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Kaisa Forsyth

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Spécialité : *Sciences de la Terre et de l'Environnement*

Par

« **Kaisa FORSYTH** »

« **La spéciation des terres rares contrôle-t-elle la phyto-disponibilité et la dissémination dans les plantes** »

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Finally, I would like to give gratitude to my friends and family who have supported me through-out my doctorate.

Kaisa Forsyth

RESUMÉ :

Les éléments terres rares (REE), un groupe de contaminants émergents ayant des applications commerciales et technologiques, partagent de nombreuses caractéristiques physiques et chimiques communes et ont été utilisés comme des traceurs efficaces de divers échantillons et processus environnementaux. Il a été démontré qu'ils augmentent dans les eaux au cours des activités minières à la suite de la dissolution des matériaux composant la roche hôte. Dans le premier volet de cette étude, la végétation spontanée et les milieux connexes (sols, sédiments aquatiques et eaux) ont été collectés dans trois anciens sites miniers portugais en Novembre 2020 afin d'évaluer la phytodisponibilité et le devenir des terres rares. Les données relatives aux eaux, sols, sédiments aquatiques et plantes ont été analysées dans le contexte : a) du contexte géochimique dominant, b) du contexte minier et c) des effets liés aux plantes. Cette étude présente les signatures des REE pour différentes espèces de plantes et relie les signatures à une source potentielle de REE biodisponibles. Les terres rares accumulées dans les tissus végétaux semblent refléter la signature des terres rares des eaux de surface dans les zones minières, en montrant un enrichissement en terres rares moyennes. Bien que les sols, les sédiments et les eaux de cette étude présentent des caractéristiques similaires, certaines plantes semblent mieux adaptées que d'autres à la translocation des terres rares légères et de l'uranium. Les REE étant facilement disponibles dans les conditions d'un site minier, cette étude montre comment la physiologie des plantes et la préférence biologique pour certaines REE contribuent au fractionnement des REE et créent une signature unique dépendant du type de plante.

Cependant si de précédentes études ont attribué la phyto-accumulation des terres rares (REE) à l'influence du substrat du sol, cette hypothèse reposait sur la quantité de REE associées au sol dans son ensemble. Or, de nombreuses études ont remis en question le fait que toutes les formes REE présentes dans le sol constituaient ou non un réservoir potentiellement biodisponible. Le consensus général est que le pool de minéraux échangeables et liés au carbonate est biodisponible. Cependant, dans la rhizosphère, les conditions géochimiques spécifiques au site et l'altération biologique qui y règne (plantes, macrofaune et microfaune) peuvent faire en sorte que d'autres réservoirs de REE du sol deviennent biodisponibles pour les plantes. Dans son deuxième volet, l'étude ici conduite caractérise le sol en quatre fractions en utilisant la méthode d'extraction séquentielle du Bureau Communautaire de Référence (BCR). La caractérisation des associations géochimiques des terres rares avec les fractions du sol ont ainsi permis de déterminer quelles phases minérales étaient les plus biodisponibles en comparant les spectres de terres rares pour les tissus végétaux collectés dans la même zone aux extractions BCR sur les sols correspondants et en observant dans quelle mesure les spectres se ressemblaient. Les résultats ont ainsi montré que les tissus végétaux souterrains reflétaient

étroitement les trois premières étapes d'extraction séquentielle et que la fraction résiduelle ou plus récalcitrante du sol présentait un spectre de REE convexe avec un appauvrissement distinct en REE moyennes (MREE) par rapport aux REE légères (LREE) et Lourdes (HREE). Ceci suggère la nature plus mobile et une biodisponibilité accrue des terres rares moyennes. Cette étude fournit en outre, la preuve que l'Eu peut être exclu par la plante, car une concentration anormale d'Eu a été observée lorsque les extractions BCR étaient normalisées par rapport aux tissus végétaux souterrains.

Le stress ferrique chez les plantes peut conduire à l'exsudation d'acides organiques de faible poids moléculaire (LMWOA) et de phyto-sidérophores. Les terres rares (REE) ont une affinité bien établie pour les groupes carboxyliques des substances organiques et peuvent former des complexes organo-métalliques solubles stables. Ces complexes sont potentiellement disponibles dans les plantes. L'objectif du troisième volet de cette étude était de stimuler le stress ferrique chez deux types de plantes caractérisées par des stratégies d'acquisition du fer différentes (Stratégie I; le colza et Stratégie II; l'orge). Outre l'imposition d'un stress ferrique, les plantes ont été exposées à des solutions de REE de 0, 1400 et 7000 ppb. Après une période de croissance de 28 jours dans les traitements susmentionnés, les paramètres de croissance et l'accumulation de REE dans les tissus ont été mesurés et comparés d'un traitement à l'autre. Si les mesures de la croissance de l'orge n'ont pas indiqué d'effet négatif induit par l'exposition aux REE, ils ont toutefois, révélé une réduction marquée de la hauteur et de la masse des tissus pour les traitements déficients en Fe. Alors que l'accumulation de REE dans les tissus végétaux était directement liée au niveau d'exposition aux REE pour les deux types de plantes, l'exposition au fer a eu un effet exacerbant sur l'accumulation de REE dans les tissus de colza. Les signatures en REE pour les plantes ont montré un léger fractionnement des La, Ce et Gd. Ce fractionnement s'est produit de la solution du système de culture hydroponique vers le tissu racinaire pour le colza et du tissu racinaire vers le tissu aérien pour l'orge.

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INTRODUCTION

The definition and origin of rare earth elements

By IUPAC (2005) definition, rare earth elements (REE) include the 15 elements of the lanthanoid series (lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu)) along with scandium (Sc) and yttrium (Y). With the exception of Pm, which is a short-lived radionuclide, the natural abundance of REE in Earth's crust is quite substantial (Taylor and McLennan, 1985). The natural abundance of Ce within Earth's crust is higher than that of Cu, and the least abundant REE, Lu, is more abundant than Ag. The misnomer of "rare" is most likely due to the fact that these lithophilic elements rarely accrue in deposits at economically viable quantities.

The research executed in this manuscript will limit the definition of REE to lanthanides since this series forms a uniform group with similar electro-magnetic properties ascribed to the unpaired electrons of the f-orbital. Though the presence of lanthanides in ores tends to coincide with the occurrence of Sc and Y deposits as well, their electron configuration varies: [Ar] 4s² 3d¹ and [Kr] 4d¹ 5s², respectively. In comparison, the lanthanide configuration can be described by [Xe] 4fⁿ 5d^{0,1} 6s², where the 4f electron shell is progressively filled with n from 0 to 14. The filling of the f-orbital also seems to induce a phenomenon labeled lanthanide contraction, observed in the contraction of the atomic radius due to insufficient shielding of the nucleus during the filling of the 4f electron shell. Ranging in atomic number from 56 to 71 and in atomic radius from 116 pm to 97.7 pm when in an 8-coordination configuration (Shannon, 1976), REE are apt at isomorphic substitution with other major elements such as Ca with an atomic radius of 112 pm. Typically trivalent REE adopt a coordination number of 8 or 9 (Hovey et al., 2023).

Natural variations in REE abundance can be described by the Oddo-Harkins rule (Cotton, 2006). In order to make REE concentrations more comparable, they are usually represented in a multielement diagram where all REE are plotted along the x-axis following the order of their respective ionic radius, with the y-axis representing log-scaled concentrations, normalized to a specific reference material. The resultant patterns are smooth and obviate any fractionation of REE in study material (Piper and Bau, 2013). Reference materials vary based on study purpose and materials. For igneous rocks, the normalization reference is the primitive mantle (i.e., the mantle prior to the extraction of the continental crust; Wood et al. 1979) or chondrites. For sediments, the references are the NASC (North American Shale Composite; Gromet et al. 1984) and the Post Archean average Australian Shale (PAAS; Taylor and McLennan 1985). The choice of reference for normalization is critical because it conditions our

ability to understand the processes we are trying to describe. Figure 1 shows a soil plotted using the raw REE concentration and the REE normalized to Upper Continental Crust (UCC) values (Taylor and McLennan 1985).

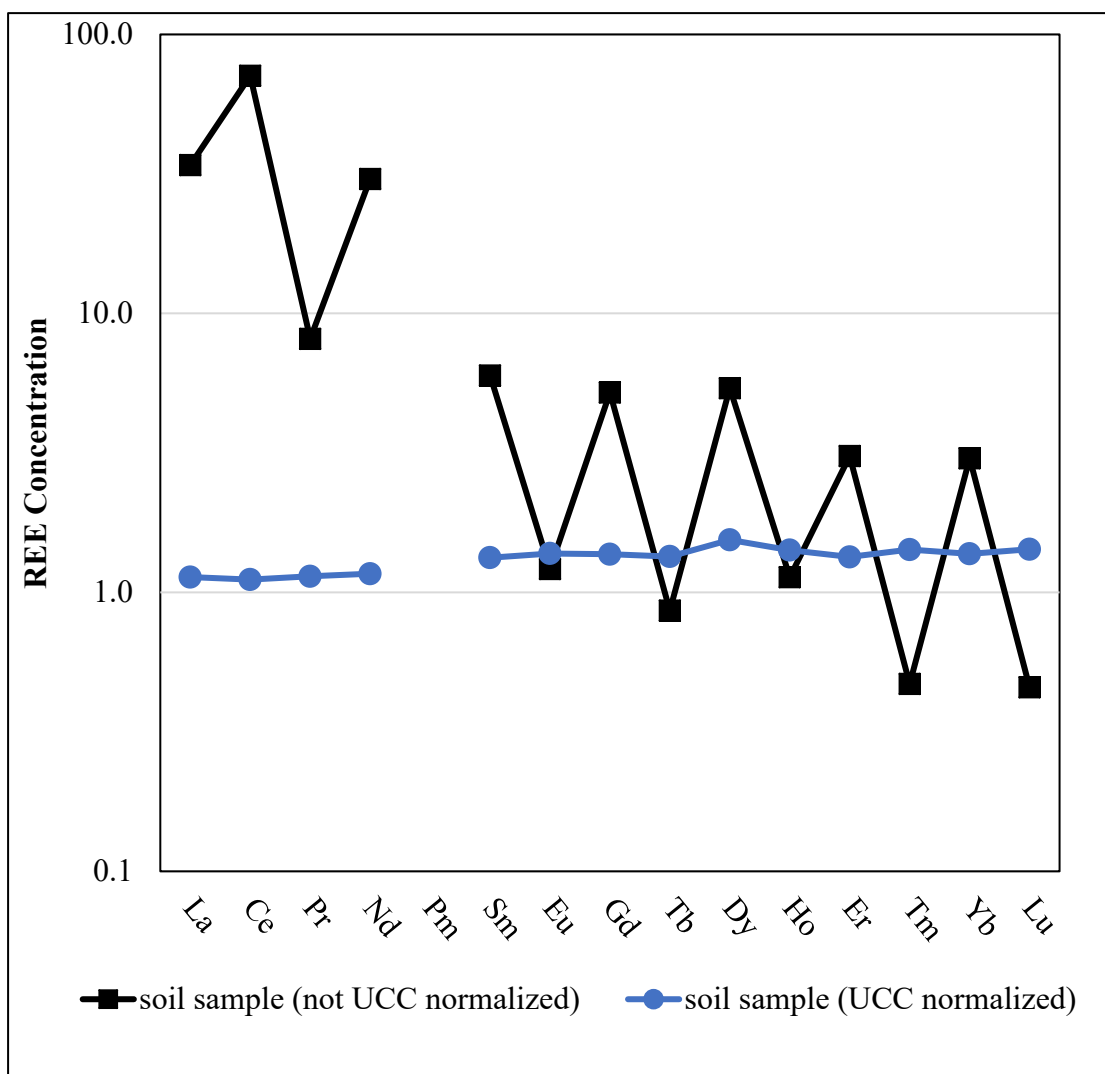


Figure 1. Demonstrates the value of normalizing REE concentrations to a reference material, in this case UCC. The line plotted using raw data contains the units $\mu\text{g g}^{-1}$ while the line plotted using UCC normalized data is a ratio of $\mu\text{g}_{\text{REE}} \text{g}^{-1}_{\text{soil}} / \mu\text{g}_{\text{REE}} \text{g}^{-1}_{\text{UCC}}$.

Slight differences in their chemical behavior enhance either enrichments or depletions amongst each other, resulting in fractionation of REE. Such remarkable behavior results from variations in the (i) increasing ionic radius from La to Lu, (ii) electronegativity and (iii) coordination number. It is therefore convenient to further classify REE by subcategories of: Light Rare Earth Elements (LREE) corresponding to La-Nd, Middle Rare Earth Elements (MREE) corresponding to Sm-Tb, and Heavy Rare Earth elements (HREE) corresponding to Dy-Lu. These three subgroups can exhibit slight differences of pattern shapes when plotted as normalized concentrations versus their atomic number, which is the usual way of representing the REE data.

In addition to categorizing REE fractionation by LREE, MREE, and HREE, fractionation of REE can be described by anomalies. While REE usually occur as stable Ln^{3+}

species in environmental conditions observed at Earth's surface, Ce and Eu can exist as Ce^{4+} and Eu^{2+} when the prevailing conditions are either oxidizing or reducing, respectively (Figure 2). The behavior of Pm is typically not included in any groupings or anomalies, since as predicted by the Mattauch isobar rule there are no stable isotopes of Pm. Small quantities of Pm can be synthesized for technological and medical applications, but there is no significant source of Pm in natural samples (Elkina and Kurushkin, 2020).

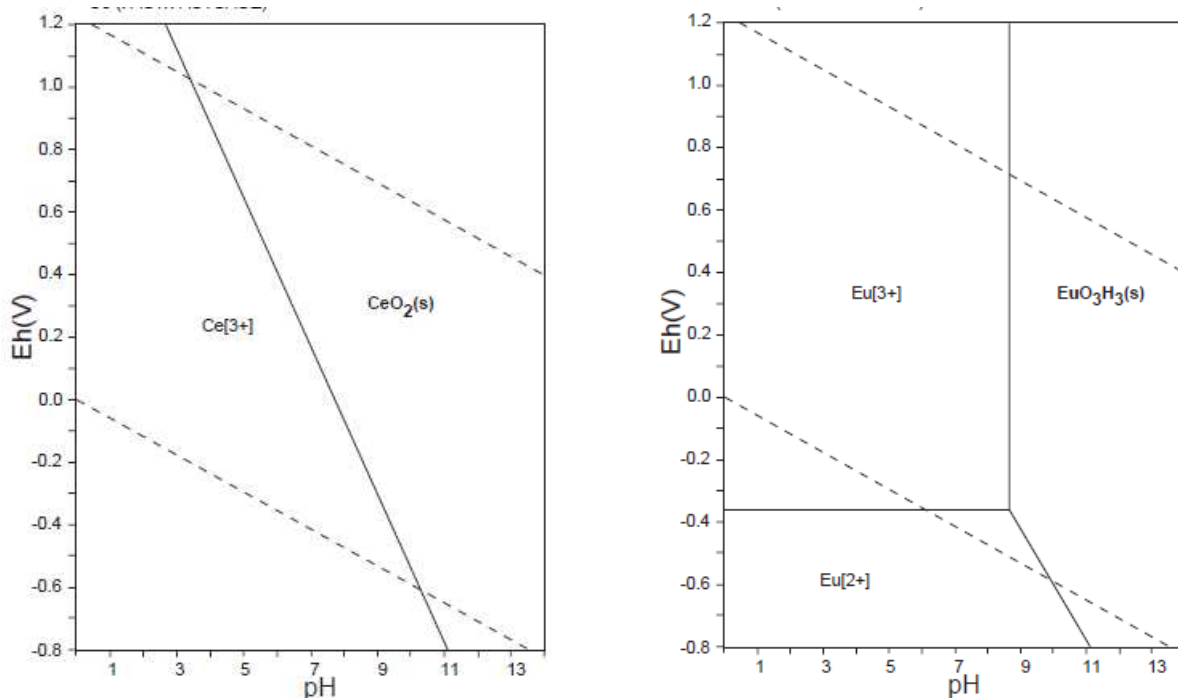


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Despite their similar chemical and physical behavior, REE can be fractionated by several processes, making them very efficient geochemical tracers. As a matter of fact, the REE composition in rocks and minerals has been used to understand and extensively describe the chemical evolution of the Earth (Henderson, 1984; Lipin and McKay, 1989; Taylor and McLennan, 1985). Rare earth element fingerprinting has had applications for geological processes in deep Earth (igneous petrology, mineral formation in ores, hydrothermal activity, etc.) (Graf, 1977; Itano et al., 2018; Mughal et al., 2020; Paoli et al., 2019; Yang, 2019) and at Earth's surface in the study of weathering or soil formation (Laveuf and Cornu, 2009; Pédrot et al., 2015; Stille et al., 2006; Viers et al., 1997). Moreover, as REE are strongly bound to organic matter, their fingerprinting aids our understanding of surface geochemical mechanisms, such as the dynamic structure of organic matter (Pédrot et al., 2010) or the identity of trace metal sources during wetland soil reduction (Davranche et al., 2011). Aside from their use for investigating natural processes, REE have also been an effective tool in assessing anthropogenic pollution sources (Perez-Lopez et al., 2010). Chemical and isotope signatures have even been

adopted in studies to identify the provenance of goods such as glasses, ceramics and wines (Degryse and Schneider, 2008; Piper and Bau, 2013; Rai et al., 2016).

While REE are efficient geochemical tracers, they are also emerging contaminants

Though few mineral deposits contain economically viable amounts of REE, certain ores can host financially feasible quantities, including monazite, bastnäsité, and xenotime (Van Gosen et al., 2017). Aside from incorporation into minerals, REE have been known to accumulate on sorption surfaces. Large amounts of ion-adsorbed REE can be found in clay deposits of Southeastern China, where REE are extracted from alumino-silicate surfaces (Van Gosen et al., 2017).

Economically, REE form a critical resource for the global economy, with 74% of REE ending up in catalysts, 10% used in ceramics and glass, 6% used in metallurgy and alloys, 4% used in polishes, and 6% used for other applications (medical, agricultural, ect) (U.S. Geological Service [USGS], 2022). Indeed, rare earth elements have an important role to play in the energy transition. For example, Nd, Pr and Dy are used for permanent magnets in wind turbines, while Y, Eu and Tb are vital elements for phosphor lamps, and La is used in rechargeable batteries in hybrid cars (Van Gosen et al., 2017). They are also notably exploited for other purposes related to technological advancement: Nd for magnets in computer hard drives; La for camera lenses; Gd as a contrast agent for Magnetic Resonance Imaging (MRI); and La and Ce in agriculture for fertilizers (Figure 3). We can see that these different applications are only growing with time.

Even with the projected demand for REE rising, global reserves of REE (130,000,000 metric tons according to USGS, 2023) would be difficult to exhaust within the next 800 years, using a projection made by Humphries (2013) which assumes an annual consumption of 160,000 tons of REE. Indeed, the ever-increasing demand for REE (Figure 4) is leading to a significant increase in mining production, which is growing exponentially (Haxel et al., 2002). Extraction of REE increased by 6.67% in the last year to meet the demand for these critical elements (USGS, 2022; USGS, 2023), leading to an emergence of elevated REE concentrations observed at Earth's surface (Li et al., 2013; Merschel and Bau, 2015).

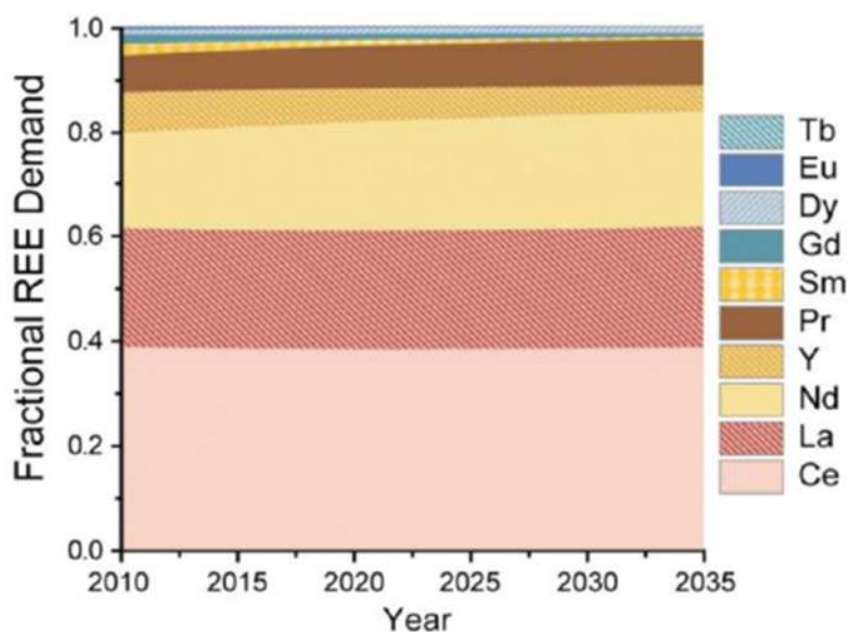


Figure 3. Increasing demand for individual REE, projections made based on historical usage of REE in industry, taken from Alonso et al. (2012).

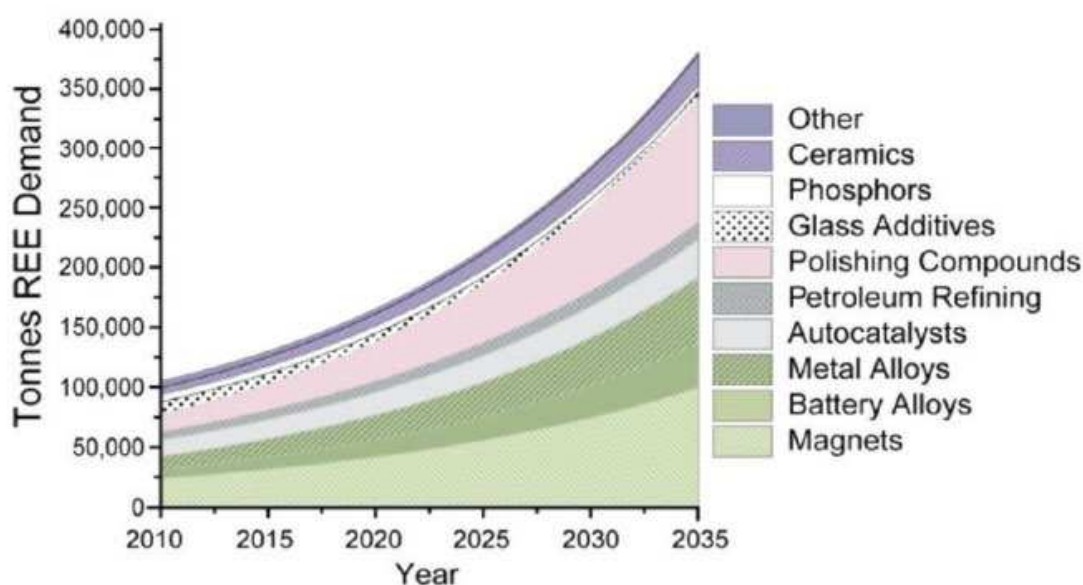


Figure 4. Alonso et al. (2012) proposed a projection of growing demand for REE by field of application, based on observations of past trends. Demand for Nd to manufacture magnets could increase 4-fold from 2010 to 2035.

Faced with this REE consumption, the question of their environmental effects arises. The contrasting use of specific rare earths has led to variations in the shape of REE spectra in different compartments of the Earth's surface, attesting to anthropogenic disturbances. Rare earth element pollution can be found in rivers, lakes and coasts internationally (Bau and Dulski, 1996; Kulaksız and Bau, 2011; 2013; Li et al., 2013; Merschel and Bau, 2015; Nozaki et al., 2000; Verplanck et al., 2005). As an example, Figure 5 shows REE spectra in the waters of the Rhine in Germany, which are particularly atypical for continental waters. Strong positive anomalies in La, Sm and Gd are observed (Kulaksız and Bau, 2013). The positive La anomaly

observed here is linked to an anthropogenic disturbance induced by an industry producing catalysts for refining oil. Increased Sm is due to contributions from an industrial discharge located 250 km upstream of the sampling site (Leverkusen). The excess of Gd is caused by discharges of contrast agents used in medical MRI.

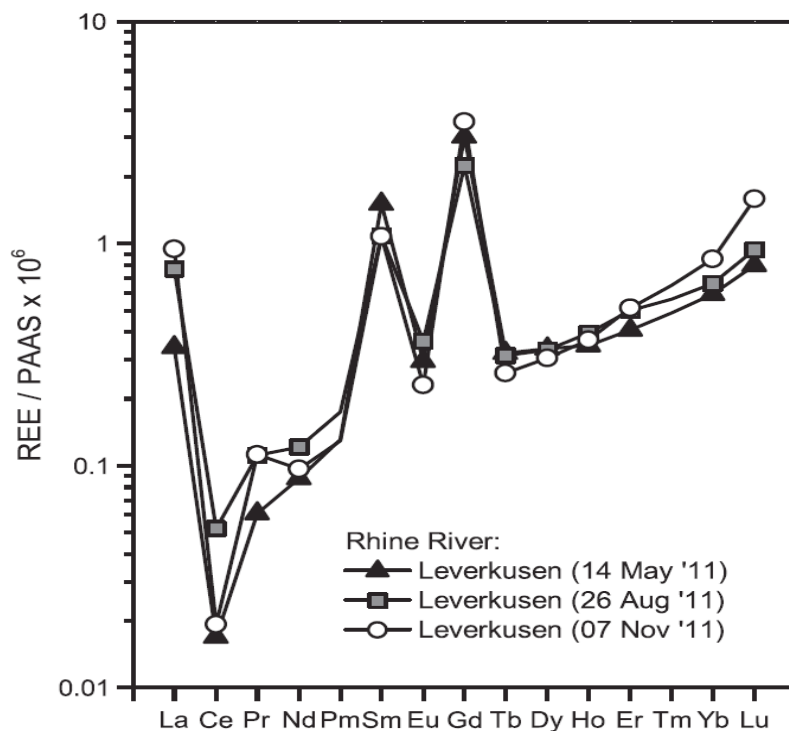


Figure 5. Anthropogenic disturbances recorded in REE signatures, demonstrating the contribution of various waste streams. Figure taken from Kulaksız and Bau (2013).

Where do they occur at the Earth's surface?

At the Earth's surface, REE can be found mainly in rocks and secondary minerals of sediments and soils with smaller amounts amassing in surface waters and biological tissues. As lithophilic elements with a ubiquitous distribution in Earth's crust, the mineral distribution of REE tends to be controlled by: silicate minerals which are associated with 43% of all REEs, followed by carbonates which are associated with 23% of all REEs, oxides with 14%, and phosphates and salts with 14% (Migaszewski and Gałuszka, 2015). Many considerations for REEs have previously revolved around their inorganic interactions, in recent years, studies have begun to focus on their interactions with soil organic matter (such as humic acids). It is estimated that soil organic matter may account for anywhere from 1.84-26.5% of the REE in the soil environment (Pang et al., 2002).

The distribution of REE in soil minerals and organic matter can produce a variety of signatures. With the composition of soils being heterogeneous in nature, soil REE signatures are an amalgamation of the signatures belonging to each mineral group. In acid-sulfate soils, jarosite minerals tended to incorporate more LREE, leading to LREE enrichment in surface waters when these minerals were transformed in oxidative dissolution (Welch et al., 2009). A

study of newly formed precipitate in passive treatment systems found that enrichment patterns for Fe and Al minerals had a MREE enrichment signature with more HREE fixed than LREE (Obregón-Castro et al., 2023). The same study also presented a MREE enrichment pattern with the preferential incorporation of LREE over HREE associated with gypsums. Ion-adsorption mines in China target the extraction of HREE, which preferentially sorbs to clay minerals over LREE, making these alumino-silicates the primary source for HREE (Wu et al., 2023).

Though typically the sorption of REE onto mineral surfaces represents a smaller pool of soil REE, this pool can be sensitive to physicochemical changes and contribute significantly to the mobilization/stabilization of REE. The surface charge of minerals can be described by their zero point of charge (zpc). Though the zpc for minerals varies, some generalizations can be made about the effective sorption surfaces for REE using average zpc reported by Sadiq (1997). The zpc for both carbonate and hydrous Fe-hydroxide minerals varies between pH 7 and 10, making them effective surfaces for REE sorption at relatively high pH, with lower stability and net cationic charges at lower pH. The zpc for aluminum oxide minerals has been recorded as approximately pH 6, making this mineral surface effective for REE sorption in slightly-acidic to circumneutral pH ranges. The zpc for clays varies widely, with ranges recorded from pH 4-8 – Sadiq (1997) reports an average zpc of 5.5. Having a wide pH range in which sites are saturated with negative charges, clays account for a significant proportion of REE chemisorption. In lower pH systems positive charges saturate surface sites, representing an environmental condition not conducive to REE sorption. The zpc recorded for manganese oxides can be between pH 2 and 5 (Davranche et al., 2008; Murray, 1973; Sadiq, 1997), making manganese oxides an important control of REE release in low pH environments. Although the zpc is a physicochemical parameter that demonstrated the relative charge of these surfaces, the importance of them is tempered by the available surface area for sorption reactions.

Soils and sediments provide a valuable sink and source of REE to the biosphere. Chemical weathering of the host rocks drives the dissolution of the primary minerals containing REE, the latter then being mobilised (Braun et al. 1998). The fate of the REE is then either to co-precipitate in secondary minerals, sorb to appropriate surfaces, or to be transported by vectors such as wind or water. As the first two processes of stabilization are well detailed in the above paragraphs, we will now focus on the mobilization of this element series at Earth's surface, the interface for geochemical processes interacting with living organisms, also known as the Critical Zone.

The concentrations and speciations of REE in freshwaters (rivers, surface waters, groundwaters and lakes) are dependent on the interactions and processes prevailing at the water/rocks or water/soil interfaces (e.g. Andersson et al., 2006; Elderfield et al., 1990; Bau and Dulski, 1996; Dia et al., 2000; Dupré et al., 1996; Elbaz-Poulichet and Dupuy, 1999; Forsyth

et al., 2023; Gérard et al., 2003; Goldstein and Jacobsen, 1988; Gruau et al., 2004; Ingri et al., 2000; Johannesson et al., 1995; 1996; 1997; 2004; Johannesson and Lyons, 1994; 1995; Johannesson and Zhou, 1999; Möller and Bau, 1993; Shiller, 2002; Sholkovitz, 1993; 1995; Viers et al., 1997). The importance of these processes is highly dependent on the physicochemical parameters of the water and soil/rock composition. Truly dissolved REE are rather low in abundance. Below pH 7-8 REE, occurring as free REE³⁺ (Takeno, 2005) are often scavenged out of solution by oxides or attracted to sorption surfaces (Davranche et al., 2004; Davranche 2008; Johannesson et al., 1996). Their solubility is largely controlled by speciation with compatible ligands in solution: PO₄³⁻, CO₃²⁻, SO₄²⁻, Cl⁻, and F⁻ (Millero, 1992), and competition with competing cations.

Though the dissolved phase has classically been defined using a cut off of 0.2 µm by filtration, many ultrafiltration studies conducted in different environments assessed that effectively REE in the dissolved fraction occur as colloid-borne particles (Elderfield et al., 1990; Dia et al., 2000; Pédrot et al., 2008; Pokrovsky et al., 2002; 2005; 2010; 2012; 2016; Sholkovitz, 1993; 1995; Tricca et al., 1999; Viers et al., 1997). Field, laboratory and modeling studies have shown the importance of humic/fulvic acids as carrier phases of dissolved REE (Dia, 2000; Chen et al., 2015; Kouhail et al. 2019; Marsac et al., 2010; Pourret et al., 2007; Tang and Johannesson, 2003). Organic phases may further inhibit REE stabilization through chemisorption to other mineral surfaces by steric hinderance, saturation of binding sites, and size exclusion or may promote REE sorption in the case of organics acting as bridging ligands – though this is limited to scenarios where soils are saturated with positive charges (Davranche et al., 2004; Davranche 2008).

Even though the surface functional groups are very diverse, ranging from acidic to basic, only two types of sites are generally modeled for REE sorption: carboxylic groups with low pK_a (5-6) and phenolic groups with higher pK_a (>8). Additionally, the coordination complex modifies the binding strength. The binding of polydentate ligands is entropically favored compared to that of monodentate ligands. Typically, a MREE enrichment is associated with the monodentate complexation while a HREE preference has been reported for stronger binding sites. Although simple carboxylic acid groups tend to form weaker monodentate complexes in high REE saturated environments, the function of stronger binding sites, associated with multidentate complexation or other anionic functional groups, is more pronounced at lower REE concentrations (Marsac et al., 2010).

What is the Bioavailable Pool of REE in Soils?

The bioavailable pool of metals in the soil has been frequently debated, with no conclusive definition. Often the limiting factor to metal availability in the environment is local

soil processes dictating physical and chemical speciation, dependent on biotic processes (e.g. biological activity within the rhizosphere) and abiotic processes (e.g. soil physical-chemical parameters), in interdependence. For REE five major processes which dictate this include: (i) the complexation mediated by inorganic ligands such as chlorides, carbonates or sulfates, (ii) the complexation by little low-weight organic ligands, such as acetic or oxalic acids, (iii) the complexation by organic colloids such as humic substances, (iv) the adsorption/desorption processes of REE with mineral surfaces such as Fe, Mn, Al-oxyhydroxides or clays and (v) the precipitation of secondary phases such as bastnäsite, cerianite, and churchite (Banfield and Eggleton, 1989; Chi et al., 1999; Wu et al., 2023).

