, non-inoculated alfalfa plants

Transfer coefficient (%)	La	Ce	Sm	Yb
M-alfalfa	60.86 \pm 55.29	181.69 \pm 167.02	120.84 \pm 127.08	71.50 \pm 78.65
NM-alfalfa	184.81 \pm 122.59	655.84 \pm 448.99	415.29 \pm 299.52	237.52 \pm 169.83
M-ryegrass	69.23 \pm 20.91A	171.26 \pm 56.18C	144.17 \pm 51.16BC	91.54 \pm 34.82AB
NM-ryegrass	72.43 \pm 11.42A	178.23 \pm 36.18C	139.33 \pm 33.32 BC	99.02 \pm 27.29AB

4. Discussion

4.1. The effects of mycorrhizal colonization on plant growth and nutrient uptake

It has been shown in the present study that arbuscular mycorrhizal inoculation with *F.mosseae* has largely improved alfalfa shoot and root biomass. However, AM inoculation of ryegrass did not significantly improve its growth with a similar mycorrhizal colonization level as alfalfa. This illustrates

that the legume plant alfalfa is more AMF dependent than the grass plant ryegrass. This result is in accordance with previous research concerning the influence of AMF on different species (Scheublin et al. 2007; Yang et al. 2016b).

With the design of the experiment (compartmented pots) the plants were not directly impacted by the presence of the four REEs, but there was probably a limited diffusion between the bottom and upper compartment. However the mycorrhizal hyphae were able to reach the REE spiked compartment with a high REE concentration (400 mg kg⁻¹). The results showed that this AM fungus was able to survive and perform symbiotic activity even in the REE contaminated conditions, which is consistent with previous studies (Joner et al. 2004; Guo et al. 2013; Chang et al. 2018; Hu et al. 2020b).

For both plant species, significantly higher P concentrations were found in mycorrhizal than non-mycorrhizal plants for both shoots and roots. The promotion of plant P uptake by AMF mycelium is a well-known effect of AMF (Smith and Read, 2008), that contributes to increase plant growth. The ability of AMF to promote plant nutrient uptake under metallic contaminated environments have been reported in many studies. For example, Vogel-Mikuš et al. (2006) reported that AMF promoted *Thlaspi praecox* Wulfen growth in a highly contaminated substrate spiked with Cd, Zn and Pb by increasing plant biomass and nutrient (P and S) uptake. Another study investigated the effect of AMF mixtures on the growth of *Metrosideros laurifolia* in a soil containing high concentrations of Ni, Co, Cr, and Mn (Crossay et al. 2019). The results showed almost doubled P concentration in plant shoots with AMF inoculated plants than the control ones. However, there has been little investigation on plant nutrient uptake by AMF in REE contaminated soils. Chang et al. (2018) studied the effect of AMF on maize plants growing in La and Cd spiked soil and found no increased N, P, K concentrations in maize in mycorrhizal treatments and even a decrease in some conditions. In an experiment studying Eu transfer to *Medicago truncatula* by *G.intraradices* (Joner et al. 2004), and where P was added in the bottom compartment, AMF inoculation increased P uptake. In our previous study using the same compartmented pots as in the present study (Hu et al. 2020b), there was no significant increase of P uptake in mycorrhizal alfalfa. However, there was no addition of a nutrient solution in the soil and the sand compartment, and likely a limitation of nutrients in the soil. In the present experiment, a nutrient solution was added in the sand compartment and after two weeks in the plant compartment explaining the different result. This increased P uptake in mycorrhizal plants compared to the non-mycorrhizal controls shows an efficient and functional symbiosis between *F.mosseae* and both plants.

4.2. Contribution of *F.mosseae* to the transfer of the different REEs to alfalfa and ryegrass

According to our knowledge, scarce information can be found concerning the AMF in terrestrial environments contaminated with REEs and the role of AMF on the transfer of REEs to plants. Our previous study carried out in Sm spiked soil found no significant difference between the mycorrhizal and the non-mycorrhizal alfalfa concerning the Sm transfer to plants. According to our

knowledge there is no previous data available on the transfer concerning both LREEs and HREEs in mycorrhizal plants, but our results show that for La, Ce, Sm and Yb they were not affected by the mycorrhizal inoculation. Previous studies using compartment devices with spiked soil showed significantly higher Cd concentrations in mycorrhizal than non-mycorrhizal roots, indicating a transport of Cd by the hyphae from soil to roots (Joner and Leyval 1997). Using also compartment devices, Joner et al. (2004) found no significant contribution of AMF to transfer of Cs and Eu to barrel medic, although there was a transfer of P to the plants indicating an efficient and functional symbiosis, as in the present study. Therefore, the behaviour of AMF towards the transfer of REEs to plant may differ from the transfer of nutrients and of transition metals. Our results on La, Ce, Sm and Yb then suggest that LREEs and HREEs were not transported by AM hyphae as observed for the LREE Eu (Joner et al. 2004).

The fungus used in our study was originally isolated from a heavy metal contaminated soil and was shown to be tolerant to high concentrations of Cd and Zn (Weissenhorn et al. 1993; Redon et al. 2008). Our previous pot study showed that *F.mosseae* was tolerant up to 200 mg kg⁻¹ of Sm and colonized well alfalfa roots with promotion of the plant growth (Hu et al. 2020b). In the present study, this metal tolerant *F.mosseae* BEG 69 strain survived in the 400 mg kg⁻¹ REE mixture condition and well colonized both plant roots. Since we did not compare different AMF species and isolates in this study for their contribution to the transfer of REEs to plants, we cannot conclude on its relative tolerance to REEs compared to other fungi. But preliminary spore germination tests using REE contaminated soils did not reveal significant difference between *F.mosseae* BEG 69 and the non-metal tolerant *F.mosseae* BEG12 (data not shown).

4.3. The transfer of the different REEs to plants

The four REEs were quantified in the roots and shoots of both plants. As reported in the literature for other plants (Hu et al. 2002b; Wahid et al. 2003; Yuan et al. 2017; He et al. 2019), there was a much higher concentration of the four REEs in the roots than in the shoots of alfalfa and ryegrass and consequently the soil-root transfer factors were higher than the soil-shoot ones. The root-shoot transfer factor in alfalfa ranged between 0.5 and 5% depending on the REEs in our study, which was lower than in the study of He et al. (2019) (10-20%).

Brioschi et al. (2013) studied the transfer of REEs from non-polluted natural sites to tree species and found that both soil-root transfer factors and leaf/needle-soil transfer factors ranged between 10⁻³ and 10⁻¹ depending on the soil and species. In our study, REEs were experimentally added in a sand compartment, but our soil-shoot transfer factors were in the same range of data. Higher soil-root transfer factors were found in our study which is likely caused by the higher availability of spiked soluble REEs in the sand than the in non spiked soil in the study of Brioschi et al. (2013) and the possible slight root induced diffusion of REEs from the bottom to the upper compartment. However, compared to our previous study with alfalfa growing in Sm spiked soil where the soil-shoot transfer factors ranged

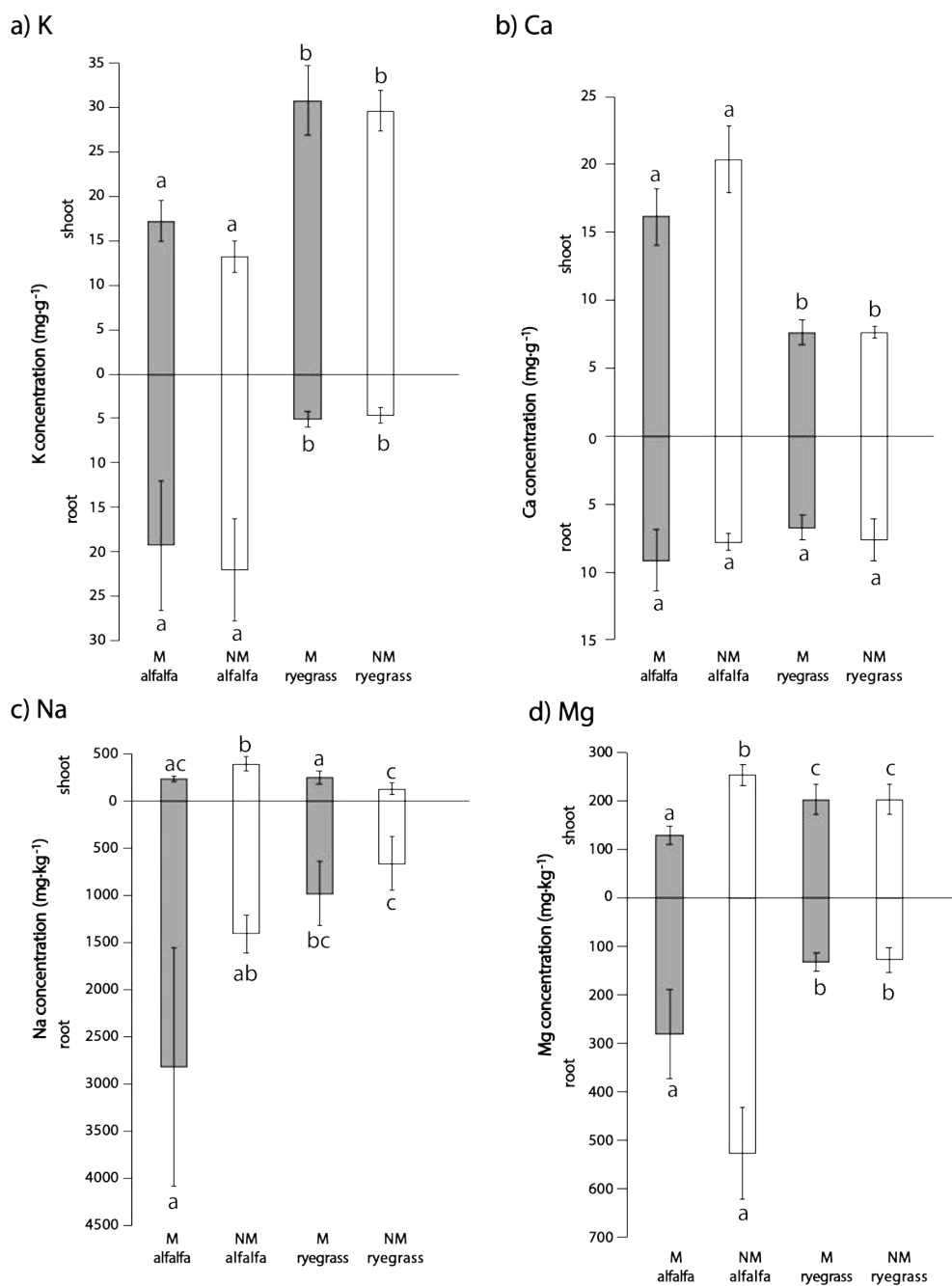
between 2 and 3.5% (Hu et al. 2020a), the soil-shoot transfer factors for Sm in the present study (0.59 to 1.26 %) were much lower due to spiking of REEs in the bottom compartment with nylon mesh separation. He et al. (2019) added REE enriched coal fly ash to different soils to study the bioaccumulation and the transfer of REEs to two legume plants, alfalfa and erect milkvetch, in pot experiments. The La, Ce, Sc, Y concentrations were significantly higher in the soils but not significantly increased in the plants. In our previous study (Hu et al. 2020b) the spiking of Sm in the soil did significantly increase the REE concentrations in the studied plant alfalfa.

