Diffusive Gradients in Thin Films (DGT) Devices as Biomimics for Bioaccessible Rare Earth Elements (REEs) in Soil

By

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ABSTRACT

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Using the bioaccessible concentration to which plant roots and microorganisms are exposed may provide a better estimation for toxicity than using total concentration. Bioaccessibility of REEs in highly organic soil were measured using Diffusive Gradient in Thin Films (DGT) and partial extractions with CaCl$_2$. The correlation of DGT accumulation to other bioaccessible determining methods and plant roots were weak. Modifications to the DGT SOP such as increasing deployment time and resupplying the labile pool to the DGT surface improved these relations. To some extent, DGT mimics the action of plants if plant uptake is limited by diffusion. Therefore, DGT is capable of serving as a predictor of plant uptake and availability of the rare earth metals. This is the first study that demonstrates the suitability of measuring REEs with DGT in soil.
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List of Acronyms

BGS: Black garden soil

DOC: Dissolved organic carbon

DGT: Diffusive gradient in thin films

OM: Organic matter

rAR: Reverse aqua regia

REE: Rare earth elements

TOC: Total organic carbon

WHC: Water holding capacity
1.0 Literature Review

1.1 Rare Earth Elements (REEs)

1.1.1 Background

Rare earth elements (REEs), also known as the lanthanides, are a group of metallic elements on the periodic table with atomic numbers 57-71 along with the 2 transition metals Sc and Y. The REEs are widely distributed in nature, and hundreds of different mineral types have been found containing them. Despite their name, they are not at all “rare” in terms of their crustal abundance. Their very similar chemical and physical properties commonly leads to several of them existing as mixtures in individual minerals\(^1\). Such minerals can include granites, silicates, carbonates and pegmatites. To date, no found mineral deposits containing only one lanthanide as a major constituent have been found\(^2\). Therefore, the occurrence of these mixtures makes the separation and purification processes considerably difficult for their various technological applications.

1.1.2 REE Properties

The properties of the lanthanide metals, their compounds and coordination complexes are influenced by the size of the ion. In most geochemical systems, REEs usually form trivalent cations with the exception of Ce (which can also be tetravalent) and Eu (which can also be divalent)\(^1,3,4\). Under very oxidizing surficial conditions Ce\(^{\text{III}}\) is readily converted to Ce\(^{\text{IV}}\) (Figure 1 (a)) which is approximately 15% smaller therefore allowing it to form highly soluble hydroxide complexes. This can lead to separation of Ce from other trivalent REEs, resulting in irregular behaviour\(^4\). In contrast, under very reducing conditions the reduction of Eu\(^{\text{III}}\) to Eu\(^{\text{II}}\) (Figure 1 (b)) results in an approximate 17% increase in atomic radius, making it nearly identical in size to
that of Sr$^{II}$. Consequently, the substitution behaviours of Eu differ greatly from the other trivalent REEs and thus irregular behavioural patterns$^4$.

![Figure 1](image.png)

**Figure 1** Eh-pH diagram showing the hydrolysis of (a) Ce(III) and Ce(IV) (b) Eu(III) and Eu(II) in the absence of other complexing ligands.

As the atomic number increases, so does the effective nuclear charge due to imperfectly shielding of the valence electrons from the nuclear charge$^5$. This shielding of the nuclear charge leads to a reduction in the atomic and ionic radii across the series. This is known as lanthanide contraction and is thought to be a result of the increasing effective nuclear charge and relativistic effects on the outer electrons$^5$.

1.1.3 REEs in the Environment

There are approximately 34 countries that have REE deposits, making them relatively abundant$^6$. Cerium for example has an average crustal concentration of approximately 66 μg/g which is more abundant than copper (Cu). The abundance of the rarest REEs, Tm and Lu, is comparable to selenium (Se) and cadmium (Cd) in most soils$^3$. Even with the crustal concentrations being high in some locations, typically REEs within these background concentration ranges have no known adverse effects on the environment and humans. However,
the recent increases in mining REEs to meet the market demand posed to hundreds of high technical applications can cause emissions that have potential to cause ecotoxicity\(^7\). These elements are used in magnets for renewable energy technologies, rechargeable batteries in hybrid vehicles, catalytic converters, electronic display screens as phosphors, etc. Their ability to meet the faster, smaller, lighter and more efficient attributes in technologies makes them a desirable asset in developing industries, especially in green technologies.

Global consumption of REEs has increased significantly to continuously enhance our technologies. Currently, China remains the dominant global supplier of REEs but they have recently prioritized its domestic markets by increasing export taxes on REEs. As a result, REEs are in short supply, and with the global demand continuously increasing the need for new suppliers is apparent to our modern way of life\(^8\). In Canada, large REE deposits have been found around Thor Lake, NT. The metals chosen for the present study, namely, Cerium (Ce), Neodymium (Nd) and Europium (Eu) in this study are light REEs (atomic numbers 57 to 64)\(^9\), will provide representative data for the broader class of REEs, considering their similar chemical behaviours. Some previous literature exists\(^3,10\) on the toxicity of these element as a result of pollution due to mining, processing and improper disposal of materials containing these elements. A study by Li et al.\(^11\) found that accumulation of REEs in the soil surrounding a tailings pond in China negatively affected the diversity if the soil macrofauna. High concentrations of REEs in soil results in a reduction in both belowground and aboveground plant biomass\(^12\). The data surrounding the toxicity of REEs is limited; therefore, this research will build on the small available database.
1.1.4 REEs in Plants

REEs are micronutrients that improve crop yield, although their essentiality to crops has not been proven. The REEs are typically applied in soluble forms to the soil, mainly as chlorides, nitrates or a complexed mix of amino acids\(^{13}\). Due to their similar ionic radius it is suggested that REEs can easily replace Ca\(^{2+}\) through isomorphic substitution. Some REEs were postulated to be taken up by plants as divalent cations but translocated as trivalent cations\(^{10}\). There is much evidence supporting the use of REEs in fertilizers to improve crop nutrition, biomass production and growth of vascular plants\(^{13,14}\). REEs can enhance respiration, water uptake, photosynthesis and increase stress tolerance by replacing Ca\(^{2+}\) \(^{15}\). However, the mechanisms of toxicity with elevated REE concentrations are not well established. Without much knowledge about the chemistry of REEs, it is difficult to understand the toxicity of REEs. The concentrations of REEs in plants are typically much lower in comparison to their total concentration in soil despite their crustal abundance. Transfer factors such as indicated by the ratio of concentration in plant to total concentration in their rhizosphere may be influenced by the relative availability of the soil elements to plants\(^{3}\).