While dissolved pools of metals are readily available (Adriano, 2001; Di Bonito et al., 2008), the physicochemical properties and the speciation of the metals, as well as the physiology of the organism affected, can influence the bioavailability of a metal. It is therefore difficult to make generalizations about the toxicity or bioavailability of metals. Nonetheless, the study of metal bioavailability is an important topic for assessing environmental and human health risks (Kubová et al. 2008; Liang et al., 2005; Pang et al., 2002; Shan et al., 2003; Wang et al., 2001a; Wang et al., 2001b; Zhang and Shan, 2001).

Many studies have been interested in assessing the environmental risk posed by multiple elements at once, which makes determining the controls of each metal an arduous affair. Due to these barriers, a series of studies were devoted to making broad definitions of metals associated with soil phases. In 1993, this led to a standardized sequential extraction scheme developed by the Community Bureau of Reference (BCR) (Ure et al., 1993). This scheme would divide the soil phases into four fractions: the exchangeable and carbonate-bound, the reducible, the oxidizable, and the residual phases. The stability of REE associated with exchange sites and carbonates is highly subject to pH-Eh changes which may alter the surface charges or cause dissolution of the host mineral, leading to mobilization of REE. In general, the exchangeable and carbonate-bound REE is considered the most labile and bioavailable pool. The reducible mineral pool accounts for REE incorporated within amorphous and slightly crystalline Fe/Mn-oxide mineral structures, whereas the oxidizable pool is composed of organic matter and oxidizable sulfide minerals. While the reducible and oxidizable mineral pools are sensitive to pH-Eh changes, these are typically more stable associations since REE is buffered by the matrix. The residual pool of REE is the most recalcitrant and dominant pool of REE in natural soils. Typically, the residual pool is composed of highly crystalline oxides and siliceous materials which are only degraded with heat, pressure, and acidity.

Transfer of Rare Earth Elements from the environment to plants

Although non-essential nutrients, plant incorporation of REE has been well documented (Pang et al., 2002; Tyler, 2004; Tao et al., 2022). In fact, REE have been reported to have hormetic effects on agricultural plants, leading to REE application on arable lands and seed treatments since 1980 (Pang et al., 2002). The reported benefits of REE have been better plant P and K nutrient status, boosted photosynthesis, regulated hemoenzyme peroxidase function, maintained structural integrity under Ca-deficient conditions where REE replace Ca^{2+} , and improved resistance to metal stress (Pang et al., 2002; Tyler, 2004). Examples proving their benefits have led to recommendations of REE-based agricultural treatments every crop cycle. Amidst growing concern for their toxicity (Pagano et al., 2015; Wakabayashi et al., 2016; Gong et al., 2021), the cumulative effects of these applications have received more attention in research.

In planta, REE have previously been implicated in the interruption of Ca, Mg, Mn, K, Cu, and Zn metabolism, reduction of root growth, and destruction of cellular integrity (Pang et al., 2002; Tyler, 2004). Natural plant samples can contain REE in undetectable quantities, although on the other end of the spectrum *Dichranopteris dichotoma* accumulate up to 3358 mg kg^{-1} in above-ground tissues (Wang et al., 1997). There is a potential for phytoremediation to play a role in controlling REE input from point sources. For successful remediation, both hyperaccumulators and plants with large biomass present useful tools (Liu et al., 2018). Classification as a hyperaccumulator being limited to plants which concentrate more than 1000 mg kg^{-1} REE in their above-ground tissues (Liu et al., 2018) hyperaccumulating plants are rare.

Physicochemical parameters pertinent to plant accumulation of REE can include pH, redox state, and ionic strength of the soil solutions. But, physicochemical conditions alone cannot explain the wide range of values for REE incorporated into plant tissue described by the literature (Wang et al., 1997; Wiche and Heilmeier, 2016; Wyttenbach et al., 1998a; Wyttenbach et al., 1998b). It is hypothesized that plant and microbial responses to nutrient deficiencies, which trigger the exudation of organic ligands, may play an important role in controlling REE bioavailability (Wiche and Pourret, 2023). The potency of simple carboxylic acids and biosynthesized organics as ligands has shown promise in the recovery and separation of REE from waste streams and even partitioning from one another (Chang et al., 2020; Han, 2020; Hovey et al., 2023). This means that plant exudates could promote the uptake of REE as well as partition particular REE, creating a unique signature.

Little work has been performed to isolate the synergistic or inhibitory effects of Fe-nutrient status on REE, though studies by Brioschi et al. (2013), Forsyth et al. (2023), and Wyttenbach et al. (1998a) have all implicated Fe in plant accumulation of REE. Plants can be

categorized into one of two iron-acquiring strategies (strategy I and strategy II), with a small number of plants capable of combining these strategies. Strategy I is typically associated with non graminaceous monocotyledon and dicotyledon plants, while strategy II is performed by graminaceous plants (Figure 6). Strategy I uses proton excretion and secondary metabolite exudation to affect the solubility of Fe(III) in the rhizosphere – Fe(III) is reduced by ferric chelate reductase and subsequently transported into the root by iron regulated transporters. Strategy II involves the production of phytosiderophores which leave the plant through mugineic acid transporters. These siderophores selectively bind Fe and collect the organo-metallic complex through the yellow-stripe transporters (Marschner and Römheld, 1994).

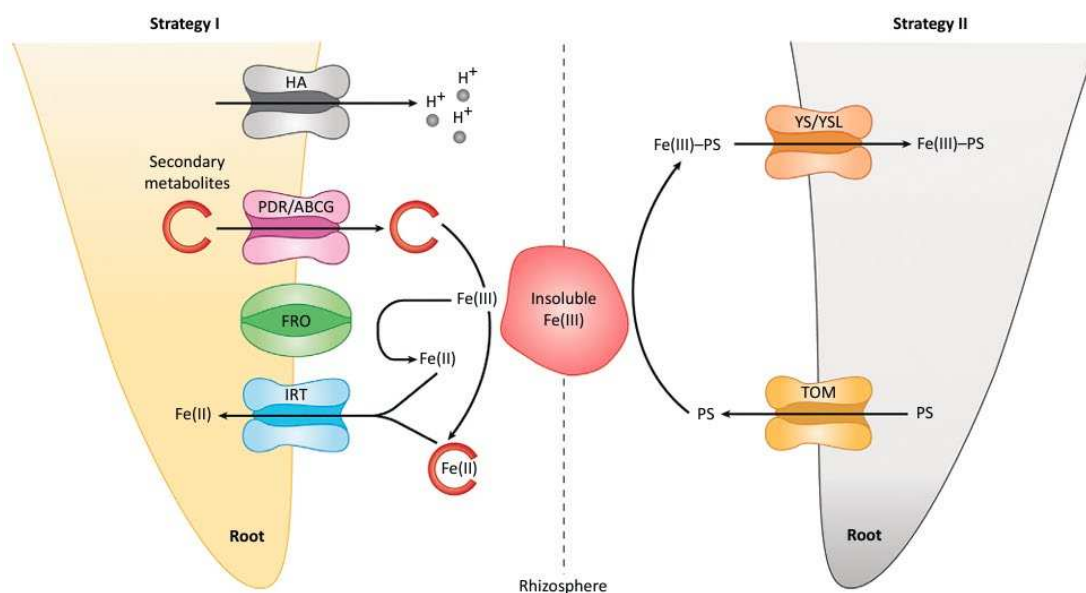


Figure 6. Fe-nutrient acquiring strategies, figure taken from Tsai and Schmidt (2017).

Main addressed issues, objectives and thesis plan

With the circulation of these elements in the biosphere increasing with utilization, it is essential to understand their fate and transport. Though many studies exist on bioremediation tactics and their effect on managing toxic elements in the environment, there are large gaps in the research of REE, especially with regards to the entire series of REE. Though their physicochemical properties are similar, the study of REE partitioning may help us better understand the mechanisms controlling REE dynamics in the context of planted environments.

The definition of a bioavailable pool has yet to be established. As a consequence, one of the main scientific obstacles to overcome is defining which soil fractions represented an available nutrient source to plants and determining which biological factors limit both the uptake and translocation of REE.

This manuscript displays a succession of three articles, with the first one published, the second submitted for publication, and the third one still in preparation for submission to

international peer-reviewed journals. It is divided into five main sections, starting with an introduction, followed by three chapters composed of the three articles and ending with a section devoted to the conclusions and perspectives arising from this study.

Chapter 1 introduces the partitioning of REE into three relevant environmental compartments: soil, surface water, and plant tissues. This study provides valuable insights into geological and biological partitioning of REE in various sample media. The study sites selected are heavily impacted by mining activity, and so represent ideal areas for the study of REE, many studies having demonstrated that REE solubility is high in such areas. This allowed us a better understanding of REE dynamics in an environment characterized by high metal mobility. Plant tissue samples collected from the mining area tended to accumulate more REE than the background samples collected from outside of the mining area. The surface waters had elevated MREE concentrations typical of mining waters, and this signature was mirrored by below-ground plant tissue. Above-ground plant tissues either maintained a MREE enrichment or showed a LREE enrichment, demonstrating a variety of signatures associated with the partitioning of REE into different environmental compartments. The material in Chapter 1 corresponds to an article published in *Frontiers in Environmental Science* (Forsyth et al., 2023).

Chapter 2 builds on the work detailed in Chapter 1 by further examining the chemical speciation of REE in soils/sediments. This study related the different pools of REE to plant availability, with results showing that MREE were incorporated into more mobile soil phases across each site. This in turn resulted in MREE seen in the below-ground tissue of plants collected from the site, exemplified by a normalization of the sum of the three sequential extraction steps to the below-ground tissue REE concentration showing little to no REE partitioning. Though there was no exaggerated REE group pattern for the BCR normalization, there was an exaggerated negative Eu anomaly. This Eu anomaly indicates that, though Eu was incorporated into more mobile soil phases, there was an exclusion of Eu from below-ground plant tissues. The results can be found in an article submitted for publication in *Chemosphere*.

Chapter 3 is dedicated to the biological partitioning of REE and the effects of nutrient deficiency on these biological processes. In order to isolate plant effects, plants were grown in a hydroponic system for 28 days. Treatments consisted of 3 REE levels, applied to two different plant types, barley and rapeseed, under either iron-sufficient or iron-deficient nutrient regimes. The growth parameters for barley were sensitive to iron status, there was an interactive effect between REE and Fe which produced a slight hormetic increase in root growth for barley. Rapeseed had no observable hormetic effect for any of the growth parameters. Both plant types accumulated REE, with an increase in tissue concentration directly related to the REE application level. Rapeseed tissues accumulated more than 10 times the amount of REE barley accumulated. Fe-deficiency did not impact the accumulation of REE in barley, but exacerbated

the accumulation of REE in rapeseed tissues. An observed fractionation of the La, Ce, and Gd was maintained in both plant types, though this partitioning occurred between the hydroponic solution and the root tissue for rapeseed and between the root tissue and the aerial tissue for barley. The work presented in Chapter 3 is under preparation for submission into an international peer-reviewed journal.

Finally, the conclusions and implications of the main results are summarized, as well as the prospects arising from this work. At the end a mechanistic model is proposed combining the conclusions from all of the preceding chapters.

CHAPTER 1: BIOCONCENTRATION AND TRANSLOCATION OF RARE EARTH ELEMENTS IN PLANTS COLLECTED FROM THREE LEGACY MINE SITES IN PORTUGAL

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Chapter 1 overviews the compartments where REEs occur in the context of three legacy mine sites. Chapter 1 is published in *Frontiers in Environmental Sciences* and can be cited as the following:

Forsyth, K., Dia, A., Marques, R., Prudêncio, M.I., Diamantino, C., Carvalho, E., Russo, D., Dionisio, I., Davranche, M., Bouhnik-Le-Coz, M., Pédrot, M. (2023) "Bioconcentration and translocation of rare earth elements in plants collected from three legacy mine sites in Portugal." Front. Environ. Sci. 11:1191909. doi: 10.3389/fenvs.2023.1191909.

CHAPTER 1: ABSTRACT

This field study aims to evaluate the phytoavailability and fate of rare earth elements (REE), a group of emerging contaminants. Spontaneous vegetation and related media (soils and surface waters) were collected from three Portuguese legacy mine sites in November 2020. Though none of the studied mines targeted the extraction of REE ores, the prevailing geochemistry of the mining environment favors their mobility, and is therefore ideal for demonstrating the behavior, fate, and cycling of REE in the environment. Data were analyzed in the context of the (i) prevailing geochemical context, (ii) the mining context, and (iii) plant effects. This study presented the REE signatures for different plant species and linked the signatures to a potential source of bioavailable REE. The REE accumulated in plant tissue seemed to reflect the REE signature of surface waters in the mining areas, showing enrichment in middle REE. Although the soils, sediments, and waters in this study had similar features, certain plants seemed better adapted to extracting particular REE over others. Light REE and Eu in particular seemed to be accumulating and translocating more, depending on plant type. This is of interest because REE share many physical and chemical characteristics and have thus been used as accurate tracers of various environmental samples. Given that REE are readily available within the field conditions of a mining site, this study shows how plant physiology and biologic preference towards particular REE contributes to the fractionation of REE and creates a unique signature dependent on plant type.

CHAPTER 1: RESUMÉ

Les éléments terres rares (REE), un groupe de contaminants émergents ayant des applications commerciales et technologiques, partagent de nombreuses caractéristiques physiques et chimiques communes et ont été utilisés comme des traceurs efficaces de divers échantillons et processus environnementaux. Il a été démontré qu'ils augmentent dans les eaux au cours des activités minières à la suite de la dissolution des matériaux composant la roche hôte. Dans cette étude, la végétation spontanée et les milieux connexes (sols, sédiments aquatiques et eaux) ont été collectés dans trois anciens sites miniers portugais en Novembre 2020 afin d'évaluer la phytodisponibilité et le devenir des terres rares. Les données relatives aux eaux, sols, sédiments aquatiques et plantes ont été analysées dans le contexte : a) du contexte géochimique dominant, b) du contexte minier et c) des effets liés aux plantes. Cette étude présente les signatures des REE pour différentes espèces de plantes et relie les signatures à une source potentielle de REE biodisponibles. Les terres rares accumulées dans les tissus végétaux semblent refléter la signature des terres rares des eaux de surface dans les zones minières, en montrant un enrichissement en terres rares moyennes. Bien que les sols, les sédiments et les eaux de cette étude présentent des caractéristiques similaires, certaines plantes semblent mieux adaptées que d'autres à la translocation des terres rares légères et de l'uranium. Les REE étant facilement disponibles dans les conditions d'un site minier, cette étude montre comment la physiologie des plantes et la préférence biologique pour certaines REE contribuent au fractionnement des REE et créent une signature unique dépendant du type de plante.

1 Introduction

The rare earth elements (REE) are comprised of 17 elements: the lanthanoid series (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu), plus Y and Sc (Pan et al., 2020). They can be characterized by similar chemical properties and closely related geochemical behaviors, resulting in similar distributions within the Earth's crust (Taylor and McLennan, 1985). Though Y and Sc are officially defined as REE by the International Union of Pure and Applied Chemistry (IUPAC) (2005), the lanthanoid series (La to Lu) share electrical and thermodynamic properties (Joshi et al., 2018) and a unique electron configuration which results in lanthanide contraction. Therefore, this paper will only consider La-Lu when referencing the REE series.

Since the initial discovery of REE in 1794, they have become a critical part of the world economy (U.S. Geological Survey, 2022). Their versatile applications in industry, medical services, agriculture, and many other domains (Bau and Dulski, 1996; Tyler, 2004; Binnemans et al., 2013; Migaszewski and Gałuszka, 2015; Van Gosen et al., 2017) have inevitably led to the elevated release of REE in the environment (Li et al., 2013; Merschel and Bau, 2015) and an increased risk of occupational exposures (Rim et al., 2013). Yet the environmental risks related to REE have so far received little attention, overshadowed as they are by economic and geopolitical considerations. Evidence suggesting their potential toxicity (Pagano et al., 2015; Wakabayashi et al., 2016; Gong et al., 2021) has consequently led to REE being categorized as 'emerging contaminants', potentially making the anomalous supply of REE in the environment a major challenge for the years to come.

Historically the management of mining wastes has posed a risk to the environment, with many sites leaving a legacy of significant pollution to the surrounding environment (Perez-Lopez et al., 2010; Li et al., 2013; Soyol-Erdene et al., 2018). Economically valuable resources have often been mined, generating major anthropogenic waste streams and spurring geogenic pollution, exemplified by Acid Mine Drainage (AMD). AMD is characterized by the oxidation of iron-sulfide minerals leading to the perpetual formation of sulfuric acid, concomitant with an increase in soluble metal concentrations (Johnson and Hallberg, 2003; Herman and Maier, 2009). Several countries such as Australia, Bolivia, China, France, Germany, India, Portugal, Romania, Spain, Sweden, and the United States (Akcil and Koldas, 2006; Casiot et al., 2009; Grawunder et al., 2014; Strosnider et al., 2014; Marquez et al., 2018, and references therein) are impacted by AMD, making it a global concern. These mine waste sites, with large quantities of metals solubilized by AMD and mining practices, provide valuable insights into the behavior of REE in the environment. Because of their ubiquitous distribution in Earth's crust, the dissolution of host-rock material during local mining activities has been shown to increase REE

in receiving waters (Ferreira da Silva, 2009; Perez-Lopez et al., 2010; Prudêncio et al., 2015; Soyol-Erdene et al., 2018), even when the resources being mined are not REE.

While there are 199 cataloged abandoned mines in Portugal, this study focuses on three sites: São Domingos, Lousal, and Quinta do Bispo. These sites have been chosen as subjects of study due to their long history of mining activity in a variety of geologic contexts with different stages of remediation efforts in place. Though the context for each of these mines is vastly different, they share previously observed high REE levels (Ferreira da Silva, 2009; Perez-Lopez et al., 2010; Cardenas et al., 2023; Obregón-Castro et al., 2023). Each site demonstrates anthropogenically driven changes to geochemistry as São Domingos and Lousal are subject to AMD evolution from waste rock piles, while low-grade uranium ores from Quinta do Bispo have been processed using *in situ* leaching (ISL). The Lousal site boasts a passive treatment system in an effort to remediate tailing leachate and AMD. Though no such treatment systems were in place at the other two sites as of November 2020, all three were monitored regularly with future remediation projects planned by Empresa de Desenvolvimento Mineiro (EDM). Since the geochemistry and background of each mine are unique, we are afforded the opportunity to study various control parameters, making them ideal targets for this investigation of the uptake and fractionation of REE by spontaneous vegetation as well as the concentration in corresponding soils and waters.

Plants potentially play a vital role in the fate and transport of REE (Tyler, 2004). Although some studies have suggested phytoremediation strategies to stabilize and remove REE from contaminated soils (Lima and Ottosen, 2021), the topic has been the subject of limited study. To date two plant types, *Dichranopteris dichotoma* (Chour et al., 2018; Liu et al., 2018) and *Phytolacca Americana* (Liu et al., 2018), have been identified as candidates for REE recovery when combined with enhanced extraction using electrokinetic methods (Lima and Ottosen, 2021).

The origin of REE fractionation in plant tissue is still subject to many questions as well. Very few authors have published on the entire REE series and their impact on the planted environment, instead opting to study a selection of REE considered representative of the LREE (light rare earth elements), MREE (middle rare earth elements), and HREE (heavy rare earth elements). However, the stability constants formed between individual REE and compatible ions vary (Millero, 1992), potentially creating differences in the bioavailability of REE in the natural environment, thus demonstrating the importance of studying the entire REE series. Moreover, while some studies have concluded that the geologic context and soil weathering are critical factors influencing plant REE uptake (Laul and Weimer, 1982; Pisciotta et al., 2017), others have suggested that a combination of plant type and geologic context is important to note in determining the phyto-availability of REE (Gałuszka et al., 2020).

Through the analysis of water, soil, and plant samples, this study presents REE signatures. The implication of each signature is discussed in the context of the environmental compartment, the geochemical background, and the mining influences. Using the bioconcentration and translocation factors, this study presents data on REE phyto-availability and fractionation.

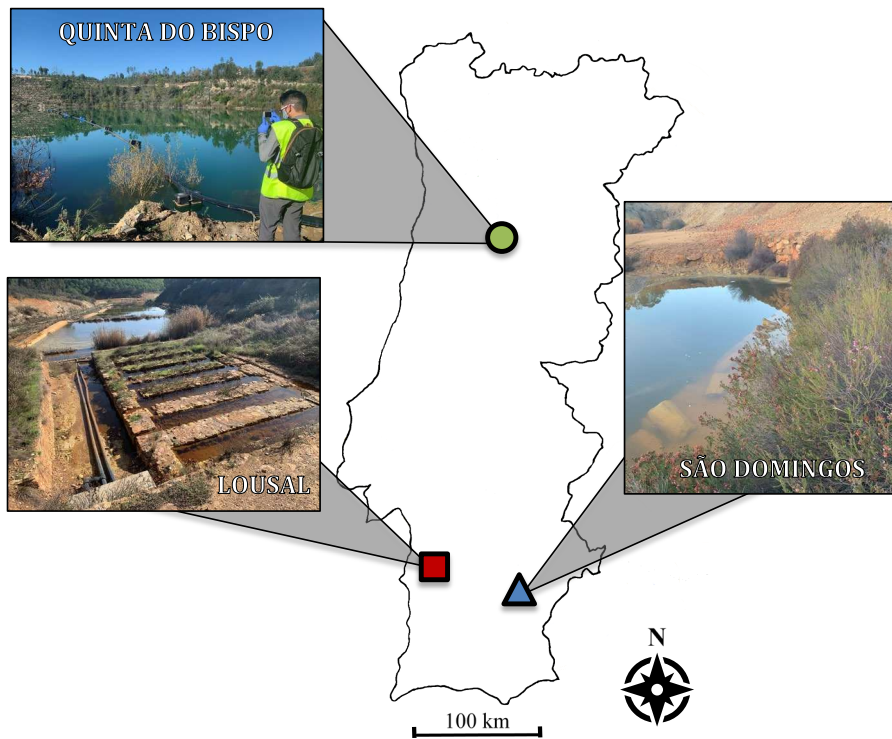
2 Materials and Methods

Nine water samples, 13 soil/aquatic sediment samples and 12 plant samples were recovered in November 2020 from three different legacy mine sites (Table 1) located in Portugal. Samples were collected from both inside and outside of mining sites to assess the legacy of mining activity. Site management and remediation is currently supervised by EDM, a State-owned enterprise, under a concession contract concluded with the Portuguese State for the environmental recovery of degraded mining areas (approved in Decree-Law nº 198-A/2001, 6/7/2001).

2.1 Field site description and collected samples

The sample locations and types are reported in Table 1 and mapped in Figure 1. The geological characteristics of the sampling sites and the descriptions of the samples collected are detailed in the following section.

São Domingos – São Domingos, located in Southern Portugal, has been an established mining area since Chalcolithic times. Copper and sulfur ores were the dominant resources extracted from São Domingos mine till its closure in 1966 (Pérez-López et al., 2010). São Domingos covers 50 km² of land in the Iberian Pyrite Belt (IPB) (Quental et al., 2002), an area infamously impacted by mining and the resulting AMD. The volcanogenic massive sulfides (VMS) in the IPB are rich in pyrite (FeS₂) with inclusions of chalcopyrite (CuFeS₂), sphalerite ((Zn,Fe)S), galena (PbS) and other polymetallic sulfide minerals. As the VMS deposits are exposed to the atmosphere, Fe(II) is oxidized to Fe(III) and S is oxidized to SO₄²⁻, forming H₂SO₄ in surface waters. The perpetual cycle of AMD has been a proven challenge for environmental recovery efforts. EDM's strategy for rehabilitation prioritizes (1) the diversion of clean waters, (2) sealing and confinement of the mine waste deposits to prevent further AMD generation, (3) passive treatment of AMD, and (4) decontamination of the downstream Mosteirão River Valley. In order to mitigate the volume of contaminated waters, diversion of uncontaminated surface waters, as part of the first intervention, has already been completed. Following remediation, EDM intends to preserve the heritage of São Domingos and promote tourism.



Sample Type	Number of Observations	
	(n)	
Water	9	
Soil	13	
Above-ground Tissue	11	
Below-ground Tissue	11	

Figure 1. Map of the sample sites with the number of observations for each sample type (n).

Table 1. Coordinates for all samples collected from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo).

Mine Site	Sample Type	Sample ID	Latitude	Longitude
São Domingos Mine	Water	Tapada Grande Reservoir, "uncontaminated"	N 37°40'10.2"	W 7°30'25.2"
	Water	Pt. 1: São Domingos, "contaminated"	N 37°39'34.0"	W 7°30'19.9"
	Water	Pt. 2: São Domingos, "contaminated"	N 37°39'11.3"	W 7°30'34.5"
	Plant/Soil	<i>Cistus ladanifer</i> ^a	N 37°40'10.2"	W 7°30'25.2"
	Plant/Soil	<i>Cistus ladanifer</i>	N 37°39'34.0"	W 7°30'19.9"
	Plant/Soil	<i>Cistus ladanifer</i>	N 37°39'37.4"	W 7°30'19.1"
	Plant/Soil	<i>Erica andevalensis</i>	N 37°39'11.3"	W 7°30'34.5"
Quinta do Bispo	Water	Pt. 1: flooded open pit mine, "contaminated"	N 40°34'42.7"	W 7°46'22.0"
	Plant/Soil	<i>Salix sp.</i>	N 40°34'37.5"	W 7°46'24.1"
	Soil	Unplanted	N 40°34'37.5"	W 7°46'24.1"
	Plant/Soil	<i>Pteridium aquilinum</i> ^a	N 40°34'39.1"	W 7°46'38.0"
	Soil	Unplanted	N 40°34'39.1"	W 7°46'38.0"
	Plant/Soil	<i>Pteridium aquilinum</i>	N 40°34'40.3"	W 7°46'16.6"
	Plant/Soil	<i>Retama sphaerocarpa</i>	N 40°34'40.3"	W 7°46'16.6"
	Soil	Unplanted	N 40°34'40.3"	W 7°46'16.6"
Lousal Mine	Water	Uncontaminated water from Albufeira da Barragem da Tapada supplemented to passive treatment	N 38°02'15.1"	W 8°25'31.1"
	Water	Pt. 1: Leachate containment for tailings, "contaminated"	N 38°02'17.2"	W 8°25'25.8"
	Water	Pt. 2: Mixing pond for leachate and diluting water	N 38°02'15.6"	W 8°25'25.4"
	Water	Pt. 3: First treatment pool lined with limestone	N 38°02'10.2"	W 8°25'36.4"
	Water	Pt. 4: 6th treatment pool with plants established	N 38°02'10.7"	W 8°25'18.3"
	Plant/Sediment	<i>Juncus acutus</i> ^a	N 38°02'15.1"	W 8°25'31.1"
	Plant/Sediment	<i>Typha Latifolia</i> ^a	N 38°02'15.1"	W 8°25'31.1"
	Plant/Sediment	<i>Typha Latifolia</i>	N 38°02'10.7"	W 8°25'18.3"
	Plant/Sediment	<i>Juncus acutus</i>	N 38°02'10.7"	W 8°25'18.3"

^a Samples collected from outside of the mining area.

The climate of São Domingos can be described as a temperate Mediterranean Köppen climate type with dry and hot summers (*Csa*) with an average annual air temperature of 17.5 °C (Estatal de Meteorología (España) and Instituto de Meteorologia (Portugal), 2011). On average 500 mm of precipitation accumulates over the course of a year (Estatal de Meteorología (España) and Instituto de Meteorologia (Portugal), 2011). Species collected from São Domingos include: *Cistus ladanifer* and *Erica andevalensis*, both were prevalent species growing in the sampling area. *Erica andevalensis* is a metallophyte species endemic to the IPB, typically found growing on tailing piles or close to AMD-impacted surface waters (Cabezudo and Rivera, 1980). Since *Erica andevalensis* is identified as a vulnerable species (Law 8/2003 on Andalusian Wild Fauna and Flora) in Junta de Andalucía: Consejería de Medio Ambiente's list of threatened flora (Santa-Bárbara Carrascosa and Valdés, 2008/2008), this plant is subject to special protections.

Lousal – Lousal is an example of a legacy mine site under remediation and culturally revitalized as an educational center (Relvas, 2014). Located in the IPB of Southern Portugal, all mining activity ended at Lousal in 1988 (Relvas, 2014) when the extraction of S, Cu, and Zn from the polymetallic massive sulfide deposit became less profitable. Long established for the extraction of VMS ores, much like São Domingos, Lousal is impacted by AMD (Ferreira da Silva, 2009). In 2010, a passive treatment system consisting of 17 basins was built as part of EDM's first phase of treatment works. The treatment system collects leachate from a sealed tailing pile. The leachate is supplemented with water from Albufeira da Barragem da Tapada reservoir during dry seasons to maintain the wetland system. Basins are structured in a cascade with a limestone barrier between each basin constituting a permeable dike and HDPE geomembrane lining underneath to contain treatment effluent until discharge into Corona Creek, a tributary of Sado River. In the first treatment stage, five basins are devoted to the neutralization, precipitation, and sedimentation of the leachate mixture. The consecutive basins are planted with macrophytes, which remediate the metal loading (Al, As, Cu, Fe, Zn) from the tailing pile (Empresa de Desenvolvimento Mineiro [EDM] and Direcção Geral de Energia e Geologia [DGEG], 2011). According to the measurements made during the sampling campaign, by the time the effluent reaches Corona Creek for discharge the pH is increased from 2.3 to 3.2.

The climate of Lousal is similar to that of São Domingos, however Lousal accumulates 600 mm of rainfall annually on average (Estatal de Meteorología (España) and Instituto de Meteorologia (Portugal), 2011). The treatment system is dominated by aquatic species such as: *Phragmites australis*, *Typha latifolia*, and *Juncus acutus*. Due to sampling constraints and the large rooting system associated with *Phragmites australis*, only *Typha latifolia* and *Juncus acutus* were collected for analysis.

Quinta do Bispo – Quinta do Bispo is a legacy uranium mine site hosted in Hercynian granite and meta-sedimentary deposits (Ramalho et al., 2009) in Northern Portugal. Unlike São Domingos and Lousal, Quinta do Bispo is not subject to the geogenic phenomenon of AMD. Quinta do Bispo was Portugal's largest open pit mine (European Commission Directorate-General for Energy, 2012). Between 1979 and 1987, 460,000 tons of ore were excavated, resulting in a 75-meter deep pit (Ramalho et al., 2009). Later, in 1992 the open pit was used to extract low-grade uranium ores (300-500 ppm) using ISL, which employs strong acids – such as sulfuric acid – to leach precious metals from ore. The exploitation of hydrothermally deposited uranium seams has left the nearby surface waters with contamination from extraction processes (Pereira, 2014). Environmental remediation of ISL practices is ongoing at Quinta do Bispo. This site was selected for study because the lanthanide series has similar physiochemical characteristics to the actinide series.

The climate of Quinta do Bispo can be described by a temperate Mediterranean Köppen climate type with a dry and temperate summer (*Csb*) characterized by an average air annual temperature of 15 °C and 1400 mm annual rainfall (Estatal de Meteorología (España) and Instituto de Meteorologia (Portugal), 2011). Due to site conditions being conducive to sample access the Quinta do Bispo site is the focus of the comparison between planted and unplanted soil environments. Species collection was limited to plants with large biomass and high prevalence on-site. Species collected included: *Salix sp.*, *Pteridium aquilinum* and *Retama sphaerocarpa*.

2.2 Sample recovery and analyses

All materials used for the recovery of major cation and trace metal analysis were prepared in a clean room and decontaminated using a 24 h nitric acid (1.5 M HNO₃) wash at 45 °C, followed by a 24 h wash with deionized ultrapure water (18 MΩ-cm) at 45 °C.

2.2.1 Water samples

In situ physiochemical water quality parameters were measured during sample collection using a Aquaread AP-2000 multimeter with an AquaPlus 2000 dynamic luminescence quenching optical dissolved oxygen (DO) combination electrical conductivity (EC) and temperature electrode, and a 3MPK1 silver chloride combination pH and oxidizing-reducing potential (ORP) probe. The ORP was converted to Eh by interpolating the half-cell potential of the reference electrode using manufacture provided potentials at variable temperatures.

For laboratory analyses, approximately 60 mL of sample were filtered through 0.2 μm polyethersulfone (PES) filters capable of resisting deterioration when exposed to low pH solutions. Filtered samples were collected and separated into two Nalgene bottles, one for

dissolved organic carbon (DOC) and anion analysis, and one for major cation and trace metal analysis. Bottles for major cation and trace metal analysis were preserved with 2% HNO₃ to maintain the integrity of the metals within the sample. The aliquots of sample reserved for dissolved organic carbon and anion analysis were kept refrigerated to preserve the integrity of analytes.

Major and trace cation concentrations were determined in water samples by ICP-MS (Agilent 7700x) using rhenium and rhodium as internal standards (Yéglicheyen et al., 2019). The international geostandard SLRS-6 was used to verify the validity and reproducibility of the results.

Dissolved Organic Carbon (DOC) and major anion concentrations of water samples were analyzed by Shimadzu TOC Analyzer, and by Dionex ICS-5000 ion chromatograph with an AS11 HC column (Dionex Method 123), respectively.

2.2.2 Plant samples

Plant samples were processed and analyzed in two mass fractions representative of the above-ground and below-ground tissues. Specimens from each species were collected within a 1-meter radius and combined to represent one sample point. The above ground tissues were freeze dried and lyophilized, while below-ground tissue was washed with deionized water (18 MΩ-cm), and then dried in a 50°C oven for 4 days, and ground using a zircon ball-mill.