In the study of Brioschi et al. (2013), an enrichment of LREEs was found in the carbonate site soil but not in the plant roots nor leaves. Slightly higher LREE compared to HREE transfer factors were observed between tree roots/trunks/leaves and soils. He et al. (2019) found more LREEs than HREEs in the soils and coal fly ash and the REEs followed the order $Ce > La > Y > Sc$, but the HREEs had higher bioaccumulation factor (plant-soil transfer factors) than the LREES with $Sc > Y > La > Ce$. In our study where four REEs were spiked at the same concentration in the sand compartment, we also found that the LREEs La and Ce were more transferred to shoots than Sm and than the HREE Yb, but only for ryegrass. La and Ce, as typical LREEs, were repeatedly reported being more bioavailable and preferred to accumulate in plant tissues (Brioschi et al. 2013; Grosjean et al. 2019, 2020; Galhardi et al. 2020). Sm which is located at the end of LREEs has been considered as middle REEs in some studies (Ding et al. 2005; Fiket et al. 2017; Martinez et al. 2018). A study carried out in 49 hardy fern species grown in a mixed peat substrate spiked with equimolar concentration of LREEs (La, Ce and Sm) and HREEs (Gd, Yb and Y) found that LREEs were preferentially transferred to the fronds regardless of species (Grosjean et al. 2020). Similar LREE preferences were also found in soya roots and leaves grown in tropical coal mining soils with more than 75% LREEs in plant roots and leaves (Galhardi et al. 2020). However, the preference of HREEs was found in soya grains (>95% HREEs) (Galhardi et al. 2020). For alfalfa, we did not find a difference in the soil-shoot or root-shoot transfer factor between the four REEs, but there was a higher transfer of Sm and Yb than for ryegrass, indicating different behaviour among plants.

For the soil-root transfer there was a significant Ce preference over La and Yb for ryegrass. In the study He et al. (2019) higher Ce concentrations were found in alfalfa and erect milkvetch plants over other REEs (La and Y). However, due to the high Ce concentrations in the soils, the Ce transfer factor was lower than other REEs. Hu et al. (2002) found no selective wheat uptake of Ce and La when they were added in combination. In the study Brioschi et al. (2013) lower Ce concentrations were found in soil water (of limestone site), causing the lower Ce concentrations in the roots of 2 out of 3 plant species collected in the site. These different results concerning Ce uptake are probably caused by the source and the speciation of the REEs in the soil and the root absorption ability of the different plant species.

5. Conclusion

The REE concentrations in alfalfa and ryegrass were higher in roots than in shoots. Significantly higher soil-root than root-shoot transfers were found, suggesting that the root absorption was stronger than the root to shoot translocation. Despite the same and high level of AMF colonization of alfalfa and ryegrass and the same promotion of P uptake, indicating that *F.mosseae* colonized and form an efficient symbiosis with both plants, a significant growth promotion was observed only for alfalfa. Therefore our results for REEs cannot be associated to a plant growth effect of the AMF. Our results showed that REE root to shoot translocation can differ between plants as observed for Yb, but was not affected by mycorrhizal colonisation. Indeed, as previously observed for Sm and alfalfa, there was no significant increased or reduced transfer of La, Ce, Sm or Yb to alfalfa and ryegrass roots or shoots due to *F. mosseae* inoculation. Since AMF could promote plant growth and nutrient uptake in REE contaminated soils, and would not contribute to REE transfer to the plants, their potential use in restoration and remediation projects could be considered. However, further experiments using historically REE contaminated soils and comparing different AMF and plants should be performed.



Supplementary Figure 5.1 a) K b) Ca c) Na and d) Mg concentration in alfalfa and ryegrass shoots and roots with (M) and without (NM) arbuscular mycorrhizal inoculation. Means followed by the same letter do not differ significantly using two-way ANOVA and Tukey (HSD) post-hoc test ($P < 0.05$). Rank transformed data were used for K, Ca, Na and Mg concentrations in plant roots.

Chapter 6. Availability and toxicity to AM fungi of REE contaminated soils from ionic mine tailings in China

Chapitre 6. Disponibilité et toxicité pour un champignon MA de sols contaminés par des REEs provenant de résidus miniers en Chine



Résumé du Chapitre 6

Les chapitres précédents de cette thèse ont étudié le transfert des REE dans l'environnement terrestre vers les plantes et leur transfert par les champignons MA dans des sols naturels dopés. Dans les sites contaminés par les REE tels que les sites miniers et les sites de résidus, les REE ont un temps de vieillissement plus long et un processus géologique et chimique plus compliqué, et la disponibilité des REE peut être différente par rapport aux sols dopés. Grâce à une collaboration avec l'université Sun Yat-sen et le projet ECOLAND, nous avons pu travailler sur des sols présentant de fortes teneurs en terres rares (jusqu'à 800 mg.kg⁻¹) issus d'un site minier de la province de Jiangxi, en Chine. Deux sols ont été prélevés dans des zones minières abandonnées depuis 12 et 16 ans, et deux autres dans des zones forestières situées à proximité et qui sont utilisées comme sols témoins (Control). L'objectif du travail présenté dans le Chapitre 6 était de déterminer la disponibilité et la toxicité des REE de sols historiquement contaminés sur des champignons AM. La biodisponibilité des REE a été déterminée en utilisant les mêmes méthodes d'extraction que dans le Chapitre 3, avec des extractions au CaCl₂ et au DTPA. La toxicité des REE de ces sols miniers a été évaluée dans deux expériences, la première par un test de germination normalisé avec deux champignons MA *F. mosseae* BEG69 (tolérant aux métaux) et BEG12 (non tolérant aux métaux), et la seconde portant sur la colonisation fongique de poireaux inoculés avec *F. mosseae* BEG12 et cultivés en pots avec 0, 20, 50 et 100% d'un des sols contaminés.

Les teneurs en REE extraites par CaCl₂ et DTPA étaient globalement élevées dans les quatre sols; mais elles sont néanmoins plus élevées dans les sols miniers abandonnés que dans les sols Control, excepté pour Ce. Les deux *F. mosseae* BEG 69 et BEG 12 sont capables de germer dans un sol minier abandonné, malgré une forte inhibition de leur germination avec 100% de ce sol. Il n'y a toutefois pas de différence significative dans la germination de *F. mosseae* BEG69 et BEG12, ce qui suggère qu'une tolérance aux métaux n'induit probablement pas de tolérance préférentielle aux REE. Enfin, la colonisation des racines de poireaux par *F. mosseae* BEG 12 était élevée dans les différents essais, sauf pour les sols miniers abandonnés et Control non dilués, pour lesquels les plantes avaient des difficultés à survivre et où la mycorhization ne pouvait donc pas être mesurée. L'isolement de champignons MA de sols miniers contenant des REE, qui pourraient être tolérants à ces REE et seraient adaptés aux conditions locales, pourrait ainsi être intéressante pour des études futures de remédiation assistée par les plantes.

