

The 'europium anomaly' in plants: facts and fiction

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Abstract

Rare earth elements (REEs) and normalized REE pattern determined in plant and soil samples represent powerful tools to trace biogeochemical processes during weathering, soil genesis and processes in the rhizosphere, and thus publications reporting rare earth elements and normalized REE pattern in soil systems and plants are increasing rapidly. Generally, a normalized REE pattern allow for the recognition of an anomalous

concentrations of an individual REE. In the literature anomalies are predominantly reported/focused for/on the redox-sensitive elements cerium (Ce) and europium (Eu) that can shift their oxidation state during interactions with organic and inorganic soil phases and biological processes affecting the elements' mobility in soil and uptake by plants. Thus positive Eu anomalies in plants are often interpreted as a consequence of reduction of Eu^{3+} to Eu^{2+} in the rhizosphere followed by a preferential uptake of Eu^{2+} . However, due to an analytical artefact in ICP-MS analysis, a false Eu anomaly may be reported. This can be avoided by using a barium (Ba) interference correction. We draw attention to the possibility of this problem and to being aware of potential occurrence when Eu anomalies are reported. Finally, we recommend (i) including information on how this potential problem was dealt with in the Materials and Methods section of articles and (ii) how to implement FAIR principles in the section (including data availability on an open repository).

Keywords

Artifact; Isobaric Correction; Europium anomaly; Interference; REEs.

Introduction

The rare earth elements (REEs) comprise a group of 16 elements from the lanthanide series including lanthanum (La), yttrium (Y) and scandium (Sc) that occur in most all soils at concentrations similar to most plant essential micronutrients, such as copper and zinc, and are therefore of increasing interest in plant and soil research (Tyler 2005; Liang et al. 2008; Liu et al. 2018). For the sake of convenience, the REE distribution in natural samples is usually expressed by normalized REE patterns where concentrations in a target material are referred to concentrations in a reference material (see Davranche et al. 2017). A distinct change in this pattern typically underlies slight differences in the REEs' chemical properties caused by the decrease in the ionic radius with increasing atomic number leading to slight

changes in the stability constants with soil organic and inorganic soil phases and differences in solution complexation between light to heavy REEs. As a consequence, these differences result in a systematic REE fractionation pattern called the 'lanthanide contraction'.

Additionally, a normalized REE pattern allows for the recognition of an anomalous concentrations of an individual REE as either a positive or negative anomaly in an otherwise smooth pattern. This type of anomaly can occur in response to the redox properties of Eu which can exist in the trivalent or divalent state (Bau 1991). The anomalous behavior of Eu is quantified *via* calculation of Eu anomaly $(Eu/Eu^*) = 2Eu_N / (Sm_N + Gd_N)$ where N equals the normalized abundance to a reference material (Figure 1) (Bau 1991; Davranche et al. 2017). REE concentrations in plants, and especially in shoot biomass are reported in an increasing number of publications (Ichihashi et al. 1992; Liang et al. 2008; Brioschi et al. 2013; Censi et al. 2014; Liu et al. 2018; Martinez et al. 2018). However, in some of publications, the analytical techniques employed are not fully documented, which may lead to questions about the validity of the results. In particular, the risk of reporting false positive anomalies in plants caused to barium (Ba) interference with europium (Eu). This Letter intends to examine this problem, to caution researchers to avoid this analytical error in their work, and to recommend a solution through improved laboratory documentation.

