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Identification of new hardy ferns that preferentially accumulate light rare earth elements: a conserved trait within fern species

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Environmental context. Rare earth elements (REEs) are strategic metals and emerging contaminants, for which accumulation in plants is poorly understood. Plant-based solutions are needed to remediate REE contaminated sites. Therefore, we screened a collection of hardy ferns and identified new REE accumulator species that preferentially transferred light REEs (LREEs) to their fronds. This study is an important step towards identifying LREE remediators and understanding REE accumulation in plants.

Abstract. Rare earth elements (REEs) include the lanthanides plus yttrium and scandium, and can be split according to their atomic mass into light (LREEs) and heavy REEs (HREEs). The increasing demand for REEs is mainly driven by new technologies, and their current low recyclability has led them to become emerging contaminants. The identification of new REE accumulators may help in determining the REE transfer mechanisms and may result in interesting candidates for phytoremediation techniques. To that end, a collection of 49 hardy fern species, grown in REE-spiked substrate, were screened for their potential in REE accumulation. REE concentrations were very low in the fronds of all *Polypodium* species, whereas all *Athyrium* species highly accumulated REEs. The REE accumulation level was more variable among the different species of *Dryopteris*, *Blechnum*, *Woodwardia*, *Cystopteris* and *Polystichum*. However, whatever the species, LREEs were preferentially transferred to the fronds over HREEs. This conserved trait was independent of the availability of different REEs in the substrate and instead may arise from specific transfer systems in ferns for the two groups of REEs. Furthermore, a correlation between REE accumulation and Ca and Al was found, which suggested the existence of common uptake pathways. Altogether, these results are of great interest for phytoremediation purposes since appropriate species can be chosen according to the

area to be remediated, and they also provide new insights into a more in-depth characterisation of the underlying REE accumulation mechanisms in ferns.

Additional keywords: *Dryopteris*, lanthanides, REE-accumulation, REE-fractionation, yttrium.

Introduction

Land pollution by metals has been widely reported and is considered as a major environmental problem. The rare earth elements (REEs) are a group of 17 metals sharing similar chemical and geochemical properties. This group contains the 15 lanthanides, as well as yttrium and scandium. The light REEs (LREEs, lanthanum to europium) and the heavy REEs (HREEs, gadolinium to lutetium) can be distinguished within the 15 lanthanides according to their atomic mass and ionic radius (Cotton 2006). The demand for REEs and the associated extraction of these elements has increased significantly; because of their unique properties, these elements are used in green energy, new technology and medical applications (Long et al. 2010; Ciacci et al. 2015). Overall, the production of lanthanides increased from 90 000 t in 2000 (Hedrick 2003) to 130 000 t of oxides in 2015 (Gambogi 2018). REEs are also commonly used as crop fertilisers to stimulate growth and production, and as a dietary supplement for cattle (Tyler 2004). Furthermore, high concentrations of REEs can be found in soils, especially around mining areas, where concentrations can reach more than 2000 mg kg⁻¹, which is more than 10 times the global average soil concentration (Ramos et al. 2016). Associated with their mining and use, a crucial lack of recyclability for these elements currently exists (Binnemans et al. 2013; Ciacci et al. 2015). The recent substantial increase in the production and widespread use of REEs has begun to raise concerns about their impact on human and environmental health (Gonzalez et al. 2014; Zhuang et al. 2017). REE transfer throughout the ecosystem is now recognised as a potential problem because many studies have shown toxicological effects on bacteria, fungi, plants and animals (Rim et al. 2013; Pagano et al. 2015; Wakabayashi et al. 2016; Ikhlayel 2017). These observations are strong arguments for considering REEs as emerging pollutants.

Environmentally friendly phytoremediation solutions (i.e. phytoextraction) may help to reduce the high levels of REEs in soils through the use of REE-accumulating plants (Padmavathiamma and Li 2007; Liu et al. 2017). In general, the REE concentrations in plants range from 0.0011 (Lu) to 0.33 mg kg⁻¹ (Ce) (Djingova et al. 2001) and can reach up to 5 mg kg⁻¹ (Ozaki et al. 1997). Although REEs are not considered essential for plants, it has been widely shown that they can be taken up and transferred to their aboveground biomass in moderate concentrations. Similar to other metals, a few plant species have been reported to accumulate REEs. The REE concentrations in *Phytolacca americana* (pokeweed) and *Carya tomentosa* (mockernut) can reach 1040 and 859 mg kg⁻¹ respectively (Wood and Grauke 2011; Yuan et al. 2018). In addition to these two angiosperms, the majority of REE-accumulating plants belong to pteridophytes (Liu et al. 2017). The two species of fern that have the greatest capacity for REE accumulation are particularly