Availability and toxicity to AMF of REEs contaminated soils from ionic mine tailings in China

Abstract

In the previous chapters, experiments have been launched using natural soils spiked with REEs in order to understand the availability of REEs in soil and their transfer to plants (Hu et al. 2020a) and the transfer of REEs from soil to plants by arbuscular mycorrhizal fungi (Hu et al. 2020b, Chapter 4). In the present chapter, we investigated historically contaminated soils with a mixture of REEs, their availability and toxicity to these AMF. Two soil samples were collected from mine tailings abandoned 12 to 16 years ago (“Aband”) and two soil samples originated from unmined forested areas (“Control”). The bioavailability of REEs in soils was analysed using the same extraction methods as in Chapter 4. *F. mosseae* spore germination tests were performed with two different isolates metal-tolerant or not, using the soil with the highest concentration of total and bioavailable REEs (Aband_2003 soil). Two other soils (Aband_2007 and Control_1 soils) were used in a pot experiment investigating the toxicity of REEs to *F. mosseae* colonization. The results showed that the DTPA and CaCl₂ extractable REEs are rather high in the four soils, and are higher in abandoned mine soils than in the control soils for all REEs except Ce. In the spore germination test, the high percentage of abandoned tailing soil inhibited the germination of both *F. mosseae* BEG 69 and BEG 12, however, both *F. mosseae* isolates are able to germinate in 100% Aband_2003 soil. There was no significant difference in germination of the metal tolerant and the non tolerant *F. mosseae* with increasing concentration of the REE contaminated soil, suggesting that transition metal tolerance does not provide REE tolerance to the fungus. REE tolerant AMF could be isolated from REE mining sites and compared to fungi from non-contaminated ones. In the pot experiment, even in the high percentage of Aband_2007 tailings soils and Control_1 soils, colonisation of *F. mosseae* BEG 12 soil by leek plants (*Allium porrum L.*) was still good, except for the 100% Aband_2007 and Control_1 soils where the plants had difficulties to survive and mycorrhization could therefore not be observed. We suggest that AMF isolation from REE mining sites which would be adapted to the local conditions might be of interest in further studies for plant assisted remediation.

Keywords

Rare earth element, *Funneliformis mosseae*, availability, toxicity, spore, colonization

Introduction

REEs are increasingly used in modern technologies and are emerging pollutants in the environment. As we have seen in the previous chapters, there is a need to better understand their availability and transfer to plants and to associated microorganisms, such as mycorrhizal fungi.

To relate the bioavailable fraction of a metallic trace element in soil, different chemical extraction methods have been proposed. A sequential extraction method has been adopted to predict the bioavailability of REEs in eight regions in China (Cao et al. 2000b). In this study, water soluble, exchangeable and bound to organic matter REEs were linked to the bioavailability of REEs, which accounted for 10 to 40% of the total REE concentrations depending on the soil type and the REEs. Single extraction methods have also commonly been employed to predict the bioavailability of REEs. Rao et al. (2010) compared different single extractants and found that EDTA and DTPA had good extraction yields and represented the available fraction of REEs in the soil. We previously compared two extractants, CaCl_2 and DTPA, to study the bioavailable fraction of Sm in spiked soils (Hu et al. 2020a) and DTPA extractable Sm was well correlated with the phytoavailable Sm. In the same experiment, an inhibition of alfalfa growth was also observed in shoots and roots in the spiked soils (Hu et al. 2020a).

The phytotoxicity of REEs has been reported in many studies, including experiments carried out in liquid media (Diatloff et al. 1995b, a, c; d'Aquino et al. 2009a), in spiked soil (Grosjean et al. 2020; Hu et al. 2020a) and in natural soils (Thomas et al. 2014; Carpenter et al. 2015; Chao et al. 2016). However, the only data on the toxicity of REEs to arbuscular mycorrhizal fungi (AMF) have been reported in our previous studies in a soil spiked with REEs and mainly focussed on the transfer of REEs to plants (Hu et al. 2020b, Chapter 6). We showed that plants were colonized by AMF and improved plant growth in these REE spiked soils, but we did not investigate real contaminated soils. It is therefore necessary to check the toxicity of REEs to AMF, notably their germination and ability to colonize plants in REE contaminated soils.

Our previous studies were carried out using spiked soils and with rather short ageing time. However, in the contaminated areas of mining and tailing sites, the REEs are with longer ageing time and more complicated geological and chemical conditions. The REEs present in the contaminated sites may have different availability compared to experimentally spiked soils. Thanks to our collaboration with the Sun Yat-sen University and the ECOLAND project, we were able to work on REE contaminated soils from a mining site in Jiangxi province, China. The general objectives were to study the bioavailability and ecotoxicity of REEs in these contaminated soils. In the present chapter, we analysed the bioavailability of REEs in soils using the same extraction methods as in our previous study (Hu et al. 2020a, Chapter 2). Meanwhile, two experiments were conducted to study the toxicity of REEs to the AMF, considering the fungal germination and colonization in the REE contaminated soils. Other

toxicology studies were performed by colleagues from LIEC UMR 7360 CNRS on aquatic organisms (Carole Cossu-Leguille, Laure Giamberini) and on soil enzyme activity (Thierry Beguiristain), but are not presented here.

Materials and methods

Soil sampling and characteristics

The soils were collected from an unreclaimed ionic REE mine tailings abandoned from 2003 to 2011 situated in the Dingnan county of Ganzhou city, Jiangxi province, China thanks to the School of Environmental Science and Engineering of the Sun Yat-sen University within the framework of Ecoland international associated laboratory. 4 soil samples were received for our studies: Aband_2003, Aband_2007, Control_1 and Control_3. Aband_2003 and Aband_2007 were both collected from abandoned mine tailings in 2003 and 2007 respectively (Chapter 2). The site Aband_2003 was covered with relatively dense and diverse vegetation while Aband_2007 had low vegetation coverage. The control soils Control_1 and Control_3 were sampled from unmined forested areas from Control site 1 and Control site 3.

The soil characteristics analysed by the Department of Environmental Science of Sun Yat-sen University (Pr Ye-Tao Tang, eesty@mail.sysu.edu.cn) are presented in Table 6.1 .

Table 6.1 Analyses of the REE mine tailing soils

			LOD	Control 1	Control 3	Aband 2003	Aband 2007
	pH	-		4.390	4.570	4.883	4.893
	TOC	%	0.040	1.890	1.927	0.277	0.177
	TN	%	0.015	0.113	0.126	0.022	0.017
	C:N	-		16.789	15.325	12.409	10.294
Major elements	Al	%	0.005	10.062	11.096	10.781	7.205
	Ca	%	0.007	0.015	0.015	0.015	0.014
	Fe	%	0.007	3.460	3.261	2.574	2.931
	K	%	0.008	3.429	4.627	5.721	4.386
	Mg	%	0.006	0.087	0.107	0.116	0.067
	Mn	%	0.008	0.024	0.065	0.031	0.055
	Na	%	0.007	0.130	0.140	0.219	0.135
	P	%	0.004	0.014	0.023	0.018	0.013
	Si	%	0.005	30.179	29.595	29.999	34.043
	S	%	0.004	0.025	0.017	0.008	0.008
	Ti	%	0.006	0.402	0.377	0.335	0.461
REEs	La	mg kg ⁻¹	0.500	54.008	185.971	240.898	99.502
	Ce	mg kg ⁻¹	0.500	136.312	254.597	231.750	185.745
	Pr	mg kg ⁻¹	0.030	13.734	39.080	43.911	21.156
	Nd	mg kg ⁻¹	0.100	53.492	145.633	152.975	76.727

Sm	mg kg ⁻¹	0.030	11.050	26.088	32.221	15.487
Eu	mg kg ⁻¹	0.030	1.910	4.977	6.831	2.875
Gd	mg kg ⁻¹	0.050	9.242	21.583	34.966	14.121
Tb	mg kg ⁻¹	0.010	1.404	2.996	5.347	2.146
Dy	mg kg ⁻¹	0.050	8.385	16.659	29.070	12.450
Ho	mg kg ⁻¹	0.010	1.673	3.133	5.458	2.409
Er	mg kg ⁻¹	0.030	4.626	7.984	14.281	6.914
Tm	mg kg ⁻¹	0.010	0.692	1.079	1.860	0.982
Yb	mg kg ⁻¹	0.030	4.657	6.559	11.333	6.600
Lu	mg kg ⁻¹	0.010	0.713	0.922	1.586	0.982
Y	mg kg ⁻¹	0.500	37.383	74.074	144.844	62.455
sum 14 REEs	mg kg ⁻¹		301.900	717.260	812.487	448.095

Around 1 kg of each soil received from China were air-dried, sieved at 2 mm and preserved in dark, cold and dry condition till the experiments. Due to the limited quantity of soil available, experiments have been designed carefully in order to make full use of the soils and launch more tests. That is the reason why the spore germination and colonization experiments were not performed with the same soils, and with more than one or two contaminated soils.

For the spore germination experiment, as a control soil, an artificial soil was also prepared according to OECD guideline 207 (1984), the composition of which is summarised in Table 6.2.

Table 6.2 Composition of the artificial soil used in the spore germination experiment

Ingredient	Reference No.	Percentage in weight %	Treatment
Peat		10%	Dried at 60 °C and sieved at 2 mm
Quartz sand	N° EINECS: 3101276	70%	Fontainebleau sand; sieved at ≤ 2 mm
Kaolinite clay	N° EINECS: 3101941	20%	
CaCO ₃	N° EINECS: 2074399	≤ 1%	Add CaCO ₃ into the soil and adjust the pH between 6 and 7

AM fungal spore germination experiment

The spore germination of two AMF was tested in the Aband_2003 soil mixed with artificial soil at different ratios. Mixed soils consisted of 0, 20%, 50%, 100% Aband_2003 soil mixed with 100%, 80%, 50%, 0 artificial soil (in weight) respectively. Two *Funneliformis mosseae* isolates were used: BEG 69 isolated from a soil with a 60-year history of industrial metal pollution (Weissenhorn et al. 1993) and BEG 12 isolated from a non-polluted soil. The spores were extracted from permanent pot cultures with leek in our laboratory and used in the experiment. The spore germination assay was launched according to the protocol ISO/TS 10832. 30 spores were carefully placed between two filtering membranes with gridlines, fixed by slide frames and placed in a Petri dish containing 80 g soil. Deionized water was added to reach 90% of soil water holding capacity (WHC) with 3 (for 0 and 100%

Aband_2003 soil) or 4 (20% and 50% Aband_2003 soil) replicates for each condition, due to the limited quantity of soil available. All 32 Petri dishes were sealed with parafilm to prevent from drying out and were incubated in a darkroom at 24°C for 15 days. After 15 days, the soil was carefully removed from the Petri plates and the filtering membranes were recovered, and carefully stained with trypan blue. The spore germination was then observed under the microscope. Spore recovery rate (the number of recovered spores/the number of original spores) and spore germination rate (the number of germinated spores/ the number of recovered spores) were calculated.