Analytical techniques

Environmental samples typically contain concentrations of REEs at trace and ultra-trace levels. Particularly in plants, concentrations of single REEs rarely exceed 1 mg/kg for the light REEs (La, Nd, Ce) and are frequently substantially lower than 1 µg/kg, especially in case of the scarcely abundant heavy REEs (e.g. Dy, Ho, Lu) following Oddo-Harkins rule. Since the 1990's, progress in REE determination in environmental samples underlies the development of instrumental analytical techniques. Among the most commonly utilized approaches for REE determinations in environmental samples are neutron activation analysis (NAA), X-ray fluorescence (XRF) spectrometry, Optical Emission Spectrometry

(ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Due to its high selectivity and sensitivity, ICP-MS is nowadays by far the most frequently used method characterized by a high sensitivity and selectivity needed for the direct determination of all REEs in complex sample matrices with supreme limits of detection (routinely parts per billion *i.e.*, $\mu\text{g}/\text{kg}$). Besides hyphenated methods coupling ICP-MS with laser ablation (LA), standard equipped ICP-MS devices typically require aqueous samples, and thus, solid environmental samples require a sample pretreatment where REEs are transferred into solution. Where total concentrations of solids are required, sample pretreatment/digestion methods for the dissolution of REE-bearing geogenic or biogenic silica are needed which can be achieved by fusion with borates or carbonates at high temperatures followed by a dissolution in acids or microwave-assisted heating of strong acids (perchloric acid, hydrochloric acid, nitric acid, hydrofluoric acid), where hydrofluoric (HF) acid is normally needed for the dissolution of sparingly soluble silicates. When HF is used, the amount of HF should be adjusted to the amount of Si in the samples because excess of F in the resulting solutions may precipitate REEs together with Ca. To overcome this problem, boric acid or digestion with HBF_4 can improve REE recovery by preventing the precipitation of Ca and REE fluorides (Krachler et al. 2002). All other procedures such as aqua regia or single acid microwave digestions without hydrofluoric acid will strongly underestimate the REE concentrations in environmental or geochemical materials, including plant material and just give pseudo-totals of the desired elements. Additionally, it is worth noting that closed vessel digestions should be preferably used to avoid losses of volatile element species.

The resulting solutions are typically not problematic for analysis by ICP-MS. The solution stability of REEs is good with HNO_3 or HCl, but fluoride, phosphate and sulfate salts and carbonate are particularly insoluble, and neutral/alkaline pH is an issue too. The typical approach for ICP-MS is to separate from the matrix using ion exchange resins and sequential elution (Yan et al. 2018). It adds complexity but improves detection and has been used for precise isotope ratio determinations (Zhao et al. 2019). Other methods for the quantification of mobile and potentially plant available fractions of REEs in soils deploy single

step or sequential extractions (Wiche and Heilmeyer 2016; Wiche et al. 2017). For ICP-MS, however, the determination of REEs just requires to introduce solutions with low concentrations of REEs (of the order of <10 mg/L) in order to measure all 16 naturally occurring REEs simultaneously without any separation from the matrix. Ions produced in high temperature plasma are identified based on their mass to charge ratio, m/z , which is characteristic for any given isotope. Each REE has at least one isotope that is free from isobaric overlaps and their sensitivity is relatively uniform from ^{139}La to ^{175}Lu .

Besides the sometimes time consuming and challenging sample pretreatment, a second more latent problem may hamper the reliable quantification of REEs by ICP-MS due to the occurrence of spectral interferences in the mass spectrum. In particular, the most important challenges with interferences encountered in ICP-MS analysis are caused by the amount of oxide formation in the plasma (Longerich et al. 1987; Jarvis et al. 1992). The REEs form a continuous group from 139 to 175 m/z , and the formation of light REEs and barium oxide can produce significant middle REE interferences. Refractory oxide ions are influenced by the plasma operating parameters. The oxide production level is close to 1% to 2% (Davranche et al. 2004; Davranche et al. 2017). Mathematical corrections are required to suppress these specific interferences and these corrections are calculated as a function of the oxide level. Previous studies have shown the importance of these corrections for accurate REE quantification (Dulski 1994; Aries et al. 2000; Raut et al. 2005a; Raut et al. 2005b). In the case of Eu, Ba which forms the BaO^+ species and the isotopes ^{135}Ba ($135+16=151$) and ^{137}Ba ($137+16=153$) isobarically interferes with ^{151}Eu and ^{153}Eu , respectively. Eventually, progress has been made with introduction of collision cell techniques and higher resolution multiple quadrupole systems which can successfully remove the influence of oxide interferences (Zhu et al. 2021).