interesting. *Pronephrium simplex* can accumulate up to 1200 mg kg⁻¹ (Lai et al. 2005, 2006), while *Dicranopteris dichotoma* can contain up to 3300 mg kg⁻¹ (Ozaki et al. 1997; Wei et al. 2001; Shan et al. 2003). It has been suggested that high concentrations of REEs in ferns may help these species adapt better to changing environments (Liu et al. 2017). Other fern species have also been mentioned as REE accumulators. Such species include *Dryopteris erythrosora* (Ozaki et al. 1997), *Blechnum orientale* (Zhang et al. 2002; Liang et al. 2014) and *Athyrium yokoscense* (Ichihashi et al. 1992), and the REE accumulation trait present in these species was identified from plants growing in non-contaminated environments. However, the number of REE-accumulating plants is limited to a relatively small number of species, and the potential of REE accumulation by ferns in a contaminated or enriched environment has rarely been studied so far.

The identification of new fern species that are able to accumulate REEs is of great interest, as it will allow for the selection of appropriate species adapted to a prospected area (soil, climatic conditions), for the determination of their use in environmental restoration and for the purpose of phytoextraction. Plant screening for new metal-accumulating species has already been done for several metals, including Cr (Shahandeh and Hossner 2000), As (Zhang et al. 2014), Pb (Salazar and Pignata 2014) and Cd (Wu et al. 2018). To identify new species that accumulate REEs and also to determine if this trait is conserved among certain genera, we have carried out a screening of fern species to determine their potential for REE accumulation. Unlike previous studies carried out under non-contaminated conditions (Ichihashi et al. 1992; Ozaki et al. 1997), in the present study, an extensive set of 49 fern species belonging to 15 different genera were screened by using soil enriched with REEs. This approach allowed us to identify new species of ferns accumulating REEs. In addition, unlike plants harvested in mining areas contaminated with LREEs, our experiment provided equimolar concentrations of specific LREEs (La, Ce and Sm) and HREEs (Gd, Yb and Y) so that the fractionation patterns in the fronds could be compared. The present study contributes to determining the global pattern of REE accumulation in plants and demonstrates that LREE preference is widely shared among ferns, which results from plant uptake or transfer mechanisms instead of REE availability in the substrate. The dichotomy between LREE preference in ferns and HREE preference in distant angiosperms, such as pokeweed or wheat, reveals the probable distinct underlying molecular mechanisms.

Experimental

Plant species, substrate preparation and growth conditions

Forty-nine fern species belonging to 15 different genera were used in the present study. The plants were purchased from a nursery specialising in hardy ferns, and the ferns were all grown with the same substrate at the nursery. The plants were first removed from their substrate and subsequently grown in an REE-spiked

substrate (peat 85 % and perlite 15 % mixture, with a fine granulation, particle size 1–7 mm: ~80 %, particle size 7–10 mm: ~20 %; data obtained from the supplier Gramoflor GmbH & Co, Germany).

The substrate was spiked with a total of 2000 mg kg⁻¹ REEs (333 mg kg⁻¹ of La, Ce, Sm, Gd, Yb and Y). Hexahydrate salts of LaCl₃, CeCl₃, SmCl₃, GdCl₃, YbCl₃ and YCl₃ (Sigma Aldrich, Saint-Quentin-Fallavier, France) were used. An REE solution was sprayed onto a thin layer of substrate (1 cm) before a thorough hand mixing to ensure proper homogenisation of the contamination. The REE-spiked substrate was then placed into 3.4-L plastic pots (20 × 15 × 15 cm). This REE-spiked substrate was used for both the first and confirmation screens.

Plants were grown under controlled conditions in a phytotron chamber (18/23 °C, 12/12 h dark/light cycles (260 μmol m⁻² s⁻¹)) and watered twice a week. The aboveground biomass was harvested after 6 months of exposure. Fronds were thoroughly rinsed with deionised water to remove potential soil contamination. The samples were dried at 70 °C for two days and used for subsequent elemental analyses.