Fungi colonization experiment

Fungi colonization experiment was launched using *F. mosseae* BEG 12 and leek (*Allium porrum* L.) plants in 2 contaminated soils (Aband_2007 and Control_1) mixed with artificial soil at different ratios.

The contaminated soil (Aband_2007 or Control_1) was mixed with artificial soil. 4 gradients were set with 0, 20%, 50% and 100% contaminated soil (in volume) respectively for both contaminated soils. Soils were distributed in the pots (around 120-190 g depending on density of the mixed soil) with 3 replicates for each condition. Deionized water was added to reach 70% of WHC. Approximately 200 *F. mosseae* BEG 12 spores were added in the pots with soil followed by the transplantation of leek seedlings which were pregerminated on sterilized vermiculite for 4-5 days for each pot. All 24 pots were then transferred to a growth chamber with 16 h photoperiod, 22 °C/18 °C day/night, 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 80% relative humidity for 8 weeks. The pots were watered every three days to maintain 70% WHC once with deionized water and once with P-deficient nutrient solution (Redon et al. 2009).

Leek shoot and root samples were harvested 8 weeks after transplantation. The shoot and root samples were rinsed 3 times with deionized water. The shoot samples were dried at 60 °C for 72h and weight. Each root sample was separated into two subsamples with both fresh weights recorded; one subsample was dried at 60 °C to calculate the total dry weight, another subsample was stained with trypan blue method to determine mycorrhizal colonization. The mycorrhizal colonization was determined according to the protocol of Trouvelot et al. (1986): 30 stained root segments were placed on slides and sealed and the mycorrhizal colonization was determined according to the description of the protocol in Chapter 2-4.5.

Chemical analysis

The bioavailable REEs in the soils was estimated using two extraction solutions: DTPA extracting solution and CaCl₂ solution. The details of the extraction solution and extraction method

were described in Chapter 3 (Hu et al. 2020a). REE concentrations were analysed by inductively coupled plasma mass spectrometry (ICP-MS), as described in Chapter 4.

Data analysis

The data were analysed using XLSTAT version 2020.3.1 (Microsoft® Excel version 16.16.22). All the analyses used a 95% significant level. Shapiro-Wilk's tests were firstly performed to check the normality. When the normality conditions were not met, a rank-transformation was performed. After, the data were analysed using one-way or two-way analysis of variance (ANOVA 2) and Tukey's honestly-significant-difference (HSD) post-hoc test as precised in the table and figure legends. The mycorrhizal colonization parameters were arcsine transformed prior to the normality tests and ANOVA 2 test.

Table 6.3 Concentration of available fraction of REEs extracted by DTPA and CaCl₂ in the four soils (mean ± SD, n=3). The percentage data below indicates the ratio of the extractable REEs to the total REEs

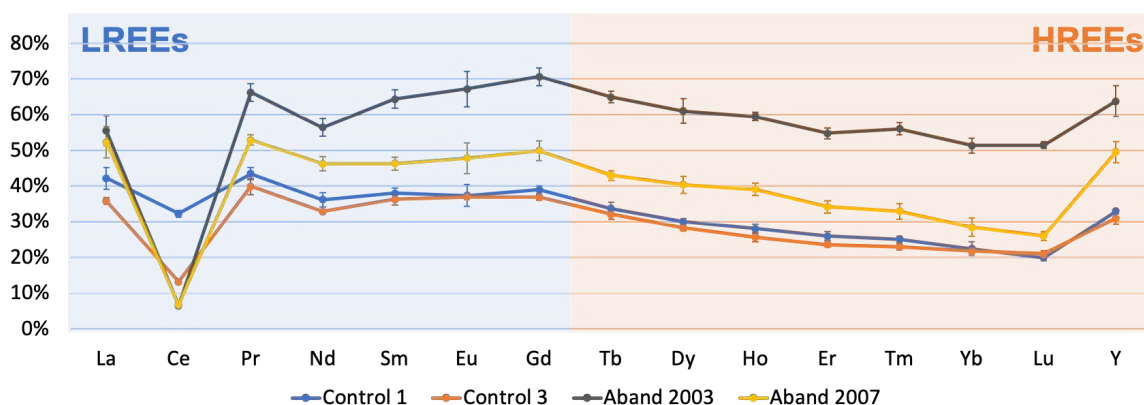
	Aband_2003		Aband_2007		Control_1		Control_3	
	DTPA extractable	CaCl ₂ extractable	DTPA extractable	CaCl ₂ extractable	DTPA extractable	CaCl ₂ extractable	DTPA extractable	CaCl ₂ extractable
Y	92.44±6.31 64%	31.64±0.88 22%	30.97±1.84 50%	10.87±0.37 17%	12.30±0.22 33%	1.71±0.14 5%	22.89±1.13 31%	4.74±0.19 6%
La	133.67±10.1 55%	52.67±4.80 22%	52.04±4.43 52%	16.63±0.30 17%	22.78±1.68 42%	3.10±0.19 6%	66.64±1.83 36%	13.91±0.41 7%
Ce	15.05±1.05 6%	4.83±0.15 2%	12.72±0.65 7%	4.32±0.13 2%	43.97±1.41 32%	4.78±0.32 4%	33.73±1.52 13%	5.27±0.01 2%
Pr	29.08±1.09 66%	8.53±0.48 19%	11.20±0.31 53%	3.54±0.04 17%	5.97±0.23 43%	0.51±0.03 4%	15.61±0.90 40%	2.16±0.10 6%
Nd	86.38±3.90 56%	25.39±0.94 17%	35.47±0.50 46%	11.05±0.61 14%	19.35±1.09 36%	1.53±0.08 3%	47.92±0.48 33%	6.24±0.19 4%
Sm	20.74±0.84 64%	6.11±0.33 19%	7.17±0.27 46%	2.22±0.08 14%	4.20±0.16 38%	0.28±0.02 2%	9.47±0.41 36%	1.03±0.05 4%
Eu	4.59±0.34 67%	1.48±0.15 22%	1.37±0.12 48%	0.38±0.01 13%	0.71±0.06 37%	0.05±0.00 3%	1.84±0.03 37%	0.20±0.01 4%
Gd	24.71±0.88 71%	8.20±0.36 23%	7.05±0.38 50%	2.37±0.08 17%	3.60±0.10 39%	0.31±0.02 3%	7.95±0.18 37%	1.08±0.04 5%
Tb	3.47±0.08 65%	1.22±0.08 23%	0.92±0.03 43%	0.34±0.01 16%	0.47±0.03 34%	0.04±0.00 3%	0.96±0.04 32%	0.14±0.01 5%
Dy	17.73±1.00 61%	6.41±0.25 22%	5.02±0.30 40%	1.79±0.05 614%	2.51±0.08 30%	0.24±0.01 3%	4.71±0.12 28%	0.72±0.03 4%
Ho	3.25±0.0 60%	1.16±0.08 21%	0.94±0.04 39%	0.34±0.01 14%	0.47±0.02 28%	0.05±0.00 3%	0.81±0.04 26%	0.13±0.01 4%
Er	7.82±0.023 55%	2.81±0.10 20%	2.37±0.12 34%	0.87±0.05 13%	1.20±0.06 26%	0.13±0.01 3%	1.89±0.04 24%	0.34±0.01 4%
Tm	1.04±0.03 56%	0.35±0.02 19%	0.32±0.02 33%	0.11±0.00 11%	0.17±0.01 25%	0.02±0.00 3%	0.25±0.01 23%	0.04±0.00 4%
Yb	5.82±0.24 51%	2.41±0.14 21%	1.88±0.17 28%	0.68±0.02 10%	1.04±0.09 22%	0.11±0.01 2%	1.43±0.04 22%	0.27±0.02 4%
Lu	0.82±0.02 51%	0.28±0.01 17%	0.26±0.01 26%	0.09±0.00 9%	0.14±0.01 20%	0.01±0.00 2%	0.19±0.01 21%	0.03±0.01 4%
Sum	454.6 51%	156.2 17%	175.4 26%	57.5 9%	123.6 20%	13.3 2%	220.5 21%	36.9 4%

Results

Analysis of available REE concentrations in the soils

The chemical analysis of the DTPA and CaCl₂ extractable REEs in the four soils can be found in Table 6.3.