Above-ground plant samples were digested on a hot-plate using an adaption of the Krishnamurty (1976) method, whereas below-ground plant samples were digested more aggressively using a microwave (Anton Paar Multiwave 7000) with a modification of the preprogrammed “organic” digestion method. Major and trace cation concentrations were determined using ICP-MS (Agilent 7700x) following an analogous protocol to the one performed on water samples, but with Tomato Leaves standard 1573a (NIST® SRM®) as a reference material for confirmation of the validity and reproducibility of results. Methodological error was determined using triplicate analysis of each sample.

2.2.3 Soil/aquatic sediment samples

Over 1 kg of Soils and aquatic sediments were collected from each site. Sampling targeted the same areas as plant roots in order to collect soils and aquatic sediments representative of the rhizosphere, the depth of collection varied based on the location of the root mass. Samples were dried at 40 °C for 48 h and sieved to recover the < 2 mm fraction. In addition to the rhizosphere soils collected for each plant, three bulk soils, identified by areas with no overlying vegetation, were collected from the Quinta do Bispo site.

Soil/aquatic sediment sample major and trace cation concentrations were analyzed by ICP-MS with alkaline fusion in the CNRS National SARM Analytical facility

(<https://sarm.cnrs.fr/index.html/>) following the procedure described in Carignan et al. (2001), whereas soil/aquatic sediment texture, pH_{water} , pH_{KCl} , cationic exchange capacity (CEC_{Metson}), and organic matter (OM) content were determined by LABOCEA. LABOCEA employed standard methods to determine extractable cations by ammonium acetate agitation at pH 7 (NF X 31-108) (Metson, 1956) and OM content by dry combustion of sample carbon (NF ISO 10694).

2.3 Data handling

In order to compare the enrichment or depletion of REE as a whole series, they are generally normalized to a standard set of values. For this study we normalized REE concentrations in soil and water samples to Taylor and McLennan (1989)'s Upper Continental Crust (UCC) values. Elements were further classified into three groups, the light REE (LREE) which range from elements La to Nd, middle REE (MREE) Sm to Tb, and heavy REE (HREE) Dy to Lu. For soil, sediment, and aqueous samples the Ce anomaly (Ce^*) and Eu anomaly (Eu^*) were calculated using a linear extrapolation of UCC-normalized REE, Equations 1 and 2 respectively:

$$Ce^* = \frac{2(Ce_{UCC})}{La_{UCC} + Pr_{UCC}} \quad \text{Equation 1}$$

$$Eu^* = \frac{2(Eu_{UCC})}{Sm_{UCC} + Gd_{UCC}} \quad \text{Equation 2}$$

For comparisons of plant samples, the bioconcentration factor (BCF) and translocation factor (TF) were calculated using Equation 3 and Equation 4, accordingly, where $[REE_{AG}]$ is the concentration of REE in the above-ground tissue ($mg\ kg^{-1}$), $[REE_{BG}]$ is the concentration of REE in the below-ground tissue ($mg\ kg^{-1}$), and $[REE_{soil}]$ is the concentration of REE in the soil or aquatic sediment collected from the plant rhizosphere ($mg\ kg^{-1}$). The resulting BCF and TF values are dimensionless ratios. The BCF is reflective of the plant's ability to accumulate an element from the soil/aquatic sediment and TF is reflective of the ability for the plant to translocate elements from the rooting system to aerial tissue. The Ce^* and Eu^* for plant samples were determined using BCF values calculated from Equations 1 and 2, instead of UCC-normalized values:

$$BCF = \frac{[REE_{AG}]}{[REE_{Soil}]} \quad \text{Equation 3}$$

$$TF = \frac{[REE_{AG}]}{[REE_{BG}]} \quad \text{Equation 4}$$

For statistical treatment, standard diagnostic tests were performed to determine whether data needed transformation. If a transformation was required, the box-cox method for transformation was used to determine the best fit. Data passing diagnostic tests were then tested

with an Analysis of Variance (ANOVA), for statistical significance the p-value was limited to $p < 0.05$. The number, n , of all datasets analyzed is reported in Figure 1. Missing data are handled as random, allowing the analysis of imbalanced datasets, particularly soils and aquatic sediments. Post-hoc testing was completed using Tukey Honestly Significantly Difference (Tukey HSD).

3 Results

In the following section the REE concentrations and associated chemistry are presented for samples representing the water, soil/aquatic sediment, and plant environmental compartments.

3.1 Water sample REE signature

In situ physicochemical water quality parameters are displayed in Table A1.1. Waters collected from outside of São Domingos and Lousal were circumneutral, pH 7.1 and pH 6.7, accordingly. Similar Eh values were observed for water collected from outside of São Domingos and Lousal, +397 mV and +360 mV, respectively. Of the water samples collected from inside of the mining sites, the open pit at Quinta do Bispo had the highest pH, 5.1. The sample collected at Quinta do Bispo still had an oxidizing Eh of +524 mV. Samples collected from the São Domingos River Valley display a pH ranging from 2.4 to 2.9 and were strongly oxidizing with an Eh ranging from +691 mV to +723 mV. Similarly, samples collected from the Lousal treatment system had a pH ranging from 2.3 to 3.0 with an Eh ranging from +615 mV to +808 mV.

The EC for the samples collected from the mining areas tended to be higher than the EC observed outside of the mining areas. The range of DOC for the samples collected ranges from 0.45 mg L⁻¹ to 9.71 mg L⁻¹. Table A1.1 presents the concentrations of anions, measured in aqueous samples. The dominant inorganic anion observed in the mining sites was sulfate, in contrast to the dominant anion outside of the mining area which was chloride. The presence of sulfate is anticipated as sulfuric acid is generated through AMD and used during ISL.

Upper Continental Crust (UCC) normalized aqueous REE patterns are displayed in Figure 2 while REE concentrations are tabulated in Table 2. The aqueous samples collected from outside of the mining area contained less REE than the samples collected from inside of the mining areas. The samples collected outside of the mining area exhibited depletion of LREE relative to both the MREE and HREE. The depletion of LREE was punctuated by a negative Ce anomaly (Ce*) for samples collected outside the mining area. Samples collected from the Lousal passive treatment system had the highest REE concentrations with 628.84 µg L⁻¹ associated with the leachate treatment tank. The sample collected from the 6th treatment pool showed a marked decrease in dissolved REE with 238.68 µg L⁻¹.

Table 2. Concentrations of REE in aqueous samples from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo). Separate sampling points are distinguished using the abbreviation pt. followed by a brief description of the sample location. Concentrations are reported in $\mu\text{g L}^{-1}$; Ce and Eu anomalies (Ce^* and Eu^* , respectively) are also reported as dimensionless values.

Description	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	ΣREE	LREE	MREE	HREE	Ce^*	Eu^*
São Domingos Mine																				
Tapada Grande Reservoir ^a	0.021	0.030	0.006	0.025	0.006	0.002	0.007	0.001	0.005	0.001	0.003	0.000	0.002	0.000	0.110	0.083	0.021	0.007	0.61	1.24
Pt. 1: São Domingos River Valley	22.7	58.2	7.71	35.1	9.46	2.39	12.2	1.93	11.8	2.35	6.46	0.855	5.18	0.748	177	124	37.8	15.6	0.99	1.02
Pt. 2: São Domingos River Valley	8.13	19.9	2.67	12.9	3.40	0.801	4.13	0.593	3.59	0.710	1.91	0.241	1.43	0.208	60.6	43.57	12.50	4.50	0.96	0.99
Quinta do Bispo Mine																				
Pt. 1: Flooded open pit	1.83	4.78	0.718	3.36	1.01	0.598	1.51	0.283	1.67	0.311	0.763	0.096	0.535	0.084	17.5	10.7	5.07	1.79	0.92	2.19
Lousal Mine																				
Uncontaminated water from Albufeira da Barragem da Tapada supplemented to passive treatment ^a	0.027	0.024	0.008	0.037	0.008	0.002	0.009	0.001	0.006	0.001	0.004	0.000	0.002	0.001	0.130	0.096	0.026	0.008	0.36	0.91
Pt. 1: Tailing pile leachate containment	91.7	227	34.5	139	36.1	8.71	35.7	4.69	25.1	4.34	11.0	1.38	8.12	1.18	629	493	110	26.0	0.90	1.14
Pt. 2: Mixing lagoon for leachate and supplemental water	28.4	76.7	11.0	44.9	11.9	3.25	13.9	1.91	10.2	1.82	4.69	0.588	3.41	0.501	213	161	41.2	11.0	0.96	1.17
Pt. 3: First treatment basin	32.9	83.7	12.0	51.5	15.9	4.49	21.0	2.94	15.6	2.75	7.02	0.878	5.16	0.756	257	180	59.9	16.6	0.94	1.13
Pt. 4: 6 th treatment basin, beginning of planted basins	30.0	82.7	11.5	49.4	13.8	3.81	17.4	2.44	13.5	2.41	6.07	0.762	4.28	0.622	239	174	51.0	14.1	0.99	1.13

^a Samples collected from outside of the mining area.

Aqueous samples collected from the AMD areas also contained higher concentrations of REE than in the open pit at Quinta do Bispo, perhaps indicating that AMD is a more potent mobilizer of REE than ISL. There were some similarities between the aqueous REE patterns, all mines showed a MREE enrichment as compared to the LREE and HREE. In addition to the MREE concavity, samples collected from São Domingos and Quinta do Bispo had a skewed pattern, with MREE>HREE>LREE. The open pit sample from Quinta do Bispo also exhibited a strong positive Eu anomaly (Eu*), not shared by the other sites.

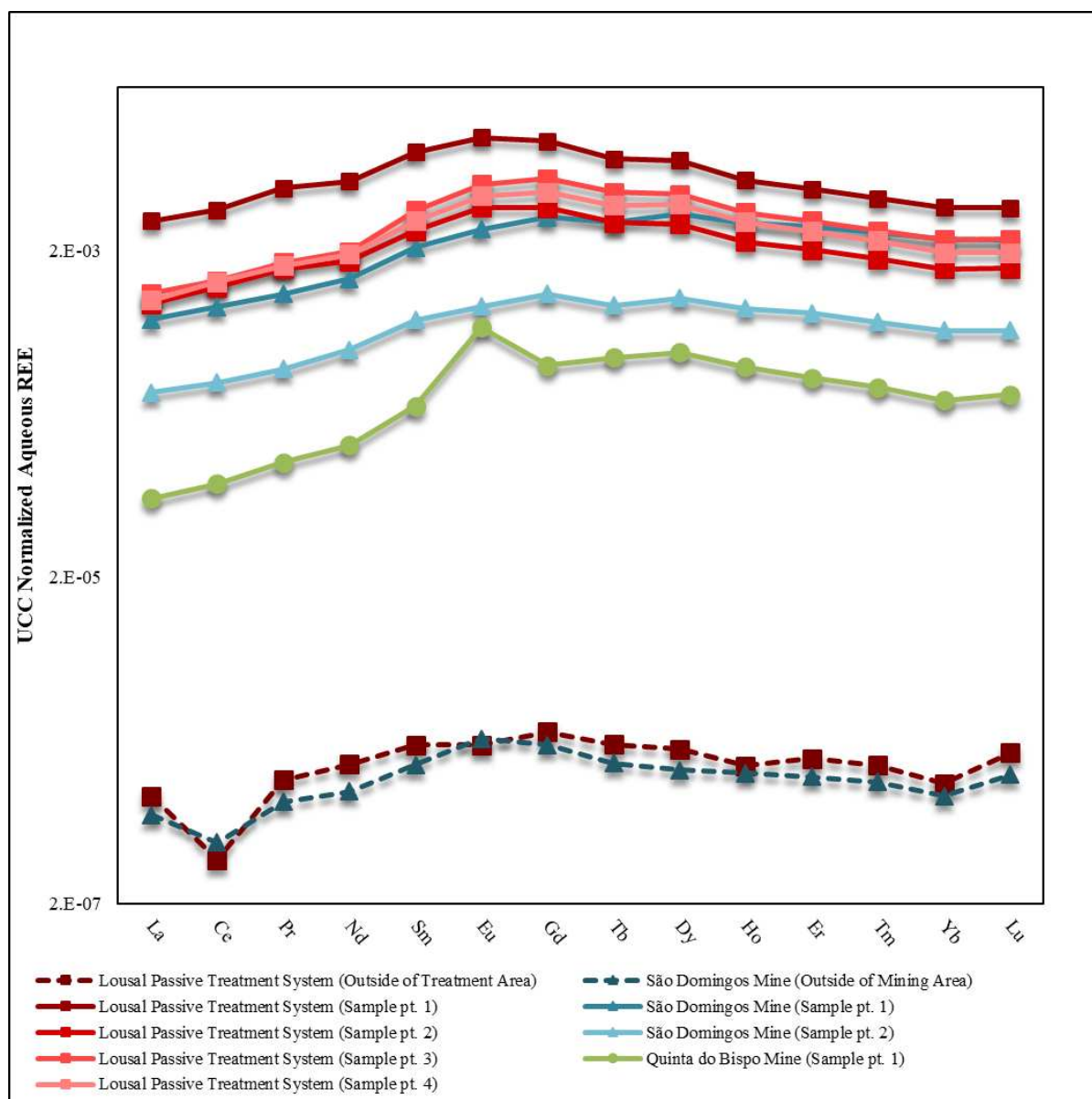


Figure 2. UCC-normalized REE patterns for aqueous samples collected from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo). Separate sampling points are distinguished using the abbreviation pt.. Solid lines represent the samples collected inside of the mine, whereas dashed lines represent samples collected outside of the mining areas. Data for samples collected in São Domingos, Lousal and Quinta do Bispo are displayed by triangle, square and circle markers, respectively.

3.2 Soil/aquatic sediment REE Signature

A summary of major cation concentrations for water and soils/aquatic sediments reported in Table A1.2 and Table A1.3, respectively. Table 3 displays the REE concentration of each individual soil. When evaluating soil REE concentrations there were three effects considered: (i) prevailing geochemical context, (ii) the mining context, and (iii) rhizosphere effects.

3.2.1 Geochemical context

All of the water and soil/aquatic sediment sample data across all of the sites were used to make a correlation matrix and determine if any particular chemical couplings controlled REE behavior. As evidenced from Pearson's correlations, HREE (Dy through Lu) in the soil have significant positive correlations ($p < 0.05$) with major cations such as Ca, Mg, and Mn. This correlation is also present in the water samples collected. Though no correlation between Fe concentration and REE was evident in soil samples, there was a correlation between Fe and REE in the water samples. These correlations are further contextualized in the results section which details how compatible cations may interact with REE.

Physiochemical characteristics of the soil/aquatic sediment were also taken into consideration for site geochemistry. Samples display contrasting pH, pH_{KCl} , $\text{CEC}_{\text{Metson}}$, and OM concentrations (Table A1.4). All of these characteristics can vary widely based on biotic and abiotic factors. The soils collected from São Domingos had high $\text{CEC}_{\text{Metson}}$ and OM, while Lousal sediments had the lowest $\text{CEC}_{\text{Metson}}$ and OM content.

3.2.2 Mining context

Based on the mining site there are distinct differences in the levels of REE enrichment observed at each site (Figure 3). Soil samples collected from Quinta do Bispo had the highest concentrations of REE (161-231 mg kg^{-1}), whereas aquatic sediment samples collected from Lousal exhibited the lowest concentrations (76-94 mg kg^{-1}) (Table 3). Statistically there was no difference between REE concentrations observed at Quinta do Bispo and São Domingos ($p < 0.05$). Similarly, there was no statistically significant difference between soils collected from São Domingos and aquatic sediments collected from Lousal ($p < 0.05$) with regards to the total REE concentrations. Quinta do Bispo and São Domingos share similarities in their environmental remediation status, since these legacy mine sites were both mitigated and maintained by EDM with environmental remediation planned in the near future. São Domingos and Lousal also share similarities, as both sites are in the IPB and subject to AMD.

Table 3. The REE concentrations for all rhizosphere soils from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo) given in mg kg⁻¹, as well as the Ce and Eu anomalies (Ce* and Eu*, respectively) given as dimensionless values.

ID Sample	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	ΣREE	LREE	MREE	HREE	Ce*	Eu*
São Domingos Mine																				
<i>Cistus ladanifer</i> ^a	16.0	26.6	4.76	20.8	5.71	1.22	6.35	1.05	6.40	1.34	3.50	0.493	3.07	0.470	97.7	68.1	14.3	15.3	0.69	0.95
<i>Cistus ladanifer</i>	32.0	63.6	7.40	27.8	5.01	1.04	3.96	0.645	4.20	0.897	2.51	0.389	2.65	0.394	152	131	10.7	11.0	0.94	1.09
<i>Cistus ladanifer</i>	34.0	71.1	8.11	30.3	5.99	1.21	5.21	0.860	5.40	1.13	3.08	0.470	3.02	0.457	170	144	13.3	13.6	0.98	1.02
<i>Erica andevalensis</i>	31.3	60.9	7.19	26.6	4.91	0.976	3.65	0.581	3.66	0.771	2.16	0.342	2.37	0.372	146	126	10.1	9.67	0.93	1.08
Quinta do Bispo Mine																				
<i>Salix sp.</i>	46.9	93.0	11.5	43.3	8.94	2.12	7.92	1.23	7.15	1.36	3.53	0.512	3.23	0.463	231	195	20.2	16.3	0.91	1.19
<i>Pteridium aquilinum</i> ^a	33.9	69.3	8.16	29.7	5.63	0.695	4.26	0.641	3.65	0.739	2.02	0.302	2.06	0.308	161	141	11.2	9.08	0.95	0.67
<i>Pteridium aquilinum</i>	38.8	76.3	9.14	33.0	6.60	0.826	5.12	0.764	4.36	0.853	2.27	0.336	2.16	0.296	181	157	13.3	10.3	0.92	0.67
<i>Retama sphaerocarpa</i>	36.0	72.1	8.45	30.7	5.97	0.747	4.47	0.654	3.73	0.745	1.98	0.294	1.92	0.267	168	147	11.8	8.94	0.94	0.68
Lousal Mine																				
<i>Juncus acutus</i> ^a	20.9	38.3	4.53	16.7	3.27	0.679	2.68	0.432	2.68	0.555	1.52	0.230	1.52	0.227	94.2	80.4	7.07	6.73	0.90	1.08
<i>Juncus Acutus</i>	14.6	30.3	3.64	14.1	2.89	0.619	2.55	0.418	2.73	0.581	1.63	0.256	1.69	0.255	76.3	62.7	6.48	7.14	0.95	1.07

^a Samples collected from outside of the mining area.

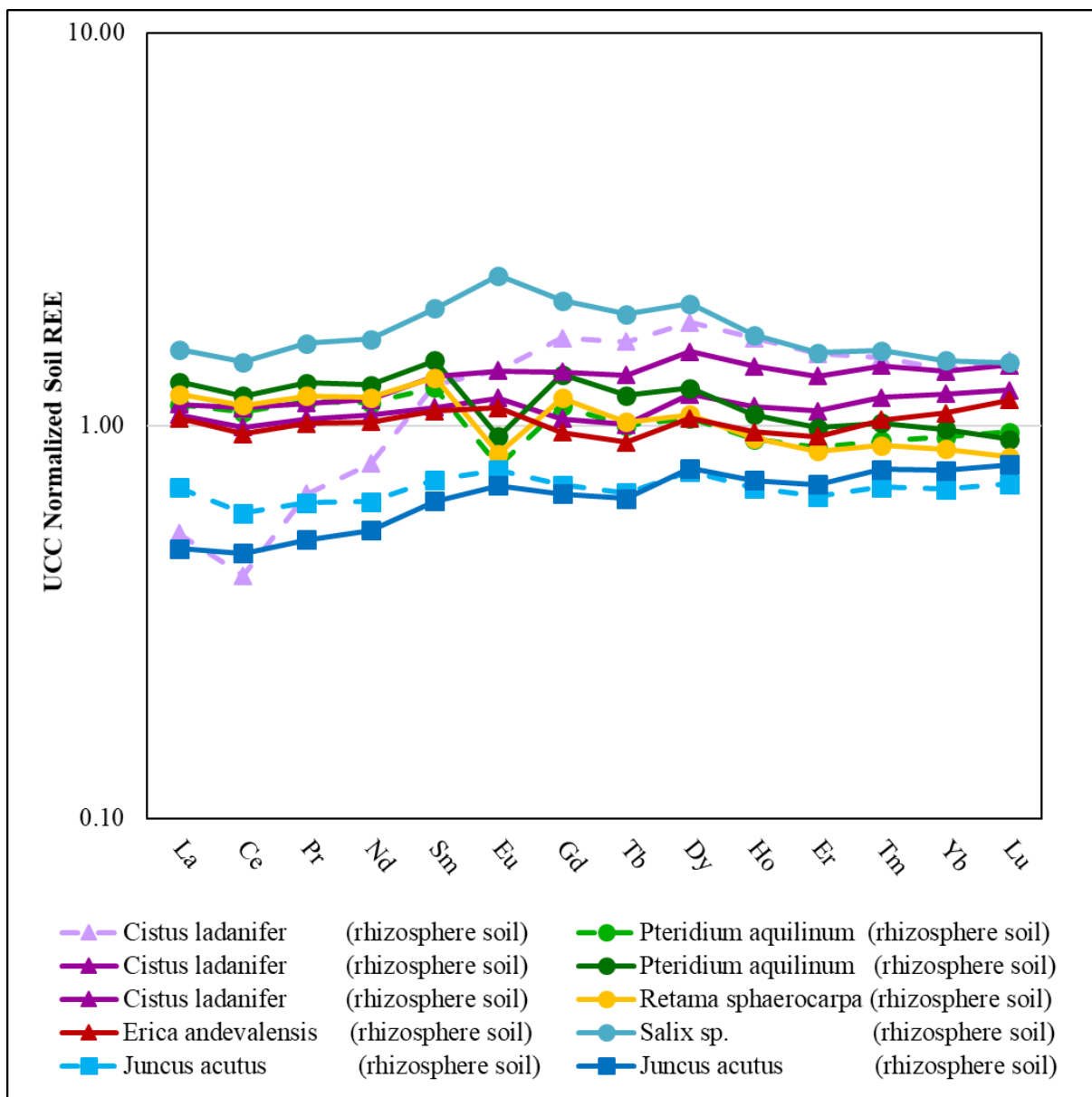


Figure 3. UCC-normalized REE patterns for different rhizosphere soils from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo). Dashed lines represent soils collected outside of the mining areas. Data from soils collected in São Domingos, Lousal and Quinta do Bispo are displayed by triangle, square and circle markers, respectively.

Both Quinta do Bispo and São Domingos had statistically significantly higher LREE and MREE concentrations than Lousal. There was no statistical difference between the HREE for each of the mining sites. This can be attributed to the large spread of HREE concentrations. For Quinta do Bispo, a majority of the REE signatures show an enrichment of LREE and MREE with lower values of HREE when normalized to the UCC. One of the samples collected from Quinta do Bispo also displayed a positive Eu*, as opposed to the other Quinta do Bispo soil samples which display a negative Eu*. The REE signature for soils collected from São Domingos shows that the LREE and MREE had lower values than the HREE, with two of the samples showing significant enrichment. All of the aquatic sediment samples collected from Lousal were depleted in LREE, MREE, and HREE with respect to the UCC. The REE

signatures for samples collected from Lousal display lower LREE values and higher MREE and HREE values.

There was no statistically significant difference for the soil LREE, MREE, HREE, or total REE concentrations among samples collected from inside or outside of the mining areas. As per the heterogeneous nature of soil, perhaps the spread of data between samples collected from inside and outside of the mining area is too large to make a distinction between the influences of the mining area without also taking into consideration the geochemical context. This is further highlighted by the fact that the REE signature for the soil sample collected from outside of Sao Domingos does not match samples collected from inside of the same site. The sample collected from outside of the mining area features depletion of the LREE and enrichment of the MREE and HREE with respect to the UCC, along with a negative Ce*. Due to sampling constraints encountered in this study there was not enough sample replication to determine the combined effects of mining and the surrounding geochemistry.

3.2.3 Rhizosphere effects

A comparison of the rhizosphere versus bulk soils was possible at the Quinta do Bispo site (Table A1.5) and shows that the rhizosphere and bulk soils display similar patterns. This comparison should also show whether plants selectively depleted or enriched REE from the soil. There was ultimately no statistical difference for the LREE, MREE, HREE, or Total REE in soils collected from the plant rhizosphere when compared with bulk soils. This would indicate that the plant does not influence the soil REE observed in the area.

3.3 Plant Sample REE Signature

When considering the responses of plant REE concentration, BCF, and TF there were three main effects investigated: (i) prevailing geochemical context, (ii) the mining context, and (iii) the plant type.

3.3.1 Geochemical context

Using the statistical testing described in section 2.3, there was no statistical difference between the Total REE BCF (Figure 4) or TF (Figure 5) observed in samples collected from Quinta do Bispo, São Domingos, or Lousal. This would indicate that the geological background did not influence the uptake of REE. However, this conclusion is difficult to support, since among the plants collected from each site there were no shared species for comparison. Therefore, a potential area for expansion of this study could be to research REE in the same plant species found at different mining sites. Such a task may prove difficult since the spontaneous vegetation is specific to the growth conditions of the individual site. In the discussion section comparisons from literature are presented in order to account for some of the differences seen across plant types collected in different geological conditions.

3.3.2 Mining context

While it is difficult to make conclusions about the effects of geochemistry on the bioaccumulation of REE in plants, there were some differences in plants collected from inside of the mining site when compared with plants collected from outside of the mining site (Figure 4).

In general, the BCF and REE concentrations (Table 4) were elevated for plants collected from inside of the mining areas, when compared with plants collected from outside of the mining areas (approximately 76% more REE in the plants recovered from inside of the mining areas), indicating an enhancement in the uptake and bioconcentration of REE from the soil into the above-ground tissue. Specifically, the MREE and HREE were taken up at a greater rate when the plant was collected from inside of the mining area (94% more MREE and 106% more HREE, as compared with the LREE which was elevated by 75%). More specifically, plants had an elevated BCF ($p < 0.05$) for Sm, Eu, Gd, Tb, Dy, Ho, Er, Yb, and Lu, when collected from inside of the mining area. However, there was also no significant difference between the TF of samples collected inside vs. outside of the mining area. In summary, mining increased the bioavailability of all REE, in particular elevating the BCF for MREE and HREE over LREE. This increased availability of REE did not translate to an increased TF.

Table 4. Concentration of REEs in above-ground and below-ground plant tissues ($\mu\text{g kg}^{-1}$) collected on mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo) as well as the Ce and Eu anomalies (Ce* and Eu*, respectively).

^a Samples collected from outside of the mining area.

Above-Ground Tissue																					
Mine Site	ID Sample	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	ΣREE	LREE	MREE	HREE	Ce*	Eu*
São Domingos Mine	<i>Cistus ladanifer</i> ^a	275	552	66.4	257	50.2	8.59	44.2	5.78	29.6	5.24	13.6	1.57	9.4	1.34	1320	1150	109	60.9	1.33	0.89
	<i>Cistus ladanifer</i>	379	809	96.0	433	80.1	16.3	74.3	9.70	50.7	9.26	24.6	3.99	21.6	3.33	2010	1720	180	113	1.03	0.91
	<i>Erica andevalensis</i>	189	460	56.9	247	76.7	13.5	55.1	6.70	34.5	6.03	14.8	1.96	12.8	1.69	1177	953	152	71.8	1.08	0.90
Quinta do Bispo	<i>Salix sp.</i>	349	521	85.4	363	86.7	38.0	89.5	12.2	63.7	11.1	26.7	3.16	18.5	2.42	1670	1320	226	126	0.75	1.70
	<i>Pteridium aquilinum</i> ^a	4700	7470	729	2260	298	43.0	212	24.9	116	18.5	43.2	5.07	27.1	3.38	16000	15200	578	214	0.95	1.21
	<i>Pteridium aquilinum</i>	9200	10500	1570	4550	518	77.7	379	47.4	218	32.2	73.7	7.72	37.5	4.07	27300	25900	1020	373	0.67	1.24
	<i>Retama sphaerocarpa</i>	503	989	109	393	75.2	10.2	62.2	8.19	40.5	6.40	15.8	2.07	10.8	1.49	2230	2000	156	77.0	1.02	1.03
Lousal Mine	<i>Juncus acutus</i> ^a	105	194	24.8	105	21.8	5.34	22.9	3.03	16.3	3.03	8.14	1.00	5.47	0.79	516	428	53.1	34.7	0.96	1.04
	<i>Typha latifolia</i> ^a	257	487	60.6	245	49.6	11.7	53.6	6.71	34.2	6.32	16.3	1.84	11.1	1.52	1240	1050	122	71.2	0.99	0.98
	<i>Typha latifolia</i>	262	574	75.0	336	87.2	22.6	108	14.9	79.6	14.8	37.9	4.52	25.1	3.61	1650	1250	232	165	0.98	1.01
	<i>Juncus acutus</i>	433	1202	130	539	107	23.9	94.2	11.6	62.1	11.4	29.7	3.78	20.6	2.79	2670	2300	237	130	1.22	1.05
Below-Ground Tissue																					
Mine Site	ID Sample	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	ΣREE	LREE	MREE	HREE	Ce*	Eu*
São Domingos Mine	<i>Cistus ladanifer</i> ^a	1920	2060	566	2600	726	128	849	126	714	140	370	45.1	259	42.5	10500	7140	1830	1570	0.65	0.80
	<i>Cistus ladanifer</i>	462	1090	142	653	192	44.4	190	24.8	125	24.0	59.0	8.8	55.7	7.77	3080	2350	451	280	1.02	0.99
	<i>Cistus ladanifer</i>	559	1120	136	526	117	20.3	95.0	16.8	89.5	14.1	42.9	3.6	32.8	4.01	2780	2340	249	187	0.95	0.89
	<i>Erica ladanifer</i>	975	2700	376	1790	580	116	431	49.8	240	42.7	128	17.5	135	22.0	7610	5850	1180	585	1.06	1.01
Quinta do Bispo	<i>Salix sp.</i>	5560	7640	1290	4730	983	263	1020	139	794	136	341	43.2	234	30.9	23200	19200	2400	1580	0.71	1.04
	<i>Pteridium aquilinum</i> ^a	5390	11100	1230	4500	784	63.5	779	83.5	491	76.8	226	29.7	185	25.0	25000	22200	1710	1030	1.04	0.57
	<i>Pteridium aquilinum</i>	5930	8670	1140	3900	638	77.7	535	76.1	449	84.1	160	24.1	119	17.1	21800	19600	1330	854	0.82	0.94
	<i>Retama sphaerocarpa</i>	700	1420	161	538	124	9.8	85.0	11.6	52.9	7.76	22.7	2.7	22.9	3.13	3160	2820	231	112	1.02	0.66
Lousal Mine	<i>Juncus acutus</i> ^a	19500	45400	5650	23600	4890	1110	4910	614	3230	569	1543	189	1115	157	112000	94100	11500	6800	1.09	0.98
	<i>Typha latifolia</i> ^a	1600	3640	491	1930	415	91.6	464	57.7	315	55.1	144	18.4	105	12.6	9340	7660	1030	651	1.03	0.90
	<i>Typha latifolia</i>	2080	6350	885	4150	1170	310	1270	167	842	150	394	50.3	291	41.7	18100	13500	2900	1770	1.09	1.11

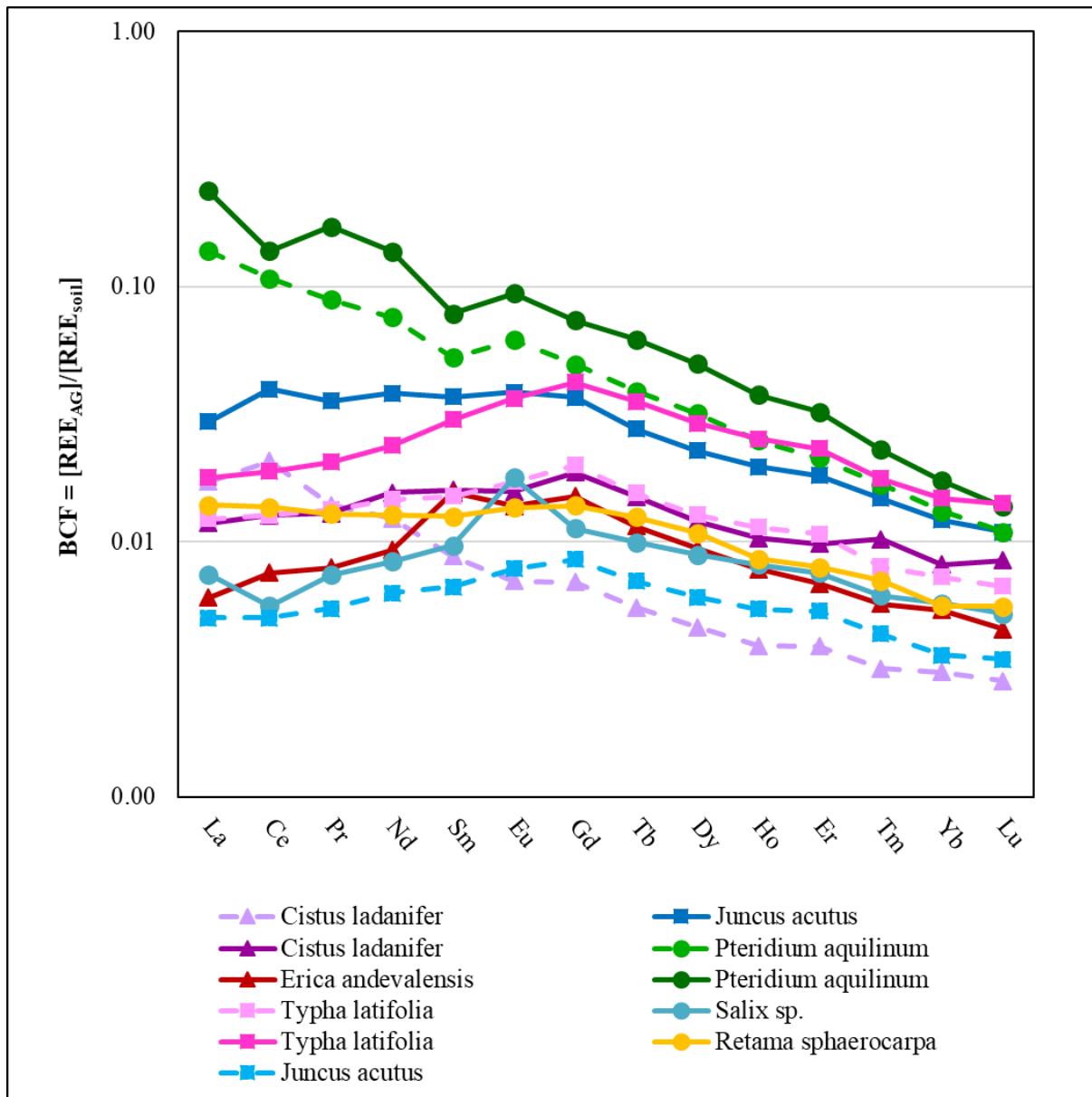


Figure 4. Plant BCF versus REE for above-ground tissue. Solid lines represent plant samples collected from inside of the mining sites while dashed lines represent samples collected from outside of the mining sites. Data from plants collected in São Domingos, Lousal and Quinta do Bispo (Portugal) are displayed by triangle, square and circle markers, respectively.