Metal bioavailability determination

The CaCl₂-extractable fraction of REEs in the substrate was obtained according to the following procedure. After plant growth, for each replicate (i.e. three pots per species), the substrate was removed from the pot and aliquots were randomly collected at five different locations of the substrate. Three grams of the 2-mm-sieved substrate were dried at 60 °C for 48 h and incubated with 50 mL of 10 mmol L⁻¹ calcium chloride under agitation (40 rpm) for 2 h at room temperature. The mixture was first filtered with ash-free filters, subsequently filtered at 0.45 μm and acidified with HNO₃ before inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Elemental analysis

The quantification of elements was performed from 250 mg of dried plant powder or from the acidified CaCl₂-extractable fraction obtained as described above. For plant samples, mineralisation was carried out using 3.5 mL of nitric acid and 1 mL of hydrogen peroxide, and samples were incubated in a heating block digestion system (DigiPREP, SCP Sciences, Courtaboeuf, France). A gradual heating mode was used to achieve a final temperature of 100 °C (total run time of 265 min). Then, ultrapure water (Millipore Milli-Q Integral 3 system, Molsheim, France) was added to a final volume of 25 mL, and the samples were filtered through a 1-μm mesh. REE concentrations were determined by using an ICP-MS instrument (X Series II Model, Thermo Fischer Scientific, Courtaboeuf, France) and other elements were analysed by an inductively coupled plasma atomic emission spectroscopy (ICP-AES) instrument (Radial ICAP 6500 Model, Thermo Fischer Scientific, Courtaboeuf, France). For plant samples, oriental basma tobacco leaves (INCT-OBTL-5, LGC Promochem, Molsheim, France) were used as a certified reference material. For CaCl₂-extracted

substrate solutions, a surface water–trace metal standard (SPS-SW1, LGC Prochem standards, United Kingdom) was used as a certified reference material. Although only six REEs were added to the substrate, all 17 REEs were analysed by ICP-MS in the bioavailable fraction. Since their concentrations were very low (more than 15 (for Dy) to 8000 (for Tm) times lower than the concentrations of the 6 REEs added), they were not considered in this study (data not shown). In the following sections, for simplicity, the term ‘REEs’ will stand for the sum of the six REEs that were used.

Statistical analyses

One-way ANOVA and Tukey HSD post hoc tests were used when normality of data (Shapiro-Wilk test) and homoscedasticity (Levene’s test) conditions were met. When at least one condition was rejected, the data were subsequently analysed using a one-way nonparametric Kruskal–Wallis test followed by Dunn’s test. The rejection level was set at 5 % in all analyses. Correlation analyses were performed using the R package Corrplot. All analyses were performed using R software (version 3.4.1). All reported values are means \pm standard deviation (s.d.).

Results

Identification of new REE-accumulating ferns

Ferns were grown for six months and the REE concentrations in fronds were analysed by ICP-MS. Of the 49 species tested, three main REE accumulation patterns were observed. REE concentrations were very low ($<10 \text{ mg kg}^{-1}$ dry weight (DW)) in 10 species, 24 accumulated moderate levels between 10 and 100 mg kg^{-1} REEs, and 15 species accumulated more than 100 mg kg^{-1} REEs in their fronds (Fig. 1). Of the ferns accumulating over 100 mg kg^{-1} , only three reached more than 200 mg kg^{-1} (Fig. 1). This was the case for *Athyrium filix-femina* var. *Victoriae*, *A. othophorum* and *Onoclea sensibilis*. At the genus level, three REE accumulation patterns were observed. REE concentrations were very low ($<10 \text{ mg kg}^{-1}$) in all *Polypodium* species, whereas all *Athyrium* species accumulated high amounts of REEs (ranging from 130 to 220 mg kg^{-1}) (Fig. 1). The REE accumulation level was more variable among the different species of the other genera. In *Dryopteris*, large disparities were measured between species (Fig. 1). Two species accumulated less than 10 mg kg^{-1} (*D. carthusiana* and *D. filix-mas*), while the other species accumulated between 23 (*D. cycadina*) and 100 (*D. marginalis*) mg kg^{-1} REEs. The known REE-accumulating species *D. erythrosora* accumulated up to 175 mg kg^{-1} . Similar to *Dryopteris*, but to a lesser extent, *Blechnum*, *Woodwardia*, *Cystopteris* and *Polystichum* showed varying levels of REE accumulation.