a) DTPA extractable REEs



b) CaCl₂ extractable REEs

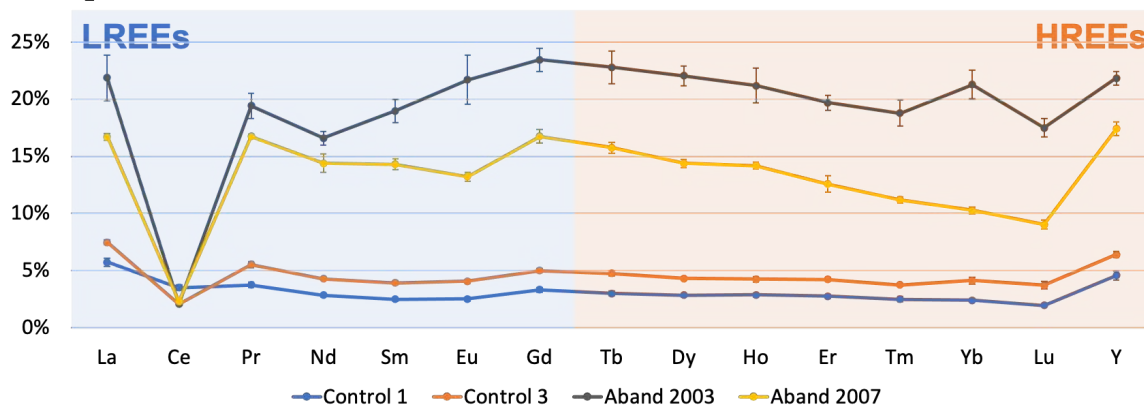


Figure 6.1 Ratios of bioavailable REEs and total REEs estimate by two extractants **a)** CaCl₂ extractant and **b)** DTPA extractant in four Chinese soils

The ratios of the available REEs to the total REEs in soils were calculated and presented in Table 6.3 and Figure 6.1. Total REE concentration in the soils ranged between 300 and 790 mg kg⁻¹ and was not significantly different between the abandoned and the control (Table 6.1). All soils had higher concentrations of LREEs, with La, Ce, then Nd and Y as the most abundant ones, than HREEs. Aband_2003 has the highest total REEs. The available REEs predicted by DTPA extractant accounted for about 30-40% of the total REEs in control soils (Control_1 and Control_3) and about 40-70% of the total REEs in the abandoned mine tailing soils (Aband_2003 and Aband_2007) (Figure 6.1). However, the available REEs predicted by CaCl₂ extractant were much lower, reaching up to 5% of the total REEs in control soils and 15-20%

in the abandoned mine tailing soils (Figure 6.1). For both extractants, significantly higher percentage of DTPA and CaCl₂ extractable REEs to total REEs ratio in the abandoned mining soils (Aband_2003 and Aband_2007) are higher than those in the control soils (Control_1 and Control_3). Compared to the other REEs, there were anomalies for Ce, with much lower percentages of DTPA and CaCl₂ extractable. This was observed in both abandoned mine tailing soils (Aband_2003 and Aband_2007), and to a lesser extent in the Control_3 soil (Figure 2).

Fungi spore germination experiment

This experiment was performed using the soil with the highest total and extractable concentrations of REEs (Aband_2003). The spore recovery rate ranged from 80% to 100% for all Petri dishes so the data were validated according to the ISO TS/10832 (data not shown). The spore germination rate decreased significantly with the increased percentage of REE contaminated Aband_2003 soil in the mixed soil for both spore fungi (Figure 6.2). The germination rates in no REE contaminated soil (100% artificial soil) ranged between 70-90% for both BEG 69 and BEG 12 and decreased to 15% for BEG 69 and less than 10% for BEG 12 in 100% Aband_2003 soil. But the two-way ANOVA indicated that there was no significant difference of germination rate between BEG 12 and BEG 69.

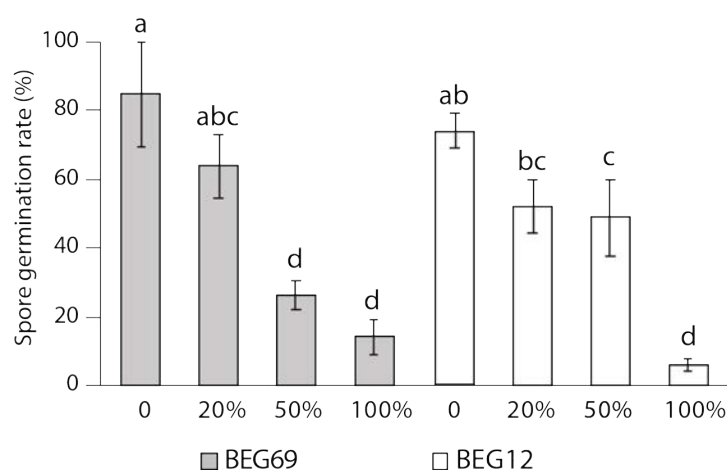


Figure 6.2 Spore germination rates of *F. mosseae* BEG 69 and BEG 12 in soils consisting 0, 20%, 50% and 100% Aband_2003 soil. Means followed by the same letter do not differ significantly among treatments using two-way ANOVA and Tukey (HSD) post-hoc test ($P < 0.05$)

AM fungal colonization experiment

The AM fungal colonization experiment was performed using two contaminated soils differing in their total and extractable REE concentrations, with higher values for Aband_2007 than Control_1. Shoot and root dry weights decreased with increasing percentage of contaminated soil and were

significantly lower in 100% REE contaminated soils than with the other 3 percentages of both soils. For Aband_2007 soil, the shoot and root dry weights were also significantly lower in 50% contaminated soil than in the control without contaminated soil (Figure 6.3).

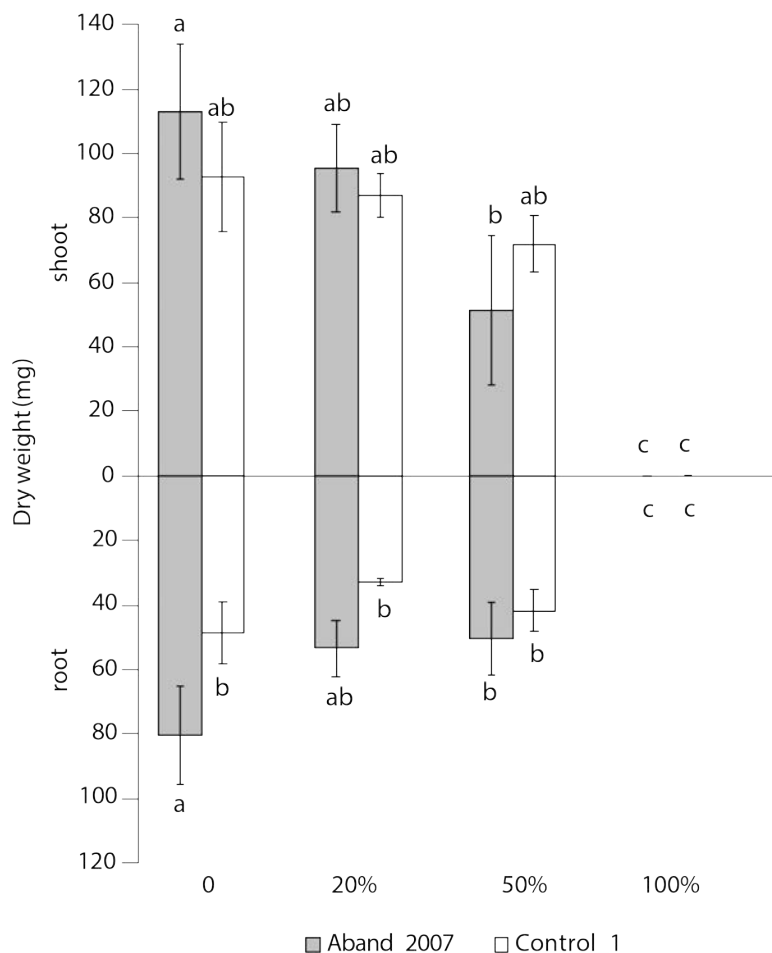


Figure 6.3 Dry weight of leek shoots and roots (total per pot) inoculated with *F. mosseae* BEG 12 in soils consisting 0, 20%, 50% and 100% Aband_2007 or Control_1 soil. Means in shoot or root followed by the same letter do not differ significantly among treatments using two-way ANOVA and Tukey (HSD) post-hoc test ($P < 0.05$)

Table 6.4 Mycorrhizal colonization of roots in the two experiments (Trouvelot et al. 1986). Data are means \pm SD. Statistical analysis using two-way ANOVA indicated no significant difference between soil dilutions.

	Mycorrhizal frequency (F%, % of roots)	Mycorrhizal intensity (M%, % of colonized roots)	Arbuscule frequency (a%, content of arbuscules in colonized part of roots)	Arbuscule intensity (A%, content of arbuscules in total root system)
A0	83.33 \pm 6.67	71.06 \pm 7.70	93.09 \pm 6.24	66.42 \pm 10.98
A20	95.56 \pm 5.09	86.22 \pm 5.99	99.26 \pm 0.56	85.58 \pm 5.99
A50	77.11 \pm 16.95	61.71 \pm 27.60	71.02 \pm 45.51	49.85 \pm 40.35
A100	-	-	-	-
C0	83.33 \pm 11.55	67.39 \pm 8.14	98.62 \pm 0.82	66.50 \pm 8.61
C20	92.22 \pm 10.72	84.01 \pm 12.28	98.07 \pm 2.05	82.53 \pm 13.44
C50	85.13 \pm 17.18	74.08 \pm 23.81	78.70 \pm 36.90	64.12 \pm 41.00
C100	-	-	-	-

High mycorrhizal colonization rates were found in both Aband_2007 and Control_1 soils. Due to the low root biomass in 100% Aband_2007 and Control_1 soils, the mycorrhizal colonization was not able to be analyzed. The mycorrhizal frequency ranged from 77 to 96% with 62 to 86% mycorrhizal intensity. Arbuscules were observed in all treatments with high arbuscule frequency between 71-99% and 50-85% arbuscule intensity. However, there was no significant difference in the four colonization parameters between the two soils and between the soil dilutions (Table 6.4).