1.2 Metal Speciation

Determining chemical speciation of contaminants through various analyses is of importance in environmental chemistry\(^{16}\). The IUPAC defines speciation as an “analytical activity of identifying and/or measuring the quantities of one or more chemical species in a sample”\(^{16}\). Metal speciation can influence its mobility and bioavailability in soil and thus its toxicity to soil organisms\(^{3}\). Therefore, the identification and quantification of metal species (including REEs) in soil samples is critical for researchers in industries, government
agencies as well as legislative bodies for monitoring and managing environmental contamination/pollution. There is plenty of previous literature reporting the speciation of common divalent, metallic cations in both aquatic and soil ecosystems\textsuperscript{16–18}. However the same cannot be said for the trivalent REEs. Determining the speciation of these data-poor metals is necessary to understand their reactivity, mobility and role they play in toxicity as well as how these factors can fluctuate in dynamic systems. It’s important to incorporate metal speciation in risk assessment and remediation strategies to minimize costs associated with testing and research without sacrificing effectiveness of the remediation processes.

**1.3 Modeling REE Chemical Reactions**

The metal ion concentrations in soil solution can be estimated using the computer speciation model WHAM (Windermere Humic Aqueous Model)\textsuperscript{7}, which was developed to stimulate the chemical reactions that occur in soil and aquatic ecosystems between inorganic elements and humic substances. It utilizes a database for cation binding to humic substances (i.e. humic and fulvic acids). WHAM is used by individuals in government agencies and industry to conduct environmental risk assessments. The output from WHAM is sensitive to the quality of thermodynamic data and input parameters, and these are frequently updated through contributions from many researchers\textsuperscript{19}. One of the faults of using WHAM for soils is that it is not capable of predicting the formation of precipitates. This is typically not an issue for aquatic media because everything is so dilute that the formation of precipitates is negligible. CHEAQS is another modeling software that has WHAM built into it, which is also capable of predicting precipitate formation. Thus, making it more applicable for the soil media used in the present study. Furthermore, such modeling programs are constantly evolving and require validation by comparison with analytical results.
1.4 Bioaccessibility and Bioavailability

Biaccessibility can be defined as the fraction of the total metal concentration that is present in the soil ecosystem which has the potential to be absorbed by an organism. Bioavailability can be defined as the fraction of the bioaccessible concentration that is taken up by an organism, thus becoming available to potentially cause harm to cellular targets\textsuperscript{20}. Generally, the adverse impact of metals on the soil ecosystem is related to the bioaccessible levels instead of the total concentration\textsuperscript{21}. Nonetheless, for remediation purposes, most environmental guidelines are based on the total concentrations which provide inadequate information about the chemical behaviour and possible fate\textsuperscript{22}.

The ratio of solid:liquid partitioning of metals in soil is expressed as Kd\textsuperscript{23}. This metal concentration can be considered ‘bioaccessible’ and is estimated via a single CaCl\textsubscript{2} extraction or by using a speciation predictive model such as WHAM\textsuperscript{19}. These methods are a single point in time (i.e. they assume a steady state) but in reality, the exposures in the environment are dynamic. Therefore, they aren’t capable of capturing the bioaccessible metal concentration that arises during exposure from metal complexes which are labile, thus contributing to the overall exposure dose.

The role of the free metal ion as a predictor of bioavailability and toxicity has been well established for many divalent cations\textsuperscript{24}. For example, the toxicity of trivalent lutetium (Lu\textsuperscript{3+}) has been successfully explained in a marine bacterium using the free ion activity model\textsuperscript{25}. Predictions made using modeling programs may sometimes deviate from the experimental results in the presence of some natural ligands such as amino acids. However, the free metal ion remains a key parameter for predicting bioavailability\textsuperscript{26}. By adapting similar methods to be used
in a different medium, a goal of the present study is to achieve similar success in estimating the bioaccessibility of Ce, Rd and Eu in soil.

1.5 Diffusive Gradient in Thin Films (DGT)

For this technique an ion-exchange resin and ion-permeable hydrogel are arranged in a commercially available assembly that exposes the hydrogel to the soil (Figure 1). The metal ions are rapidly immobilized after diffusing through the ion-permeable gel. Assuming that the resin sink is not saturated and the concentration of dissolved and easily exchangeable metals in the solution stays constant, a linear concentration gradient will be established in the ion-permeable gel\textsuperscript{17}.

![Diagram of DGT device cross-section](image)

**Figure 2** A schematic of a DGT device cross-section\textsuperscript{27}.

The diffusive gel layer only has a small effect on the diffusion of simple inorganic species (such as metal ions) and as the appropriate diffusion coefficients (D) are determined in the gel, calculation of the DGT concentration ($C_{\text{DGT}}$) using Eq. 1.1 is reliable\textsuperscript{28}. The total metal bound by the binding layer ($M$, calculated using Eq. 1.2) during a certain deployment time ($t$) is influenced by the thickness of the diffusion layer ($\delta^{mdt}$) and the sampling area ($A_s$)\textsuperscript{28,29}. Where
the $V_{NO3}$ is the volume of NO$_3$ used to elute the metals from the resin layer which is influenced by the elution factor ($fe$).

$$c_{DGT} = \frac{M \delta^{mdl}}{DA_p t} \quad \text{(Eq. 1.1)}$$

$$M = \frac{Ce(V_{gel} + V_{HN03})}{fe} \quad \text{(Eq. 1.2)}$$

This method can provide kinetic information of metal ion release from solid phase to the solution in soils, in a time frame that is relevant to both acute and chronic exposure\textsuperscript{30}. To date, this method has not been used to infer the speciation of REEs or other trivalent metals in soil. To interpret the DGT measurements in soils it is useful to consider two differences from the use of DGTs in aquatic systems\textsuperscript{31}.

Figure 3 Concentrations of ionic species in a DGT device and adjacent pore water during deployment for (a) the sustained case, (b) the intermediate case and (c) the diffusive only case. The wavy line illustrates the diffusive pathway of the mobile species\textsuperscript{29}.

It is assumed that due to a lack of mixing in soils, pore water concentrations adjacent to the DGT device becomes depleted (Figure 3 (b))\textsuperscript{31}. Without a mechanism of metal resupply other than diffusion, the zone of depletion adjacent to the DGT device becomes progressively larger.
over time. Consequently, the $C_{\text{DGT}}$ which is driven by the concentration gradient through the gel layer also progressively decreases over time$^{29,31}$. In the case in which the diffusive and desorption rates of uptake match the initial flux into the DGT, it would allow their solution concentrations at the DGT interface to be sustained at a similar level to those in the bulk solution (Figure 3 (a)). In contrast, for some ions with a very low flux from the solid phase, the diffusion from the soil pore water is the only metal available to the DGT device (Figure 3 (c))$^{27,29,31}$.

The DGT determined concentration ($C_{\text{DGT}}$) will be less than or equal to the concentration of labile species found in the bulk pore water ($C_{\text{Soln}}$). DGT measurements can be interpreted as a ratio ($R$) of $C_{\text{DGT}}$ to the concentration in solution (bulk pore water) using Eq. 1.3$^{29,31}$:

$$R = \frac{C_{\text{DGT}}}{C_{\text{Soln}}}, \quad 0 < R < 1$$

$R$ can be obtained experimentally to characterise elemental concentration dynamics during a deployment as one of three cases (illustrated in Figure 3):

(a) **Sustained Case ($R > 0.8$).** The pore water concentration adjacent to the DGT device is sustained throughout the deployment. This can occur if there is rapid mixing or if the resupply from the solid phase is rapid in comparison to the removal rate into the DGT. The DGT measured concentration can be interpreted as the concentration of labile metal species in the pore water. The theoretical upper limit for the ratio of 1 is never really achieved as it implying that there is an instantaneous and infinite resupply to the DGT device$^{31,32}$.