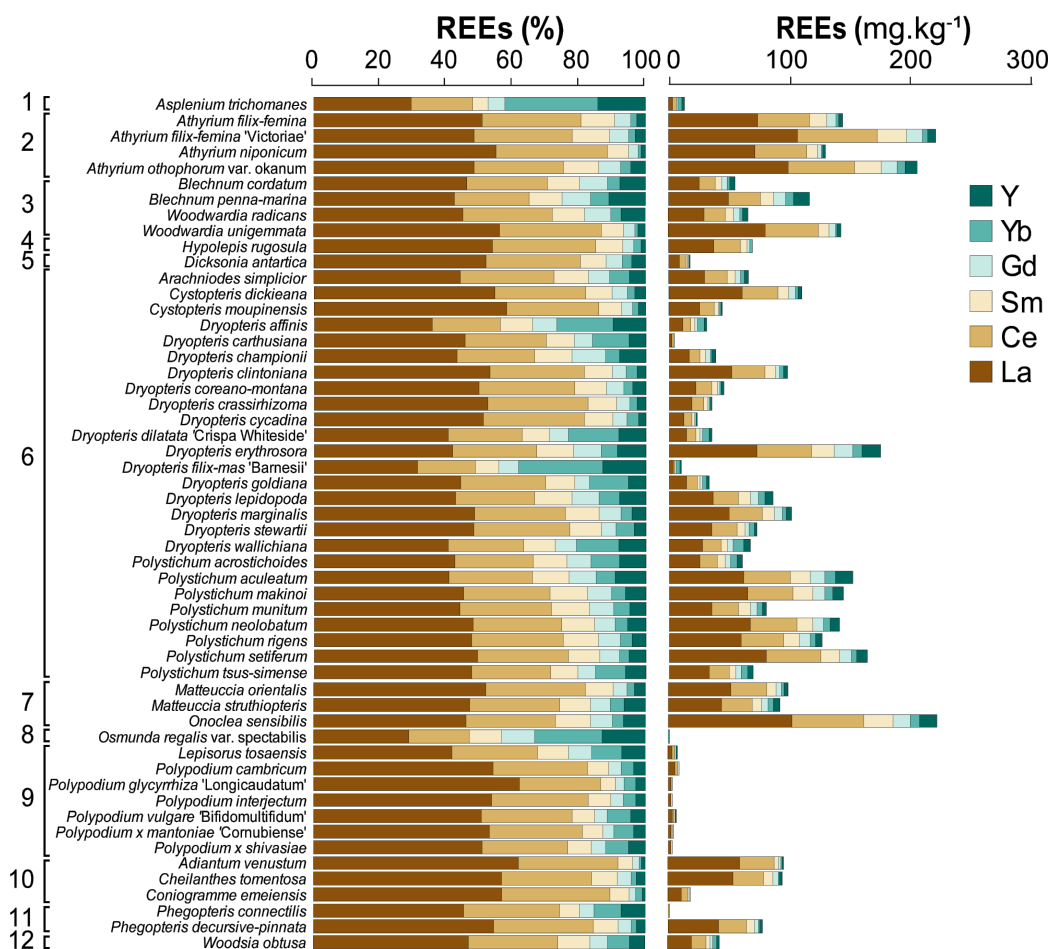


Fig. 1. Screening for accumulation potential of six REEs among fern species. On the left, the accumulation pattern of the six REEs in the fronds of 49 species is expressed as a percentage of the various REEs (La, Ce, Sm, Gd, Yb and Y) on the total of the six REEs accumulated. Species are organised by family: (1) *Athyriaceae*, (2) *Blechnaceae*, (3) *Dryopteridaceae*, (4) *Onocleaceae*, (5) *Polypodiaceae*, (6) *Pteridaceae* and (7) *Thelypteridaceae*. On the right, the accumulation of the six REEs in fern species is expressed as mg kg^{-1} DW of the above ground biomass. For the first screening, a single plant was tested for each species.

In the primary screening, we tested 49 species of hardy ferns. The species tested belonged to seven families, namely, *Athyriaceae*, *Blechnaceae*, *Dryopteridaceae*, *Thelypteridaceae*, within which some species have been previously shown to accumulate REEs (Liu et al. 2017), as well as *Onocleaceae*, *Polypodiaceae* and *Pteridaceae* (Fig. 1). However, none of the 49 species have been previously tested for their REE accumulation capacity, with the exception of *Dryopteris erythrosora*, which has been reported as an REE-accumulating species (Ozaki et al. 2000). The second screening included 14 species, which represented 28 % of the ferns tested in the first screening. This second screening was carried out in triplicate to confirm the results obtained from the first screening. We focussed our attention on 4 genera (*Athyrium*, *Dryopteris*, *Polypodium* and *Polystichum*) to represent different accumulating patterns (Fig. 2a). This second dataset confirmed the first screening, with *Athyrium filix-femina* and *A. niponicum* being species which strongly

accumulated REEs, whereas the *Polypodium* species proved to be poor REE accumulators. Finally, a significant variation in REE concentrations was again observed among *Dryopteris* species (Fig. 2a).