3.3.3 Plant type

Pteridium aquilinum showed the greatest affinity for REE, accumulating 27.3 mg kg^{-1} of REE in the above-ground tissue. Similar to other studies, many of the plant types collected show higher concentrations of REE associated with the below ground-tissue as opposed to the above-ground tissue (Babula et al., 2009), with the exception of *Pteridium aquilinum* which accumulated more La, Ce, and Pr in the above-ground plant tissue than in the below-ground plant tissue. Of the plants collected with replication (*Pteridium aquilinum*, *Cistus ladanifer*, *Typha latifolia*, and *Juncus acutus*), plant type played a statistically significant role ($p < 0.05$) in the BCF of La, Ce, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, and Yb. *Pteridium aquilinum* had a

significantly higher BCF for all of the aforementioned elements except for Ho. For Ho, *Pteridium aquilinum* had similar rates of accumulation to *Typha latifolia*, but had higher accumulation rates when compared to *Juncus acutus* and *Cistus ladanifer*. *Typha latifolia* also had a higher BCF when compared to *Cistus ladanifer* for Dy and Ho, and a higher BCF when compared to *Juncus acutus* for Tm.

Of the REE accumulated in *Pteridium aquilinum*, the LREE tended to concentrate at a higher rate than MREE, and both LREE and MREE tended to concentrate more than the HREE. Lanthanum was three orders of magnitude more concentrated than Lu. Similarly, the *Cistus ladanifer* sample collected from outside of the mine exhibited the same downward trend of LREE>MREE>HREE. This trend was not conserved across all plant types. *Typha Latifolia* and *Erica andevalensis* demonstrated a MREE concave pattern instead, MREE>LREE=HREE. The BCF of *Juncus acutus* and *Retama sphaerocarpa* displayed a pattern where LREE=MREE>HREE.

Three plant samples had a positive Eu*: the *Salix sp.* sample and both of the *Pteridium aquilinum* samples. All of these samples were collected from Quinta do Bispo. This anomaly accounts for the initial soil Eu* as the plant tissue is normalized to the native soil when evaluating the BCF. Additionally, there was a positive Eu* for the TF, so though modest amounts of Eu* were incorporated into the below-ground tissue from the soil, there was a preferential transport and accumulation of Eu into the aerial plant tissue.

Moreover, the *Cistus ladanifer* sample collected from outside of the São Domingos mining area had a positive Ce* in the above-ground tissue and negative Ce* in the below-ground plant tissue, which is matched with a high TF for Ce. The total concentration of Ce in the plant tissue was not in fact inordinately high and when normalized to UCC values shows no such anomaly. This observation is matched with soil and water samples collected from the same area showing Ce depletion as well. In this particular sample, Ce was accumulated in the above-ground tissue of the plant at a higher rate and the root anomaly is an artifact of less plant-available Ce in the rhizosphere.

There are three distinct groups of TF rates (Figure 5). The highest TF is seen for both of the *Pteridium aquilinum* (collected from both inside and outside of the Quinta do Bispo mining area), *Cistus ladanifer* (collected from inside of the São Domingos mining area), and *Retama sphaerocarpa* (collected from inside of the Quinta do Bispo mining area). The middle group consisted of the *Cistus ladanifer* collected from outside of the mining area, *Typha latifolia*, *Salix sp.*, and *Erica andevalensis*. The TF of the middle group was 3 times lower than the highest TF group. The lowest TF was observed in *Juncus acutus*, which had large quantities of REE associated with the below-ground tissue. Low TF resulted in low accumulation of REE

in the above-ground tissue. The TF for *Juncus acutus* was 177 times less than the TF recorded for plants in the highest TF group. This observation indicates that a majority of the REE remained stabilized within the below-ground tissue of *Juncus acutus*. Plant type had a significant effect on the TF of total REE and LREE, but had no effect on the MREE and HREE. REE was preferentially translocated in the following order: LREE>MREE>HREE for select plants, particularly *Cistus ladanifer* and *Pteridium aquilinum* samples. Many of the other plant samples have no relative slope regarding the TF for LREE, MREE, and HREE.

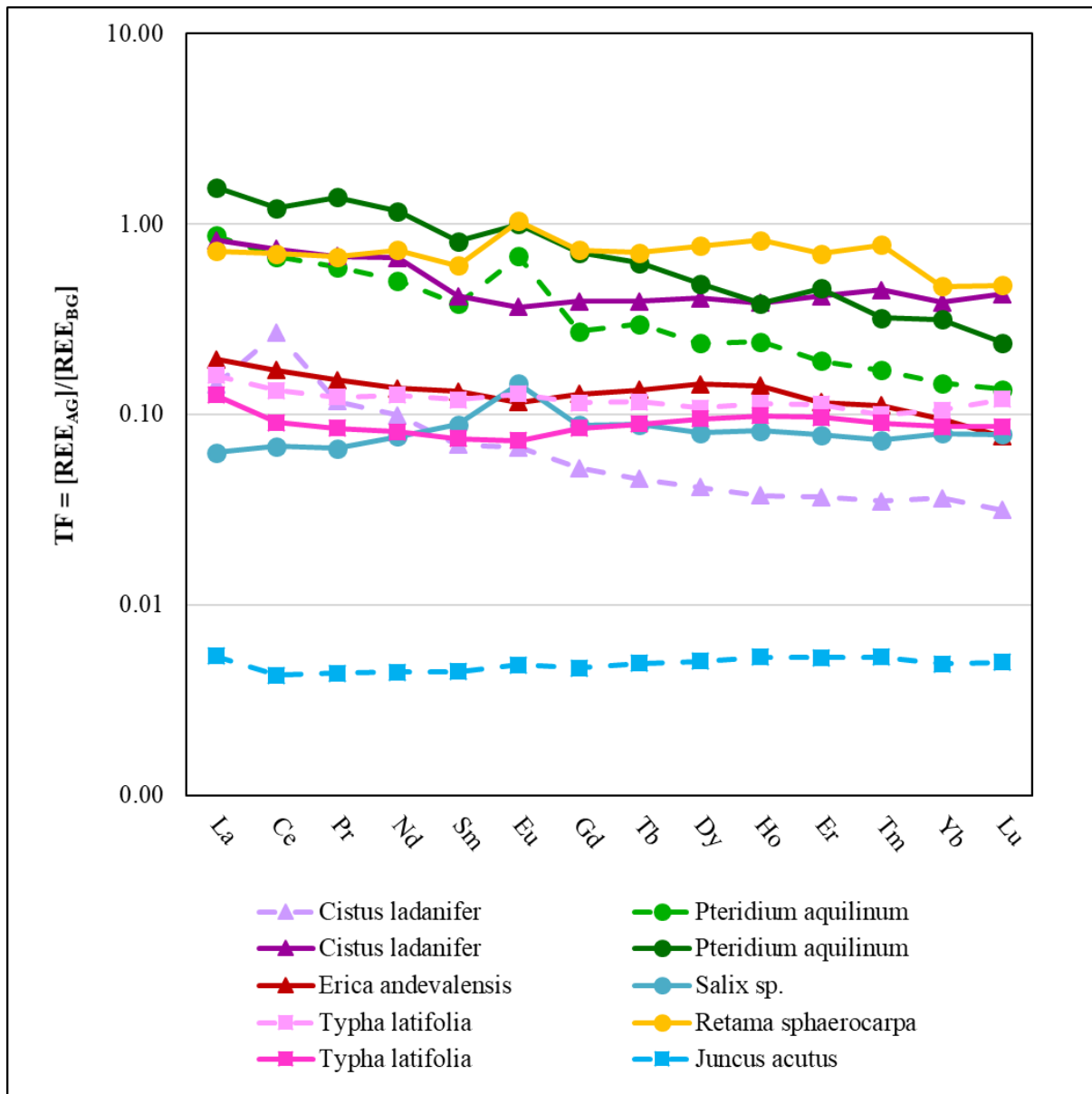


Figure 5. Plant TF versus REE. Solid lines represent plant samples collected from inside of the mining sites while dashed lines represent samples collected from outside of the mining sites. Data from plants collected in São Domingos, Lousal and Quinta do Bispo (Portugal) are displayed by triangle, square and circle markers, respectively.

4 Discussion

4.1 Geochemical and mining context

Though none of the studied mines targeted the extraction of REE ores, REE can be distributed throughout silicate, carbonate, oxide, phosphate, and oxyhydroxide (Migaszewski and Gałuszka, 2015) minerals, which are susceptible to dissolution under low pH conditions or changes in reduction/oxidation conditions (Cao et al., 2001; Mihajlovic et al., 2017). The MREE enrichment in aqueous samples is a typical signature of AMD (Perez-Lopez et al., 2010). The pH range and Eh observed for both São Domingos and Lousal are also consistent with the geochemical context of AMD (Dold, 2014). The pH observed in Quinta do Bispo was relatively high when compared with the pH of São Domingos and Lousal. This is most likely due to the fact that sulfate mineral oxidation or AMD is negligible in the surrounding geology (Carvalho et al., 2005) and the ISL methods employed at Quinta do Bispo ended in 1999 (European Commission Directorate-General for Energy, 2012). The saturation of cations and anions in waste streams, represented by the EC measured in aqueous samples, favors the precipitation of metals (Nieto et al., 2007), and is therefore also a parameter used to evaluate the precipitation/dissolution dynamics at these sites. Low pH, high EC, and elevated sulfate concentrations were indicative of the geogenic and anthropogenic dissolution of primary minerals inside of the three legacy mine sites. These geochemical changes were induced by AMD processes or ISL practices.

In addition to dissolution, processed REE were susceptible to sorption and desorption processes, which were evaluated in this study based on the CEC_{Metson} and OM content of soils and aquatic sediments. A soil with higher CEC_{Metson} and OM content has a high metal loading capacity, whereas a soil with a low CEC_{Metson} and OM content has less potential metal-sorbing surfaces, thus the metal holding capacity of soils collected from São Domingos is higher and the metal holding capacity of aquatic sediments collected from Lousal is lower. The sediments collected from Lousal had lower concentrations of REE indicative of the lower metal holding capacity of these sediments relative to the other sampled sites. As a result, Lousal also displayed the highest aqueous REE concentrations.

All of the samples collected from inside of the mining sites are considered impaired simply on the basis of having low pH ($pH < 6.5$) (Baker et al., 1990). As Baker et al. (1990) reported, biological diversity is lost starting at $pH < 6.5$, and an interruption of nutrient cycling at $pH < 5.0$. While pH directly impacts the ecology of a system, the increased metal solubility and bioavailability caused by acidification (Baker et al., 1990; Cao et al., 2001; Batista et al., 2013) can also induce toxicity (Baker et al., 1990; Plante, 2007; Batista et al., 2013).

Inorganic anions typically associated with REE complexation include: SO_4^{2-} , PO_4^{3-} , Cl^- , CO_3^{2-} , and F^- (Millero, 1992; Vaziri Hassas et al., 2021). As stated in the results, the mining areas were dominated by sulfate. A study by Zhimang et al. (2000) implicates sulfates in the

bioavailability of REE to plants, mainly showing that REE accumulation in below-ground tissue is limited by sulfate. Secondary sulfate salts such as jarosite and alunogen have been shown to be temporary stabilizers of polyvalent cations (including REE) in AMD (Ferreira et al., 2021). Subsequent solubilization of metals associated with jarosite, alunogen, and other secondary sulfate minerals has been implicated as a cause for delayed revegetation (Ferreira et al., 2021). This demonstrates that plants growth in mining environments is dependent on the prevailing geochemistry, as primary and secondary minerals constitute a source or sink for potentially toxic metals.

Aside from sulfates being a sink for REE, sulfates have also been implicated in the selective partitioning of REE in soils and waters (da Silva et al., 2009; Welch et al., 2009; Grawunder et al., 2014; Soyol-Erdene et al., 2018). REE have even been suggested as tracers of mining pollution due to their unique MREE-enriched signature in mining wastes (Merten et al., 2005; Perez-Lopez et al., 2010). This signature is presumably due to sulfate and sulfate intermediates showing higher stability in complexation with MREE (Perez-Lopez et al., 2010) and precipitation/sorption processes occluding LREE (Liu et al., 2019) and HREE (Byrne and Li, 1995) from solution, paired with dissolution/desorption of MREE-enriched Fe and S minerals (Welch et al., 2009; Perez-Lopez et al., 2010; Grawunder et al., 2014). The occurrence of a correlation between REE and Fe, as seen in the water samples collected in this study, may indicate that Fe-mineral dissolution contributes to the soluble REE in these systems (Cao et al., 2001; Merten, 2005; Prudêncio et al., 2015; Prudêncio et al., 2017; Riley and Dutrizac, 2017; Liu et al., 2022). Many geochemical factors may contribute to the observed high REE concentrations and unique MREE enrichment in AMD surface waters, a detailed study on REE speciation is still needed.

The DOC concentrations anticipated in AMD waters should be low as acidic pH promotes an increase of organic aggregation, inducing a decrease of their colloidal stability (Pédrot et al., 2009; Johnson and Hallberg, 2003). Though limited amounts of DOC and OM are associated with AMD systems, no broad generalizations can be made about the fractionation of REE by organics, which is specific to the type of organic matter. Organic matter forms a significant sink for REE, both as a sorbing surface and as a bridging ligand (Byrne and Li, 1995; Pourret et al., 2007). Organic ligands make a more stable complex with HREE, causing HREE to deplete from solution into complexes with sediment surface ligands (Byrne and Li, 1995). More complex organics, such as humic substances, have been shown to produce a fractionation of MREE enrichment over both LREE and HREE, specifically in natural systems characterized by a high metal loading like the sites studied (Pourret et al., 2007; Pédrot et al., 2008).

The oxidation state of REE is generally trivalent, with the exception of Eu^{2+} and Ce^{4+} . In this study, a negative Ce^* was associated with samples collected from outside the mining areas. Geochemically, the waters collected from outside of the mining area are less oxidizing and have a higher pH. Therefore, the speciation of metals may vary between waters collected from inside of the mining area versus those collected from outside of the mining area. The negative Ce^* observed in surface waters collected from outside of the mining areas is indicative of preferential Ce^{4+} sorption to the solid phase, which is typical of circumneutral pH waters under oxidizing conditions (Bau, 1999).

The positive Eu^* in liquors and ores associated with the open-pit can be attributed to the hydrothermal deposit where the Eu^{2+} oxidation state is preferentially incorporated into high-temperature hydrothermal seams over the other REE, leading to enriched Eu concentrations (Sverjensky, 1984; Danielson et al., 1992). The proceeding ISL processes increased mobilization of all REE contained in the Eu-enriched ore, accordingly producing an enrichment of Eu in the remaining liquor and analogous waste rock. This mechanism explains the positive Eu^* seen in the open-pit water sample and the *Salix sp.* rhizosphere soil, which was collected from the waste rock pile. All other soil samples collected from Quinta do Bispo had a negative Eu^* . Consequently, the conclusion can be drawn that the soils collected from the surrounding material were depleted in Eu as it was partitioned into the hydrothermal seam. Thus, in the soil samples collected from the surrounding areas, there is a negative Eu^* corresponding to this partition.

4.2 Plant Influences

In order for a plant type to be considered an REE hyperaccumulator, the REE concentration in the above ground-tissue must exceed $1000 \mu\text{g g}^{-1}$ (Liu et al., (2018)). None of the plants collected in this study met the hyperaccumulator definition. Liu et al. (2018) acknowledges the importance of plant biomass, as high biomass can accrue significant amounts of REE. The concentrations of REE observed in the collected plants do not show any particular elevation or depletion when compared with other studies of plant REE (Wytenbach et al., 1998a; Liang et al., 2008; Migaszewski and Gałuszka, 2015). The *Pteridium aquilinum* plant species did accumulate more REE than any other plant type: though this figure represents 6-53 times the amount accumulated by the other plant samples in the present study, the pteridophyte sample is not remarkably high. Pteridophytes are generally known to accumulate much higher concentrations of REE over other plant types (Tyler, 2004). *Pteridium aquilinum* also tended to concentrate certain REE at a higher level in the above-ground tissue than in the below-ground tissue. Wang et al. (1997) made a similar observation in *Dicranopteris dichotoma*, another

pteridophyte, which accumulated more REE in the above-ground tissue than the below-ground tissue.

As mentioned in the materials and methods section, the BCF is reflective of biological occlusion or assimilation of particular REE from the surrounding media. The closer the BCF value is to 1, the closer the plant concentrations reflect the soil concentrations. In this study, any uptake of REE by the plant was insignificant in comparison with the soil pool. This was proven by a comparison of bulk and rhizosphere soils which showed no depletion or enrichment dependent on plant growth, and further supported by the BCF which for most plants was much less than 1.

The BCF can also be looked at in terms of slope and anomaly, indicating the effects of soil speciation and plant species selectivity towards the assimilation of particular REE into the above-ground tissue. Ultimately this study showed that for certain plant types (*Pteridium aquilinum*), above-ground tissues were enriched in LREE relative to the surrounding soil. This finding is congruent with previous research, which demonstrated that LREE tend to be enriched in the above-ground tissue of plants compared to the MREE and HREE (Fu et al., 1998; Wang et al., 1997; Liang et al., 2008; Gonzalez et al., 2014). This may have to do with the plant's affinity to uptake LREE (Brioschi et al., 2012) and porewater chemistry restricting available HREE (Millero, 1992; Byrne and Li, 1995; Fu et al., 1998; Tyler, 2004; Brioschi et al., 2012). However, this pattern was not maintained across all plant types. In fact, *Typha latifolia* and *Salix sp.* showed an MREE-enrichment compared with LREE and HREE, supported by Wyttenbach et al. (1998a).

Salix sp. and *Pteridium aquilinum* had positive Eu* in their BCF signatures. Positive Eu* in plant tissue has been observed in other studies (Durães et al., 2014; Krzcuik and Gałuszka, 2019; Krzcuik and Gałuszka, 2020). There are a couple of to-date unconfirmed hypotheses on why plants may have Eu*. Potentially Eu³⁺ is transformed to Eu²⁺ and is taken up by plants under the redox conditions of the rhizosphere. This may be a result of diurnal patterns, which dictate oxygen supply to the roots (Krzcuik and Gałuszka, 2020), or it may be related to the acquisition of other essential nutrients; such as Fe, which is reduced at the root dermis (Marschner and Römheld, 1994; Wyttenbach et al., 1998a). In both scenarios, the supply of Eu and the redox conditions are a vital control on the bioavailability of Eu. Many authors (Wang et al., 2012; Brioschi et al., 2013; Durães et al., 2014; Thomas et al., 2014) have suggested that the origin of positive Eu* in plants is the result of Eu²⁺ substitution for an essential plant nutrient such as Ca²⁺ due to the similarity in ionic radius (Shannon, 1976). All of the above-ground tissue REE concentrations positively correlate with above-ground tissue Ca²⁺ concentrations for this study.

The investigation of the TF can be used to make conclusions about the transfer and relative accumulation of REE in and to different parts of the plant. In this study, the REE transfer from below-ground tissue to above-ground tissue did not relate to the geochemistry or mining context. It was only reflective of plant type differences. The subsequent enrichment in the LREE seen in the *Cistus ladanifer* and *Pteridium aquilinum* plants is then an artifact of the preferential translocation of LREE into the above-ground tissue. Fu et al. (1998) posited that preferential uptake and translocation of REE in a pteridophyte was related to plant Si processes and that *in situ* recrystallization of Si would allow for substitution of MREE and HREE occluding the larger ionic radius of LREE, leaving the LREE more mobile and able to reach the distal and aerial tissues. Evidence supporting this may be seen in the strong positive correlation between all of the REE and Si (Table A1.6) among all samples collected in this study.

Combining the information presented on the BCF enrichment of MREE with the lack of a TF MREE enrichment, it can be concluded that the MREE fractionation into the above-ground plant tissue, observed in *Typha latifolia* and *Salix sp.*, originates from the preferential uptake of MREE in the below-ground tissue. While the total bioavailable pool of metals can include the soil (water-soluble, ligand-exchangeable, and organic or carbonate bound metals), the dissolved metals in water are typically thought of as readily bioavailable (Adriano, 2001; Di Bonito et al., 2008). These dissolved concentrations could explain the elevated MREE in plants, as the enrichment of the MREE in plant tissue mirrors the enrichment of MREE in surface waters impacted by mining.

The information presented may show that the MREE enrichment seen in the roots is an artifact of the soluble REE pool in these mine sites, whereas the LREE enrichment and Eu* in the above ground plant tissue demonstrates plant-specific fractionation. Thus, this study provides evidence that the REE pattern displayed by each plant may result from an interaction between the plant type and growth medium. This conclusion is contrary to one posited by Laul and Weimer (1982). Laul and Weimer (1982) studied a selection of biological samples and concluded that the REE patterns closely resembled their growth medium when normalized to chondrites, however the REE patterns for plants were never normalized to the soil media to confirm enrichment or depletion, which would have obviated any preferential uptake of specific REE. Wang et al. (1997) similarly found that the concentration of REE in most plants collected from sites with REE-rich ores had similar patterns to the soils they were collected from, but also notes an accumulation of LREE over HREE. Pisciotta et al. (2017) made a similar conclusion that the plant tissue reflected the geochemistry of the collection site, however this conclusion was based on one plant type in one geochemical context. Another study by Krzcuik and Gałuszka (2020) showed that plants generally had a similar pattern for uptake regardless of

the land usage, proving quite the opposite of earlier studies by Laul and Weimer (1982) and Pisciotta (2017), since this would mean that regardless of geochemical background, plants had a certain physiological signature for REE-uptake. In the context of Krzcuik and Gałuszka (2020) it should be noted that researchers did find some differences based on collection of samples inside or outside of AMD areas. A study conducted by Barbera et al. (2021) actually showed that a planted system when spiked with REE did not necessarily lead to increased uptake of REE into the above-ground tissue. Barbera et al (2021) instead shows increased REE concentration in the below ground tissue with limited transfer of REE into the above-ground tissue. Wyttenbach et al. (1998a) similarly refuted the idea that the growth media was completely responsible for REE fractionation in above-ground tissues. Thus, there is evidence to support claims from each of these previous studies and create a more cohesive theory that both geochemical background and plant type contribute to REE patterns in plant tissues. The results from the present study seem to indicate that some plant types may be reflective of the area geochemistry, whereas others may show increased fractionation of soil REE, which demonstrates the importance of capturing many plant species in differing contexts.

5 Conclusions

Using samples from three Portuguese legacy mine sites, this study reaffirms previous findings that AMD waters tend to be enriched in MREE as compared with LREE and HREE. Through the coupled recovery of water, soil and plant samples, this research contributes to a growing body of work proving that (1) the substrate plays a role in the fractionation of REE into plant tissue and (2) the plant type can also contribute to REE fractionation.

Samples collected from inside of the mining sites had elevated dissolved REE concentrations in water samples, matched with a higher BCF in plant samples. This demonstrates the importance of the dissolved metal pool on the bioavailability of REE. Further sampling and identification of chemical speciation is recommended as the signatures for REE indicate some differences amongst soil types leading to differences in REE bioavailability. There were also no statistical differences in the BCF for plants based on the site in which they were collected from. This combined with the elevation of MREE in plant tissue may indicate that the dissolved REE pool plays a more significant role in plant REE uptake than the soil mineral REE. The concentration of REE in the soils collected from the rhizosphere and bulk soils were not statistically different, showing that the soil pool of REE in the plant rhizosphere was not significantly depleted when compared with unplanted soils.

Differences in uptake may be due to plant physiology as well. This hypothesis is supported by the fact that although the soils, aquatic sediments, and waters in this study had similar features, certain plants, such as *Pteridium aquilinum*, seemed better adapted to

extracting particular REE over others. Though it was anticipated that plant types commonly used in passive treatment systems, such as *Juncus acutus*, would perform best in extracting REE, very little REE was associated with the above-ground mass of these plants. Though the REE share physiochemical properties, there was observed preferential transfer of LREE and Eu into the aerial parts of certain plants. Thus, the unique geochemical context in which MREE is enriched in the dissolved phase, when combined with the preferential translocation of LREE and Eu based on plant type, creates each plant's REE signature.

CHAPTER 2: HOW DOES THE ASSOCIATION OF RARE EARTH ELEMENTS IN SOIL POOLS RELATE TO PLANT CONCENTRATIONS? A SEQUENTIAL EXTRACTION STUDY

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Chapter 2 is awaiting review and submitted to Chemosphere. A brief description of the geochemical background and the fractionation of REE into different soil phase is explained for each site. This fractionation is then related to the fractionation in below-ground plant tissue.

CHAPTER 2: ABSTRACT

Previous studies have attributed the phyto-accumulation of Rare Earth Elements (REE) to the influence of the soil substrate. However, this assumption has been predicated on the amount of REE associated with the soil as a whole. Many studies have called into question whether or not all forms of REE in the soil form a potentially bioavailable pool. The general consensus is that the exchangeable and carbonate bound mineral pool is bioavailable. However, within the rhizosphere, the geochemical conditions which are site specific and biological weathering (plant, animal and microbes) may cause other pools of REE in the soil to become plant available. For this reason, this study characterizes the soil into four fractions using the Community Bureau of Reference (BCR) sequential extraction method. The geochemical associations of REE with soil fractions allowed this study to determine which mineral pools were most bioavailable by comparing REE patterns for plant tissues collected in the same area to BCR extractions on compatible soils and seeing how well these patterns resemble each other. Results showed that below-ground plant tissue closely reflected the first three sequential extraction steps and that the residual or more recalcitrant fraction of the soil displays a convex REE pattern with distinct depletion of the Middle REE (MREE) when compared with the Light REE (LREE) and Heavy REE (HREE). This demonstrates the more mobile nature and increased bioavailability of MREE. Additionally, this study provides evidence that Eu may be excluded by the plant, as there was an anomalous concentration of Eu when the BCR extractions were normalized to below-ground plant tissue.

CHAPTER 2: RESUMÉ

Des études antérieures ont attribué la phyto-accumulation des terres rares (REE) à l'influence du substrat du sol. Toutefois, cette hypothèse reposait sur la quantité de REE associées au sol dans son ensemble. De nombreuses études ont remis en question le fait que toutes les formes REE présentes dans le sol constituaient ou non un réservoir potentiellement biodisponible. Le consensus général est que le pool de minéraux échangeables et liés au carbonate est biodisponible. Cependant, dans la rhizosphère, les conditions géochimiques spécifiques au site et l'altération biologique qui y règne (plantes, macrofaune et microfaune) peuvent faire en sorte que d'autres réservoirs de REE du sol deviennent biodisponibles pour les plantes. L'étude ici conduite caractérise le sol en quatre fractions en utilisant la méthode d'extraction séquentielle du Bureau Communautaire de Référence (BCR). La caractérisation des associations géochimiques des terres rares avec les fractions du sol ont ainsi permis de déterminer quelles phases minérales étaient les plus biodisponibles en comparant les spectres de terres rares pour les tissus végétaux collectés dans la même zone aux extractions BCR sur les sols correspondants et en observant dans quelle mesure les spectres se ressemblaient. Les résultats ont ainsi montré que les tissus végétaux souterrains reflétaient étroitement les trois premières étapes d'extraction séquentielle et que la fraction résiduelle ou plus récalcitrante du sol présentait un spectre de REE convexe avec un appauvrissement distinct en REE moyennes (MREE) par rapport aux REE légères (LREE) et Lourdes (HREE). Ceci suggère la nature plus mobile et une biodisponibilité accrue des terres rares moyennes. Cette étude fournit en outre, la preuve que l'Eu peut être exclu par la plante, car une concentration anormale d'Eu a été observée lorsque les extractions BCR étaient normalisées par rapport aux tissus végétaux souterrains.

1 Introduction

Rare Earth Elements (REE) are technology critical elements (Van Gosen et al., 2017). Increasing evidence of their toxicity and prevalence in the biosphere indicate they pose a risk to human and environmental health (Gong et al., 2021; Li et al., 2013; Merschel and Bau, 2015; Pagano et al., 2015; Rim et al., 2013; Wakabayashi et al., 2016). These 14 elements of the lanthanide series typically adopt a +3 oxidation state, with the exception of Ce, which can be oxidized to +4 and Eu, which can be reduced to +2. Another shared attribute is the filling of the 4f electron shell, which results in insufficient shielding of the nucleus, ultimately inducing the contraction of the atomic radii. Due to lanthanide contraction, REE are adept at isomorphic substitution with similarly sized elements such as Ca (Shannon, 1976). Minor differences in how these elements interact with surfaces and compatible ligands contribute to their partitioning and fractioning in the soil. It is therefore convenient to categorize them as light REE (LREE) (La to Nd), middle REE (MREE) (Sm to Tb), and heavy REE (HREE) (Dy to Lu).

With complex biogeochemical interactions dictating the bioavailability of not just REE, but all elements, in rhizosphere soil, predicting the origin of elements found in plants is a challenge. Brioschi et al. (2013) theorizes that the preferential uptake of LREE can be summarized by the following: (1) preferential precipitation of HREE over LREE into Fe-oxyhydroxide minerals (meaning that they exclude this fraction from being bioavailable) and (2) preferential uptake of free and uncomplexed cations (LREE) by the plants. Although there is a general consensus that the dissolved pool and easily exchangeable cations are bioavailable and pose the largest threat to local ecology (Liang et al., 2005; Pérez-López et al, 2008), often this pool is minimal in comparison with the sum of elements found in plant tissue (Liang et al., 2005). Plants affect nutrient solubility and in turn weather the surrounding soil media (Stille et al, 2006) through manipulations of pH, redox potential, and production of biogenic exudates (Liang et al, 2008; Wyttenbach et al, 1998a). Manipulation of the rhizosphere conditions even extends outside of the plant, with symbiotic microbes playing a major role in promoting nutrient extraction and limiting the impacts of metal toxicity to the plant (Sessitsch et al., 2013; Kong and Glick, 2017). This would suggest that the definition of a bioavailable pool would need to be expanded to include REE associated with other pools of soil minerals and organic matter. The contribution of each of these mineral pools to REE as a whole in the soil is described in Migaszewski and Gałuszka (2015) who estimated that 43% of all REE are associated with silicate minerals, 23 % with carbonates, 14 % with oxides, and 14 % between phosphates and oxyhydroxides. While the contribution of REE from soil organic matter was not quantified by Migaszewski and Gałuszka (2015), other studies have sited the significance of the organic pool as contributing between 1.84 and 26.5% of the REE in soil (Pang et al., 2002).

As a lithophilic group of elements, the solubility of REE is usually limited. Exceptions to this include environmental conditions where low pH is developed, and metal solubility is high (Baker et al., 1990; Perez-Lopez et al., 2010; Prudêncio et al., 2015; Soyol-Erdene et al., 2018). In addition to geochemical conditions triggering REE release, many authors theorize a biological or organic influence on the solubility of REE through the production of low molecular weight organic acids (LMWOA) and humic/fulvic acids (Dia, 2000; Pourret et al., 2007; Tang and Johannesson, 2003; Tyler, 2004; Wiche and Pourret, 2023). Thus far conclusive evidence of which processes dictate the solubility and thus bioavailability of REEs has yet to be provided.

The Community Bureau of Reference (BCR) sequential extraction is a tool typically used for environmental risk assessment and predicting the origin and fate of polyvalent cations in soil (Ure et al, 1993). Through selective partitioning of the soil, we are able to identify metals associated with the (1) exchangeable and carbonate bound, (2) oxidizable, (3) reducible, and (4) residual soil phases. Knowledge of the distribution of REE with these pools provides clues as to their behavior and fate. Many studies have attempted to use BCR and other soil extraction schemes to predict the toxicity and bioavailability of REE and other trace metal contaminants (Kubová et al. 2008; Liang et al., 2005; Pang et al., 2002; Shan et al., 2003; Wang et al., 2001a; Wang et al., 2001b; Wyttenbach, 1998a; Zhang and Shan, 2001). Kubová et al. (2008) posted a study using BCR extractions to compare the bioavailability of nine different elements in plants. Ultimately, they concluded that sequential extraction alone is not a pertinent predictor of bioavailability but could be used qualitatively to determine relative amounts of plant-available elements.