Discussion

The four mine tailing soils contained high concentrations of REEs (330 to 800 mg/kg) compared to the Chinese soils average (<200 mg/kg, Hu et al. 2006). CaCl₂ extraction reflecting easily bioavailable elements (Houba et al. 2000) and DTPA extraction as well showed a net difference between abandoned mine tailing (Aband) and unmined forested areas (Control), with lower values for Control_1 & 3 (2 to 4% for CaCl₂) than Aband 3 (9 to 17%) suggesting that REE extraction has increased REE availability. According to the chemical analysis of the total and available REEs, the Aband_2003 soil had both highest total REEs and total available REEs. Therefore, the Aband_2003 was chosen for the soil toxicity test to AMF spores. The Control_1 and Control_3 had different total REEs but the available REEs were at the same level. Therefore, one mine tailing soil (Aband_2007) and one control soil (Control_1) were chosen for the spore colonization experiment.

The spore colonization indicates that the 2 experimental soils (Aband_2007 and Control_3) were not suitable for leek growth. The phytotoxicity of REEs have been commonly reported in previous studies with different plants (Thomas et al. 2014; Carpenter et al. 2015; Chao et al. 2016; Grosjean et al. 2020; Hu et al. 2020a). At the same time, both the Control_3 soil and the Aband_2003 soils have higher total and available REEs than the Control_1 and the Aband_2007 soil and similar low pH level. It is reasonable to deduce that all 4 soils are not suitable for leeks to grow.

In the 100% Aband_2003 soil, the germination rate for both BEG 12 and BEG 69 fungi were rather low, indicating that these REE contaminated soils were toxic to the spores by inhibiting the germination rate. At the same time, both BEG 12 and BEG 69 fungal spores germinated in all soil conditions, and still germinated at rather high REE concentrations. Previous studies also reported that REEs are toxic to fungi such as *Penicillium simplicissimum* and *Aspergillus japonicus* (Bergsten-Torralba et al. 2020). Our previous studies showed that *F. mosseae* can successfully form symbiotic activities with alfalfa (*Medicago sativa* L.) grown in 100 mg kg⁻¹ Sm spiked soil with a high mycorrhizal colonization (Hu et al. 2020b). Guo et al. (2013) have carried out experiments on maize (*Zea mays* L.) grown in REE mine tailing soils collected from Bayan Obo mining area and inoculated with *F. mosseae* and *Glomus versiforme*. They have found high mycorrhizal colonization and mycorrhizal symbiotic activity with total REE concentrations higher than 950 mg kg⁻¹.

In the spore germination experiment, two *F.mosseae* isolates were compared, one isolated from a metal-contaminated soil and metal-tolerant, the other one from a non-contaminated soil. However, there was no significant difference in the germination rate between the fungi. *F. mosseae* BEG 12 originated from a non-contaminated soil was able to germinate in the 100% Aband_2003 soil and colonized leek roots growing in 50% Aband_2007 and Control_1 soils. Previous spore germination experiment carried out in Cd contaminated soil using the same method (ISO 10832) showed a lower spore germination rate for BEG12 (less than 10% at 10 mg kg⁻¹ Cd in the soils) than BEG69 (Weissenhorn et al. 1993). Although the mechanisms involved in AMF improved plant tolerance to metal stress have been investigated (Joner and Leyval 1997; Joner et al. 2000; Wu et al. 2016; Hu et al. 2020c), it was mainly done at the mycorrhizal plant level. But little has been done on the tolerance of AMF isolates to different metals, and nothing on REEs. For the yeast *Saccharomyces cerevisiae*, a recent study on toxicity of Yttrium showed a response pattern similar to nickel stress (Grosjean et al, 2018). Our result suggests that the AMF tolerance to REEs has no relationship with their tolerance to transition metals. Therefore, it would be needed to study the toxicity of REEs to different AMF and their tolerance to REEs as well as the mechanisms involved at the molecular level.

The low germination rates of the two *F.mosseae* fungi at high percentages of REE contaminated soil are highly caused by the toxicity of the REEs but the low pH of the soils could also contribute to it. Wang et al. (1993) have found that significantly fewer mycorrhizal fungi spores presented in pH 5.5 soil and no spores were found in the most acidic plots. The lack of growth of leeks in 100% contaminated soils may also be caused by the low pH of the soils. The 50% dilution of the contaminated soils by ISO soil allowed the plant to grow, possibly by increasing the pH, as well as decreasing the REE concentration. Higher availability of the REEs were found in acidic soils in our previous experiments (Hu et al. 2020a, Chapter 4). For 2 weeks' ageing soils, the DTPA extractable Sm accounted for 30-40% of the total Sm for the acidic soil and around 10-20% for the neutral soil (Hu et al. 2020a). In the present study, the DTPA extractable REEs accounted for 20-40% for two control soils and Abond_2007 soil which correspond to our results in the spiked acidic soil. The DTPA extractable REEs reached 50-70% for Abond_2003, even higher than the spiked soils, which may be related to the lower pH of Abond_2003 (4.88) than the acidic Maron soil (5.34) in Hu et al. (2020a) and the different soil properties.

Many studies indicated the beneficial effect of mycorrhizal symbiotic activities brought to plants, especially in metallic contaminated soils (Chen et al. 2004, 2007b; Hovsepyan and Greipsson 2004; Da Silva et al. 2005; Janoušková et al. 2005; Redon et al. 2008; Zhang et al. 2010; Merlos et al. 2016; Chang et al. 2018). For REE contaminated soils, there are indications that AMF could also help the plant to cope with the presence of toxic REE concentrations (Joner et al. 2004; Chang et al. 2018; Hu et al. 2020b). Therefore AMF could be considered as an help in environmental remediation and

ecological friendly strategies using plants. AMF could be isolated from contaminated sites and therefore be already adapted to the REE contaminated environment and have higher survival ability and activity than AMF isolated from non-contaminated areas. These adapted AMF could be further tested with different plants before setting up remediation projects on site.

Conclusion

The abandoned mining site soils had higher percentage of bioavailable REEs than control soils suggesting that the mining process has increased the availability of the REEs. But there was no significant difference in leek growth between abandoned and control soil. All 4 chinese REE mining soils collected had a low pH, high REE concentrations and high percentages of available REEs, which could explain the lack of growth of leek on these soils without dilution in the pot experiment. Probably for the same reasons, high percentage of these soils can also reduce the germination rates for both *F. mosseae* fungi BEG 69 of metal-tolerant origin and BEG 12 of non-metal-tolerant origin. However, even in the 100% contaminated soil, both spores can germinate at a comparatively low rate. Indigenous plants and AMF collected from these mining sites could be investigated to study the interactions between plants and AMF in REE contaminated soils. In order to better promote plant growth on such REE contaminated mining sites, AMF extraction is suggested in order to obtain some fungi that are already adapted to the local environment and therefore could be tested for plant assisted land remediation.

Chapter 7. General discussion, conclusion and perspectives

Chapitre 7. Discussion générale, conclusion et perspectives



7.1 General discussion

7.1.1 What are the bioavailability of REEs in the soil and their effects on plants?

It has been reported in naturally grown plants, that the uptake of REEs depended primarily on the concentration of REEs, their speciation and their adsorbing ability in the soil (Brioschi et al. 2013). Therefore, in order to understand the transfer of REEs, the first step was to study the bioavailability of REEs in the soil and their effect on the model plant and the first question [“What are the bioavailability of REEs in the soil and their effects on plants?”](#) has been drawn. In this study, we chose the [plant alfalfa \(*Medicago sativa*\)](#) in the REE transfer studies due to its [rapid growth, common presence internationally and its response to AM colonization in many previous studies](#) (El-Kherbawy et al. 1989; Joner and Leyval 1997; Chen et al. 2007a; Campanelli et al. 2013; Gao et al. 2017). In the earth crust, the abundance of LREEs are much higher than HREEs (Voncken 2016), therefore, we wanted to start our research with one model REE which [represented the LREEs](#), and Sm was ideally [located in the middle of LREEs and HREEs](#) and has [both parts’ properties](#). Two soils with [different properties, and pH](#) were chosen, with [two different spiking concentrations](#). In order to avoid the high availability of the freshly spiked Sm, the spiked soils were stored for a [2 weeks’ ageing](#) period before the transplantation and plant growing period. During the experiment, [two single extractants](#) were applied to measure the extractable Sm in the soil to predict the plant-available fraction of Sm, since there was little information on the best chemical extractants reflecting REE plant availability.

This experiment enabled us to observe the decrease of bioavailability of Sm after the spiking and the inhibition of plant growth in Sm spiked soils. However, in the acidic Maron soil, the alfalfa could not survive due to the concurrent effects of high availability of Sm and the unsuitable pH value for growth. However, it is very difficult to attribute the low growth to one of the causes because there was a causal relationship between the low soil pH and the high availability of Sm. Our results showed that DTPA extraction was correlated with Sm uptake in alfalfa, while CaCl₂ extractable Sm was below the detection limit for the unspiked Maron soil and all the treatments of Bouzule soil. In the Chinese REE mine tailing soils (Chapter 6), the CaCl₂ extractable REEs accounted for 2-17% of the total REEs while the DTPA extractable REEs accounted for 20-51%. Soils with lower pH (Maron and Chinese REE mine tailing soils) tend to have higher extractable REEs but plants have difficulties to grow in these soils.

Our results confirm that the amount of REEs taken up by plants differs from species to species. The concentrations of total REEs in alfalfa and ryegrass were both less than 20 mg kg⁻¹ in shoots and few hundreds mg kg⁻¹ in roots depending on the spiking REEs.