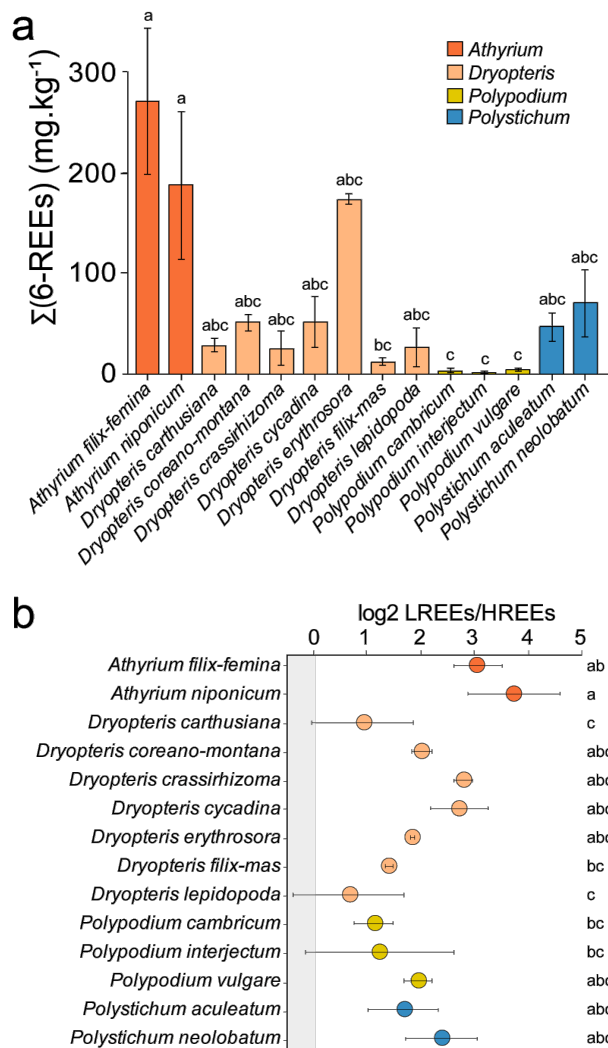


Fig. 2. Concentrations and fractionation of REEs in aboveground parts of ferns in the second screening. (a) Total concentrations of the six REEs in fern species harvested at the second screening ($n = 3$, mean \pm s.d.). (b) REE fractionation in fronds. Log₂ ratio of LREEs (La, Ce, Sm) to HREEs (Gd, Yb, Y) are given ($n = 3$, mean \pm s.d.). Bars topped by the same letter do not differ significantly between species ($P < 0.05$, ANOVA followed by the Tukey HSD, or Kruskal–Wallis followed by the Dunn test).

Preferential accumulation of LREEs

To investigate whether preferential accumulation of different REEs in the fronds of these ferns occurred, we analysed the fractionation pattern of REEs in different species. Interestingly, a similar behaviour was observed among all fern species studied in the first and second screenings (Fig. 1, Fig. 3). LREEs accumulated more than HREEs and accounted for between 70 % and 90 % of the total REE content (Fig. 1,

Fig. 3), with the exception of *Dryopteris filix-mas* and *D. affinis*, which accumulated a little less LREEs (Fig. 1). This general LREE enrichment in ferns was confirmed during the second screening from the LREE/HREE ratios (Fig. 2b). The two *Athyrium* species accumulated 8 times more LREEs than HREEs, while in the *Polystichum* and *Polypodium* species, these ratios were 4 and 2 respectively (Fig. 2b). In *Dryopteris* species, the LREE/HREE ratios ranged from 2 (*D. lepidopoda*) to 8 (*D. crassirhizoma*). The highest REE-accumulating species of *Dryopteris* (*D. erythrosora*) had a ratio of 4 (Fig. 2b). In general, higher concentrations of La were found in all species, followed by Ce, Sm, Gd, Y and Yb (Fig. 3). However, in *Polypodium* species, Yb was more abundant than Gd and Sm, which was similar to some *Dryopteris* species (Fig. 1, Fig. 3c-d).