While the geochemical speciation of REE is not a good predictor of bioavailability on its own, this tool is a useful predictor of soil REE fractions vulnerable to solubilization, which pose a bioavailable pool. Based on previous studies it is likely that these elements are geochemically partitioned before entering plant tissue (Brioschi et al., 2013; Forsyth et al., 2023; Gonzalez et al., 2014; Tyler, 2004). Rare earth elements are a physiochemically uniform group of elements which should have similar biogeochemical reactions (Gonzalez et al., 2014), however slight differences in their interactions with compatible ligands, redox chemistries, and atomic radius lead to fractionation of individual elements with different phases. Therefore, it is interesting to use a sequential extraction scheme to compare the lability and recalcitrance of REE against each other. This information can then be used to determine which elements are primed towards phytoavailability and under which conditions.

The current literature relating REE chemical speciation within soils to plant phytoavailability is limited. Many studies have only been able to quantify a subset of REE instead of

the entire series (Shan et al., 2003; Wang et al., 2001b; Wytttenbach et al., 1998a; Xinde et al., 2000; Zhang and Shan, 2001) and often the focus of these studies is on above-ground tissue rather than below-ground tissue (Wang et al., 2001b; Wytttenbach et al., 1998a; Xinde et al., 2000). However, biological partitioning of REE from below-ground tissue to above-ground tissue has been well documented (Forsyth et al., 2023; Liang et al., 2008, Tyler, 2004). The partitioning of REE into above-ground tissue, while not observed across all specimens, seems to be related to plant species endogeneity rather than the bioavailability of REE (Liang et al., 2008; Tyler et al., 2004). For this reason, the current study focuses on relating the chemical speciation of REE in soils to the below-ground tissue rather than the above-ground tissue, with further studies planned for isolating the mechanisms and effects of biological partitioning of REE *in planta*.

This study uses a BCR sequential extraction scheme to complement a study by Forsyth et al. (2023). Forsyth et al. (2023) investigated the origin of REE in plant tissues collected from three distinct legacy mine sites in Portugal. These three study sites were selected and characterized based on their geochemical background as mining sites. Generally, the geochemistry at these sites promotes the dissolution of polyvalent cation-bearing minerals. As a ubiquitous element in Earth's crust (Taylor and McLennan, 1985), incidental release of geogenic REE can be promoted by the conditions formed in mining sites (Ferreira da Silva et al., 2009; Perez-Lopez et al., 2010; Prudêncio et al., 2015; Soyol-Erdene et al., 2018). Results from Forsyth et al. (2023) showed that the bioaccumulation of REE was higher in areas impacted by mining activity. This combined with the fact that REE patterns for plants closely resembled the soluble REE pattern observed in mining drainage, while soils showed no such MREE enrichment, provoked a research question on defining the soil pools vulnerable to solubilization and subsequent plant uptake. Soil and aquatic soil samples used in this study were the same as those used in Forsyth et al. (2023). The BCR extraction information is therefore normalized to parity plant tissue and soil/sediment concentrations recorded in Forsyth et al. (2023).

2 Materials and Methods

2.1 Site Description

Samples were originally collected from three mine sites in November 2020: Lousal, São Domingos, and Quinta do Bispo (Portugal). A detailed description of their collection and processing can be found in Forsyth et al. (2023), which outlines a comprehensive survey of the REE concentrations and their associations in the surface waters, soils/sediments, below-ground and above-ground plant tissues. The following section details the current knowledge on

mineralogy in these sites and describes the procedures used for determining the chemical fractionation of soils/sediments described in Forsyth et al. 2023.

Lousal – Lousal mining operations were situated along the Sado Valley Fault, an area of volcanic deposition with major hydrothermal alteration (Morais et al, 2020) resulting in Volcanic Sedimentary Complexes (VSC) emblematic of the Iberian Pyrite Belt (IBP). The area features bimodal volcanic rocks with massive sulphide lenses (dominantly composed of pyrite with lesser amounts of chalcopyrite, galena, sphalerite, pyrrhotite, marcasite, bournonite, tetrahedrite, arsenopyrite, cobaltite, magnetite, and gold (Luís et al, 2011) and carbonate veins. Theorized mantle melting is assumed to be responsible for characteristic enrichment of Eu and Tb paired with depletion of Sc, Al, and Ti in the felsic and intermediate geology (Morais et al, 2020). Tertiary shales and quartzites from the Corona formation (Luís et al, 2011) and other Post-Mesozoic materials overlay a Paleozoic basement (Oliveira et al, 2001) in the area surrounding the passive treatment system. In proximity to a confined waste-rock deposit, which produce acid mine drainage (AMD) and acid leachates, the passive treatment system at Lousal successfully treats effluents for sulphates, arsenic, aluminum, iron, copper, and chrome before confluence with the nearby Corona River (Empresa de Desenvolvimento Mineiro [EDM] and Direcção Geral de Energia e Geologia [DGEG], 2011). Metal enriched effluents progress through the 17 basin-treatment system, whereas the pH slowly increases along the reach causing the precipitation of secondary minerals with further stabilization of metals supported by the aerobic wetland system. Lousal was the only study site which contained a passive treatment system for AMD effluent at the time of sample collection.

Two aquatic sediment samples were collected from the Lousal site: L-1 which corresponds to sediment collected from a stock pond outside of the mining area, filled with fresh water from Albufeira da Barragem da Tapada reservoir and L-2 which is an aquatic sediment collected from the 6th treatment basin, where the aerobic wetland basins begin.

São Domingos – Similarly to Lousal, São Domingos is hosted in the IPB of Southern Portugal. Exploitation of iron-sulfide ores rich in copper, lead, zinc, and sulfur over the course of time has led to the continual formation of AMD at this site (Álvarez-Valero et al., 2008). Though the dominant ore composition is that of pyrite and with heterogeneous arrangements of chalcopyrite, galena, and sphalerite (Durães et al., 2017), the long-established usage of this site has led to the development of many secondary minerals along the São Domingos River Valley. Secondary minerals are typically formed from the supersaturation of various metal species associated with AMD. They include Fe-oxide gossans (goethite and hematite) along with sulfate salts such as alunogens, and the presence of calcites, clinoclors, gypsums, jarosites, and schwertmannites (Durães et al., 2017; Ferreira et al., 2021). Much of the site mineralogy

has been characterized previously by Álvarez-Valero et al. (2008) for the identification of different wastes and their composition and Pérez-López et al. (2008) who performed a sequential extraction on the materials characterized by Álvarez-Valero et al. (2008) to identify the risk posed by various trace and major elements. Neither group described the rare earth element contents.

Four soil samples were collected from the São Domingos River Valley: SD-1, SD-2, and SD-3. The soil corresponding to SD-1 was retrieved from outside of the mining area close to the Albufeira da Tapada Grande reservoir. The soil around Albufeira da Tapada Grande reservoir represents the background lithology (Quental et al., 2002), weathered from shales of the Represa Formation, felsic and mafic volcanic rocks, and black shales (Pereira et al., 2008). Soils SD-2 and SD-3 were collected from along the São Domingos River Valley in areas previously identified by Pérez-López et al. (2008) as being landfilled over with heavily siliceous material. Despite the use of a relatively inert material (up to 90% quartz according to Pérez-López et al., 2008), vegetation in the area is still sparse, presumably due to the presence of secondary precipitates on surface soils after runoff (Ferreira et al., 2021).

Quinta do Bispo – Unlike São Domingos and Lousal, Quinta do Bispo is hosted in granite and metasediment of the Hercynian formation (Ramalho et al., 2009). Previous studies have characterized the plutonic igneous and metamorphic rock hosting Quinta do Bispo as: (1) Coarse-grained, (2) Medium-grained, and (3) Medium-fine-grained monzonitic two-mica granites (Matos-Dias and Costa, 1972 as referenced in Barbosa, 2012). Deposits of uranium oxides and uranium phosphates are associated with hydrothermal deposition into stockworks (Barbosa, 2012). Metasomatic albitization of feldspars and chloritization of biotites is evident in cores taken from the area (Barbosa, 2012). Previous studies of the area have attributed Eu anomalies to the albitization of plagioclase minerals (Cardenas et al., 2023; Forsyth et al., 2023), where Ca^{2+} and Eu^{2+} incorporated in plagioclases are metasomatically displaced resulting in positive Eu anomalies corresponding with the uranium ores while negative Eu anomalies correspond to the altered material.

While manganese oxides, pyrite, and arsenopyrite have been associated with the mineralogy of Quinta do Bispo, the dominant ore mineralogy is autunite and torbernite (Cardenas et al., 2023). Uranium was extracted from these ores between 1979 and 1987. Originally ores were exported from the Quinta do Bispo site and processed at Urgeiriça (Pereira et al., 2014). However, after 1992 the open pit at Quinta do Bispo was used for in-situ leaching (ISL) of low-grade ores, thus the site is impacted by acidic waste waters which are isolated in the open pit awaiting treatment.

Seven soil samples were collected from the Quinta do Bispo site, corresponding to three distinct geological units. Samples QB-1 and QB-1b represent soils collected from outside of the mining area, though there was no exploitation of uranium ores the material is similarly medium-grain granite. Samples QB-2 and QB-2b represent a large waste-rock pile on-site and thus should reflect the uranium enrichment of the exploited material. Samples QB-3, QB-4, and QB-4b correspond to the alluvium formed by an ephemeral stream and thus are reflective of weathering from materials in the surrounding drainage basin. Samples QB-1b, QB-2b, and QB-4b are further distinguished from the other samples collected at Quinta do Bispo as these samples represent bulk material which was not collected from a plant rhizosphere. These samples were collected in order to compare the rhizosphere environment to the bulk soil.

2.2 BCR Extraction Procedure

Chemical speciation was determined using a three-step BCR extraction scheme standardized by the Standard Measurements and Testing Program of the European Community (Ure et al. 1993). The BCR extraction partitions the soil into three fractions defined as the “easily exchangeable and carbonate bound” (Exch.), “reducible mineral” (Red.), and “oxidizable mineral and organic matter” (Oxid.) soil phases. The soil fraction which remains can then be calculated using Equation 1, where the sum of the elements associated with the three sequential extraction steps is subtracted from the total concentration of elements in the soil to recover the remaining soil REE fraction. The remaining soil REE was referred to as the residual (Resid.) and describes a much more recalcitrant pool of REE.

$$REE_{Resid.} = REE_{soil/aquatic\ sediment} - (REE_{Exch.} + REE_{Red.} + REE_{Oxid.}) \quad \text{Equation 1}$$

All materials were prepared in a clean room and decontaminated using a 24 h nitric acid (1.5 M HNO₃) wash at 45 °C, followed by a 24 h wash with deionized ultrapure water (18 MΩ-cm) at 45 °C. Approximately 0.5 g of soil was added into a decontaminated 50 mL centrifuge tube, with the reagents and agitation times are reported in Table 1. The supernatant of each extraction was separated by centrifugation and filtered through a 0.2 μm polyethersulfone (PES) filter before analysis. Reagent carbon was digested from each of the sequential extractions in preparation for ICP-MS analysis. Concentrated HNO₃ was used to digest the carbon associated with the “exchangeable and carbonate bound” and “oxidizable minerals and organic material” extractions, while a combination of HCl and HNO₃ was used to digest the “reducible mineral” extractions.

Table 1. Modified sequential extraction procedure (Ure et al., 1992).

Targeted Phase	Reagent	Time
BCR1: Exch.	40 mL of 0.11 M acetic acid solution	16 h
BCR 2: Red.	40 mL of 0.5 M hydroxylamine hydrochloride adjusted to pH 1.5	16 h
BCR 3: Oxid.	Predigesting at 85 °C using 20 mL 8.8 M hydrogen peroxide 50 mL of 1 M ammonium acetate adjusted to pH 2	N/A 16 h

*N/A signifies a time which was subject to the rate of sediment digestion and not confined to any particular time window.

2.3 Rare Earth Element analysis

Sequential extractions were analyzed for major and trace cation concentrations by ICP-MS (Agilent 7700x) using rhenium and rhodium as internal standards (Yéghicheyan et al., 2019). The international geostandard SLRS-6 was used to verify the validity and reproducibility of the results. Similar protocols were used and described by Forsyth et al. (2023) to obtain REE concentrations in soils, sediments, and plant tissues.

Elements were categorized as light REE (LREE) (La-Pm), middle REE (MREE) (Sm-Tb), and heavy REE (HREE) (Dy-Lu). Since both Ce and Eu can exhibit fractionation and anomalous concentrations due to their additional oxidation states, this behavior is described using a linear extrapolation of soil-normalized REE (Equations 2 and 3 respectively), where Ce^* represents the cerium anomaly and Eu^* represents the europium anomaly, the greater this value varies from 1, the more anomalous the concentration is.

$$Ce^* = \frac{2(Ce)}{La+Pr} \quad \text{Equation 2}$$

$$Eu^* = \frac{2(Eu)}{Sm+Gd} \quad \text{Equation 3}$$

3 Results and Discussion

3.1 REE Speciation in Mining Soils

The REE speciation is expressed as a percentage of the total soil REE and is shown in Table 2. Figures 1a-1c demonstrate the REE speciation for each soil sample (data were normalized to the total soil REE and plotted on a log-scale). These figures can be used to interpret the fractionation of individual REE in the soils. An interesting pattern across all of the soil phases is an enrichment in MREE. This artifact is further exemplified by Figure 1d which plots the percentage of residual REE in the soil. Figure 1d shows depletion of MREE in the residual fraction, demonstrating the more labile nature of MREE when compared with LREE and HREE. For all of the samples collected a majority of REE is associated with the Resid. phase demonstrating the more recalcitrant and conservative behavior of these elements. Pang et al. (2002) reported the average amount of REE associated with the residual fraction was between

45.2% and 94.7%, with many authors reporting that in natural soils the majority of soil REE is bound to the Resid. phase (Cao et al., 2022; Pang et al., 2002; Pédrot et al., 2009; Xinde et al., 2000; Zhang et al., 2023), the exception of course being soils associated certain mining waste streams (Khorasanipour and Rashidi, 2020).

Table 2. The percentage of soil REE extracted by each step of the BCR in samples from Lousal, São Domingos and Quinta do Bispo mine areas. Total soil REE information was taken from Forsyth et al. (2023).

	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Th	U	LREE	MREE	HREE	Total REE	Ce*	Eu*
BCR 1: exchangeable and carbonate bound																						
<i>SD-01^a</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.96	0.77
<i>SD-02</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	1.02	1.18
<i>SD-03</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.99	1.13
<i>QB-02</i>	3%	2%	3%	3%	4%	9%	6%	5%	5%	5%	5%	4%	4%	4%	0%	28%	2%	5%	4%	3%	0.58	1.81
<i>QB-02b</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	17%	0%	0%	0%	0%	0.52	1.68
<i>QB-01^a</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0.98	1.80
<i>QB-01b^a</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	9%	0%	0%	0%	0%	1.09	2.26
<i>QB-03</i>	1%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	0.72	1.32
<i>QB-04</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%	0%	0%	1.14	1.42
<i>QB-04b</i>	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	9%	0%	0%	0%	0%	0.95	1.42
<i>L-01^a</i>	0%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	0%	1%	1%	1%	1%	1%	1.06	1.18
<i>L-02</i>	2%	2%	2%	2%	3%	3%	3%	2%	2%	1%	1%	1%	1%	1%	0%	4%	2%	2%	1%	2%	1.25	1.08
BCR 2: reducible minerals																						
<i>SD-01^a</i>	35%	31%	29%	29%	26%	22%	33%	30%	30%	28%	29%	24%	22%	20%	0%	2%	31%	29%	25%	30%	0.95	0.75
<i>SD-02</i>	1%	2%	2%	3%	5%	7%	7%	6%	5%	4%	3%	3%	2%	2%	0%	2%	2%	6%	3%	2%	1.03	1.06
<i>SD-03</i>	5%	6%	5%	6%	8%	8%	11%	8%	7%	6%	6%	4%	4%	3%	0%	1%	6%	8%	5%	6%	1.22	0.85
<i>QB-02</i>	10%	11%	13%	15%	21%	43%	28%	30%	32%	28%	29%	25%	23%	21%	0%	26%	12%	28%	26%	14%	0.95	1.74
<i>QB-02b</i>	14%	9%	13%	14%	14%	24%	18%	18%	20%	18%	18%	15%	12%	10%	0%	29%	12%	17%	15%	12%	0.70	1.49
<i>QB-01^a</i>	2%	2%	1%	2%	2%	5%	3%	3%	4%	3%	3%	3%	2%	2%	0%	21%	2%	3%	3%	2%	1.04	1.91
<i>QB-01b^a</i>	1%	1%	1%	1%	1%	3%	2%	2%	2%	2%	2%	1%	1%	1%	0%	21%	1%	2%	1%	1%	1.20	2.16
<i>QB-03</i>	14%	12%	12%	11%	9%	13%	11%	11%	10%	9%	7%	6%	4%	3%	0%	10%	12%	10%	6%	12%	0.89	1.36
<i>QB-04</i>	4%	5%	3%	3%	3%	4%	4%	4%	5%	4%	4%	3%	2%	2%	0%	11%	4%	4%	3%	4%	1.32	1.28
<i>QB-04b</i>	11%	11%	8%	8%	7%	11%	10%	11%	11%	10%	9%	7%	6%	5%	0%	37%	10%	9%	8%	10%	1.12	1.33
<i>L-01^a</i>	8%	8%	9%	10%	14%	13%	13%	10%	8%	7%	7%	5%	5%	4%	0%	4%	9%	12%	6%	9%	1.00	0.97
<i>L-02</i>	25%	36%	32%	35%	35%	36%	33%	26%	21%	18%	17%	14%	13%	12%	0%	9%	33%	30%	15%	31%	1.24	1.05

	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Th	U	LREE	MREE	HREE	Total REE	Ce*	Eu*
BCR 3: oxidizable minerals and organic matter																						
<i>SD-01^a</i>	16%	11%	23%	27%	32%	27%	29%	24%	22%	18%	19%	17%	18%	17%	3%	8%	18%	27%	18%	20%	0.55	0.89
<i>SD-02</i>	0%	1%	1%	1%	2%	3%	3%	2%	2%	1%	1%	1%	1%	1%	1%	6%	1%	2%	1%	1%	1.02	1.08
<i>SD-03</i>	2%	3%	3%	4%	6%	6%	7%	5%	4%	3%	3%	2%	2%	2%	1%	6%	3%	6%	3%	3%	1.29	0.96
<i>QB-02</i>	2%	2%	3%	3%	5%	7%	5%	5%	5%	4%	4%	3%	4%	3%	2%	13%	2%	5%	4%	3%	0.83	1.45
<i>QB-02b</i>	4%	4%	5%	6%	6%	9%	8%	8%	8%	7%	7%	6%	5%	4%	3%	12%	4%	7%	6%	5%	0.80	1.30
<i>QB-01^a</i>	1%	2%	2%	2%	3%	6%	4%	3%	3%	3%	2%	2%	2%	2%	2%	44%	2%	3%	2%	2%	1.28	2.03
<i>QB-01b^a</i>	1%	1%	1%	1%	1%	3%	1%	1%	1%	1%	1%	1%	1%	1%	1%	26%	1%	1%	1%	1%	1.49	2.27
<i>QB-03</i>	5%	10%	8%	9%	10%	15%	12%	11%	11%	9%	9%	7%	6%	5%	8%	34%	9%	11%	8%	9%	1.59	1.38
<i>QB-04</i>	3%	4%	4%	4%	6%	9%	7%	7%	7%	6%	6%	5%	4%	4%	3%	46%	4%	7%	5%	4%	1.23	1.31
<i>QB-04b</i>	6%	9%	7%	8%	10%	14%	12%	12%	12%	10%	10%	8%	7%	6%	6%	47%	8%	12%	9%	8%	1.31	1.24
<i>L-01^a</i>	4%	3%	2%	3%	3%	4%	3%	3%	2%	2%	2%	1%	1%	1%	5%	18%	3%	3%	2%	3%	0.94	1.12
<i>L-02</i>	3%	4%	4%	5%	6%	7%	6%	4%	3%	3%	3%	3%	3%	3%	10%	22%	4%	5%	3%	4%	1.19	1.11
Residual: recalcitrant soil mineral pool																						
<i>SD-01^a</i>	48%	58%	47%	43%	42%	51%	38%	45%	48%	53%	52%	59%	60%	63%	96%	90%	50%	43%	56%	49%	1.22	1.26
<i>SD-02</i>	99%	98%	97%	96%	92%	90%	90%	92%	94%	95%	95%	96%	97%	97%	98%	92%	98%	92%	96%	97%	1.00	0.99
<i>SD-03</i>	93%	91%	92%	90%	86%	86%	83%	86%	89%	91%	91%	93%	94%	94%	99%	92%	91%	86%	92%	91%	0.98	1.02
<i>QB-02</i>	85%	86%	82%	78%	70%	41%	61%	60%	58%	63%	62%	68%	69%	73%	98%	33%	84%	62%	66%	80%	1.03	0.63
<i>QB-02b</i>	82%	87%	82%	80%	79%	67%	74%	74%	72%	75%	75%	79%	83%	85%	97%	42%	84%	75%	78%	83%	1.06	0.87
<i>QB-01^a</i>	97%	96%	97%	96%	95%	89%	93%	93%	93%	94%	94%	95%	96%	97%	98%	30%	97%	94%	95%	96%	1.00	0.94
<i>QB-01b^a</i>	98%	98%	98%	98%	98%	94%	97%	96%	97%	97%	97%	98%	98%	98%	98%	44%	98%	97%	98%	98%	0.99	0.97
<i>QB-03</i>	80%	78%	80%	80%	80%	71%	77%	77%	79%	82%	84%	87%	89%	91%	92%	54%	79%	78%	86%	79%	0.97	0.90
<i>QB-04</i>	93%	91%	93%	93%	91%	87%	88%	88%	88%	90%	91%	92%	94%	94%	97%	42%	92%	89%	92%	92%	0.98	0.97
<i>QB-04b</i>	83%	80%	84%	84%	82%	74%	77%	76%	77%	79%	81%	84%	87%	89%	94%	7%	82%	79%	84%	82%	0.96	0.93
<i>L-01^a</i>	88%	88%	88%	86%	82%	82%	83%	87%	89%	91%	91%	92%	93%	94%	95%	77%	88%	84%	92%	88%	1.00	1.00
<i>L-02</i>	70%	58%	61%	58%	56%	55%	59%	67%	74%	78%	79%	82%	83%	84%	89%	66%	61%	62%	81%	62%	0.88	0.96

^a Samples collected from outside of the mining areas.

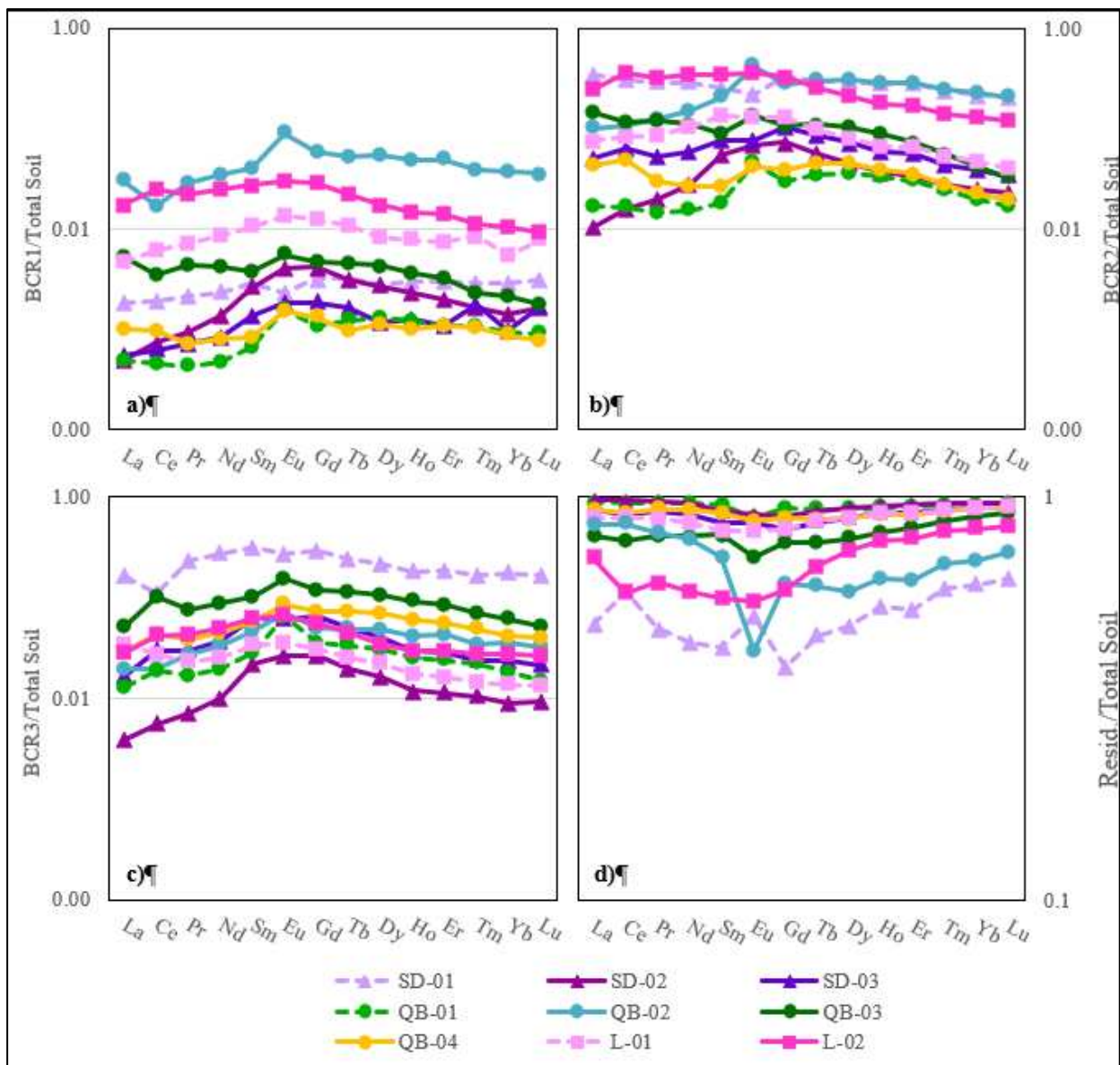


Figure 1. Plots of each REE on a log scale for the a) Exch. phase b) Red. phase, and c) Oxid. phase after normalization to the soil/sediment as a whole. Plot d) expresses the Resid. REE as a fraction of the total soil/sediment REE on a log-scale. Dashed lines correspond to samples taken from outside of the mining areas. Samples collected from São Domingos are presented using a triangular marker, samples collected from Quinta do Bispo are presented using a circular marker, and samples collected from Lousal are presented using a square marker.

3.1.1 Lousal

In Figure 2 a) the REE associated with the sediments collected from inside and outside of the passive treatment system at Lousal is displayed. Of the samples collected from inside of the mining areas, the aquatic sediment collected from Lousal had the least amount of REE associated with the recalcitrant residual fraction, which is not surprising since the sediment collected from the treatment system was a composite of growth medium for the plants, eroded material from the surroundings, and newly formed precipitates. There was a marked change in the fractionation of REE in all the soil phases, which showed preferential incorporation of LREE into the Exch., Red., and Oxid. phases of the sediments collected from inside of the

treatment basin. This observation is most notable in the reducible phase which demonstrated an increase in LREE from 9% to 33%. Similarly, a study of another passive treatment system installed by EDM in Jales, Portugal made an association between REE and Fe-oxide ochre precipitates formed in the limestone treatment basins (Prudêncio et al., 2015). Ochre-precipitates seemed to scavenge more LREE, causing an increase in the observed LREE/MREE ratio. Subsequently this ochre precipitate was vulnerable to changes in hydrology and chemistry leading to seasonal similarities in LREE/MREE ratios between effluents and precipitates (Prudêncio et al., 2015). Obregón-Castro et al. (2023) investigated similar samples from Lousal, finding that a majority of the materials found were Fe oxyhydroxides with a large proportion of jarosites. MREE with a slight enrichment of LREE over HREE has been recorded in jarosite minerals (Obregón-Castro et al., 2023; Welch et al., 2009). Therefore, in this study we attribute the increased percentage of REE associated with the exchangeable to the formation of jarosites in sample (L-02) which is further supported by the characteristic increase in the ratio of LREE/MREE observed in Obregón-Castro et al. (2023) and Welch et al (2009). The Exch., Red., and Oxid. phases also shared a slight positive Ce*, ranging from 1.19-1.29. The highly oxidizing nature of the AMD wastes may promote Ce oxidation and cause enrichment of Ce in the secondary minerals observed and the high organic content of the wetland area may trap Ce as well, this is further reflected in the development of a slightly negative Ce* in the residual phase (0.88).

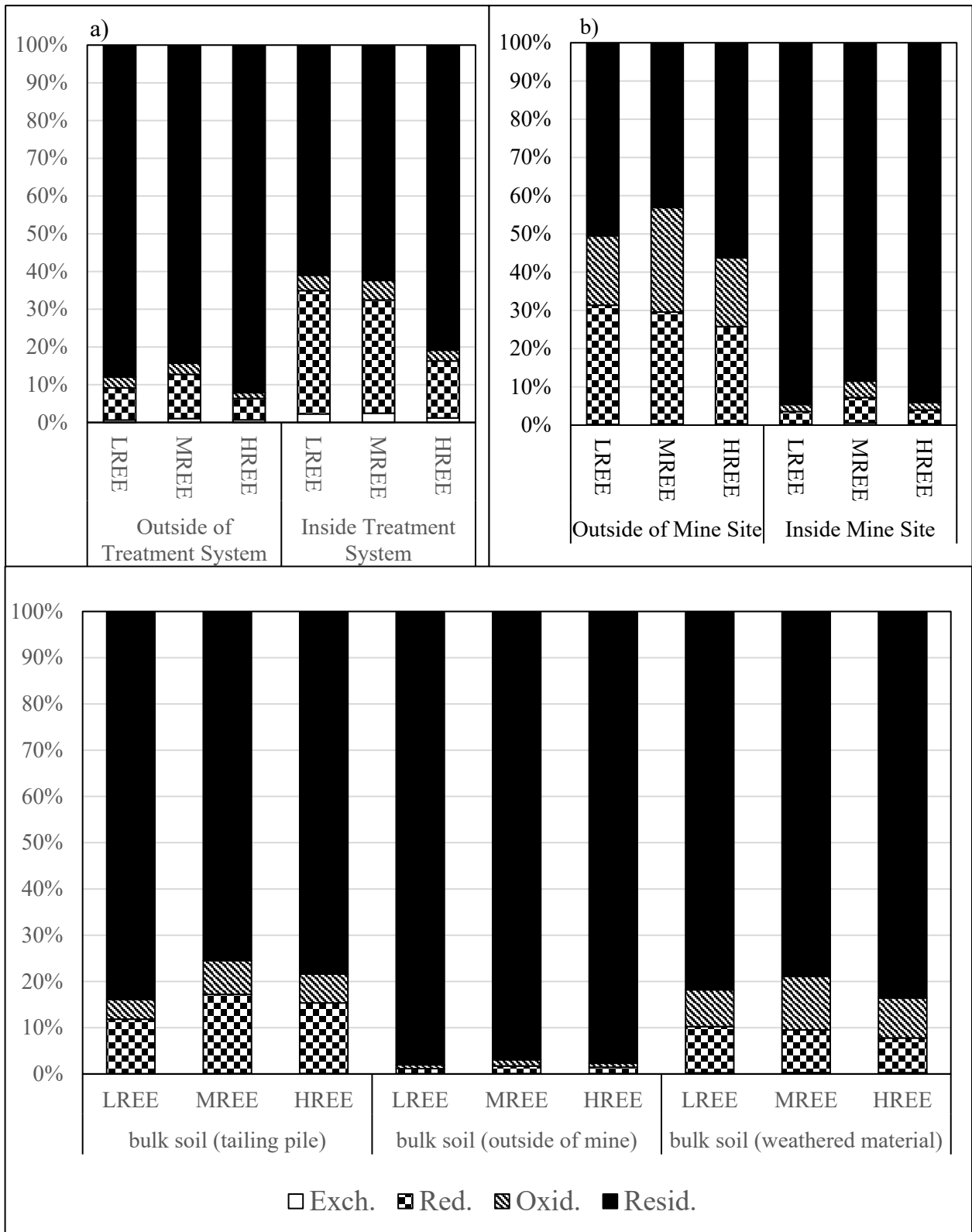


Figure 2. a) Presents the REE speciation for samples collected inside and outside of the Lousal passive treatment system. b) Compares the REE speciation of samples collected from inside and outside of the São Domingos River Valley. c) Demonstrates the REE speciation for a tailing pile, outside of the mine, and weathered material around the mine at Quinta do Bispo.

3.1.2. São Domingos

As in Lousal, the residual phase is the largest REE pool (Figure 2b), but also displayed a significant variation in the REE bearing soil phases when comparing outside and inside the mining area. The percentage of REE associated with the Red. and Oxid. phases was greater in the sample collected from outside of the mining area than for the samples collected from inside of the mining area. Presumably, the lower proportion of REE associated with the Exch., Oxid., and Red. soil phases for samples collected from inside of the mining site can be attributed to the dissolution of REE-bearing minerals by the development of AMD in the area. Soil samples collected from the São Domingos River Valley had a similar fractionation of REE across all of the BCR steps with a skew concave pattern showing MREE>HREE>LREE. This was in contrast to the sample collected from outside of the mining area, which displayed a MREE enrichment for the Oxid. soil phase but had a LREE>MREE>HREE pattern for the Red. phase.