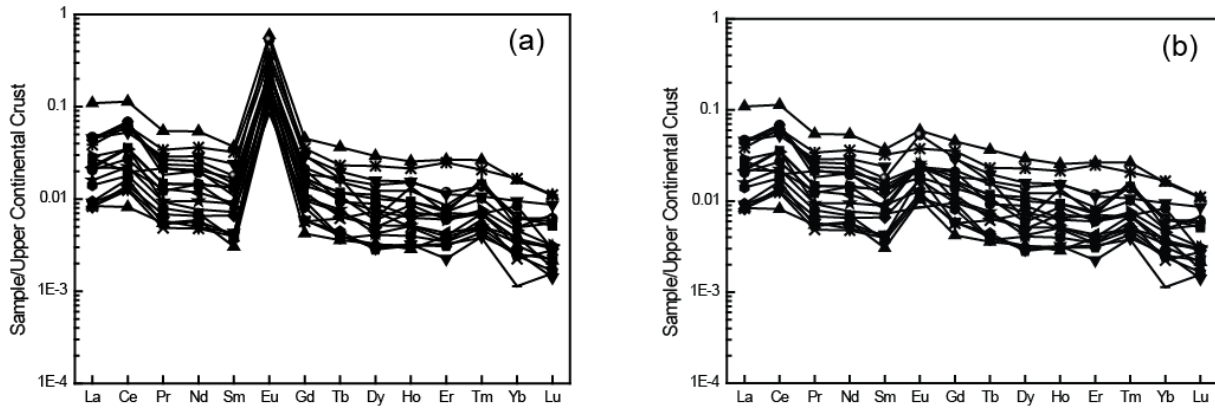


Figure 1 Example of Upper continental crust (UCC)-normalized REE patterns in *Anisopappus chinensis* leaves sampled at Mikembo (Democratic Republic of Congo), (a) without Ba interference correction (modified after (Pourret et al. 2014)) and (b) with Ba interference correction (modified after (Pourret et al. 2019)). The UCC values are from McLennan (2001).

Specific behavior of europium during absorption by plants

At low to very low Ca concentrations in the soil, REE concentrations have been described to decrease simultaneously with the appearance of a strong Eu anomaly in the biomass of plants growing on such soils (Stille et al. 2006). The shoot biomass is then characterized by positive Eu anomalies, as has been reported by Ding et al. (2006) for soil-grown wheat and Brioschi et al. (2013) for plants growing on granite-derived soils. Ding et al. (2006) posited that such Eu anomalies were caused by precipitation of Eu-enriched phosphate particles in the different plants organs, whilst Stille et al. (2006) and Brioschi et al. (2013) hypothesized that this anomaly was a linked to the strong depletion of Ca in the soils where plants may suffer from Ca-deficiency (Brioschi et al. 2013). Gao et al. (2003) have shown that Eu can be absorbed and bound to the inner membranes of different plant cell organelles. Transport proteins that can mediate REE uptake by plant cells have not yet been fully characterized, but it can be readily hypothesized that ion channels could be involved in passive REE diffusion. Most studies to date have revealed that plants preferentially accumulate light REE