(b) **Partially Sustained Case ($0.2 < R < 0.8$).** In this situation, there is a resupply of the solute from the solid phase, but it is insufficient to fully sustain the pore water
concentrations. The R value represents the quantitative measure of the capacity of the solid phase to resupply the pore water in response to depletion induced by the DGT sink\textsuperscript{31,32}.

**(c) Unsustained Case (R < 0.2).** There is no resupply of solutes to the pore water. Therefore, the only supply to the DGT device is by the diffusion of solutes through the pore water, which becomes progressively depleted. The exact value of $R_{\text{diff}}$ depends on the diffusion coefficient of the solute in the soil, the DGT device and the deployment time\textsuperscript{31–33}.

Of all the components in solution, DGT does not measure large colloids or kinetically-inert organically complexes, due to size and mobility factors (Figure 3). The DGT does measure the contribution from exchangeable solid phase pool. Depending on the soil, this can lead to a poor correlation between $C_{\text{DGT}}$ and $C_{\text{Soln}}$, especially for a limited range of metal concentrations. However, it is more likely for $C_{\text{DGT}}$ and $C_{\text{Soln}}$ to be well correlated if the proportions of colloid formations and inert complexes are not significant components of the soil solution\textsuperscript{34}. For soils amended to different concentrations of metal, $C_{\text{Soln}}$ may correlate with free ion activity. Consequently, the DGT, soil solution and free ion concentrations may be well correlated with plant uptake\textsuperscript{28}.
1.6 DGT as a Biomimic

Accumulation of metals by DGT devices is hypothesized to be correlated to plant accumulation due to the similar limitation of diffusion. DGT mimics the removal of metals similar to a plant by having a chelating resin layer behind the diffusive layer that is in contact with soil solution. This induces a sink for ions in the soil, which results in a concentration gradient in the soil solution that is adjacent to the device window\textsuperscript{35}. Specifically, in the case of low concentration exposures, it is likely that the diffusion of the metal ion to the roots is rate limiting to uptake, as the rate of uptake by the roots is greater than the rate of diffusion through the surrounding soil\textsuperscript{17}. DGT uses diffusive transport as well as exchange between solid phase and solution in the adjacent soil (Figure 3(a)). The transport of ions to the DGT device occurs solely by molecular diffusion in the soil solution\textsuperscript{35}, whereas plants have to take into account the

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**Figure 4** Illustration of what is measured by DGT in soil solution (blue). The DGT measurements reflect dynamic supply (red arrows) which does not include colloid due to size exclusion (vertical dotted line) rather than the rate of metal release. The proportion of organic complexes measured by DGT is predominately determined by their diffusion rates (size), but slow rates are still important. DGT can also assess metals from the solid phase depending on both pool size and rate of release. However the tightly bound solid phase metals are not considered to be accessible within the DGT deployment timeframe\textsuperscript{25}. 

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diffusion and mass flow as the mechanisms by which ions are supplied to the plant (Figure 4 (b)). The supply from the solid phase is incorporated in the concept of “buffer power”, which can be described as the relative exchange in ion concentration in the solid phase exchange pool and the soil solution\textsuperscript{36}. The buffer power concept implies that there is an instantaneous exchange of ions between solid and solution phases that is not limited by the rates of sorption or desorption within the time frame of diffusional transport\textsuperscript{35}. Labile metal complexes will likely not contribute to metal accumulation in plants under non-rate limiting conditions. However, they will contribute to metals accumulated by the DGT devices. This implies that the metal accumulation in the DGT devices will be greater than the accumulation in the plant if there is competition for biouptake by other cations in the soil\textsuperscript{37}.

\[
\text{Resin} \quad \xrightarrow{\text{Binding}} \quad \text{Dissolved (diffusion layer)} \quad \xrightarrow{\text{Diffusion, } D_s} \quad \text{Sorbed to solid phase (} C_s \text{)} \quad \xleftrightarrow{\text{(De) sorption}} \quad \text{Dissolved (} C_L \text{)} \quad \xrightarrow{\text{Diffusion, } D_s} \quad \text{Soil}
\]

\[
\text{Soil} \quad \xrightarrow{\text{(De) sorption reactions controlled by first order kinetics:}} \quad \text{C}_L \xleftarrow{\text{k}_i} \text{C}_r \xrightarrow{\text{k}_d} \text{C}_s
\]
The DGT devices have been utilized in various media including fresh water systems, marine aquatic systems, waste water, sediments and soil. In aquatic environments they have been used to measure and monitor levels of REEs. There are numerous studies that have established good relationships between plant and DGT metals, therefore demonstrating the usefulness of DGT as a tool to determine the availability of various trace metals in soil. For example, DGT was suggested as a tool for measuring and monitoring the efficiency of As phytoextraction and for predicting bioavailable As and Sb in contaminated soils in comparison to chemical extractions. Although there are many sources stipulating the practicality of DGT as predictor for trace metal toxicity and its affiliation with phytotoxicity, it has yet to be proven effective for the trivalent REEs in soil.

2.0 Objectives

The objective of this research is to determine whether DGT devices can predict bioaccessibility of Ce, Nd and Eu in an organic soil better than a single extractant method.
using a CaCl$_2$ reagent. The corresponding null hypothesis would be there is no difference between the bioaccessible concentrations determined by DGT and CaCl$_2$ extractions. These novel data were compared to existing data on plant tissue concentrations.

3.0 Materials and Methodology

3.1 Glassware Preparation

All laboratory equipment was soaked overnight in 10-20% HNO$_3$ (certified ACS grade, Fisher Scientific) prior to use. It was then rinsed three times with deionized water and then rinsed three times with 18 MΩ water (certified ACS grade, Fisher Scientific) before being air dried. The Teflon vessels used for the reverse aqua regia (rAR) procedure were washed with Sparkleen detergent (Fisher Scientific), then washed in a dishwasher with neodisher® Laboclean A8: Dr. Weigert soap before following the acid wash procedure.

3.2 Range-finding Test

3.2.1. Soil Preparation and Basic Soil Properties

A large quantity of an uncontaminated, commercially available black garden soil (BGS) was purchased from Greenhorizons Cambridge, ON in Fall of 2016. The BGS was air dried, gently ground and sieved (3mm polypropylene sieve) prior to analysis. After being oven dried to remove moisture, soil samples were submitted to the University of Guelph Lab Services for inorganic and organic carbon quantification (total organic carbon, TOC) as well as determination of the soil texture profile. A pore water sample was also extracted and sent to the Centre for Cold Regions and Water Science in Waterloo, ON for dissolved organic carbon (DOC) quantification.
The pH of the soil was determined in-house using the water slurry method\textsuperscript{50}. This involves mixing 2.0 g of oven dried BGS with 20.0 mL of 18 MΩ water for 30 min. The mixed sample was then left to sit for an hour prior to determining the pH with a pH meter (Mettler Toledo SevenExcellence\textsuperscript{TM} Benchtop pH/Conductivity Meter).