It would seem that for samples collected from outside of the mining area the Ce* and Eu* is predominantly controlled by the Resid. soil phase (Fig. 1) which was enriched in Ce (Ce*=1.22) and Eu (Eu*=1.26) relative to the status of these elements in the soil as a whole. Slightly negative Eu* was associated with each of the BCR fractions. The Ce was depleted in the Oxid. phase, where there appears to be a significant negative Ce* of 0.55, which was paired with . a negative Ce* associated with the soil and surface waters, as reported in Forsyth et al., 2023. The presence of a negative Ce* in the reducible phase may demonstrate Ce preferential oxidation and thus depletion in the reduced minerals. The negative Ce* could also result from stable Ce complexes made with microbially mediated organic acids, which has been shown to produce a negative Ce* for mineral surfaces and increase Ce mobility (Kraemer et al., 2017).

For the soil sample collected from the mining area, a positive Ce* was associated with both the reducible minerals and oxidizable minerals. Pédrot et al. (2015) looked at the combined effects of soil organic matter (SOM) and Fe-oxides on the behaviour of REE and found that Ce* were mainly controlled by the Fe-oxide phase. Similarly, the oxidizable phase formed in the mine site would scavenge any oxidized Ce, therefore producing a positive Ce* associated with the Red. phase. In this study the Oxid. phase of soils collected from the mining site is dominated by volcanogenic sulfide minerals and low in organic matter. Similar positive Ce* have been observed in sulfides, which may have an origin in oceanic waters and sediments enriched in Ce (Otake et al., 2021).

The difference in the observed geochemistry across studies and sites may be due to the geochemical conditions in which the soil phases were formed. As indicated in the Materials and Methods section, the soils collected from inside and outside of the mining area differ in origin and pedogenesis, this is high-lighted by the observations made using BCR extraction. Although

these soils are not comparable they did however, add valuable insight into the geochemistry established inside and outside of the mining area.

3.1.3. Quinta do Bispo

There were three distinct soil groups sampled at the Quinta do Bispo site: the low grade ore pile, the weathered materials collected in an ephemeral stream below Quinta do Bispo, and a representation of the surrounding lithology from outside of the mining area for comparison. Figure 2c displays the distribution of LREE, MREE, and HREE in bulk soil collected from these three different sampling points. While the total concentrations of LREE, MREE, and HREE in the soils collected from Quinta do Bispo were not statistically different (Forsyth et al., 2023) there appears to be distinguishable differences with the geochemical associations of these REE groups at the three sampling points. As mentioned before, the surrounding area is granite with metasomatic deposits of uranium. The sample collected from outside of the mining area appears to reflect the surrounding lithology, the Resid. fraction accounted for 89%-98% of the REE. Across all Quinta do Bispo sites, the Resid. phase hosted the largest proportion of REE. The samples collected from the alluvium and the ore pile contained similar proportions of Resid. REE. However, the alluvium contained a lower proportion of REE associated with the Red. fraction (10% instead of 15%) and almost twice as much Oxid. REE proportionally.

Strong positive Eu^* are seen in all of the Quinta do Bispo soils through out all sequential extraction steps, showing that Eu^{2+} is assimilated into more easily labile minerals (Fig. 1a-1c), rather than in the recalcitrant Resid. fraction (Fig. 1d). However, because the Resid. represents such a large pool of REE, a negative Eu^* was only observed in samples QB-02 and QB-02b which represent the ore body which was enriched in hydrothermally deposited Eu.

The uranium content was also measured for all samples. This was done in order to confirm differences in the geochemistry of the samples collected and assess the risks associated with uranium release in this area. Only the samples collected from the ore pile contained appreciable amounts of Exch. U (17%-28%). Additionally, the ores contained a smaller percentage of U associated with the Oxid. phase as compared with the other two soils. Unlike REE, the dominant pool of U was not always the Resid. phase. All of the Resid. U for soil samples contained similar concentrations (30%-54%) with the exception of the bulk soil sample collected from the alluvium (7%). This sample also contained a larger proportion of Red. U. Potentially, the conditions developed in the ephemeral hydrology have encouraged a secondary phase of U associated with reducible minerals. This should be further investigated as changes in geochemistry (through anoxic conditions or pH driven dissolution) would make these sediments vulnerable to U release.

3.2. Bioavailability Fingerprinting

Exemplified by a study from Vermeire et al. (2016), abiotic and biotic processes weathering soils, sediments, and host rocks can change the speciation and total amount of REE bioavailable in the top soil horizons. The following section describes the geochemical associations of REE in the soils and attempts to explain the observed partitioning in the context of biological weathering processes.

The bulk soils and rhizosphere soils were compared at Quinta do Bispo. For both bulk and rhizosphere soils, the Resid. phase represents the most important component ranging from 62 to 98% for individual REE. In Forsyth et al. (2023) there was no statistical difference found amongst the rhizosphere and bulk soil REE concentrations. However, data recovered from the BCR extractions show slight differences between rhizosphere and bulk soil phases, displayed in Figure 3. Rhizosphere samples show a 0.77-1.94% increase in the amount of REE associated with the Exch. phase, this corresponded to a 1.69 $\mu\text{g kg}^{-1}$ increase in the total soil REE concentration. Since the Exch. phase represents such a small proportion of soil REE, this is not very significant. However, there was also a 4.02 and 4.24% increase in the Red. phase for the MREE and HREE, respectively. The LREE associated with the Red. phase only increased by 0.12% which meant only a 0.28 $\mu\text{g kg}^{-1}$ increase in total rhizosphere soil REE concentrations. The observed change in soil phases may be due to carboxylic acids effecting the solubility of REE in the rhizosphere, as theorized by Wiche and Pourret (2023).

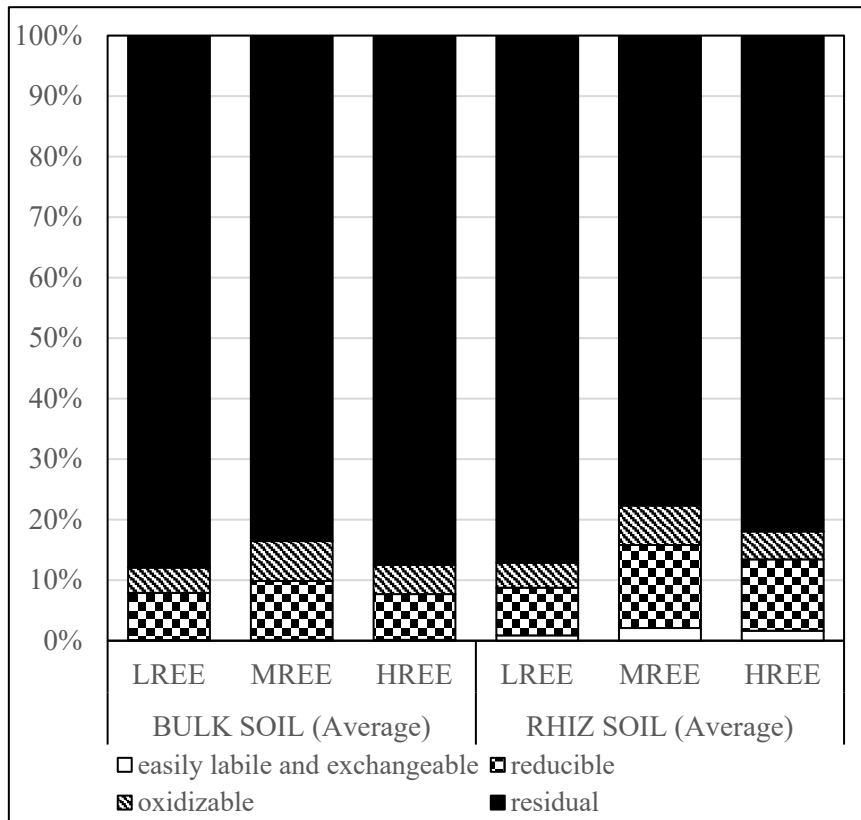


Figure 3. Presents the REE speciation of rhizosphere soils (Rhiz. soil) and compares it to bulk soils collected from Quinta do Bispo.

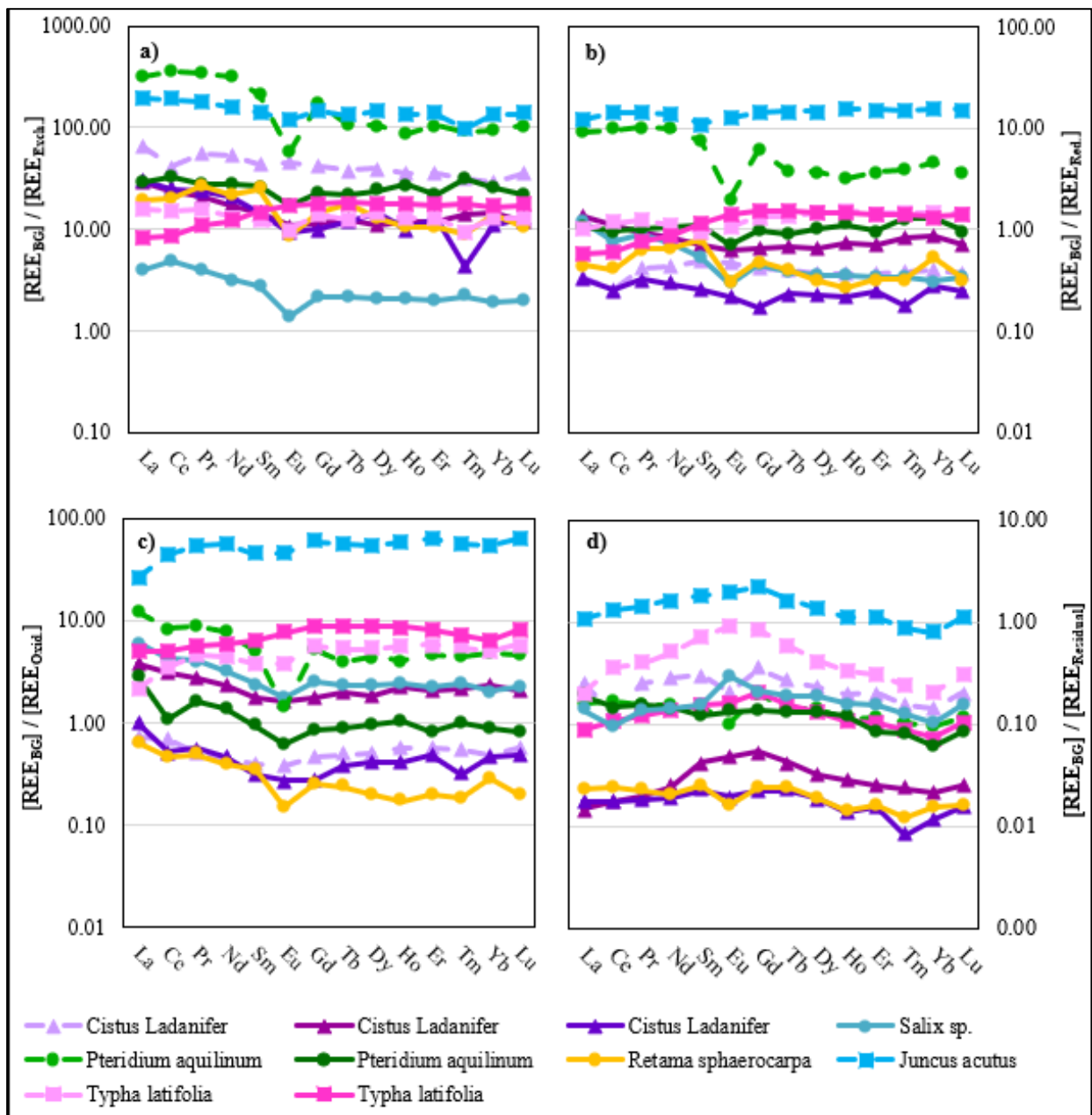


Figure 4. Concentration of REE associated within the a) Exch., b) Red., c) Oxid., and d) Resid. soil phases normalized to the BG plant tissue REE concentrations. São Domingos are presented using a triangular marker, samples collected from Quinta do Bispo are presented using a circular marker, and samples collected from Lousal are presented using a square marker.

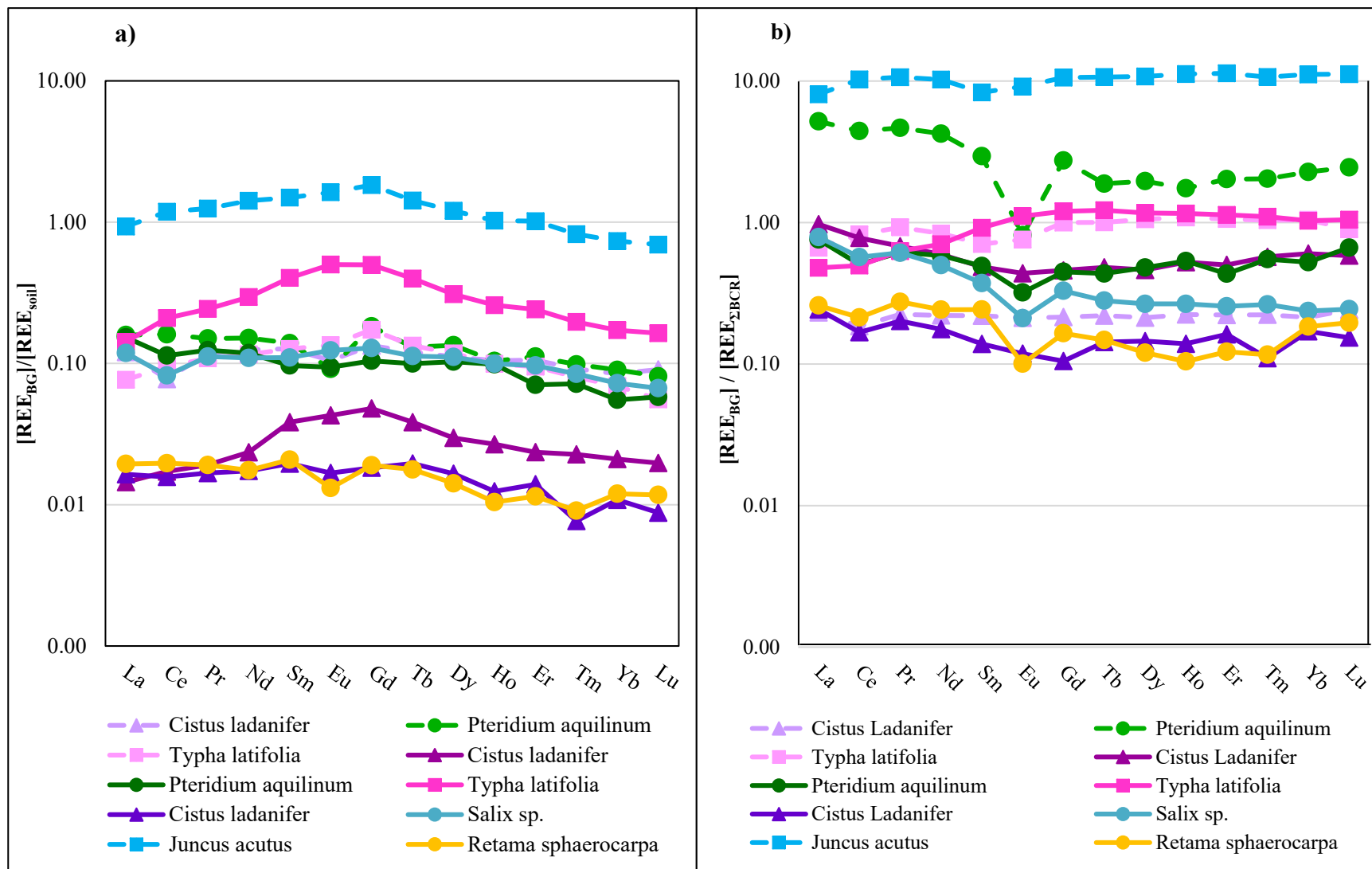


Figure 5. a) Data taken from Forsyth et al. 2023, where the BG tissue is normalized to the total soil/sediment REE whereas b) demonstrates the BG normalized to the sum of all three soil phases identified by sequential extraction. São Domingos are presented using a triangular marker, samples collected from Quinta do Bispo are presented using a circular marker, and samples collected from Lousal are presented using a square marker.

When the below-ground tissue (BG) associated with the soils was normalized to the BCR extractions (Figure 4a-4d), the patterns were smoother with an obviated negative Eu* in samples collected from Quinta do Bispo. This paired with the observed positive Eu* in Figures 1a-1d demonstrates that the mobility of Eu in these soils was high, but that the plant uptake of Eu* was relatively low by comparison. Europium has a particular affinity for carboxylic and phosphate functional groups limiting the solubility of Eu into plant tissue by sorption onto microbial mass or other surfaces in the rhizosphere (Texier et al., 2000; Tyler, 2004). It is interesting to note that the only site which had anomalous Eu in soils and plants was the Quinta do Bispo site. Accumulation of REE in plant tissues collected from Quinta do Bispo seem to indicate the presence of an interaction between the effects of geology and biology. According to the BCR extraction Eu was incorporated into less recalcitrant soil pools, however accumulation into plant tissues seems to have been limited in comparison to the potentially labile pool.

In the context of AMD, based on the geochemical observations it would be fair to assume that (1) many of the Exch. REE would be mobilized by changes in surface charges caused by the lower pH, (2) acid driven dissolution of reducible minerals is predicted to contribute to REE mobilization, and (3) oxidation of the Oxid. phase, which decomposed the mineral while forming AMD and therefore also mobilizes REE incorporated in the Oxid. phase. Thus, Figures 5a and 5b indicate that the below-ground tissue (BG) reflects the same REE fractionation observed through the first three sequential extraction steps as there is little to no slope or concavity observed with the normalization (Figure 5b) as compared with the normalization of BG to the total soil (Figure 5a). Samples collected from Quinta do Bispo do however have negative Eu* and slight depletion in HREE. The normalization of the BG tissue to the BCR extractions also changed the scale of observations, with all but one of the plants demonstrating values deviating only one order of magnitude from 1.00. This can be taken as qualitative evidence that the BCR extractions reflect fairly well REE uptake by the investigated plants.

This study examined the REE geochemistry to determine the source of REE in spontaneous vegetation around mining sites. These mining sites are characterized by the dissolution of the oxidizable and reducible phases. Though the insight provided here is valuable in determining the controls of REE fractionation in plant roots collected from such environments, further studies would ideally capture a variety of geological contexts and pH conditions. Additionally, further studies should be planned to determine biological factors controlling or limiting plant uptake of REE.

4 Conclusions

The soil is a complex medium and it is difficult to predict the bioavailability of a nutrient based on chemical extractions alone. Some studies have tried to address this topic by comparing different chemical extraction methods and determining which extractions best correlate with plant uptake of REE. This study was able to quantify all REE associated with different soil phases and make conclusions about the REE patterns associated with soil/sediments and the below-ground plant tissues. The REE in the soil is mainly associated with the residual fraction, which is depleted in MREE. This in turn means that an enrichment was observed in the “exchangeable and carbonate bound”, “reducible mineral”, and “oxidizable mineral and organic” phases, which were more vulnerable to desorption and dissolution processes promoted by the geochemical conditions accrued from mining activity. As a result, all three sequential extraction steps were operationally defined as plant available in this system.

The concentration of REE in below-ground plant tissue is similar to the sum of REE associated with the three sequential extraction steps. This was demonstrated by the normalization of the below-ground tissue to the operationally defined available REE, which showed that the shape and scale of the REE patterns was smoother and distributed around 1.00. However, this statement is made with a caveat for Eu as there was a distinct negative Eu* for the Quinta do Bispo samples. There were negative Eu* in the below-ground tissue normalizations combined with positive Eu* for the total soil normalizations, meaning that there appears to be an exclusion of Eu from plant tissue though there is plenty of Eu available in less recalcitrant soil phases.

While this study was useful for determining the potential soil origins of REE in plant tissues, there are still areas to explore relating to REE bioavailability. Prospective research should include determining the biological triggers for REE uptake in plants. This may include examining plant nutrient acquisition and how this relates to the availability of REE to the plant.

CHAPTER 3: DOES THE IRON-ACQUIRING MECHANISM OF PLANTS INFLUENCE THE UPTAKE AND TRANSLOCATION OF RARE EARTH ELEMENTS INTO PLANT TISSUES?

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Chapter 3 is in preparation for an international peer reviewed journal. Following the themes of the first two chapters, this chapter outlines the physiological plant processes which may contribute to plant uptake of REE.

CHAPTER 3: ABSTRACT

Iron stress in plants can lead to the exudation of low molecular weight organic acids (LMWOA) and phyto-siderophores. Rare earth elements (REE) have a well-established affinity towards the carboxylic groups of organics and can form stable soluble organo-metallic complexes. These complexes are potentially plant available. The aim of this research was to stimulate iron stress in two different plant types (barley and rapeseed) with differing iron-acquiring strategies. In addition to imposing iron stress, plants were exposed to 0, 1400, and 7000 ppb REE solutions. After a 28-day growth period in the aforementioned treatments growth parameters and REE tissue accumulation was measured and compared across treatments. Measurements of barley growth did not indicate any negative effect induced by REE exposure but did indicate a marked reduction in height and tissue mass for Fe-deficient treatments. Rapeseed root weight was also negatively impacted by Fe-deficiency, while aerial tissue weights demonstrated differences between the highest REE treatment level and the control. While the REE accumulation in plant tissue was directly related to the REE exposure level across both plant types, iron-status had an exacerbating effect on the accumulation of REE in rapeseed tissues. The REE signatures for plants showed a slight fractionation of La, Ce, and Gd. This fractionation occurred from the solution of the hydroponic system to the root tissue for rapeseed and from the rooting tissue to the aerial tissue for barley.

CHAPTER 3: RESUMÉ

Le stress ferrique chez les plantes peut conduire à l'exsudation d'acides organiques de faible poids moléculaire (LMWOA) et de phyto-sidérophores. Les terres rares (REE) ont une affinité bien établie pour les groupes carboxyliques des substances organiques et peuvent former des complexes organo-métalliques solubles stables. Ces complexes sont potentiellement disponibles dans les plantes. L'objectif de cette étude était de stimuler le stress ferrique chez deux types de plantes caractérisées par des stratégies d'acquisition du fer différentes. Outre l'imposition d'un stress ferrique, les plantes ont été exposées à des solutions de REE de 0, 1400 et 7000 ppb. Après une période de croissance de 28 jours dans les traitements susmentionnés, les paramètres de croissance et l'accumulation de REE dans les tissus ont été mesurés et comparés d'un traitement à l'autre. Si les mesures de la croissance de l'orge n'ont pas indiqué d'effet négatif induit par l'exposition aux REE, ils ont toutefois, révélé une réduction marquée de la hauteur et de la masse des tissus pour les traitements déficients en Fe. Alors que l'accumulation de REE dans les tissus végétaux était directement liée au niveau d'exposition aux REE pour les deux types de plantes, l'exposition au fer a eu un effet exacerbant sur l'accumulation de REE dans les tissus de colza. Les signatures en REE pour les plantes ont montré un léger fractionnement des La, Ce et Gd. Ce fractionnement s'est produit de la solution du système de culture hydroponique vers le tissu racinaire pour le colza et du tissu racinaire vers le tissu aérien pour l'orge.

1 Introduction

Rare earth elements (REE) are composed of Sc, Y, and the lanthanide series on the periodic table of elements. High demand exists for these elements in medical, technological, and agricultural fields (Migaszewski and Gałuszka, 2015). A Mineral Commodity report published in 2023 by the United States Geological Survey (USGS) highlights their unique electromagnetic and optical properties, appraising their status as critical in these industrial applications, with no recognized effective alternatives (USGS, 2023).

With their high demand, REE have recently increased circulation in the Critical Zone. Kulaksız and Bau (2013) have traced Gd pollution of surface waters to waste from medical contrast agents. Elevated REE concentrations have been identified in mining waste streams, a phenomenon which is not limited to REE mines as these elements are ubiquitous in Earth's crust (Ferreira da Silva, 2009; Perez-Lopez et al., 2010; Prudêncio et al., 2015; Soyol-Erdene et al., 2018). Mass application of REE fertilizer in China covered 16 to 20 million hectares in 1995 (Pang et al., 2002), constituting about 10% of China's reported agricultural lands in 2022.

Though the definition of REE typically includes Sc and Y, the physicochemical properties of these elements – which are a product of their electron configurations – vary from that of the lanthanide series. Therefore, this work will restrict the definition of REE to the elements of the lanthanide series. Further classifications as light REE (LREE), middle REE (MREE), and heavy REE (HREE) help to describe their fractionation in environmental media due to slight differences in their physicochemistry. While REE dominantly adopt a +3 oxidation state, Ce⁺³ can lose an electron, becoming Ce⁺⁴ in oxidizing conditions whereas Eu⁺³ can gain an electron, becoming Eu⁺² in reducing conditions. Both Ce and Eu can display anomalous behavior in accordance with their alternative oxidation states.

REE have a strong affinity towards soil organic matter, which has been well established (Wiche et al., 2017a), with REE even being known to associate with the functional groups of microbial (Takahashi et al., 2005; Texier et al., 2000) and plant (Ding et al., 2005) cell walls. In particular in mining areas impacted by acid mine drainage, REE have a propensity to mobilize from ores and waste rock piles; as a result studies have documented their enhanced accumulation in plant tissues (Forsyth et al., 2023; Krzcuik and Gałuszka, 2020). Long-term application of REE in an agricultural context has led to increased REE concentrations observed in plant tissues (wheat) with no increase observed in background soil concentrations (Liang et al., 2005b), demonstrating that plants may be an important sink for REE. Though REE are not essential plant nutrients, smaller doses of REE stimulate photosynthesis and chlorophyll production and can also promote plant resistance to stress (Tao et al., 2022). Larger doses of REE however, also play an antagonistic role in interrupting Ca²⁺ and Mg²⁺ processes leading

to inhibition of photosynthesis and reduction in root elongation and growth (Tao et al., 2022; Tyler, 2004). The physiological plant functions which limit or promote phyto-extraction remain poorly understood.

Given that differences are observed across plant species grown in the same media, a natural conclusion is that plant type influences REE uptake (Forsyth et al, 2023; Wiche and Heilmeier, 2016; Wyttenbach et al., 1998a; Wyttenbach et al., 1998b). Which factors differentiate these plant species and their affinity for REE? Few have studied the exact relationship between REE in plant tissues and nutrient acquisition strategies. Wiche and Pourret (2023) recently posited a relationship between exogenic organics in the rhizosphere and REE uptake, relating this to P-acquisition. There is indeed literature to support this hypothesis, with a study by Shan et al. (2003) asserting that low molecular weight organic acids (LMWOA) were a better predictor of plant available REE than classic sequential extraction schemes, and studies by Han et al. (2004) and Wang et al. (2004) successfully enhancing plant uptake of REE using LMWOA treatments.

Other organics associated with Fe acquisition potentially play a role in REE uptake. Bau et al. (2013) and Kraemer et al. (2017) have previously demonstrated that microbial siderophores such as desferrioxamine B (DFOB) were capable of increasing the solubility of Ce and inducing fractionation of REE, as DFOB has a lower affinity for LREE. The relationship between Fe-nutrient acquisition and REE solubility has implications for plant uptake of REE, since the soluble and exchangeable REE is regarded as plant available (Liang et al., 2005b). However, authors have largely dismissed the effects of Fe acquisition strategy on REE uptake after a study by Wiche et al. (2017b) who found that the addition of the siderophore DFOB and citrate resulted in decreased accumulation of REE in *Phalaris arundinacea* grown in sand, indicating a potential detoxification by expulsion and exclusion of organo-REE complexes. Even so, previous studies of plant Fe acquisition have demonstrated that microbial siderophore production and exogenous organics have an indirect effect on Fe uptake by plants and for certain plant types only phyto-siderophores such as, deoxymugineic acid (DMA) and mugineic acid (MA) can shuttle plant available Fe across root transporters (Römheld and Marschner, 1986). With many field studies relating Fe-bearing minerals phases to REE concentrations in plants (Brioschi et al. 2013; Wyttenbach et al., 1998a), the topic requires further examination of the physiological plant mechanisms for REE accumulation as they relate to iron uptake.

The aim of this work is to identify the effects of Fe-acquiring nutrient strategies on the uptake and translocation of REE in plants. A set of hydroponic experiments were planned using two plant types. Plants can be categorized by their Fe-acquiring strategies (Marschner and Römheld, 1994; Marschner et al., 1986):

- I. Strategy I involves the acidification of the rhizosphere environment and the exudation of LMWOA which influence the solubility of Fe-bearing minerals (Marschner and Römheld, 1994). Chelated Fe-complexes are then reduced by iron-chelate reductase and Fe²⁺ is transported in planta through divalent metal transporters.
- II. Strategy II requires the exudation of phyto-siderophores such as MA and DMA, these organics have a high affinity for iron and form stable complexes with a variety of metals (Römheld, 1991; Shenker et al., 2001; Treeby et al., 1989). Phyto-siderophore complexed iron is then transported through yellow stripe and yellow stripe-like transporters in the root tissue.

Strategy I is generally employed by non-graminaceous monocotyledon and dicotyledon plants, whereas Strategy II is typically performed by graminaceous plant types. A combination of both strategies is possible and has been notably observed in *Oryza sp.* (Wairich et al., 2019).

2 Materials and Methods

2.1 Plant Preparation

All seeds used for the investigation were washed in a 2% (v/v) H₂O₂ solution for 10 minutes and then rinsed three times with deionized water (18 MΩ-cm). Two plants species were selected to represent Strategy I and Strategy II Fe-nutrient acquisition.

Rapeseed (*Brassica napus*, cv *Samourai*) was selected to represent Strategy I Fe-nutrient acquisition. The Fe-nutrient acquiring strategies for rapeseed have been well established, and recent studies focused on Brassica species have also implicated other root exudates such as Coumarins in Strategy I Fe-acquisition (Sarashgi et al., 2021). Seeds were germinated in a 1/10th Hoagland solution for 13 days in a climate chamber using a fixed temperature of 24°C with no photoperiod. 5 rapeseed sprouts were then placed into separate hydroponic pots for each treatment scheme.

Barley (*Hordeum vulgare L.*) was selected to represent Strategy II Fe-nutrient acquisition. As a well-documented phyto-siderophore producer, barley is commonly cultivated to produce standards for mugineic acid (Kawai et al., 2008; Shi et al., 1988; Sugiura and Nomoto, 1984; Takemoto et al., 1987). Seeds were germinated in a 1/10th Hoagland solution for 10 days in a climate chamber using a fixed temperature of 24°C with no photoperiod. Three barley seedlings were then added into each hydroponic pot and 5 pots were designated to each treatment scheme.

2.2 Hydroponic Solution Composition

All laboratory materials used for hydroponic systems were cleaned using a 7.3M HNO₃ rinse followed by copious amounts of deionized water (18 MΩ-cm). Hydroponic materials were then wiped down using ethanol and air dried to sterilize surfaces from foreign organisms. Each plant type was

subjected to six different treatment schemes made in a background nutrient solution with a total volume of 3.2 L. Macronutrients supplied to the hydroponics were scaled to 1/200th and micronutrients were scaled to 1/1000th of the solution described in Hoagland and Arnon (1950) (Table 1), with adaptations according to iron treatment.

Table 1. Hydroponic nutrient solution recommended in Hoagland and Arnon (1950).

Macronutrients	molar concentration (M)
KNO ₃	0.6
Ca(NO ₃) ₂ •4H ₂ O	0.4
MgSO ₄ •7H ₂ O	0.2
NH ₄ H ₂ PO ₄	0.1
Fe-EDTA	1.453 mM
Micronutrients	molar concentration (M)
H ₃ BO ₃	2.860
MnCl ₂ •4H ₂ O	1.810
ZnSO ₄ •7H ₂ O	0.220
CuCl ₂ •2H ₂ O	0.080
NaMoO ₄ •2H ₂ O	0.020

Three of the treatment schemes were supplied with sufficient Fe, while three of the treatment schemes were Fe-deficient with no Fe-EDTA added. In addition to the Fe treatment, three levels of REE were supplied (1) 0 ppb REE, (2) 1400 ppb REE, and (3) 7000 ppb REE. The REE treatments were composed of equal concentrations of all 14 REE. Pang et al. (2002) and references therein report the inhibition of plant growth above a concentration of 1400 ppb when testing La, Ce, and Yb. The 1400 ppb REE modality was reflective of this reported toxicity threshold. The higher REE dose was a 5-fold increase in REE concentrations over the lower dose.

2.3 Plant and Hydroponic Solution Analysis

All sample collection materials were decontaminated using a 24 h 1.5 M HNO₃ wash at 45°C, followed by a 24 h deionized water (18MΩ-cm) wash at 45°C. Hydroponic solutions were collected at the start and finish of the experiment in order to perform a mass balance. Aliquots of the hydroponic solution were filtered through a 0.2 µm filter and separated into two containers, one for pH, dissolved organic carbon (DOC) and ion chromatography (IC) analyses and another preserved with 2% HNO₃ for major and trace metal analysis by ICPMS. The filters for the initial hydroponic solution were sent to scanning electron microscopy (SEM) with EDS analysis to examine precipitates formed and determine their composition before commencing plant exposure. The pH was measured using a standard combination probe. Measurements of DOC were made using a Shimadzu TOC Analyzer whereas major anions and low molecular weight organic acids were measured using a Dionex ICS-5000 with an AS11

HC column (Dionex Method AN 123). Major and trace metals were measured by an Agilent 7700x ICP-MS with rhenium and rhodium as internal standards (Yéghicheyan et al., 2019). Quality control was verified using international geostandard SLRS-6.

Plants were harvested after 28 days of exposure and separated into two distinct masses: root tissue and aerial tissue. Plant tissues were frozen and lyophilized prior to microwave digestion (Anton Paar Multiwave 7000). Major and trace metal analysis was then performed by ICPMS, using an analogous plant tissue quality control verification standard (Tomato Leaves standard 1573a, NIST® SRM®).