(e.g., La to Eu + Sc) (LREE). This suggests that most plants discriminate between cations differing by their ionic radius and charge. Calcium and Eu have almost identical ionic radii (Shannon 1976), which means that Eu is likely to competitively replace Ca ions in biological systems (Evans 1983). Although, a biological role for lanthanides is not known, previous studies have shown that Eu can substitute Ca in plants, in particular in soils with elevated Eu/Ca ratios (Zeng et al. 2003; Shtangeeva and Ayrault 2007). Moreover, positive Eu anomalies in plant shoots suggest that Eu^{3+} can form stable organic complexes thereby replacing Ca^{2+} in some biological processes, such as in xylem fluid during normal nutrient fluxes. The possibility that Eu mobility in these fluids can be enhanced by its reductive speciation to Eu^{2+} cannot be ruled out. In rice, a positive Eu anomaly has been found in the aerial plant parts, whereas HREE enrichment (e.g., Gd to Lu + Y) has been observed in grains. A positive Eu anomaly has also been found in rice roots suggesting Eu accumulation in plant nutrition fluxes (Liang et al. 2008). However, these results were not found in hydroponic studies performed by Martinez et al. (2018) on REE accumulation in rice plants. Whether or not Eu may be a Ca physiological substitute remains unknown.

Studies using biological enrichment indices have shown that the different enrichment patterns of REE may occur in different plant species and because of different growth environments. Fractionation of REEs with both variable valences (*i.e.*, Ce and Eu) and stable valences (*i.e.*, La and Lu) can be easily observed in plants owing to the combined effects of physical, chemical, and biological factors. REEs have a dual effect on the physiological and biochemical reactions in the plant development, Zeng et al. (2003) showed that Eu^{3+} could replace Ca^{2+} in plants (e.g. *Amaranthus caudatus*) and promote Ca transport across plasma membrane. Therefore, possibly some Ca, which plays a key role in plant cellular walls and membranes, might have been replaced by Eu^{3+} . The concentration of free and active Ca^{2+} in the cytoplasm of plant cell must remain low and is controlled by the precipitation of oxalate crystals which allows the storage of biologically inactive Ca and probably also of Eu in older plant organs.

A false europium positive anomaly?

The presence of a major positive Eu anomaly, as shown in Figure 1a, is highly suspicious and is caused by an analytical artifact due to insufficient correction for the BaO interference. This analytical quality of some of the literature that report positive Eu anomalies in plant material samples should be scrutinized, especially if information on any REE pre-concentration method; previously shown to have a significant positive impact on isobaric interferences (Zhu and Itoh 2021); is not provided in the Materials and Methods and suggests that we cannot rule out Ba oxide interference. As we have emphasized, Eu analysis in low-REE samples suffer from important interferences of BaO on the Eu signal, often leading to false positives in normalized patterns. Barium is ubiquitous in soils and all plants contain a small quantity of it usually in concentrations around 4 to 50 $\mu\text{g}/\text{kg}$ dry weight of plants (Chaudhry et al. 1977). If Ba concentrations are not reported, it is impossible to know whether this interference may have occurred. The authors should make sure that the Eu-signal is real (rule of thumb: $\text{Ba}/\text{Eu} < 1000$), and that the reported Eu values are not a false positive (Shabani et al. 1992). The Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) method has gained in sensitivity over recent years (routinely low parts per million *i.e.*, $\mu\text{g}/\text{g}$), and does not suffer from the same problematic Eu interferences, although other spectral interferences may still occur, it offers the opportunity to cross validate detection issues (Amaral et al. 2017; He et al. 2017). Such detail procedural descriptions are not usually written in the methods section.

Concluding remarks

In summary, the Eu anomaly may be reported because of an analytical artifact in ICP-MS analysis. This error can be avoided by using isobaric corrections considering Ba and alternative detection strategies are increasingly available. Not all studies using ICP-MS for REE analysis of soil and plant material samples provide comprehensive information on how

this error was avoided or dealt with. We draw attention to the possibility of this problem and recommend being cautious, recognizing its potential occurrence and to take appropriate measures during analysis. The use of material reference certified for REE (Gills 1995) may be part of the solution and making analytical data available considering FAIR principles (Pourret et al. 2020) another part. Further, we also propose that FAIR principles should be used to document laboratory methods. Detail procedures, including the failures in experiment, usually are not described sufficiently in common journal articles, due to the editorial guidelines. The usage of electronic laboratory notebook could be an option. The successes and failures would contribute to the development of knowledge base in REE tests in plants, including those related to Eu.

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