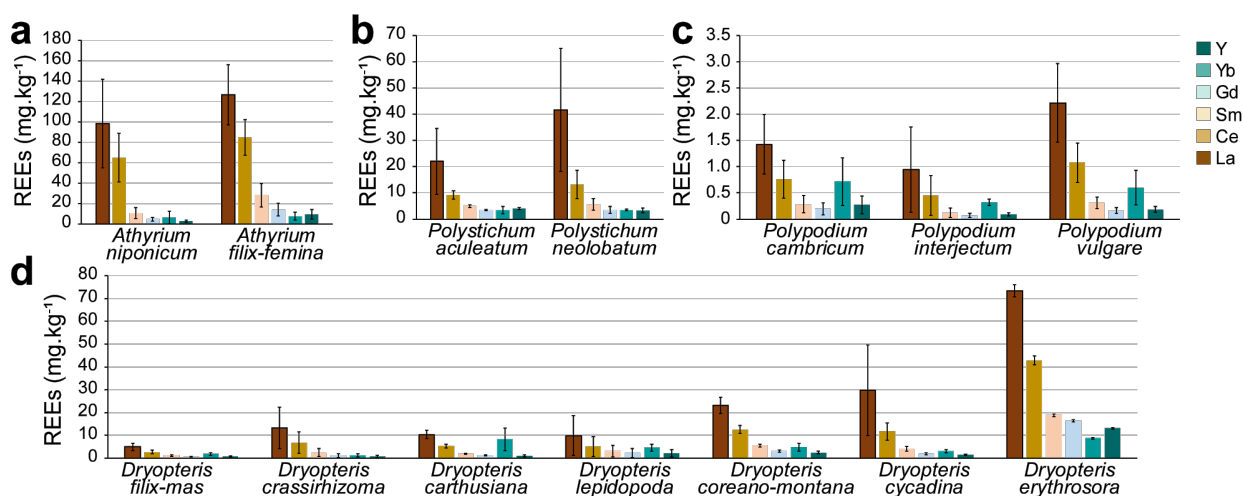


Fig. 3. Detailed concentrations of the six REEs in selected ferns in the second screening. REE concentrations in (a) *Athyrium*, (b) *Polystichum*, (c) *Polypodium* and (d) *Dryopteris* species. Plants were harvested after 6 months of growth in REE-contaminated substrate ($n = 3$, mean \pm s.d.).

Since this preferential accumulation of LREEs could ensue from a higher bioavailability of LREEs compared with that of HREEs, we analysed the pH and the REE concentrations in the CaCl_2 -extractible fraction of the substrate from the pots of an REE-accumulating species (*D. erythrosora*) and a non-accumulating species (*D. filix-mas*) (Fig. 4a). First, the substrate of the two species had very close pH values of 5.71 ± 0.11 for *D. filix mas* and 5.78 ± 0.06 for *D. erythrosora* (not significantly different). Second, the total REE extractible fraction was very low ($<1 \text{ mg kg}^{-1}$) in comparison to the total amount provided (2000 mg kg^{-1}). Only 0.05 to 0.25 mg kg^{-1} of each REE was retrieved in this fraction. Overall, the sum of the six REEs added was 0.53 mg kg^{-1} and 0.63 mg kg^{-1} for *D. filix mas* and *D. erythrosora* respectively in the bioavailable fraction, while the corresponding non-contaminated planted pots contained 0.030 mg kg^{-1} of these six REEs (data not shown) i.e. ~ 20 times lower. Moreover, the 10 other REEs naturally present in

the substrate (contaminated or not) accounted for a total composition of between 0.0036 mg kg⁻¹ and 0.0057 mg kg⁻¹ (data not shown), being 147 and 110 times lower than the six REEs added. Thus, the bioavailable REE-background of the substrate was very low and nearly all of the REEs bioavailable for the plants arose from the six REEs added. The REE extractible fraction did not vary significantly between the two species of fern. However, the extractible fraction of Yb and Y were similar and were ~3 to 4 times higher than that of the four other REE species (La, Ce, Sm and Gd) (Fig. 4a). These results led us to determine the REE bioaccumulation factors for the two plants. The values for this parameter were much higher for *D. erythrosora* compared with *D. filix-mas*, especially for La, Ce, Sm and Gd (Fig. 4b). The values decreased in the order of La > Ce > Sm > Gd > Y > Yb in the two species, with the exception of Yb in *D. filix-mas*.