7.1.2 Effect of an AM fungus on plants growing in an REE contaminated soil and on the transfer of REEs to the plant?

With the knowledge of the first experiment, we had data on the Sm availability and the plant growth in different soils. With our research interest in the AMF and the previous studies concerning the role of AMF to metallic trace element transfer but no information concerning role of AMF to the REEs transfer, we posed a question [“What are the effects of an AM fungus on plants growing in a REE contaminated soil and on the transfer of REEs to the plant?”](#). In order to have the good colonization, [good root growth is needed](#). Therefore, we chose to continue our study with the [less acid Bouzule soil](#). The [same plant and element Sm](#) were used as in the previous study (Chapter 4). An AM fungus *Funneliformis mosseae* BEG 69 isolated from a soil with a 60-year history of industrial metal pollution (Weissenhorn et al. 1993) reported with [metal tolerance](#) was used (Joner and Leyval 1997; Joner et al. 2000; Tonin et al. 2001). Two experiments were designed to answer this question:

1. To study the role of *F. mosseae* on Sm transfer and on the plants growing in Sm contaminated soil, a pot experiment was performed with different concentrations of spiked Sm with or without *F. mosseae* inoculation
2. To precise the Sm being transferred to plants by AM hyphae, a compartmented experiment has been launched. Compartmented pots have been successfully adopted in previous studies concerning the transfer of metallic trace elements (Joner and Leyval 1997; Joner et al. 2004). Based on these previous work, we designed and tested compartmented pots.

At the beginning, we designed and built compartmented pots with lateral compartments (Joner et al. 2004) The Sm was spiked in the lateral compartment with sand and the main compartment contained soil, plants and 200 *F. mosseae* spores. The nylon mesh separated the two compartments that only *F. mosseae* fungi hyphae can pass through. The preliminary tests showed a low transfer of Sm from lateral compartmented pots to plants. Therefore, we used radio-labelled ^{151}Sm in order to be able to detect the Sm transfer at a low detection limit (DL). Unfortunately, maybe due to the quenching problems or the low transfer, the signals we detected were still below the DL. Meanwhile, we also found that with the lateral compartmented pots, sometimes we did not have roots close to the lateral compartments and likely low amount of hyphae.

In the end, we built a new compartmented pot (Chapter 4) separating vertically the spiking compartment and the soil compartment with plant. And the transfer of the Sm was analysed using ICP-MS technique, because the detection limit of this technique allowed to quantify REEs in plant parts. We decided to use two types of nylon mesh 37- μm mesh and the air mesh (37-1000-37 μm) in between which only AM hyphae can pass through to get access to the spiked contamination in the bottom

compartment. For this experiment, we observed that with the air mesh, the transfer of Sm to plants shoots and roots was very low and data difficult to interpret. Therefore in Chapter 5, we decided to use only 37- μm mesh.

In these two experiments, we observed significant growth promotion with the colonization of AMF in the Sm spiked soil although the promotion was less significant at the high Sm concentration (200 $\text{mg}\cdot\text{kg}^{-1}$). In the Chapter 6, the spore germination rate was low in the mine tailing soil with high REE concentration (up to 900 $\text{mg}\cdot\text{kg}^{-1}$). High concentration of REEs is highly likely toxic to AMF especially at the germination stage and further affect the functioning of the AMF to the plant growth. For both experiments, no significant difference was found in Sm transfer between the mycorrhizal and the non-mycorrhizal plants, indicating no significant transfer of the Sm from spiked soil to alfalfa by *F. mosseae*. The previous studies on metallic trace elements (MTEs) suggested increasing concentrations of MTEs such as Cu, Ni, Pb and Zn in plants (Killham and Firestone 1983) or decreasing concentrations of MTEs (suggested by more studies) with the promotion of plant biomass (Weissenhorn et al. 1995; Joner and Leyval 2001a). REEs have then different transfer by AMF to plant roots compared to these MTEs.

Notably, the improvement of P content in mycorrhizal plant shoots was found in these two experiments, the same improvements of P nutrition has been reported in many studies in different MTE contaminated soils and was believed having a causal relationship with the promotion of plant growth (Chen et al. 2007b; Smith and Read 2008; Smith and Smith 2011b).

Ca^{2+} is an element that has been many discussed in previous studies and it was reported that LREEs uptake by plants involved Ca^{2+} channels (Gonzalez et al. 2014; Yuan et al. 2017). Ca was also analyzed in this study, but we did not see a relationship with REE uptake, and Ca uptake was not significantly affected by AMF in either experiment.

7.1.3 Effects of an AM fungus on plants growing in a soil with multiple REE contamination and on the transfer of these REEs to plants?

The two previous questions helped us to understand the bioavailability of Sm in the soil and the effect of *F. mosseae* on plant growth and on Sm transfer in a contaminated soil. However, natural soils and earth crust normally contain multiple REEs. Many studies mentioned that the behavior and the transfer are different between the HREEs and the LREEs (Brioschi et al. 2013; Grosjean et al. 2019; Gallhardi et al. 2020). In order to better simulate the natural environment and study the transfer of different REEs including LREEs and HREEs, we designed the experiment to answer our question “[What are the effects of an AM fungus on plants growing in a soil with multiple REE contamination and on the transfer of these REEs to plants?](#)”. Considering the natural abundance of REEs, we chose two abundant LREEs La and Ce, plus the element Sm and an HREE Yb. The compartmented

experimental design was the same as in the Chapter 5 with only [one type of nylon mesh](#). The study was carried out using two plant species including alfalfa and a grass species ryegrass inoculated or not with the same [F. mosseae BEG 69](#).

This experiment allowed us to quantify the transfer of the 4 elements in the different plant parts and obtain the transfer efficiency. Significantly higher concentrations of the 4 elements were found in the roots than in the shoots with higher soil-root transfer factor than root-shoot transfer factor. Meanwhile, this experiment also allowed us to observe the different transfer between different elements and between two plant species. Significantly higher LREEs La and Ce were transferred to shoots than Sm and than the HREE Yb in ryegrass but not in alfalfa. Moreover, we are able to compare the effect of AMF on different plants. The dry weight of alfalfa was significantly increased by *F.mosseae* inoculation. Notably, for both plants, P uptake was significantly improved in the mycorrhizal plants compared to the non-mycorrhizal ones. But, as in the previous chapter for Sm, there was no significant transfer of the 4 REEs to alfalfa and ryegrass roots or shoots caused by *F. mosseae* inoculation. Previous study indicates that REE contaminated area have almost no vegetation (Chao et al. 2016). So, the finding of the improvement of P uptake in mycorrhizal plants and biomass increase of mycorrhizal alfalfa indicated a potential application of AMF in REE contaminated sites remediation. Some plants have a strong ability to concentrate REEs from soil to their leaves up to 1000 mg kg⁻¹ (*Dicranopteris dichotoma*, *Blechnum orientale*, van der Ent et al., 2018). Considering such plants combined with mycorrhizal symbiosis might be interesting to improve total REE extracted.

7.1.4 What are the bioavailability of REEs in historically contaminated mining tail soils and their effects on AMF?

After all the experiments carried out using spiked natural soils, we did extra studies using soils collected from an REE mining area thanks to our collaboration with the Sun Yat-sen University and the Ecoland project. However, due to the limited quantity of soil available, a limited number of studies could be performed. In the previous study, we found that the presence of REEs inhibited the plant growth (Chapter 3) and the colonization with *F.mosseae* promoted plant growth even at high concentrations of spiked REEs (100 to 400 mg kg⁻¹)(Chapter 4 & 5). Therefore, we posted the 4th question [“What are the bioavailability of REEs in historically contaminated mining tail soils and their effects on AMF?”](#). It was important to test the survival and functioning of AMF in the REE contaminated soils.

Two toxicology tests were performed focussing on 1) AMF spore germination stage of [two F. mosseae \(BEG69\) and \(BEG 12\)](#) following the protocol ISO/TS 10832 and 2) on root colonization using [a pot experiment](#) with [leek plants](#), the typical host plant in AMF spore culturing.

The percentage of extractable REEs in these contaminated mining tail soils (2-17% for CaCl_2 and 20-51% for DTPA) are at the same level as in acidic Maron soil (25-40% for DTPA depending on the ageing time) but higher than in the Bouzule soil (8-20 for DTPA depends on the ageing time) in Chapter 3. The availability of REEs in the mine tailing soils are therefore very high. REEs released from mining wastes and industrial wastes are either solubilized during the acid drainage procedure for the mining wastes or the REE extraction procedure for the industrial wastes (Dev et al. 2020). Due to extraction procedure, REE bioavailability could increase and then decrease with time because of ageing effect. This could explain the higher availability of REEs in the abandoned mine soils compared to the unmined ones.

AMF germination decreased with increasing concentration of the REE mining soil, but both fungi can germinate even in 100% mining site soils. Although the two *F.mosseae* isolates differed in their tolerance to metals, there was no significant difference in their germination rate with increasing concentration of REEs. The root colonization by *F.mosseae* was not decreased by the increased concentration of REE mine soil. Meanwhile, leek plants showed difficulties to grow in 100% mining site soils but not in 50% mining site soil. However, the inhibitions were caused by the concurrent effects of low soil pHs and the high total and available REEs.

7.2 Perspectives

The 3 years' PhD time flew quick plus the loss of laboratory working time caused by the COVID-19 pandemic and the quarantines. We had very limited time to figure out the REEs and their transfer in the soil-AMF-plant system. There are many possibilities to make this project/study go further.