2.4 Data Analysis

Measurements of plant height (cm), root tissue mass (g), aerial tissue mass (g), root tissue REE content ($\mu\text{g g}^{-1}$), and aerial tissue REE content ($\mu\text{g g}^{-1}$) were made. These measurements were used to perform analysis of variance (ANOVA) with Tukey HSD post-hoc testing. Data which did not pass preliminary diagnostics were transformed according to the best fit λ derived from Box-cox. Each of the physical parameters were put in a model to compare by plant type, with statistical significance limited to $p < 0.05$. Since there are inherent differences in the physical characteristics of each plant type another model was made, separated by plant type for the analysis of REE and Fe status treatment effects. Again, statistical significance was limited to $p < 0.05$.

3 Results and Discussion

3.1 REE in the Hydroponic Solution

At the beginning of the study an aliquot of hydroponic media was taken from each treatment to confirm the amount of REE added and perform a mass balance at the end of the study. The REE contents were not in agreement with the nominal concentrations. Residue from the filtrate went to SEM. Results of SEM (Figure 1) confirm the loss of REE from the dissolved phase upon initiation of the experiment. Figure 1 shows evidence of phosphate precipitates being formed with approximately 2% inclusions of REE. Bryne et al. (1996) and Ding et al. (2005) have reported phosphate precipitates as significant to REE interactions at circumneutral pH. As a major component of the nutrient solution, phosphate was able to decrease REE solubility at the beginning of the experiment. By the end of the study, 35-100% of the REE initially added was accounted for in the plant tissue. This indicates that though there was an initial precipitation of REE-phosphates, potent plant exudates were able to liberate REE, which increased their phytoavailability.

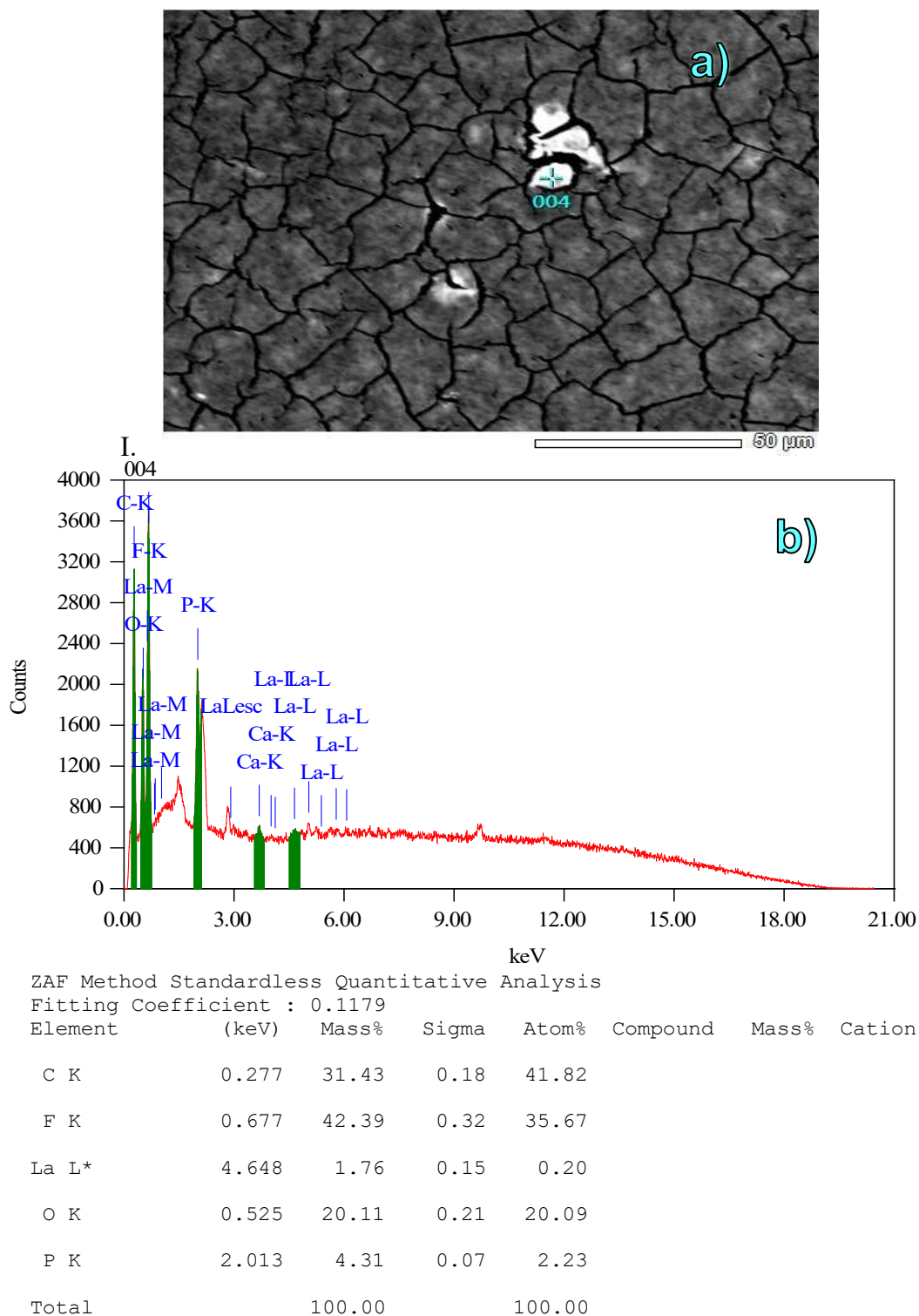


Figure 1. (a) SEM imaging of phosphate precipitates formed, similar results were produced for all samples. The sample represented by the image is the Fe-sufficient, 1400 ppb REE treatment. (b) The blue point selected in image (a) was analyzed for composition.

3.2 Effects of treatments on plant growth

Qualitatively, the effect of iron status could be confirmed in treatments by visual observation (Figure A2.1 and A2.2 in Appendix 2), yellowing of aerial plant tissue was indicative of Fe-deficiency

related chlorosis (Sugiura and Nomoto, 1984). Quantitative measurements of the plant height (Figures 2a and 2b), root tissue weight (Figures 2c and 2d), and aerial tissue weight (Figure 2e and 2f) were used to measure relative plant health and compare treatments (Table 2). Barley measurements had a smaller spread and were more self-consistent than rapeseed. Rapeseed growth was severely impacted by the highest REE treatment levels (7000 ppb), by the end of the study, with 40% of the plants subjected to the 7000 ppb REE treatments dead at the end of the 28-day experiment.

Table 2. Average height and weight measurements, treatment modalities include 0, 1400, and 7000 ppb REE and Fe-deficient (D) or Fe-sufficient (Fe).

Plant type	REE level, ppb	D/Fe	n	Height, cm	Root mass, g	Aerial mass, g
Barley	0	D	5	25.9	0.1603	0.1827
		Fe	5	31.2	0.2218	0.3674
	1400	D	5	24.2	0.1127	0.1913
		Fe	5	35.9	0.2505	0.4019
	7000	D	5	26.2	0.2024	0.2516
		Fe	5	32.6	0.2301	0.3908
Rapeseed	0	D	3	8.6	0.0128	0.0567
		Fe	3	11.6	0.0681	0.2533
	1400	D	5	4.5	0.0018	0.0071
		Fe	5	9.7	0.0494	0.2494
	7000	D	3	2.5	0.0011	0.0037
		Fe	3	5.3	0.0049	0.0242

There was a large variation in the height of rapeseed plants (Figure 2b) which resulted in no statistical difference amongst plants based on iron or REE treatment at a $p < 0.05$ level. If the level of significance was decreased to $p < 0.1$, there was an effect of REE on height measurements ($p = 0.0876$). Iron status was a significant factor for barley height ($p < 0.0001$) (Figure 2a), with no effects attributed to the REE level. This demonstrates differences in the stressors for these two plant types.

Iron status played a significant role in the development of root tissue for barley ($p < 0.0001$) (Figure 2c) and rapeseed ($p = 0.0436$) (Figure 2d), Fe-deficiency resulted in less root mass as compared with Fe-sufficient treatments. Typically, Fe-deficiency induces root elongation and increased root density leading to increased phyto-siderophore production (Schmidt 2001; Garrid et al., 2006). The weight of barley roots also showed a significant interaction between REE level and iron status, where Fe-sufficient plants supplied with a low to high dose of REE resulted in larger root weights than the iron deficient treatments supplied with no or low REE concentrations. This may demonstrate a hormetic effect of REE to barley roots, dependent on plant iron status. A potential explanation for the change in root morphology associated with the combined effects of REE and iron status could be REE stress induced detoxification. Phyto-chelates have been associated with detoxifying actions in the rooting zone (Kinnersley, 1993) and thus an increase in the amount of phyto-siderophore excretion corresponding to

root elongation could represent a stress response to REE. This would be antithetical to research which demonstrates root growth is inhibited by high REE levels. However, REE levels when considered separately did not significantly affect the weight of barley roots at a $p < 0.05$ level ($p = 0.0860$).

The weight of barley aerial tissue (Figure 2e) was only influenced significantly by the iron-status ($p < 0.0001$), iron-deficiency caused lower weight measurements than the Fe-sufficient treatments. For rapeseed aerial tissue (Figure 2f), increasing REE exposure levels had a deleterious effect on aerial tissue weight ($p = 0.0018$). However post-host analysis showed that the weight of the aerial tissue was only significantly different between the control – with no REE – and the highest REE treatment level (7000 ppb). The average aerial tissue weight of the control was 10-16 times larger than the average tissue weight of the high REE treatment level.

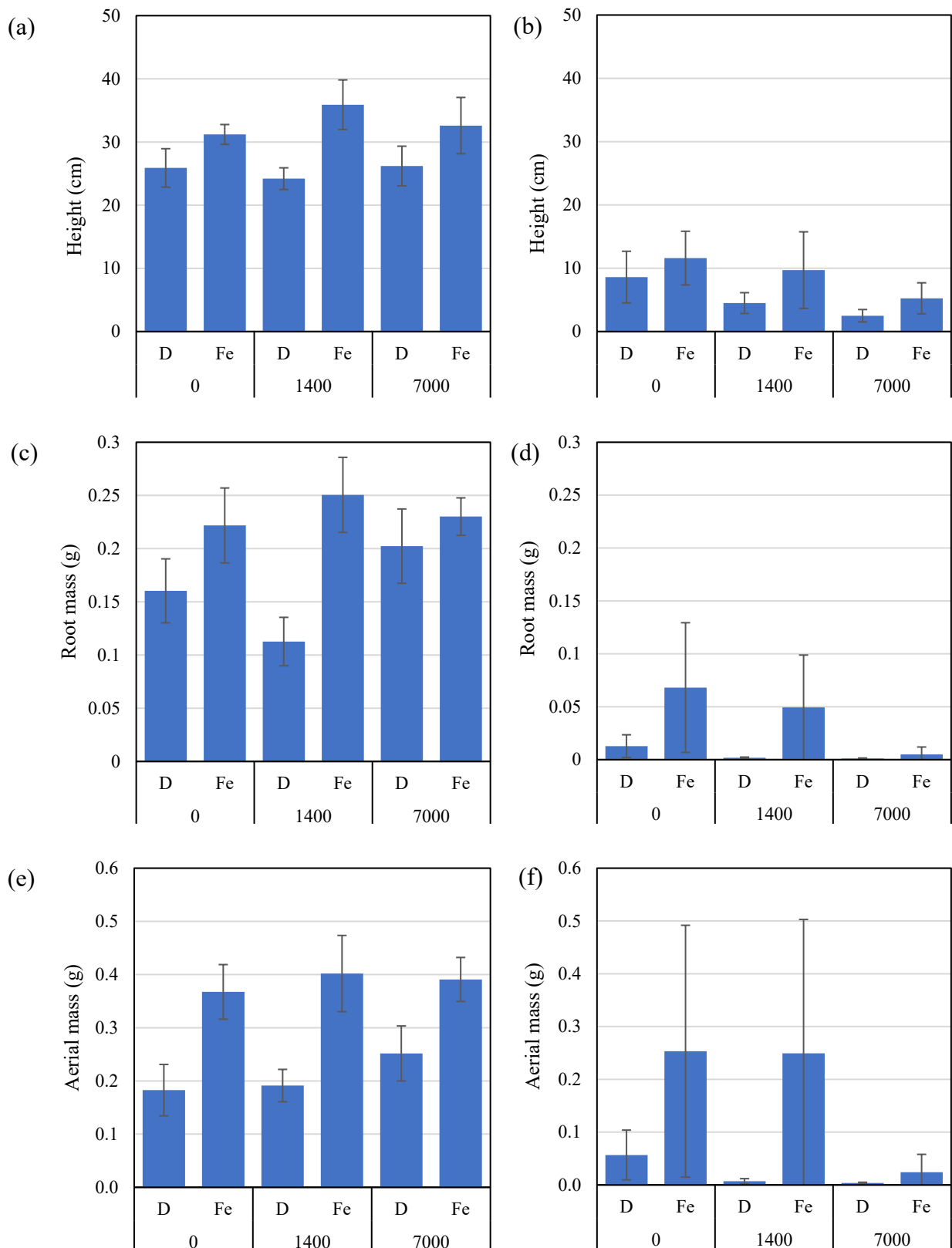


Figure 2. The aerial tissue height (a and b), root tissue mass (c and d), and aerial tissue mass (e and f) measured for barley (a, c, and e) and rapeseed (b, d, f) under Fe-sufficient (Fe) and Fe-deficient (D) nutrient regimes with application of 0, 1400, or 7000 ppb REE. Error bars represent the 95% confidence interval.

3.3 Effects of treatments on plant REE uptake

For both plant species, there is an increase in REE concentrations in tissues with increasing REE exposure (Table 3). There is an order of magnitude or more differentiating the bio-accumulated quantities between the two plant species, both for the root and aerial parts. There was no significant difference observed in the concentration of REE in root tissue amongst plant types ($p=0.1160$) (Figures 3a and 3b), however barley accumulated less REE in aerial tissue than rapeseed ($p<0.0001$) (Figure 3c and 3d). Results can be confirmed by a similar study from Wiche and Heilmeyer (2016), who concluded that non-gramineous plants, and specifically rapeseed, were more adept at REE uptake than grasses selected in the study.

For barley plants, REE concentrations in the root and aerial tissues (Figure 3a and 3c) could only be attributed to REE treatment level ($p<0.0001$ and $p<0.0001$, respectively), where the highest REE treatment (7000 ppb) produced significantly higher tissue concentrations than the lowest REE treatment level (1400 ppb) and both treatments produced higher concentrations than the control. The Fe status of barley had no observable effect on the concentration of REE in the root and aerial tissues. This would indicate that iron status, while important to the physical parameters pertaining to barley growth, did not impact uptake of REE and therefore REE is likely not complexed by siderophores and taken up through yellow stripe-like transporters. While the predicted specificity of siderophores to REE complexes is similar to those of other metals (Cd, Zn, Cu, Pb) which complex with siderophores (Hernlem et al., 1999), this does not directly translate to metal uptake. In the case of Cd, a study by Shenker et al. (2001) found that the solubility of Cd could be influenced by complexation with phyto-siderophores, but plant uptake of Cd was limited.

The concentration of REE in rapeseed root tissue and aerial tissue (Figure 3b and 3d) were affected by iron status ($p=0.0002$ and $p=0.0029$, respectively) and REE exposure level ($p<0.0001$ and $p<0.0001$, respectively). Fe-deficiency resulted in higher REE concentrations than Fe-sufficient treatments. As an example, among the highest REE treatment levels, Fe-deficiency resulted in a 1.6-fold increase in the REE concentrations associated with the roots and a 10-fold increase in the REE concentrations associated with aerial tissue. In addition to iron-status, a direct relationship was established between REE exposure level and REE plant tissue concentration, meaning an increase in REE exposure was reflected by an increased tissue concentration.

There was also an interactive effect in accordance with iron status and REE application for rapeseed ($p=0.0018$), where Fe deficiency enhanced the accumulation of REE in root tissue with increasing REE level. A study by Johnson and Barton (2007) found that the addition of LaCl_3 to Fe-deficient *Cucumis sativus L.* caused a notable decrease in proton secretion and an 87% reduction in ferric chelate reductase activity as compared with the Fe-deficient control. This study demonstrates the interruption of Strategy I by the addition of REE. This would most likely be a mechanism adapted by

the plant to control REE uptake in Strategy I plants. Though our study did not follow the activity of ferric chelate reductase, we were able to demonstrate a link between Fe-Deficiency and REE accumulation in the roots of a Strategy I plant.

Table 3. Average REE concentration recorded in aerial and root tissues for the treatment modalities: 0, 1400, and 7000 ppb REE with either Fe-deficient (D) or Fe-sufficient (Fe) status. Note that at the 1400 ppb REE level, 100 ppb of each individual REE was added to the initial hydroponic system and at the 7000 ppb treatment level, 500 ppb of each individual REE was added to the initial hydroponic system.

Plant	REE level, ppb	Fe/D	n	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Total REE	
Aerial Tissue																			
Barley	0	D	5	0.29	0.30	0.27	0.30	0.29	0.28	0.28	0.26	0.29	0.26	0.28	0.26	0.28	0.26	3.9	
		Fe	5	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.53
	1400	D	5	38	36	37	37	37	36	37	37	37	39	39	40	39	38	38	527
		Fe	5	22	22	21	21	21	21	21	22	22	23	23	23	23	23	24	310
	7000	D	5	142	144	140	141	142	142	142	145	149	150	152	152	149	147	143	2037
		Fe	5	173	171	170	174	173	171	173	173	169	174	170	176	173	177	176	2421
Rapeseed	0	D	3	1.7	1.7	1.6	1.6	1.7	1.7	1.8	1.7	1.9	1.8	1.8	1.6	1.5	1.4	23	
		Fe	3	0.43	0.39	0.34	0.35	0.34	0.34	0.38	0.35	0.35	0.33	0.32	0.30	0.29	0.30	4.8	
	1400	D	5	125	121	118	118	116	116	118	116	117	115	117	116	115	114	1642	
		Fe	5	49	42	39	39	37	38	40	39	40	42	43	43	43	44	579	
	7000	D	3	3040	2400	2201	2171	1960	1978	2169	2089	2108	2167	2238	2186	2134	2163	31002	
		Fe	3	1732	1444	1360	1337	1293	1293	1376	1333	1365	1367	1410	1392	1374	1398	19473	
Root Tissue																			
Barley	0	D	5	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.43	
		Fe	5	0.08	0.09	0.08	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	1.2
	1400	D	5	0.99	1.1	0.79	0.84	0.85	0.86	0.93	0.88	0.93	0.92	0.96	0.88	0.91	0.90	13	
		Fe	5	0.44	0.37	0.33	0.34	0.32	0.32	0.35	0.33	0.34	0.34	0.35	0.33	0.33	0.33	4.8	
	7000	D	5	2.6	2.2	1.8	1.8	1.7	1.8	2.0	1.9	2.0	2.0	2.1	1.9	1.9	1.9	28	
		Fe	5	1.7	1.5	1.4	1.4	1.4	1.4	1.5	1.4	1.5	1.5	1.6	1.5	1.5	1.5	21	
Rapeseed	0	D	3	0.13	0.11	0.10	0.10	0.09	0.08	0.09	0.08	0.09	0.09	0.09	0.07	0.07	0.06	1.3	
		Fe	3	0.10	0.08	0.06	0.07	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.82	
	1400	D	5	23	22	21	21	20	20	21	20	21	21	21	21	20	20	292	
		Fe	5	3.2	3.1	3.0	3.1	3.0	3.0	3.1	3.0	3.0	3.0	3.0	3.1	3.0	3.0	43	
	7000	D	3	496	373	336	331	293	296	334	323	328	344	358	348	331	338	4830	
		Fe	3	37	33	32	32	32	32	34	33	34	36	37	37	37	38	483	

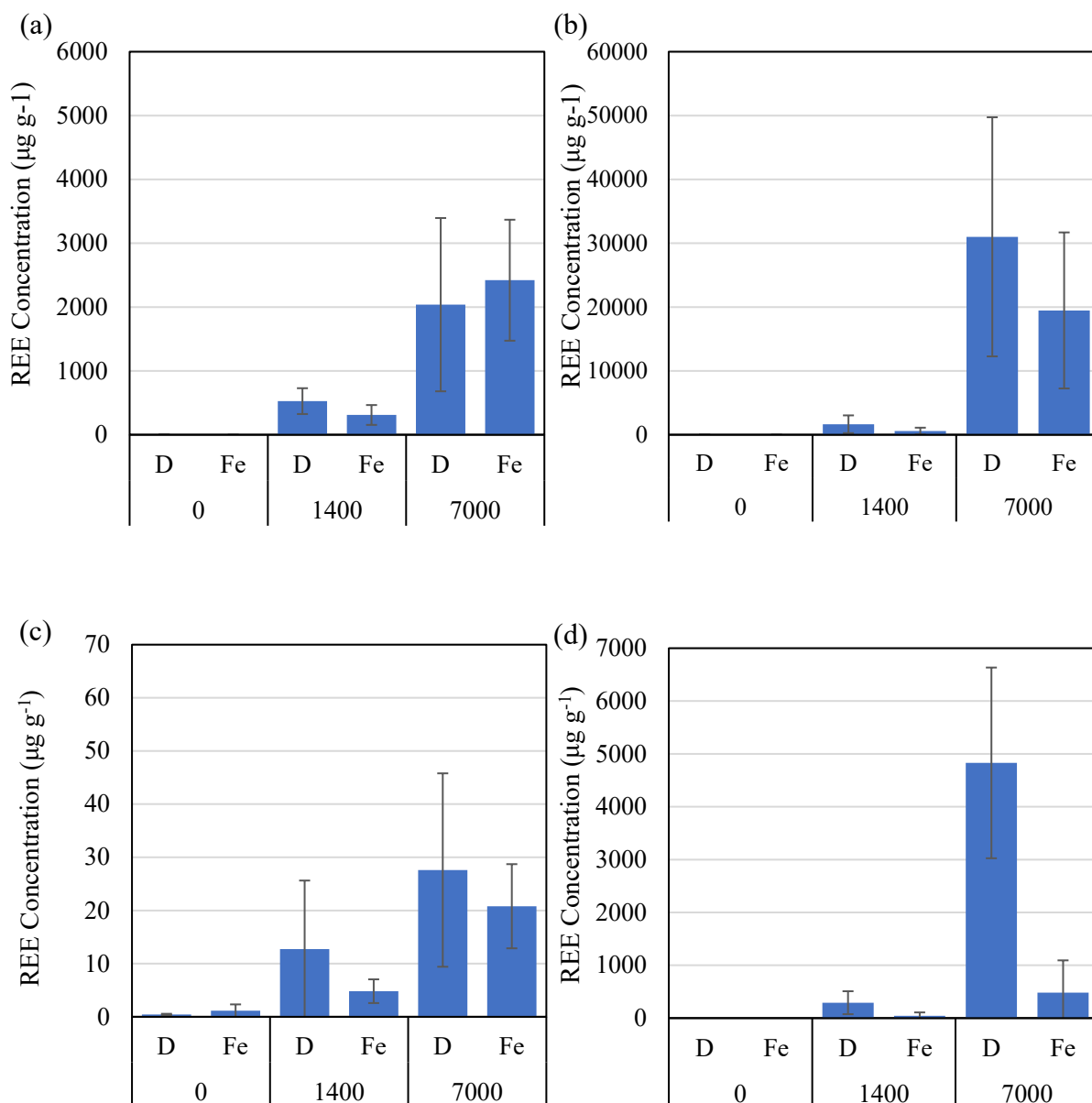


Figure 3. The Concentration of REE in (a) root-tissue of barley, (b) root-tissue of rapeseed, (c) aerial-tissue of barley, and (d) aerial-tissue of rapeseed. Treatments consist of (D) iron-deficient or (Fe) iron-sufficient with three levels of REE application: 0 ppb, 1400 ppb, and 7000 ppb. Error bars represent the 95% confidence interval.

3.4 Bioaccumulation and translocation patterns

Since all of the REE were added in equal amounts at the beginning of the study, it was possible to interpret the bioconcentration and translocation patterns for individual REE. The bioconcentration factor (BCF) was calculated by normalizing the root tissue concentration to the nominal REE concentration of the treatment. The translocation factor (TF) was calculated similarly by dividing the aerial tissue concentration by the root tissue concentration.

The pattern exhibited in the BCF for barley (Figure 4a) has a relatively flat line with no slope, each of the lines are clustered relatively close together. The TF for barley (Figure 4b) however does show slight preferential translocation of La, Ce, and Gd from the root tissue to the aerial tissue. The Fe-deficient, low REE treatment contains a Ce anomaly which is not observed in the other treatments.

For rapeseed, fractionation occurs from the solution to the root tissue as demonstrated by the BCF pattern (Figure 5a). La, Ce, and Gd demonstrate an increased fractionation by BCF, exaggerated by iron-deficient plant modalities with high concentrations of REE. The TF (Figure 5b) for rapeseed shows no relative slope or fractionation of REE.

The observed differences in BCF and TF fractionation among plant types demonstrates that there was no discernment in REE uptake by barley roots, but once in planta REE was fractionated by transportation to aerial tissue. Conversely, the fractionation of REE in rapeseed roots seems to bear some affinity for the three particular REE mentioned above, with elevated levels of La, Ce, and Gd; however, this fractionation is carried through to the aerial tissue and no additional fractionation was observed between plant compartments. The evident constraint of REE fractionation in the root tissue of rapeseed may be linked to REE association with phosphate and organic functional groups limiting transport from the root surface to the xylem (Ding et al, 2005). As for the particular pattern obtained, Liang et al., (2005a) observed similar fractionation and cited another study made by Sun et al. (1999) who showed that La, Gd, and Yb had strong affinities for EDTA present in the hydroponic solutions leading to an increase in aerial REE concentrations. Sun et al. (1999) only studied La, Gd, and Yb, and thus it is difficult to attribute the fractionation of these elements to EDTA without further studies of the stability constants formed between EDTA and the other REE. However, the aerial tissues in the present study (Figure 4b) demonstrated more exaggerated fractionation patterns for the Fe-deficient treatments. These treatments were devoid of EDTA, meaning the fractionation cannot be attributed to EDTA complexation in these treatments. Instead, a more fitting hypothesis was posed by Ding et al. (2006) who attributed fractionation of REE between roots and aerial tissue of a Strategy II plant to REE complexation by organic ligands in the plant.

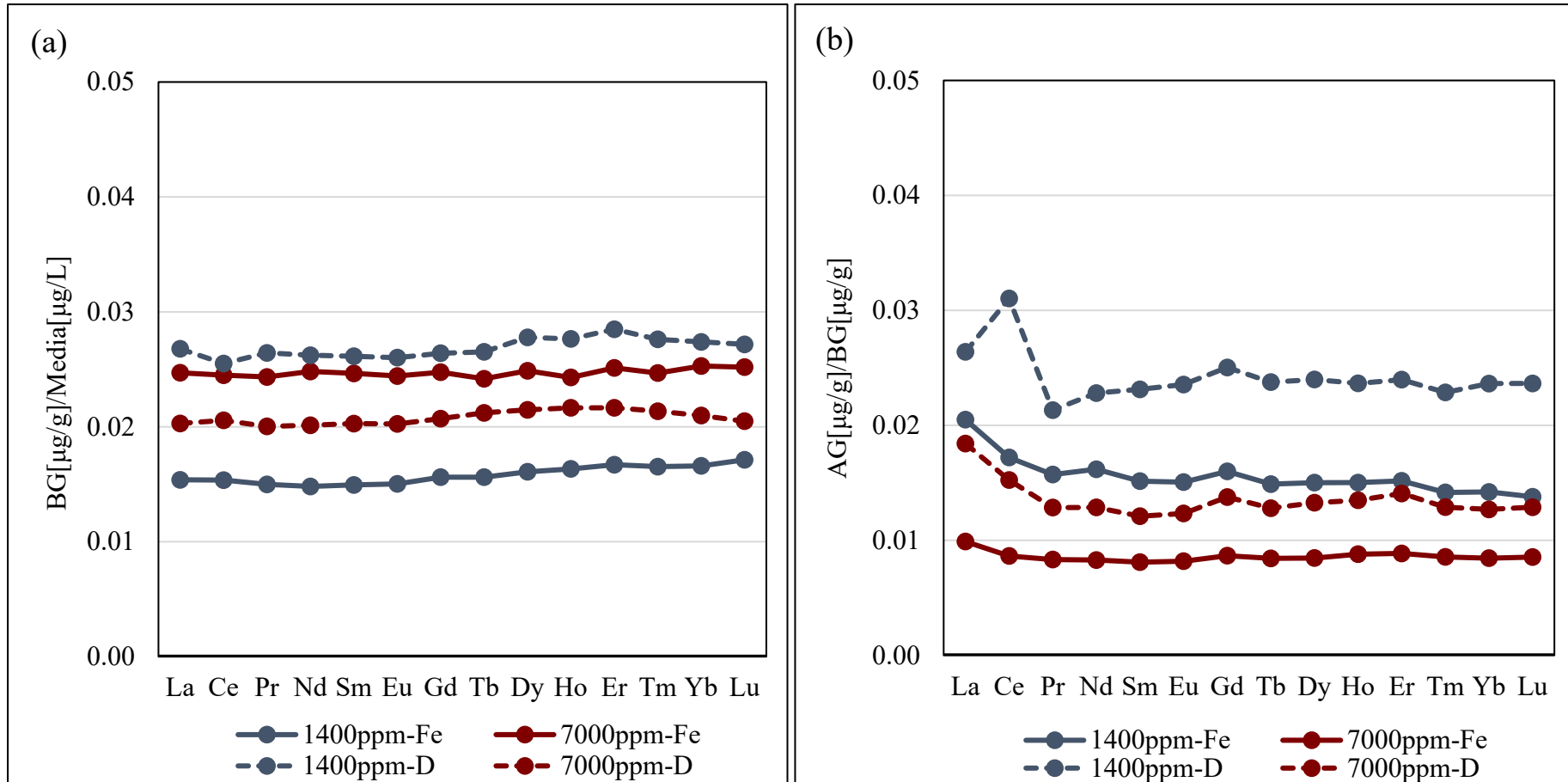


Figure 4. Plots pertain to barley fractionation and accumulation of REE by the (a) BCF and (b) TF. Dashed lines represent iron-deficient treatments (D), whereas solid lines represent iron-sufficient plant treatments. Blue lines plot the plant response to the low REE treatment (1400 ppb) and red lines plot the plant response to the high REE treatment (7000 ppb).

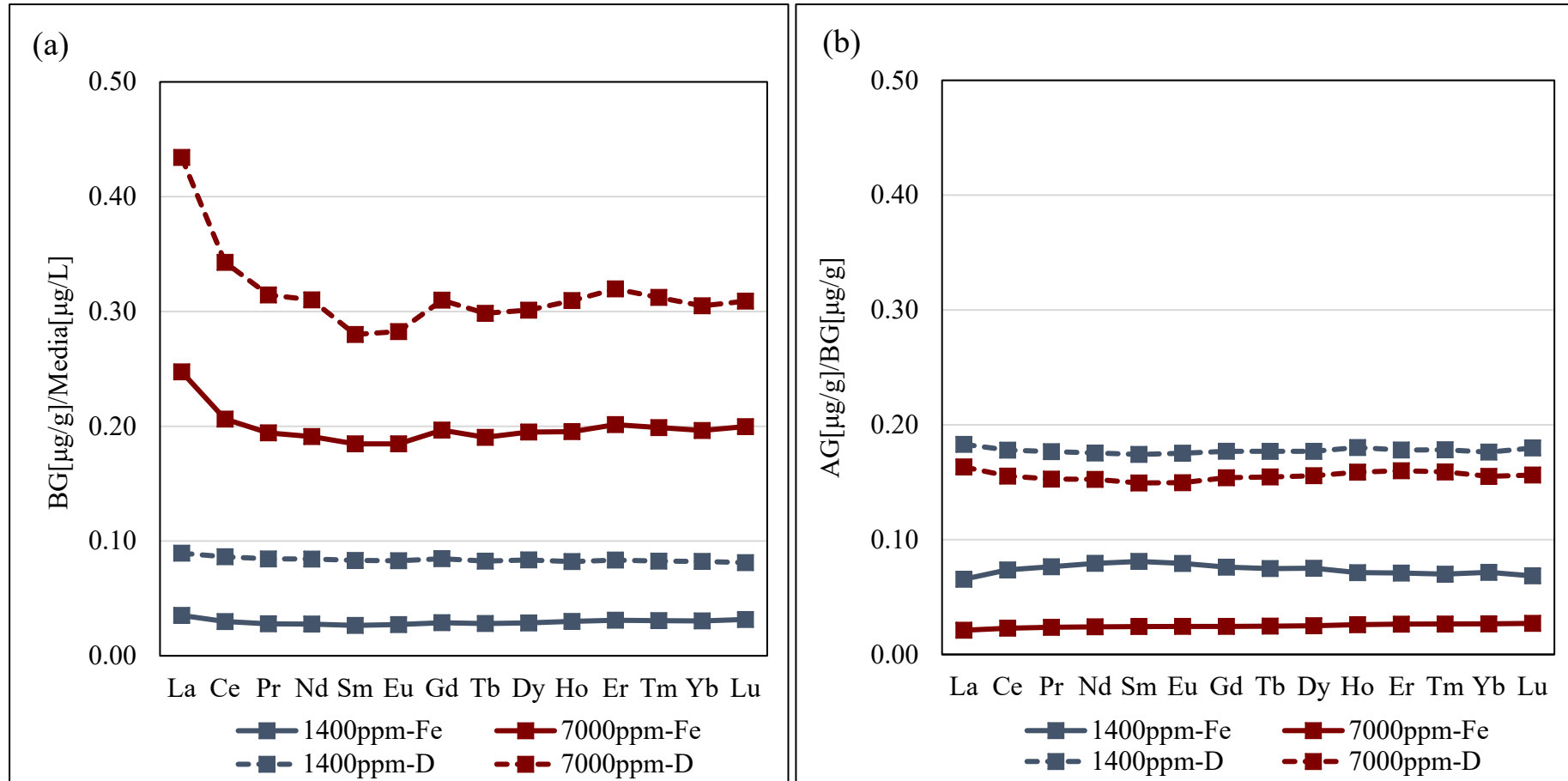


Figure 5. Plots pertain to rapeseed fractionation and accumulation of REE by the (a) BCF and (b) TF. Dashed lines represent iron-deficient treatments (D), whereas solid lines represent iron-sufficient plant treatments (Fe). Blue lines plot the plant response to the low REE treatment (1400 ppb) and red lines plot the plant response to the high REE treatment (7000 ppb).