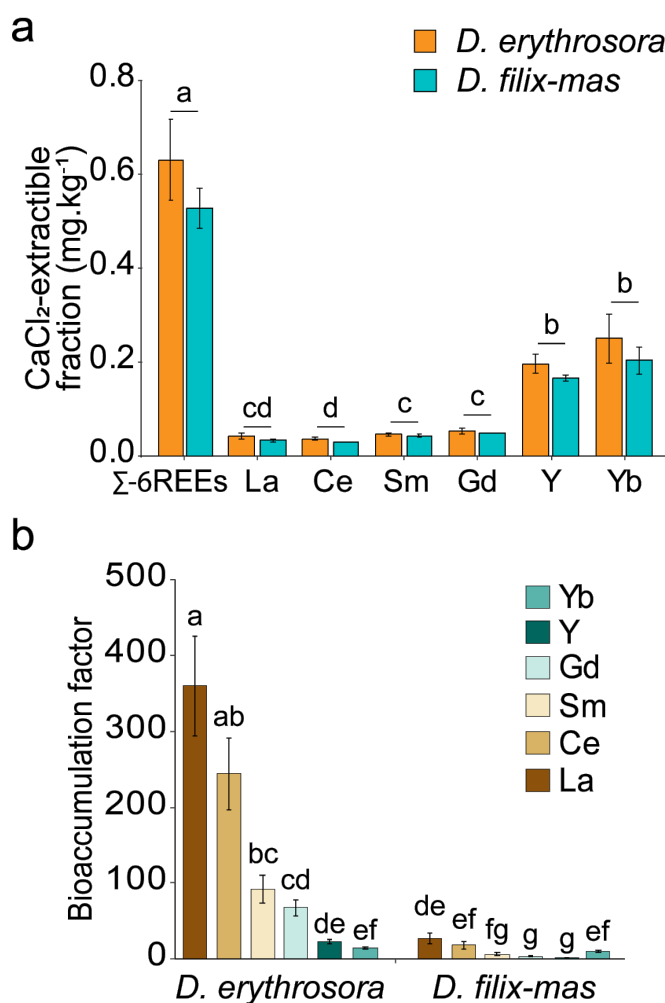


Fig. 4. (a) CaCl₂-extractible fraction and (b) bioaccumulation factor of REEs of two contrasted *Dryopteris* species. (a) CaCl₂-extractible fraction of the REEs in the substrate enriched with 2000 mg kg⁻¹ REEs (333 mg kg⁻¹ of 6 REEs). Σ(6-REEs) represents the sum of the six REEs. (b) Bioaccumulation factor (ratio of the accumulation in fronds over the extractible fraction in the substrate) of *D. erythrosora* and *D. filix-mas* ($n = 3$, mean \pm s.d.). Different letters denote significant differences between the REE species ($P < 0.05$, ANOVA, Tukey HSD).

Padmavathiamma and Li 2007; Liu et al. 2017). Relatively few plant species accumulating REEs are currently known; however, most of them are ferns (Ichihashi et al. 1992; Ozaki et al. 1997; Wei et al. 2001; Zhang et al. 2002; Shan et al. 2003; Lai et al. 2005, 2006; Liang et al. 2014), which could represent a yet unexplored reservoir of REE accumulators. Therefore, we screened a large panel of 49 fern species, all hardy and ornamental ferns, on a substrate spiked with six REEs (La, Ce, Sm, Gd, Yb and Y). The screening of hardy and ornamental plants has several advantages (Wu et al. 2018). For example, the use of non-edible plants restricts their entry into the food chain (Liu et al. 2008; Jelusic and Lestan 2015; Wu et al. 2018). Moreover, associated with their hardiness, these plants can be grown over several seasons and in diverse environments and climates. Furthermore, greater cultivation skills and knowledge of such plants has been developed, which help in their phytoremediation application (Liu et al. 2008; Jelusic and Lestan 2015; Wu et al. 2018).

Unlike previous studies (Ozaki et al. 1997), the present work is the first screening of ferns grown on an REE contaminated substrate to determine their ability to accumulate REEs. Using this screening strategy, we identified new REE-accumulating species. Of these, the species that accumulated the most ($>200 \text{ mg kg}^{-1}$) were *Onoclea sensibilis*, followed by two species of *Athyrium*, namely, *A. othophorum* and *A. filix-femina* ‘Victoriae’. The already known REE-accumulating species *D. erythrosora* ranked fourth, with more than 175 mg kg^{-1} accumulated in its fronds. *Athyrium yokoscense* (Ichihashi et al. 1992) has been previously reported as an REE-accumulating fern, thus it was noteworthy to find that other *Athyrium* species were also able to accumulate REEs. Similarly, two *Blechnum* species were able to accumulate moderate amounts of REEs in our study (55 mg kg^{-1} and 115 mg kg^{-1} for *B. cordatum* and *B. penna-marina* respectively); so far, only a single species, *B. orientale*, has been reported in the literature (Zhang et al. 2002; Liang et al. 2014). In addition, we identified here, for the first time, new plant REE accumulators belonging to other fern genera, including several *Polystichum* species, *Onoclea sensibilis* (Onocleaceae) and *Woodwardia unigemmata* (Blechnaceae), all accumulated more than 100 mg kg^{-1} REEs in their fronds.