Firstly, in our study in Chapter 3, the alfalfa plants used to study the REE transfer were sensitive to the acidic environment, the same as the leek plants in the AMF colonization studies in Chapter 6. Therefore, it is very difficult to draw a conclusion on the toxicity of REEs to plants or to observe the AMF colonization in plant roots. In the future, more plant species should be tested and the ideal model plant should be with rapid growth, commonly distributed, tolerant to a wide range of pH, sensitive to REE contaminations and responsive to AMF. Then studies could be carried out in acidic environment to see the toxic effect of REEs to plants in acidic soil. The toxic effect can be caused mainly by the high dose of REEs, not the low pH. In this case, the mycorrhizal colonization can also be observed in the roots. Plants collected on REE contaminated sites and adapted to the sites may also be good candidates to study the transfer of REEs to plants.

Secondly, Smith and Read (2008) pointed out that the promotion of plant growth by AMF is correlated with the P uptake. However in Chapter 5, no significant P concentration promotion was found in the mycorrhizal plants, it was only significantly higher for P contents while in Chapter 6, significantly higher P concentrations were found in the mycorrhizal plants. This is likely caused by the lack of

nutrient addition in Chapter 5. In further studies, plants should be given nutrient solutions (no or low P) and P concentration and other nutrients would be given more attention.

Thirdly, there was no significant difference in the root concentration of LREEs and HREEs in Chapter 6. However, there was a large variance among the data. Further studies could focus on more REEs and varying concentrations and include more replicates so that the pattern of the uptake of the different REEs from soil to roots and from roots to shoots could be seen more clearly.

Fourthly, we observed no effect of AMF on the REE transfer from soil to plants, whereas previous studies on MTE transfer to plants found some effects of AMF, which means that the transfer mechanism of REEs is different from MTEs. Studies concerning the comparison of the transfer of MTEs and REEs affected by the presence of AMF could be performed. Transfer mechanisms of REEs from soil to plants, and from soil to AMF should be further investigated, especially at a molecular level to have a deeper understanding of the interactions between plants and AM hyphae. It was initially planned to use the NanoSIMS technique to localize the spiked Sm in the mycorrhizal plant roots and the fungi hyphae. Due to the lack of apparent transfer of Sm by the AM hyphae and to a lack of time, the localization using this technique was not done but would be an interesting and complementary approach to consider in the future.

Fifthly, the REE contamination in the mining and mine tailing sites has become a severe problem, and within the LabEX R21 project, some REE hyperaccumulators have been found. Another issue might be to inoculate AMF to the roots of the hyperaccumulators to improve the growth and or the accumulating effects.

Last but not the least, the AMF used in our studies are able to survive in the REE mining soils and colonize plants like alfalfa and ryegrass. However, in other REE contaminated sites, these fungi may not grow or colonize well indigenous or introduced plants and the introduction of these fungi with foreign origin may cause the environmental issue. In order to get the most adapted AMF strains and be more ecologically friendly, AMF isolation and purification from REE contaminated sites to be restored using phytoremediation (revegetation) could be a promising project.

7.3 Conclusion

This PhD work is the first consistent work on interactions between REE and mycorrhizal plants and the role of AMF on REE transfer to plants. A general inhibition of plant growth was found in soils with high bioavailable REEs, but the tested AMF was relatively tolerant to REE contaminated soils, colonized well the plant roots. and in the same environment, mycorrhizal colonized plants grow better than the non-mycorrhizal ones. The bioavailability of REEs is influenced by the concentrations of REEs in the soils, soil pH, organic matters and so on but was not influenced by AMF inoculation. The inoculation of AMF did not affect the transfer of REEs from soil to plants. This PhD work has therefore

opened possibilities for further studies on the transfer of REEs in the terrestrial environment, considering different AM fungi and their relationships with REEs, for fundamental as well as applied research.

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Abstract

Mobility and transfer of rare earth elements from soil to plants by arbuscular mycorrhizal fungi

Rare earth elements (REE) are a group of strategic metals that have been widely used in modern technologies in the recent decades. However, due to the corresponding REE emission from industries and the over-exploitation, large amounts of anthropogenic rare earth can accumulate in the environment, and be phytotoxic. Arbuscular mycorrhizae (AM) benefit to plants in metal-contaminated soils by improving their survival and growth and alleviating metal toxicity, but little information is available about soil contaminated by rare earth elements. The objective of this PhD project is to understand the transfer of REEs from soil to plants and especially the role of AM fungi on plant growth and REE transfer to plants in REE contaminated soils. Experiments were launched using a model legume plant alfalfa (*Medicago sativa*), a model REE samarium (Sm), and a metal-tolerant *Funneliformis mosseae* fungus in a growth chamber. We first studied the bioavailability and transfer of an REE to *Medicago sativa* grown on two contaminated soils differing in their chemical characteristics. The results showed that DTPA extractable Sm was well correlated with Sm uptake in alfalfa shoots. Although the soil to plant transfer factor was low, alfalfa biomass was reduced when the soils were spiked with 100 to 200 mg kg⁻¹ of Sm. Then the hypothesis was drawn that arbuscular mycorrhizal fungi might protect the plant against REE toxicity. Therefore, a pot experiment was launched to study the role of AM fungi on alfalfa growth and a compartment experiment was performed to study the transfer of Sm to alfalfa via AM fungal hyphae. The biomass of alfalfa grown on Sm-spiked soil was significantly higher following arbuscular mycorrhiza inoculation. P content was also higher in mycorrhizal than nonmycorrhizal plants, but there was no significant Sm transfer to the plant by *F.mosseae*. Since there are often multiple REEs in contaminated soils, including light (LREE) and heavy (HREE) REEs, a compartment experiment was launched using 4 REEs, alfalfa and ryegrass, which confirmed that there was no transfer of the 4 REEs to alfalfa plants by *F.mosseae*. Finally, an REE mining soil collected from China was used to analyze the toxicity of REEs to AM fungal spores and to leek plants inoculated or not with the AM fungus, using spore germination assays and a plant growth experiment. The high concentration of REEs significantly inhibited plant growth and spore germination rate, and the fungus tolerated relatively high REE concentrations, but there was no significant difference in REE tolerance between two isolates of *F.mosseae*. Other fungi and plants should be tested, and field experiments performed, but our results suggest that arbuscular mycorrhizal plants might be considered in phytoremediation of REE-contaminated soils.

Résumé

Mobilité et transfert de terres rares du sol aux plantes par les champignons mycorrhiziens à arbuscules

Les terres rares (REE) sont un groupe de métaux stratégiques qui ont été largement utilisés dans les technologies modernes au cours des dernières décennies. Cependant, en raison des émissions de REE liées à ces industries et à la surexploitation, de grandes quantités de terres rares d'origine anthropiques peuvent s'accumuler dans l'environnement et être phytotoxiques. Les mycorrhizes à arbuscules (MA) sont bénéfiques pour les plantes dans les sols contaminés par les métaux en améliorant leur survie et leur croissance et en atténuant la toxicité des métaux, mais peu d'informations sont disponibles sur les sols contaminés par des REE. L'objectif de cette thèse est de comprendre le transfert des REE du sol aux plantes et en particulier le rôle des champignons MA sur la croissance des plantes et le transfert des REE aux plantes dans les sols contaminés par des REE. Des expériences en chambre de culture ont été réalisées en utilisant la luzerne (*Medicago sativa*) comme plante modèle, le samarium (Sm) comme REE modèle et un champignon mycorrhizien *Funneliformis mosseae* tolérant aux métaux. Nous avons d'abord étudié la biodisponibilité et le transfert du Sm à *Medicago sativa* cultivée sur deux sols artificiellement contaminés différant par leurs caractéristiques chimiques. Les résultats ont montré que le Sm extractible au DTPA était bien corrélé à l'absorption de Sm dans les pousses de luzerne. Bien que le facteur de transfert sol-plante soit faible, la biomasse de luzerne a été réduite lorsque les sols ont été enrichis de 100 à 200 mg kg⁻¹ de Sm. Nous avons ensuite émis l'hypothèse que les champignons mycorrhiziens à arbuscules pourraient protéger la plante contre la toxicité des REE. En conséquence, une expérience en pot a été menée pour étudier le rôle des champignons MA sur la croissance de la luzerne et une expérience en compartiments a été réalisée pour étudier le transfert de Sm à la luzerne via les hyphes fongiques MA. La biomasse de la luzerne cultivée sur un sol enrichi en Sm était significativement plus élevée après l'inoculation par *F.mosseae*. La teneur en P était également plus élevée dans les plantes mycorrhizées que dans les plantes non mycorrhizées, mais il n'y avait pas de transfert significatif de Sm vers la plante par *F.mosseae*. Puisqu'il y a souvent plusieurs REE dans les sols contaminés, y compris des REE légers (LREE) et lourds (HREE), une expérience à compartiments a été réalisée en utilisant 4 REE, la luzerne et le ray-gras, qui a confirmé qu'il n'y avait pas de transfert des 4 REE aux plants de luzerne par *F.mosseae*. Enfin, un sol minier contaminé par des REE prélevé en Chine a été utilisé pour analyser la toxicité des REE vis à vis des spores de champignons MA et pour des plants de poireaux inoculés ou non avec *F.mosseae*, en utilisant des tests de germination des spores et une expérience de croissance des plantes. La forte concentration de REE a considérablement inhibé la croissance des plantes et le taux de germination des spores, mais le champignon MA tolérait des concentrations relativement élevées de REE, et il n'y avait pas de différence de tolérance aux REE de deux isolats de *F.mosseae*. D'autres champignons et plantes devraient être testés et des expériences sur le terrain devraient être effectuées, mais nos résultats suggèrent que des plantes mycorrhizées par des champignons à arbuscules pourraient être prises en compte dans la phytorestauration de sols contaminés par les REE.