Pseudo-total soil concentrations of Ce, Nd, Eu and soil nutrients (Mg, Al, P, S, K, Mn, Fe, Zn) were determined using a modified version of the U.S. EPA Method 3051a\textsuperscript{51}, in which the modification consisted of the use of an oven (110°C) rather than a microwave. This procedure requires the use of 0.5 g DW of soil which is massed into a Teflon digestion vessel with the addition of 3.0 mL of hydrochloric acid and 9.0 mL of nitric acid. After a predigestion period of 2 days, the lids were tightened and the vessels were placed in an oven at 105°C overnight. For each digestion batch a NIST SRM 2711a (Montana II Soil, National Institute of Standards Technology), NIST SRM SU-1b (Nickel-Copper-Cobalt Ore, Natural Resources Canada) and a blank were included. Once the vessels cooled, the samples were filtered through a 2.5 μm Whatman\textsuperscript{®} filter paper (Grade 42) and diluted to a total volume of 25.0 mL using 18 MΩ water in a 25.0 mL volumetric flask. All samples were then analysed using ICP-OES (Varian Vista Pro ICP OES with an axial viewed plasma).

Calcium chloride extractions were performed as a means to determine the bioaccessibility of Ce, Nd and Eu in the soil. 0.01M of CaCl\textsubscript{2} was prepared by dissolving 1.47 g of calcium chloride dehydrate into 1.0 L of 18 MΩ water. Then 25 mL of the 0.01 M CaCl\textsubscript{2} was added to 2.5 g DW of soil and shaken for 2 h on a rotary shaker\textsuperscript{52,53}. The samples were then filtered through a 2.5 μm Whatman\textsuperscript{®} filter paper (Grade 42) before analysis via ICP-MS (Bruker 820-MS ICP-MS with a CETAC ASX-520 autosampler).
3.2.2 Soil Amendment

The BGS was amended with four concentrations of metal nitrates for Ce (as \(\text{Ce(NO}_3\text{)}_3 \cdot 6\text{H}_2\text{O}\)), Nd (as \(\text{Nd(NO}_3\text{)}_3 \cdot 6\text{H}_2\text{O}\)) and Eu (as \(\text{Eu(NO}_3\text{)}_3 \cdot 5\text{H}_2\text{O}\)) ranging from crustal concentrations to concentrations expected to cause toxicity (Table 1). Metal nitrates were chosen due to their solubility properties and to minimize toxicity due to the counter ion.

**Table 1** Nominal REE concentrations of the REE-amended soils for the range-finding test (in mg of metal / kg of dry soil).

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Low</th>
<th>Low-Medium</th>
<th>Medium-High</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce</td>
<td>0</td>
<td>160</td>
<td>2500</td>
<td>5000</td>
<td>7400</td>
</tr>
<tr>
<td>Nd</td>
<td>0</td>
<td>110</td>
<td>1000</td>
<td>2500</td>
<td>5000</td>
</tr>
<tr>
<td>Eu</td>
<td>0</td>
<td>150</td>
<td>1100</td>
<td>3600</td>
<td>5000</td>
</tr>
</tbody>
</table>

Approximately 1.1 kg of air-dried BGS was massed into a sealable bag for each treatment prior to amendment. To obtain the appropriate concentrations listed above for each treatment the necessary amount of each metal nitrate was dissolved in 1.5 L of 18 M\(\Omega\) water prior to getting added to bag of soil. Afterwards, the soils were mixed by hand and allowed to age for a 14 days in an open system (allowing some evaporation to occur) while getting mixed bi-daily to ensure thorough homogeneity. After the 14-day aging period, the pH, CaCl\(_2\) bioaccessibility and pseudo-total metal concentrations were reanalysed the same way they were prior to amendment.

3.2.3 Range-finding DGT Test

The amended soil was added to three plastic cups (50 g DW) of each treatment. The soil was then prepared for DGT deployment as outlined by Zhang\(^{31}\). This involved wetting each soil sample to 60% of the water holding capacity (WHC) with 18 M\(\Omega\) water and letting it sit for 48 h
prior to wetting it to 90% WHC (to give a slurry consistency) for an additional 24 h. Afterwards, the DGT devices were removed from their individually wrapped packaging and gently inserted into the surface of the soil (recording the time to the nearest minute) using a rocking and rotating method to avoid the presence of air bubbles at the interface of the DGT’s exposure window. The DGTs were removed from the soil after 24 h (recording the time to the nearest minute) and rinsed with 18 MΩ water. The casting was broken with the use of a flathead screwdriver which allowed removal of the resin layer which was added to a 15.0 mL falcon tube containing 1.0 mL of strong acid (1.0 M HNO₃). The resin was eluted for 24 h before adding 5.0 mL of 18 MΩ water to dilute the sample for analysis of REEs via ICP-MS by the University of Guelph Lab Services.

The DGT devices have a short shelf life and for some experiments, they were past their expiry date, so they were revived prior to use following the procedure developed by Zhang³¹. This involved soaking the devices in 18 MΩ water for 4-6 h. In the meantime, 1.0 L of 0.01 M sodium nitrate was cleaned overnight by mixing it with approximately 7.0 g of Chelex-100 on a stir plate. The DGT devices were then soaked in the NaNO₃ (that was decanted from the Chelex-100 beads) overnight. The devices were then deployed in the soils that were prepared following the procedure outlined in section 3.2.3.

3.3 Time Dependant Studies

3.3.1 Soil Amendment

New batches of soils were amended (8 REE concentrations plus a control) to be used for all experiments from this point on, (Table 2) following the same procedure outlined in section 3.2.2. However, the addition of potassium nitrate was also done to balance out the nitrates levels
across all treatments (Table 2). This involved dissolving the required amount of KNO₃ into 1.0 L of 18 MΩ water before mixing it in each treatment.

Table 2 Nominal REE concentrations of the REE-amended soils with corresponding KNO₃ concentrations.

<table>
<thead>
<tr>
<th>Element</th>
<th>Treatment</th>
<th>Reference</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce</td>
<td>[REE] (mg/kg) / [KNO₃] (mg/kg)</td>
<td>0.00 / 17320</td>
<td>1000 / 15150</td>
<td>2000 / 12990</td>
<td>3000 / 10820</td>
<td>4000 / 8660</td>
<td>5000 / 6490</td>
<td>6000 / 4330</td>
<td>7000 / 2160</td>
<td>8000 / 0.00</td>
</tr>
<tr>
<td>Nd</td>
<td>0.00 / 16820</td>
<td>1000 / 14720</td>
<td>2000 / 12620</td>
<td>3000 / 10510</td>
<td>4000 / 8410</td>
<td>5000 / 6308</td>
<td>6000 / 4210</td>
<td>7000 / 2100</td>
<td>8000 / 0.00</td>
<td></td>
</tr>
<tr>
<td>Eu</td>
<td>0.00 / 11980</td>
<td>750 / 10480</td>
<td>1500 / 8980</td>
<td>2250 / 7490</td>
<td>3000 / 5990</td>
<td>3750 / 4490</td>
<td>4500 / 2990</td>
<td>5250 / 1500</td>
<td>6000 / 0.00</td>
<td></td>
</tr>
</tbody>
</table>

3.3.2 Time Dependant Study

The time dependant DGT tests were completed using only the highest treatment concentrations from each of the elements (8000 mg/kg Ce, 8000 mg/kg Nd and 6000 mg/kg Eu). The soil preparations and DGT deployments were performed the same way as described in section 3.2.3. However, the devices were deployed for six different times (24 h, 48 h, 72 h, 1 wk (168 h), 2 wk (336 h) and 4 wk (672 h), in triplicate.