4 Conclusion

Barley growth factors seemed to be more resistant to REE application and were more sensitive to iron status. A hormetic effect resulting in increased root tissue mass was attributed to high REE doses for Fe-sufficient barley treatments. Potentially REE causes stress to barley roots stimulating root elongation as a means of detoxification. In agreement with their varying tolerances towards REE levels and iron status, results demonstrated that rapeseed (a Strategy I plant) accumulated more REE in root and aerial tissues than barley (a Strategy II plant). For both plant types REE level had a direct effect on tissue concentrations. The iron status of rapeseed also contributed to an observed increase root and aerial tissue REE concentrations. Rapeseed root REE concentrations increased with REE exposure level and were enhanced by iron deficiency.

When observing the fractionation of particular REE, there seemed to be higher amounts of La, Ce, and Gd partitioned from the solution media to the roots for rapeseed, with no fractionation occurring in planta. The fractionation of La, Ce, and Gd also occurred in barley but was not observed between the root tissue and the hydroponic media, rather it was demonstrated in the translocation of these elements from the roots to the aerial tissue.

While this study was able to show significant differences in how these plants reacted to iron deficiency and REE-application it is difficult to draw conclusions about nutrient acquiring strategies from just one example of each strategy. This research could be expanded to include more species and see if the observed patterns are maintained. Ultimately this research contributes to a new and expanding field examining the effect of plant nutrient status on metal uptake. Evidence of plant nutrient deficiencies playing a synergistic role in the uptake of REE also has interesting implications for ecotoxicology, agriculture, and environmental remediation. Ostensibly calcareous soils where iron and phosphate have limited solubility could exacerbate the accumulation of REE in plant tissues. Future works could also expand on the topic of nutrient limitations and organo-metal complex interactions with plants.

CONCLUSIONS AND PERSPECTIVES

1 Conclusions

Rare earth elements are emergent contaminants, and as their commercial use expands it will be necessary to understand their circulation in the Critical Zone and to develop control measures dedicated to recycling them or handling them as wastes. Yet models describing the mechanisms of REE bioavailability and uptake by plants have hitherto been lacking as these non-essential metals have only recently come under scrutiny for their toxicity.

It is widely accepted that a major limit to element availability in the planted environment is the chemical speciation, and solubility in particular, of the element in question. Though these lithophilic elements tend to be stabilized in recalcitrant soil mineral pools, their incorporation into Fe/Mn-oxides and organic soil pools make them sensitive to release in certain contexts. A theme built across all of the research presented in this manuscript is that the bioavailability of REE was reliant on (i) the geochemical context and (ii) the biological context. This was observed in the **first chapter**, which characterized the environmental compartments where REE are observed; in the **second chapter**, which examined the REE-bearing phases of field samples and related plant uptake to the conditions which may lead to REE release; and the in **last chapter**, which consisted of a hydroponic laboratory test aiming to isolate the effects of Fe nutrient acquisition on plant uptake of REE.

Beginning with the comprehensive characterization of the REE content in the water, soil, and plant samples collected from legacy mine sites, the conducted study demonstrated REE signatures as a confluence of geological and biological factors. As is emblematic of AMD sites, MREE enrichment was observed in surface waters. Plant roots contained analogous MREE enrichment patterns, thus plant uptake evidently resembles soluble REE signatures. The tested plant types expressed a variety of rates at which REEs were assimilated and translocated into tissues, revealing a biological factor, which contributed to uptake in addition to the influence of geochemical factors. Soils, as a very large source and sink of metals, had similar concentrations of REE when comparing the effects of mining activity and the presence or absence of plants. This was contrasted by the knowledge that plants collected from inside of the mining areas had higher REE concentrations than plants collected from outside of the mining area, indicating that mining activity promoted the uptake of REE. Therefore, a natural conclusion to chapter one was that there was a distinction to be made between the REE-bearing phases in soils collected from inside of the mines and outside of the mines, resulting in variable REE plant availability.

Elemental speciation is an important factor to consider when determining the availability of REE. **Within the framework of chapter one, chapter two further explored the differences between soils, which engendered the observed MREE enrichment signature in plants.** The origin of soluble MREE was traced to a combination of mineral pools, which were deemed soluble in the mining context. The exchangeable and carbonate bound REE-bearing phases are typically classified as vulnerable to environmental changes and therefore mobilizable. While the reducible and oxidizable soil phases are not typically considered as labile, the perpetual formation of AMD in mining areas is wrought by the oxidation of oxidizable soil minerals (particularly iron-sulfides) whereas the drop in pH associated with AMD is responsible for the dissolution of many reducible minerals. As a whole, the provided dataset established that operationally defining all three phases as plant-available produced signatures without slopes or REE group enrichments, with the exception of Eu, and the scale was distributed around 1.00. Ultimately this shows us that whereas speciation is important, the geochemistry of the site as a whole must be considered in predicting which REE-phases are phytoavailable.

Following the conclusions of the preceding chapters, and of other field studies citing the importance of Fe in the biogeochemical cycling of REE, the third chapter is dedicated to understanding the influence of phyto-chelates on REE availability. This is in line with current research in the field, as other authors have recently been hypothesizing occurring links between Fe acquisition strategies and REE uptake. A hormetic effect was documented for the mass of the Strategy II plant, REE levels stimulated root growth for the Fe-sufficient treatments. The Strategy I plant experienced a fractionation of La, Ce, and Gd between the hydroponic solution and the root tissue, while the Strategy II plant experienced fractionation of the same elements between the rooting tissue and the aerial tissue. Though the concentration of REE in the Strategy II plant was dependent upon REE exposure, Fe status did not play a significant role in REE accumulation, indicating that the phyto-siderophores related to iron stress did not impact REE uptake. However, results for the Strategy I plant did establish a link between REE concentration and Fe status, reflected in the root and aerial tissues. This would mean that though siderophores are potent metal chelates and form stable complexes with REE, the exudates associated with Strategy I provided more plant-available REE.

2 Implications

When considered separately these studies provide their own valuable conclusions, taken together they suggest certain behaviors of REE in a planted environment as regards the uptake of REE. future studies will definitely have to address the indicated gaps in our knowledge.

Chapter one and two focused on characterizing the geochemistry of the sampling areas. While it is difficult to study soil as a medium due to its heterogeneity, breaking the soil down into differing REE-bearing phases allowed us to make comparisons in greater depth of the bioavailable REE pool and to fingerprint the origin of REE in plant tissue. This method seems to have also highlighted discrepancies between elements, particularly in the case of Eu, which was present in more labile soil pools but however, excluded from the below-ground plant tissue. One of the hypotheses, which spurred the hydroponic study, was that plants may parenthetically uptake REE through the activation of Fe stress. With the exception of grasses, plants cannot absorb Fe(III), assimilation requires reduction of Fe(III) to Fe(II). This has interesting implications for Eu, specifically, which is potentially reduced and transported through analogous divalent cation transporters. The results of the second study, however, are in direct contradiction to this theoretical model and the results from the hydroponic experiment demonstrate neither promotion nor impediment of Eu uptake.

For many plant-relevant nutrients, electrochemistry and valency seem to be vital parts of nutrient assimilation. Specified divalent uptake channels are meant to exclude the toxic trivalent compounds. Since Eu is the only REE which can be found in both the trivalent and divalent oxidation states, an assumption upon initiation of the experiments was that Eu may be reduced and preferentially assimilated into the plant through divalent transporters. For all of the pH ranges relevant in this study, the reduction-oxidation potential of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ coupling was higher than the reduction-oxidation potential for the $\text{Eu}^{3+}/\text{Eu}^{2+}$ coupling. This meant that the energy required for the plant to perform Fe^{3+} reduction is dissimilar to the range in which Eu^{3+} is reduced to a divalent cation. Limitations to Eu uptake, then, can likely be attributed to its physical speciation with soil organics in the rhizosphere rather than any physiological exclusion of Eu from the plant.

Ln^{3+} are all similarly attracted to negatively charged functional groups present on the root surface and readily complex with them. A similar mechanism has previously been proposed wherein the conductance of major divalent metals is suppressed by Ln^{3+} blocking Ca^{2+} uptake channels. The observed increase in root mass corresponding to an interaction between REE-level and Fe-status in barley plants is perhaps a symptom of Ln^{3+} blocking important uptake channels resulting in root elongation. Root elongation results in new, active divalent cation uptake sites and increased phyto-siderophore exudation. Any partitioning from media to root is then a product of limitations induced by the cell wall as a barrier. Translocation to the above ground parts is strictly attributed to the *in planta* partitioning occurring in xylem sap.

The mechanistic model presented in Figure 1 summarizes a possible interpretation of the performed research. The implications of this work are significant to the fields of

ecotoxicology, as they may provide an explanation for how, the confluence of REE speciation and nutrient status alter the toxicity of REE. The implications present for phyto-remediation may show that organic amendments can influence the solubility of REE. However, this solubility does not translate to uptake of REE across all plant types and caution should be used when dictating treatments. Though the research has yet to be developed, this work may potentially contribute to our understanding of REE behavior in an agricultural setting and the hormetic effects achieved through REE application.

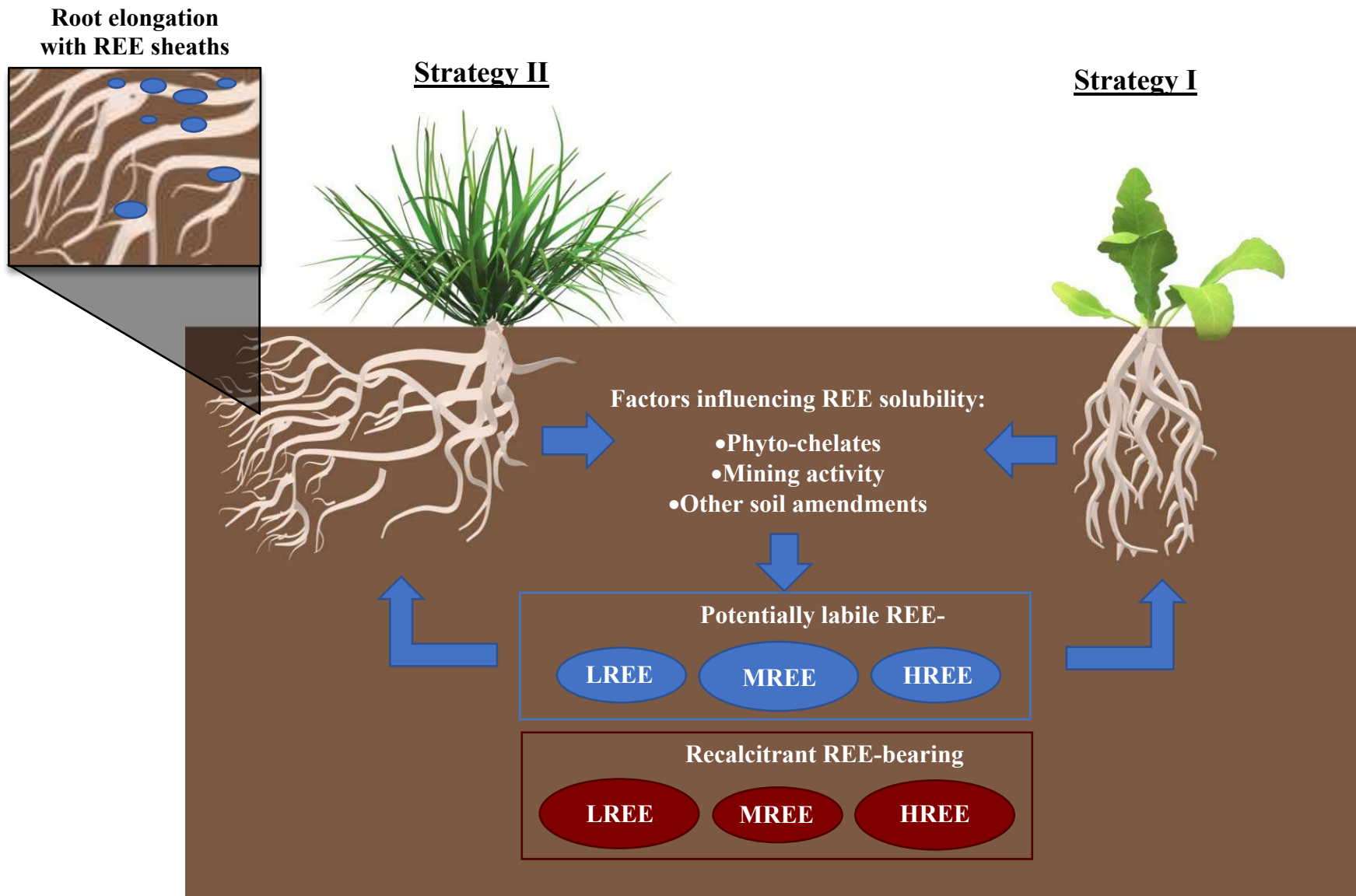


Figure 1. Mechanistic model for REE uptake by plants characterized by different Fe-acquiring strategies. Blue arrows correspond to interactions between environmental compartments with regards to REE movement.

3 Perspectives

This study demonstrated potential difference by examining one plant species from each acquisition style. This is not sufficient to establish a pattern across strategies. A variety of species should instead be used. If a pattern is indeed proven across Fe-acquiring strategies, this sets a precedent for further studies pertaining to particular soil environments.

This study was performed in environments where iron and phosphorus were readily available due to the natural abundances of these elements in the area and favorable geochemistry. Compounding factors may include the presence or absence of other essential plant nutrients and their form or physical speciation. Specifically, case studies examining the mobility of REE in more alkaline soils would be interesting as iron and phosphorous bioavailability is limited and plant response to these conditions could have make REE more soluble leading to plant uptake. This statement has significant implications for future research regarding REE bioavailability. While a few studies have started to focus on the phosphorus and iron status of plants, no consistent model has however, been derived.

There are currently several limitations to work attending to plant nutrient status. Commercially sold phyto-siderophores are priced high and methods for synthesizing and refining phyto-siderophores are time-consuming. As having phyto-siderophore compounds is a prerequisite to their detection, these factors limit the work of scientists who find the topic worth exploring. Improvement in techniques for refining and detecting phyto-siderophores would open the field to more study.

Negatively charged functional groups on organic matter make them important geochemical compartments for ligand-exchange with cationic metals. Given the proven affinity of REE for organic matter; characterization and understanding of this heterogenous material is essential to completing rhizosphere models. Further study of phyto-siderophores and LMWOA interactions in the environment would help to improve the geochemical modeling of rhizosphere interactions. This body of work merely scratches the surface of phytogenic organics, which may influence REE mobility and uptake, leaving plenty of room for expanding into study of other soil organics (secondary microbial metabolites, humic and fulvic acids, and organic amendments for agricultural applications).

With the expansion of research centered around plant response to environmental stressors, geochemical models should be developed and improved as a tool for scientists to determine REE speciation in the rhizosphere under a variety of stressors. Speciation of REE is relevant for evaluating REE phyto-avaialability. Completing the research proposed above will contribute to developing these models and tools.

Supplementary Material

**BIOCONCENTRATION AND TRANSLOCATION OF RARE
EARTH ELEMENTS IN PLANTS COLLECTED FROM THREE
LEGACY MINE SITES IN PORTUGAL**

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Table count:6

Table A1.1 *In situ* physicochemical water quality parameters for samples in mine areas from Portugal (São Domingos, Lousal and Quinta do Bispo). Separate sampling points are distinguished using the abbreviation pt. followed by a brief description of the sample location. DO, EC, TDS and DOC refer to Dissolved Oxygen, Electrical Conductivity, Total Dissolved solids and Dissolved Organic Carbon, respectively. Major anion constituents for water samples include fluoride (F⁻), chloride (Cl⁻), sulfate (SO₄²⁻), and nitrate (NO₃²⁻). For all samples collected phosphate (PO₄³⁻) and nitrite (NO₂⁻) were below detection limit (DL) and are thus excluded from the table.

Mine Site	Description	pH	Eh (V)	DO [mg/L]	EC [μS/cm]	TDS [mg/L]	TOC [mg/L]	F ⁻ [mg/L]	Cl ⁻ [mg/L]	SO ₄ ²⁻ [mg/L]	NO ₃ ²⁻ [mg/L]
São Domingos Mine	Tapada Grande Reservoir ^a	7.12	0.397	7.72	358	232	8.47	0.37	61.8	16.7	1.24
	Pt. 1: São Domingos River Valley	2.37	0.723	10.5	2760	1790	2.16	1.64	77.4	1720	9.52
	Pt. 2: São Domingos River Valley	2.86	0.691	9.09	1500	975	6.06	0.75	103	789	5.01
Quinta do Bispo	Pt. 1: Flooded open pit	5.08	0.524	9.84	954	620	0.45	1.54	8.53	504	<DL*
Lousal Mine	Untaminated water from Albufeira da Barragem da Tapada supplemented to passive treatment ^a	6.74	0.360	8.63	846	549	7.01	0.27	146	99.6	<DL*
	Pt. 1: Tailing pile leachate containment	2.30	0.808	11.7	3910	2540	9.17	2.09	28.6	2880	17.3
	Pt. 2: Mixing lagoon for leachate and supplemental water	2.93	0.615	9.47	1620	154	2.22	1.03	98.6	722	3.64
	Pt. 3: First treatment basin	2.89	0.745	9.62	2030	1320	2.56	1.69	104	1030	4.46
	Pt. 4: 6th treatment basin, beginning of planted basins	2.98	0.680	10.1	2230	1450	2.51	1.52	112	1170	4.62

^a Samples collected from outside of the mining area.

*Anion measurements below detection limit.

Table A1.2 Major cation concentrations ($\mu\text{g L}^{-1}$) for aqueous samples collected from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo). Separate sampling points are distinguished using the abbreviation pt. followed by a brief description of the sample location.

Mine Site	Description	Si	Fe	Mn	Mg	Ca
São Domingos Mine	Tapada Grande Reservoir, "uncontaminated"	828	28.4	2.71	9760	13600
	Pt. 2: Soa Domingos, "contaminated"	40000	60200	7680	64900	85200
	Pt. 3: Soa Domingos, "contaminated"	13100	27500	4420	43000	55500
Quinta do Bispo	Pt. 1: flooded open pit mine, "contaminated"	16600	37.8	2930	33000	119000
Lousal Mine	Pt. 1: Albufeira da Barragem da Tapada water stocked for diluting before passive treatment, "uncontaminated"	354	5.58	24.6	26100	38600
	Pt. 2: Leachate containment for tailings, "contaminated"	13600	266000	11600	83700	230000
	Pt. 3: Mixing pond for leachate and diluting water	8000	21100	8300	42000	48000
	Pt. 4: First treatment pool lined with limestone	12100	28100	16900	58500	94400
	Pt. 5: 6th treatment pool with plants established	7720	7030	17000	65400	158000

^a Samples collected from outside of the mining area.

Table A1.3 Major element concentrations (g kg⁻¹) for rhizosphere soil and sediment samples from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo).

Mine Site	ID Sample	Si	Fe	Mn	Mg	Ca
São Domingos Mine	<i>Cistus monspeliensis</i> ^a	200	45.2	1.53	16.0	12.5
	<i>Cistus monspeliensis</i>	282	77.7	0.238	6.60	1.67
	<i>Cistus monspeliensis</i>	295	47.8	1.83	5.30	3.91
	<i>Erica andevalensis</i>	216	83.3	0.273	3.85	1.00
Quinta do Bispo	<i>Salix sp.</i>	289	24.1	1.14	6.52	11.7
	<i>Pteridium aquilinum</i> ^a	331	12.6	0.472	2.27	2.02
	<i>Pteridium aquilinum</i>	324	16.5	0.247	2.74	1.82
	<i>Retama sphaerocarpa</i>	333	12.9	0.407	2.30	1.72
Lousal Mine	<i>Juncus acutus</i> ^a	361	33.8	0.252	3.87	1.34
	<i>Juncus acutus</i>	324	43.5	0.524	3.86	2.59

^a Samples collected from outside of the mining area.

Table A1.4 A summary of general soil characteristics from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo) (pH_{water} , pH_{KCl} , Cation Exchange Capacity ($\text{CEC}_{\text{Metson}}$), and Organic Matter (OM)).

Mine Site	pH_{water}	pH_{KCl}	$\text{CEC}_{\text{Metson}}$	O.M. (%)
São Domingos Mine	3.3 - 5.8	3.1 - 4.8	10.3 - 10.8	2.1 - 4.8
Quinta do Bispo Mine	5.4 - 6.0	4.2 - 5.0	6.2 - 10.2	0.3 - 4.7
Lousal Mine	4.2	3.8	4.4	0.5

Table A1.5 A comparison of REE concentrations in the rhizosphere soil versus in the bulk soil (mg kg^{-1}), as well as the Ce and Eu anomalies (Ce^* and Eu^* , respectively) (dimensionless value) for Quinta do Bispo mine (Portugal).

ID Sample	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Ce^*	Eu^*
Rhizosphere																
<i>Salix sp.</i>	46.9	93.0	11.5	43.2	8.94	2.12	7.92	1.23	7.15	1.36	3.53	0.512	3.23	0.463	0.91	1.19
<i>Pteridium aquilinum</i> ^a	33.9	69.3	8.2	29.7	5.63	0.695	4.26	0.641	3.65	0.739	2.02	0.302	2.06	0.308	0.95	0.67
<i>Pteridium aquilinum</i>	38.8	76.3	9.1	33.0	6.60	0.826	5.12	0.764	4.36	0.853	2.27	0.336	2.16	0.296	0.92	0.67
<i>Retama sphaerocarpa</i>	36.0	72.1	8.4	30.7	5.97	0.747	4.47	0.654	3.73	0.745	1.98	0.294	1.92	0.267	0.94	0.68
Bulk																
N/A	51.4	96.6	11.9	43.2	8.14	1.11	6.02	0.819	4.32	0.781	2.00	0.281	1.81	0.258	0.89	0.74
N/A ^a	41.2	84.6	9.8	35.6	6.82	0.721	5.04	0.742	4.33	0.870	2.36	0.364	2.39	0.356	0.96	0.58
N/A	36.6	73.2	8.5	30.7	5.89	0.822	4.55	0.634	3.60	0.673	1.77	0.259	1.69	0.243	0.95	0.75

^a Samples collected from outside of the mining area.

Table A1.6 Major element concentrations ($\mu\text{g g}^{-1}$) in above-ground and below-ground plant tissues collected from mine areas in Portugal (São Domingos, Lousal and Quinta do Bispo).

Mine Site	ID Sample	Above-ground tissue				
		Si	Fe	Mn	Mg	Ca
São Domingos Mine	<i>Cistus monspeliensis</i> ^a	643	381	228	2750	6640
	<i>Cistus monspeliensis</i>	1080	1220	1540	1920	4210
	<i>Erica andevalensis</i>	393	1040	814	2370	6310
Quinta do Bispo	<i>Salix sp.</i>	305	97.7	984	6040	11400
	<i>Pteridium aquilinum</i> ^a	1570	706	397	2360	6320
	<i>Pteridium aquilinum</i>	3240	246	937	4510	17600
	<i>Retama sphaerocarpa</i>	361	226	363	1650	2950
Lousal Mine	<i>Juncus acutus</i> ^a	1000	103	1170	1580	2880
	<i>Typha Latifolia</i> ^a	280	193	2510	2630	9220
	<i>Typha Latifolia</i>	388	5230	2920	2410	7750
	<i>Juncus acutus</i>	1270	68.6	438	1720	2330
Mine Site	ID Sample	Below-ground tissue				
		Si	Fe	Mn	Mg	Ca
São Domingos Mine	<i>Cistus monspeliensis</i> ^a	2650	938	92.0	1160	5560
	<i>Cistus monspeliensis</i>	2770	775	153	449	6890
	<i>Cistus monspeliensis</i>	2030	323	287	629	3230
	<i>Erica andevalensis</i>	2520	1860	654	564	784
Quinta do Bispo	<i>Salix sp.</i>	2840	834	127	2680	8820
	<i>Pteridium aquilinum</i> ^a	3920	1820	127	1920	1920
	<i>Pteridium aquilinum</i>	3310	855	117	1960	2580
	<i>Retama sphaerocarpa</i>	1140	283	135	1360	859
Lousal Mine	<i>Juncus acutus</i> ^a	2890	26100	1890	2520	2350
	<i>Typha Latifolia</i> ^a	1340	1110	1140	2420	4010
	<i>Typha Latifolia</i>	<DL*	29400	338	1800	3880

^a Samples collected from outside of the mining area.

*Major element measurements below detection limit (DL).

Supplementary Material

**DOES THE IRON-ACQUIRING MECHANISM OF PLANTS
INFLUENCE THE UPTAKE AND TRANSLOCATION OF
RARE EARTH ELEMENTS INTO PLANT TISSUES?**

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Figure count: 2



Figure A2.1 Picture demonstrating visual differences between treatment modalities applied to rapeseed. Notice that the Fe-deficient (D) treatment has yellowing and less voluminous aerial tissue when compared to the Fe-sufficient (Fe) treatments.

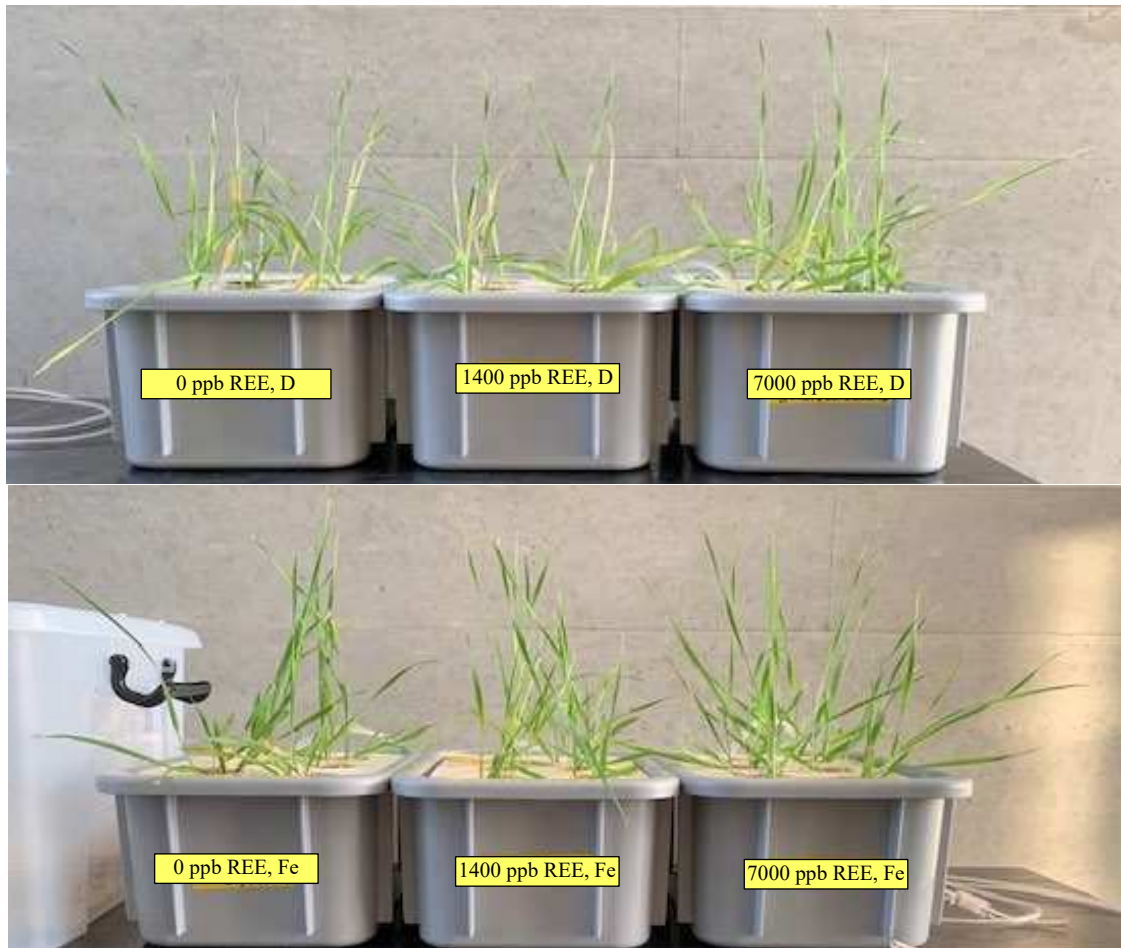


Figure A2.2 Picture demonstrating visual differences between treatment modalities applied to barley. Notice that the Fe-deficient (D) treatment has yellowing and sparser leaf density when compared to the Fe-sufficient (Fe) treatments.

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Titre : La spéciation des terres rares dans les sols contrôle-t-elle leurs phyto-disponibilité et dissémination dans les plantes?

Mots clés : végétaux, sols, terres rares, spéciation, bioconcentration, translocation

Résumé : Grâce à des études de terrain et de laboratoire portant sur des échantillons d'eaux, de sols et de végétaux, ce travail a permis d'analyser les déclencheurs (paramètres et processus) (1) géochimiques et (2) biologiques de l'absorption des terres rares (REE) par les plantes. Ces éléments ont d'abord été examinés à l'aide d'échantillons de terrain prélevés sur trois anciens sites miniers au Portugal. Les sites miniers, où les REE et d'autres métaux sont facilement solubilisés par les drainages miniers acides (DMA), constituent des cibles idéales pour identifier les effets de la physiologie des plantes et la sélectivité biologique des plantes pour des REE particulières. Les plantes prélevées sur ces sites ont montré des signatures en REE similaires aux concentrations de REE dissoutes présentes dans les eaux de surface locales; ce qui nous a conduit à nous interroger sur la nature des interactions à l'interface minéral-eau, responsables de la solubilisation des REE dans ces systèmes. En utilisant le schéma d'extraction séquentielle de type BCR pour caractériser les REE associées au sol, l'étude réalisée a ensuite permis de comparer la signature des REE dans les tissus végétaux aux signatures dans différentes phases organiques et minérales du sol.

Des études antérieures ont affirmé que seules phases minérales dissoutes ou échangeables et liées aux carbonates contiennent des REE potentiellement biodisponibles. Notre étude a démontré que les fractions oxydables et réductibles peuvent contribuer de manière unique au pool de REE solubles dans les systèmes de DMA. L'un des résultats de cette étude a montré une augmentation mineure de la quantité de REE échangeables et liées au carbonate dans les échantillons prélevés dans la rhizosphère par rapport aux échantillons prélevés dans les sols globaux. Un nombre croissant de spécialistes du domaine, cite l'importance des matières organiques exogènes associées à l'acquisition de nutriments par les plantes, en influençant la solubilité et l'absorption des métaux nutritifs traces dans la rhizosphère. Nous avons ainsi proposé hypothèse selon laquelle le stress nutritif subi par la plante pourrait induire une exsudation de phyto-chélatants qui augmenterait l'absorption et la bioconcentration des REE. Cette hypothèse permet d'expliquer les différences observées dans l'absorption des REE selon les différents types de plantes et schémas d'acquisition des nutriments.

Title: Does soil rare earth element speciation control their phyto-availability and dissemination within plants ?

Keywords: plants, soils, rare earth elements, speciation, bioconcentration, translocation

Abstract: Through field and laboratory studies of water, soil, and plant samples, this work used novel sites and approaches to examine (1) geochemical triggers and (2) biological triggers of REE uptake in plants. This was examined using field samples collected from three legacy mine sites in Portugal. Mining sites, where REE and other metals are readily solubilized by acid mine drainage (AMD), constitute ideal targets for identifying the effects of plant physiology and biological preference towards particular REE. Plants had similar signatures to soluble REE concentrations observed in local surface waters, leading the study to question which mineral-water interface interactions were responsible for soluble REE in these systems. Using a BCR sequential extraction scheme to characterize the soil associated REE, the conducted study then compared the signature of REE in plant tissues to the signatures in different soil organic and mineral pools.

Previous studies have asserted that only dissolved or exchangeable and carbonate bound mineral pools host potentially bioavailable REE. Our study demonstrated that oxidizable and reducible fractions may uniquely contribute to the soluble REE pool in AMD systems. One of the results from this chapter showed a minor increase in the amount of exchangeable and carbonate bound REE in samples collected from the rhizosphere as opposed to samples collected from bulk soils. A growing number of specialists in the field, cite the importance of exogenic organics associated with plant nutrient acquisition in influencing the solubility and uptake of trace nutrient metals in the rhizosphere. For this reason, a hypothesis was formed that nutrient stress to the plant may induce phyto-chelate exudation which would increase uptake and bioconcentration of REE. This study did indeed demonstrate differences in REE acquisition across plant types and nutrient acquiring schemes.