3.4 Zone Depletion Study

The zone depletion DGT tests were completed using 10 replicates of the lowest (1000 mg/kg Ce, 1000 mg/kg Nd and 750 mg/kg Eu) and 10 replicates of the highest (8000 mg/kg Ce, 8000 mg/kg Nd and 6000 mg/kg Eu) treatment concentrations. The soil preparations and DGT deployments were performed the same way as described in section 3.2.3. However, the devices were deployed for 4 wk (672 h) to correspond to the typical duration in ECCC plant toxicity
protocols\textsuperscript{54}. At 2 wk, the DGT devices were temporarily removed from five replicates to mix the soil to replenish the metal supply in the area surrounding the device. The DGTs in the remaining 5 replicates were transplanted into new soil (freshly prepared for deployment at the 2 week time following the sample protocol as described in section 3.2.3) as an alternative method to replenish the metal supply adjacent to the DGT device.

3.5 REE Concentration Dependant Study

The full studies were conducted using the 9 treatment concentrations outlined in Table 2 for each metal. Five replicates of each Ce treatment were prepared and the DGT devices were deployed following the procedure mentioned in section 3.2.3. However, the DGTs were deployed for 72 h to more closely mimic the duration of a toxicity test. To mimic the actual plant growth associated with the corresponding plant toxicity experiments, the Nd and Eu DGT tests were deployed for a 4 wk period. Afterwards, the metal concentration in all samples were analysed using the ICP-MS.

3.6 Statistical Analysis

All graphing and regression analyses were performed in SigmaPlot v13. Various curvilinear regression relationships were fit to the data; the best fitting model was selected based on the minimized of sum of squares, corrected for the terms in the model.

4.0 Results and Discussion

4.1 Soil Properties

The soil texture was 36% silt, 35% clay and 29% sand (Table 3). Altogether, this results in a clay loam soil profile. The pH of the soil and the extracted pore water was slight lower than
neutral. After amendment, the pH ranged from 6.3-6.8 with the higher concentrations (for all metals) being slightly more acidic. This indicates that the soil is well buffered and that pH is not the predominant influencing factor for REE accumulation in the present study. Referring back to Figure 1, it can be concluded that the three REEs of interest are in fact in their trivalent oxidation state.

Soil TOC was approximately 30% which is over a magnitude higher than typical Canadian field soils. This can lead to higher complexation capabilities and a reduction in bioavailable metal\textsuperscript{21}. Therefore, the need to use higher metal concentrations to overcome these consequences was necessary. The pore water had 371 mg DOC/L which further confirms the high organic nature of the BGS. When this DOC value is inputted into CHEAQS it is predicted that none of the metal is precipitated, rather it all gets complexed with OM; 90\% bound to fulvic and 10\% bound to humic. The high organic content also influenced the WHC which exceeded 100\%. Soil organic matter may act as a sponge, with the ability to absorb 90\% of its weight in water\textsuperscript{55}.

Table 3 Soil characteristics of the BGS soil prior to amendment.

<table>
<thead>
<tr>
<th>Characteristic Property</th>
<th>Soil pH</th>
<th>Pore-water pH</th>
<th>WHC</th>
<th>TOC</th>
<th>DOC</th>
<th>Gravel</th>
<th>Silt</th>
<th>Clay</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGS</td>
<td>6.8</td>
<td>6.7</td>
<td>114%</td>
<td>29%</td>
<td>370 mg/L</td>
<td>4.1%</td>
<td>36%</td>
<td>35%</td>
<td>29%</td>
</tr>
</tbody>
</table>

The BGS soil (prior to amendment) was digested to analyse for total background ions and nutrients naturally present in the soil (Table 4). A CaCl\textsubscript{2} extraction was also conducted on the pre-amended BGS soil to quantify the bioaccessible fractions of the same background ions and nutrients in the soil (Table 4). All values are expressed as mg of the element per kg of dry soil.
As expected, the bioaccessible fraction of each element was much lower than the total. This further justifies the importance of using bioaccessible values in toxicity determination procedures rather than the total concentrations, although their integration into regulation is limited\textsuperscript{20,56}.

**Table 4** Nutrient concentrations of the BGS soil prior to amendment determined via CaCl\textsubscript{2} extractions.

<table>
<thead>
<tr>
<th>Element</th>
<th>Al</th>
<th>Mn</th>
<th>Mg</th>
<th>Fe</th>
<th>K</th>
<th>P</th>
<th>S</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Total] (mg/kg)</td>
<td>10600</td>
<td>268</td>
<td>7340</td>
<td>9700</td>
<td>2850</td>
<td>1200</td>
<td>4530</td>
<td>53.9</td>
</tr>
<tr>
<td>[Bioaccessible] (mg/kg)</td>
<td>0</td>
<td>2.5</td>
<td>600</td>
<td>3.6</td>
<td>540</td>
<td>3.6</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

Despite the fact that Al has the highest total abundance in the soil, none of it appears to be bioaccessible. This implies that all the Al is complexed with the OM in the soil and/or is precipitated into insoluble Al-hydroxides, making it unavailable for organisms to take up. The elements of highest bioaccessible abundance are Mg and K, which are both essential macronutrients to plants and are within normal fertility levels for healthy crop systems\textsuperscript{57}.

**4.2 REE Range-finding Study**

The DGT devices were deployed for a duration of 24 h as suggested by the SOP in triplicates. Once the labile metals were eluted from the resin layers and analysed via ICP-MS they were plotted against the total metal concentrations determined by ICP-EOS following a rAR acid digest (Figures 6-8). The bioaccessible metal concentrations were determined by dividing the metal mass accumulated by the resin (calculated using eq 1.2) by the mass of the resin (0.026 g) to give an overall concentration in mg/kg. All bioaccessible metal concentrations determined via DGT were calculated in this manner.
Figure 5 The \([\text{Ce}]\) accumulated on the DGT resin over 24 h. The total concentrations are expressed as mg of metal per kg of dry soil, whereas the bioaccessible metal concentrations are expressed as mg of accumulated metal per kg of resin. The bi-directional error bars represent the standard deviations.
Figure 6 The [Nd] accumulated on the DGT resin over 24 h. The total concentrations are expressed as mg of metal per kg of dry soil, whereas the bioaccessible metal concentrations are expressed as mg of accumulated metal per kg. The bi-directional error bars represent the standard deviations.
Figure 7 The [Eu] accumulated on the DGT resin over 24 h. The total concentrations are expressed as mg of metal per kg of dry soil, whereas the bioaccessible metal concentrations are expressed as mg of accumulated metal per kg of resin. The bi-directional error bars represent the standard deviations.

Data for Ce and Nd were fit with a sigmoidal curve; however the Eu data fit a quadratic curve. As the total concentration of Ce and Nd increased, so did the bioaccessible concentration, as expected. The Eu accumulation by the resin is an order of magnitude lower than Ce and Nd. As well, unlike Ce and Nd, Eu did not seem to show a simple dose response relationship (Figure
7), something which requires a larger range of doses to explain (as seen in concentration dependent study, section 4.4).

Despite the theory of all the metals being complexed with humus, there is still REE accumulation by the resin. If the modeling predictions are true, this suggests that some of the complexes are in fact labile and are capable of diffusion through the DGT and accumulating on the resin layer. This would comply with previous studies\textsuperscript{34,40,58} suggesting that complexes with large enough diffusion rates (smaller sizes) are indeed able to diffuse through the DGT. The remaining metal could be present as larger, less mobile complexes in solution or bound to soil solids such as OM and clay minerals. This could be further investigated using sequential extractions\textsuperscript{59}.

4.3 REE Time Dependant and Zone Depletion Studies

The results from the range-finding test suggested that some of the parameters in the original SOP warranted investigation; specifically, the deployment time. The purpose was to determine if the DGT devices should be deployed for longer than 24 h since plant toxicity protocols have a much longer growth period. DGTs were therefore deployed at a single concentration for various times (24 h, 48 h, 72 h, 1 wk (168 h), 2 wk (336 h), and 4 wk (672 h)) the results shown in Figures 9-11.
Figure 8 Accumulated [Ce] on the DGT resin over time (24-672 h) for the 8000 mg/kg treatment (circles). Additional DGTs were deployed for 672 h in which the labile pool was resupplied by soil mixing (triangle) or repotting into freshly prepared soil (square) at 336 h. The error bars represent the standard deviation of accumulated metal by the DGT.
Figure 9 Accumulated \([\text{Nd}]\) on the DGT resin over time (24-672 h) for the 8000 mg/kg treatment (circles). Additional DGTs were deployed for 672 h in which the labile pool was resupplied by soil mixing (triangle) or repotting into freshly prepared soil (square) at 336 h. The error bars represent the standard deviation of accumulated metal by the DGT.
For these metals, the rate of uptake decreases over time and reaches a plateau between 336 h and 672 h. This reduction in sorption rate with deployment time is congruent with the behaviour of lanthanide complexes in solution. Prior to 336 h, the DGT accumulation is linear, congruent with a plant’s uptake response. The plateauing could be a result of either zone depletion of the metal supply adjacent to the DGT’s exposure window or to saturation of the DGT’s resin. As the ions are progressively removed from the surrounding pore water it leads to a state of disequilibrium between the free ions in the pore water, their complexes and ions adsorbed onto the soil solid phases. The dissociation of labile complexes within the diffusion and
resin layers of the DGT device, and in the adjacent pore water, in response to the progressive reduction of free ion, results in a reduction in diffusive rate of complexes towards the resin\textsuperscript{29}. When DGT devices were exposed to 1:1 soil-water mixtures and saturated soil samples (containing Co, Pb, Cd and Cu) for various time\textsuperscript{17}, it was discovered that the accumulation of the metals in the saturated samples significantly decreased after 48 h, whereas the 1:1 samples continued to accumulate the metals linearly. This again suggests depletion in the saturated soil samples and that the re-supply rate and diffusion from the solid phase can be incapable to meet the DGT demand\textsuperscript{17,60,61}.

![Graph](image)

**Figure 11** Resupply rate of Ce into solution from the solid phase with respect to deployment time (h).
The resupply rate, \( R \), was calculated for Ce (same soils as those used for Figure 8): the rates ranged from \( 4.85 \times 10^{-11} - 1.20 \times 10^{-10} \). Referring back to Figure 2, it can be concluded that Ce can be categorized as the unsustained case. This implies that diffusion is the major process supplying the DGT with solute\(^{29}\). In other words the rate of diffusion of the labile species from the solid phase to soil solution is lower than the diffusion from the soil solution into the DGT. This will eventually lead to the progressive depletion of the labile pool over time as the labile species diffuse into the DGT.

To determine what could be causing the reduction in accumulation rate, two different metal resupply methods were investigated. The 2 wk deployment time is when the DGT accumulation reaches a plateau, so metals were reintroduced to the DGT’s surroundings at that time. Five replicates of each treatment had the metals resupplied to the surface by thoroughly mixing the soil. The other five replicates of each treatment had the metals resupplied by repotting them into new pots of metal-amended soil. There was no significant difference between the accumulation at 4 wk with or without resupplying the metals to the DGT interface (Figures 9-11).

When comparing the two different resupply methods, there was no difference with the exception of the Eu 750 mg/kg treatment (Table 5). When comparing the controls to the resupply methods, it can be seen that for the lowest concentrations of both Ce and Nd there was no difference. However, either resupply method at the higher REE concentration increased the mass of REE on DGT. This implies that the resin can accumulate more metals when they are reintroduced adjacent to the DGT device.
Table 5 The differences in concentration (μg/g) between the zone depletion resupply methods (repotting versus mixing) against the control for the lowest and highest treatments of each metal. Where * represents the level of significance.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control – Repotting, μg/g</th>
<th>Control – Mixing, μg/g</th>
<th>Repotting – Mixing, μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce 1000 mg/kg</td>
<td>-18.7</td>
<td>-8.31</td>
<td>10.4</td>
</tr>
<tr>
<td>Ce 8000 mg/kg</td>
<td>-572*</td>
<td>-576*</td>
<td>-3.84</td>
</tr>
<tr>
<td>Nd 1000 mg/kg</td>
<td>-10.2</td>
<td>-20.3</td>
<td>-10.1</td>
</tr>
<tr>
<td>Nd 8000 mg/kg</td>
<td>-399*</td>
<td>-428*</td>
<td>-28.8</td>
</tr>
<tr>
<td>Eu 750 mg/kg</td>
<td>-13.4*</td>
<td>-8.38*</td>
<td>5.04</td>
</tr>
<tr>
<td>Eu 6000 mg/kg</td>
<td>169*</td>
<td>-203*</td>
<td>-33.7</td>
</tr>
</tbody>
</table>

* p ≤ 0.001

Another modification that may influence the usefulness of DGT as a biomimic is the thickness of the diffusion layer and/or the resin layer. Although not evaluated in the present study, when DGT devices containing various diffusive layer thicknesses (0.24-1.95mm) were deployed for 24 h in soils (containing Co, Cd, Ni, Cu, Pb or Zn), it was determined that there was a larger flux demand by the DGT when thinner membranes were used. Using thicker membranes is likely to maintain a steady-state concentration in the soil solution, by keeping DGT demand to a minimum. Garmo et al. found similar trends when they deployed DGT devices in solutions containing varying lanthanides. They concluded that increasing the thickness of the diffusive layer allows for increased diffusion (larger contribution) of the complexes, making it ideal for longer deployment times. However, in contrast to the previous study, they found that increasing the thickness of the resin gel increased the uptake, especially for lanthanides that form less labile complexes (slowly dissociating).

4.4 Concentration Dependant Study – REE Bioaccessibility via DGT

The results from the time dependant test suggested performing a concentration dependant test for Ce, in which the DGT devices were deployed for 72 h in pots with 9 different treatments...
Bioaccessible Ce was also quantified by CaCl$_2$ extraction for comparison. When the bioaccessible concentrations via DGT and CaCl$_2$ were plotted relative to the pseudo-total concentrations in the soil (Figure 13), CaCl$_2$ identified more soil Ce as bioaccessible than did DGT ($p < 0.001$).

![Figure 12 Bioaccessibility of Ce (deployed for 72 h) determined by DGT (circles) and CaCl2 extractions (triangles) with respect to [Ce] in the soil. Both curves were fit with a sigmoidal curve. The bi-directional error bars represent the standard deviations.](image)

Variation in the relationship between bioaccessible and the total metal could be explained by variation in DOC$^{62}$, as was seen when 30 Swedish Cambisols were compared and contrasted. It was found that 46-74% of the variability could be explained by the presence of DOC. This is due to the fact that stable and immobile OM establishes an efficient barrier to mobility and transport$^{63}$. However, the main physical and chemical characteristics of soils have a
role in REE retention and release. Such factors may include high content of clay minerals$^{64,65}$, high levels of sulfates and phosphates in the soil solution$^{66,67}$ as well as concentrations and lability of REE-containing Fe-Mn-hydroxy compounds$^{68}$. The pH of the soil also influences the stability of REE complexes with OM. In more alkaline conditions the stability of the REE complexes has been shown to be very high$^{69}$. As the pH decreased in the soil, it resulted in a consequential increase in REE release and an increase in REE concentrations in soil solution$^{62,68}$. Therefore only a limited quantity of REEs exists in the water-soluble state in the soil, which is available for uptake by plant roots and biota. Perhaps an uncontaminated field soil with a lower OM content should be considered for future studies to improve these relationships or using a wider range of soils with different characteristics.
Figure 13 Bioaccessibility of Nd (deployed for 4 wk) determined by DGT (circles) and CaCl2 extractions (triangles) with respect to [Nd] in the soil. Both curves were fit with a sigmoidal curve. The bi-directional error bars represent the standard deviations.
A concentration dependant DGT test was also performed for Nd and Eu, however, the devices were deployed for a duration of 4 wk (Figures 14 and 15). This was to correlate with the time at which plants are grown in correspondence to the ECCC plant toxicity protocol\textsuperscript{54}. The results show that the DGT device tends to underestimate [REE] in comparison to those determined by CaCl\textsubscript{2}. Similar to Ce, the p-value of Nd and Eu are both <0.0001 indicating that there is a significant difference between bioaccessibility determined by DGT and CaCl\textsubscript{2} extraction.

\textbf{Figure 14} Bioaccessibility of Eu (deployed for 4 wk) determined by DGT (circles) and CaCl\textsubscript{2} extractions (triangles) with respect to [Eu] in the soil. Both curves were fit with a sigmoidal curve. The bi-directional error bars represent the standard deviations.
Both methods of determining bioaccessibility have been previously shown to be good tools to explain metal ecotoxicity. However, the parameters are different between them (Table 6); DGT is dependent on diffusion whereas CaCl$_2$ extractions are based on ion-exchange. The single extractant method of CaCl$_2$ has been proven to work well for the trace metals in most soils (peat based soils are an exception) but it has also been proven to overestimate the availability by partially extracting the DOC bound metals$^{56}$. Peat based soils have a higher OM much like the BGS used in the present study, therefore limiting its reliability for this particular study. Another potential limiting factor of this extraction method is that the reagent used is composed of a divalent calcium cation (Ca$^{2+}$) and two monovalent chloride anions (Cl$^{-}$) in which the Ca$^{2+}$ typically exchanges with the metal species of interest. However, REEs are trivalent and may not exchange as readily with Ca$^{2+}$ as the divalent trace metals. The binding of REEs to Cl$^{-}$ is also possible, thus resulting in an underestimation of bioaccessible metal. However, for the REEs of interest the chloride complexation potential is relatively low and may not contribute much to the underestimation of these metals.

**Table 6** Summary of pros and cons of the 2 bioaccessibility determination methods (DGT and CaCl$_2$ extractions) used in the present study.

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGT can be applied to a wide range of soils</td>
<td>CaCl$_2$ is not effective on high organic soils</td>
</tr>
<tr>
<td>CaCl$_2$ is easy and cost effective</td>
<td>DGT is expensive and requires training</td>
</tr>
<tr>
<td>DGT integrates over time like an organism and accounts for environmental variability</td>
<td>CaCl$_2$ is a single point measurement</td>
</tr>
<tr>
<td>CaCl$_2$ has extensive published data</td>
<td>DGT is a newer technique</td>
</tr>
<tr>
<td>DGT accounts for abiotic processes (i.e. labile complexes, buffered solid-phase pool and the diffusion flux)</td>
<td>DGT only accounts for abiotic factors</td>
</tr>
</tbody>
</table>
4.5 Validation of the Range-Finding Study with Congruent Radish Phytotoxicity Studies

The bioavailable metal concentrations were collected by a colleague and quantified by reverse aqua regia digestion after a 4 wk growth period. The concentrations accumulated in the radish (*Raphanus sativus*, ‘Cherry Belle’) roots tissues were plotted against the bioaccessible concentrations that were determined via DGT (Figures 16-18). Roots accumulated at least a magnitude greater concentration of Ce and Nd than what was accumulated by the DGT devices. This magnified accumulation could be due to the presence of not easily washable soil particulates or surface dust contamination on the roots\(^3\), therefore, making it difficult to report “true” root accumulated metal concentrations. Maize and mungbean grown in solution culture accumulated 20-150 times higher La in their roots than in their shoots\(^70\). There are many studies available that have shown a reduction in REE concentrations in the order of root > leaf > stem > grain/fruit for a variety of crops\(^14,71-73\). Shoot accumulation was looked at however; the REEs accumulated more in the roots.
Figure 15 Bioavailable [Ce] accumulated by the radish roots (after 4 wk growth) of the range-finding test with respect to the bioaccessible [Ce] accumulated by the DGT resin for a 24 h deployment period. A 1:10 line has been included for ease of reference and the bi-directional error bars represent the standard deviations.
Figure 16 Bioavailable [Nd] accumulated by the radish roots (after 4 wk growth) of the range-finding test with respect the bioaccessible [Nd] accumulated by the DGT resin for a 24 h deployment period. A 1:30 line has been included for ease of reference and the bi-directional error bars represent the standard deviations.
Figure 17 Bioavailable [Eu] accumulated by the radish roots (after 4 wk growth) of the range-finding test with respect to the bioaccessible [Eu] accumulated by the DGT resin for a 24 h deployment period and the bi-directional error bars represent the standard deviations.

Accumulation of Eu by the radish roots failed to show any relationship with the Eu concentrations accumulated by the DGT resin (Figure 18). This could be a result of the behavioural differences relative to the other REEs. As mentioned in Sec 1.1.2, Eu can be found in the divalent redox state rather than the typical trivalent state, thus causing it to have a larger atomic radius$^4$. The increased size in atomic radius is due to the presences of the larger divalent ions in the lattice which influences the stability of its half-filled 4f$^7$ orbital making it more reactive. The data indicate that there may be a mismeasurement with the second Eu treatment, however it is not possible to go back and re-measured.
4.6 Validation of the Concentration Dependent Study with Congruent Durum Wheat and Tomato Phytotoxicity Studies

Similar to section 4.4, there were corresponding phytotoxicity studies completed independently in the same soil system using durum wheat (*Triticum durum*, ‘Kyle’) and tomato (*Solanum lycopersicum*, ‘Bonnys best’). When the bioavailable concentrations were plotted with respect to the bioaccessible concentrations, the roots accumulated more metal than the DGTs by 3-10 fold (Figures 19-21). The durum wheat roots accumulated five times more metal in comparison to the tomato roots for all three metals of interest. However, the radish tends to accumulate slightly more than the wheat roots for Nd. This suggests that plant physiology influences the accumulation rates of the metals in conjunction with metal speciation and concentration.

There are transfer factors (i.e. the ratio of concentration in the plant tissue/total concentration in the rhizosphere) that may provide insight about the relative availability of soil metals to plants. In previous studies, the transfer factors for REEs (including those tested in the present study) for forest plants, vascular plants and fungal plants were all low (0.02-0.09)\textsuperscript{74,75}. A recent study\textsuperscript{76} looked at the transfer factors in beech root tips in an organic Podzolic soil which decreased from 0.10-0.09 moving across the periodic table from Y to Tb. However Eu was an exception, as it had a transport factor of 0.30. This suggests that Eu is at least partially taken up as a divalent cation, as it has a similar transfer factor as other divalent cations in comparison to the trivalent cations\textsuperscript{76}. 
Figure 18 Bioavailable [Ce] accumulated by the durum wheat roots (circles) and tomato roots (triangles) from the concentration dependant study with respect to the bioaccessible [Ce] accumulated by the DGT resin. A linear regression was also plotted in which the tomato line is bolded. The equation for wheat is $y=23.5+9.9x$, $R^2=0.76$ and tomato is $y=15.8+4.8x$, $R^2=0.59$. A 1:10 and 1:5 line included for ease of reference and the bi-directional error bars represent the standard deviations.
Figure 19 Bioavailable [Nd] accumulated by the durum wheat roots (circles) and tomato roots (triangles) from the concentration dependant study with respect to the bioaccessible [Nd] accumulated by the DGT resin. A linear regression was also plotted in which the tomato line is bolded. The equation for wheat is $y=157+1.7x$, $R^2=0.01$ and tomato is $y=37.8+2.5x$, $R^2=0.63$. A 1:3 line included for ease of reference and the bi-directional error bars represent the standard deviations.
Figure 20 Bioavailable [Eu] accumulated by the durum wheat roots (circles) and tomato roots (triangles) from the full study test with respect to the bioaccessible [Eu] accumulated by the DGT resin. A linear regression was also plotted in which the tomato line is bolded. The equation for wheat is $y=119.5+2.9x$, $R^2=0.06$ and is tomato $y=56.3+5.2x$, $R^2=0.22$. A 1:5 line included for ease of reference and the bi-directional error bars represent the standard deviations.

Radish roots accumulated 10-30 fold more than the resin, when the DGTs were deployed for 24 h. When the deployment time was increased to 72 hr for Ce (Figure 19), wheat and tomato roots accumulated 5 and 10 fold more Ce, respectively. Finally, when the deployment time was increased to 4 wk for Nd and Eu (Figures 20 and 21) the wheat and tomato roots accumulated 3-5 fold more metal than the resin. Therefore, increasing the deployment time improves the correlation between the accumulation in the plant roots and on the DGT resin.

As mentioned above, the uptake period during plant growth for toxicity testing is typically several weeks longer than the traditionally used 24 h DGT deployment timeframe.
However, the period in which the plant exploits a particular volume of soil can be shorter due to the root growth and localized uptake along the root\textsuperscript{77,78}. The roots themselves have an effect on the uptake of metals; thinner roots can withstand a larger diffusion rate in comparison to thicker roots, but the root radius isn’t as significant as the root’s overall size (expressed as a per weight basis)\textsuperscript{79} since smaller roots tend to have a larger surface area. However, root hairs have been shown to have the greatest influence assuming they are long enough to surpass the depletion zone around the roots\textsuperscript{80}. The plants themselves can alter the soil in the rhizosphere and this affects metal speciation. All of these factors can further explain why the DGT-determined accumulation of REEs may not correspond well with root uptake. It is obvious that DGT does not account for any of these biotic factors. Therefore, it can only be used to predict differences in uptake solely related to the soil properties.

5.0 Conclusion

The objective of this research was to determine the reliability of using DGT as a predictive tool for REE bioaccessibility in soil in relation to a single extracted method (i.e. CaCl$_2$ extraction). For the case of Ce, Nd and Eu, significantly ($p < 0.001$) greater metal was accumulated in the plants compared to the DGT as indicated by the slope of the linear correlation being consistently less than one. Both methods have their pros and cons in terms of evaluating bioaccessibility of REEs in soil. The soil conditions used for the experiment may not have been broadly representative. Using a soil with a lower OM content may improve correlation by reducing the REE colloid formation and thus the overall variation. Reducing OM would lead to a corresponding decrease in the quantity of tightly bound complexes and colloids which could also lead to an increase in diffusion from the solid phase to the soil solution therefore potentially better sustaining the DGT demand.
When the time of deployment is extended it is necessary to also resupply the labile metals to rhizosphere adjacent to the DGT device. It is possible to improve this correlation further by increasing the diffusive layer within the DGT. This will also prolong the resin from becoming saturated when the DGT devices are exposed to media contaminated with higher concentrations for prolonged deployments. When comparing REE accumulation in the DGT to plant roots, it is obvious that the roots accumulate significantly more. There is variability between the plant species as well as the metals species implying that accumulation is influenced by metal concentration in the soil, metal species (ie. ratio of labile : bound/colloidal species) and plant physiology. However, deploying the DGT devices in the soil for longer periods of time to correspond to the duration of plant growth improved the relation between plant and DGT accumulation.

To some extent, DGT mimics the action of plants if plant uptake is limited by diffusion. Therefore, as shown in the present study, DGT is capable of serving as a predictor of plant uptake and availability of the rare earth metals as has been shown with the trace metals. Especially in the cases that plant uptake is limited by the supply of metals to the membrane surface, instead of the transport across the membrane. The DGT integrates over time while taking into considering variation in the environment that can influence the change in exposure. This is useful in addressing the typical uncertainties in risk assessment. In terms of risk assessment, DGT can also be utilized as a monitoring tool in the field to provide accurate metal contamination information and even the success of contaminant containment methods.
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