In several cases, different species of the same genus have a similar accumulation potential. This was particularly true for the *Athyrium* species, which accumulated high REE concentrations, in contrast to the *Polypodium* species that had very low REE concentrations. A possible explanation could be a conserved mechanism of REE uptake and/or translocation in *Athyrium* that could be lacking in *Polypodium* species. Such a conserved trait for metal accumulation has been reported for *Carya* species that all accumulate REEs in their shoots (Wood and Grauke 2011) and also for Ni hyperaccumulation in several species of *Alyssum* and *Cochlearia* (Krämer 2010). Conversely, it was interesting to note the differences in REE accumulation between different species of a given genus. This was notably the case for *Dryopteris*: *D. erythrosora* was the highest accumulator in this genus, and *D. filix-mas* was the lowest accumulator in this genus. This

variation in REE accumulation capacities between *Dryopteris* species may be correlated with the presence of specific mechanisms of REE absorption and/or translocation, which are not common to all *Dryopteris* species. A greater REE accumulation capacity was related to a higher ploidy level in *Carya sp* (Wood and Grauke 2011). These species native to xeric habitats could benefit from REE absorption since these elements might be involved in drought tolerance. This hypothesis also fits with the ‘low resource requirement hypothesis’ according to which polyploids exhibit lower growth rate and a higher anti-oxidant capacity to result in a better stress resistance (Deng et al. 2012). Therefore, plants with higher ploidy levels may be able to better cope with REEs compared with diploids. However, this was not the case in our study, since the REE accumulator *D. erythrosora* is triploid (Hori et al. 2016) and the non-accumulator *D. filix mas* is tetraploid (Sessa et al. 2012). This suggests the existence of other mechanisms involved in the observation of the REE accumulation trait in ferns, not linked to the ploidy level but possibly related to higher expression levels of non-specific REE-uptake and transfer systems.

A preferential accumulation of LREEs has been previously reported in REE-accumulating ferns (Ozaki et al. 1997; Wei et al. 2001; Wang et al. 2005). However, those studies were based on samples taken from ferns grown naturally in mining areas that were highly enriched in LREEs. Indeed, in these areas, LREEs are usually 3 to 60 times more abundant than HREEs, which result in an obvious bias when comparing the accumulation pattern of LREEs versus that of HREEs. To circumvent this bias in the present study, we exposed ferns to equal amounts of each REE. Nevertheless, we observed a preferential accumulation of LREEs (La, Ce, Sm) in fronds compared with HREEs (Gd, Yb, Y), which ranged from 2 to 8 times more LREEs than HREEs. More specifically, REE accumulation increased with the increase in ionic radii of the REE species (La > Ce > Sm > Gd > Y > Yb). This preferential accumulation of LREEs is unlikely to result from their bioavailability, as Yb and Y were 3 to 4 times more extractible than La, Ce, Sm and Gd. Moreover, although the bioaccumulation factors were much lower for the non-accumulator *D. filix mas*, the same trend was observed and LREEs were also slightly more bioaccumulated. These statements support the hypothesis that an LREE import and transfer mechanism would be exacerbated in the REE-accumulating species and that it would also exist to a lesser extent in the non-REE-accumulating species. In species other than ferns, under equal amounts of each REE, the opposite pattern has been observed, with a preferential accumulation of HREEs, as shown in pokeweed (*Phytolacca americana*) (Yuan et al. 2017), wheat (*Triticum aestivum*) (Ding et al. 2005, 2006) and soybean (*Glycine max*) (Ding et al. 2007).

The difference in REE fractionation between pteridophytes and angiosperms suggests various mechanisms of root-to-shoot transfer and selective enrichment. It has been shown that in wheat and soybean grown in hydroponic culture, the HREE enrichment observed in the leaves was mostly to the result of a larger transfer of HREEs complexed with organic ligands when compared with free LREE ions (Liang et

al. 2008). Indeed, it appears that there is an increase in the stability of organic acid-REE complexes with increasing atomic number (Suzuki et al. 1980; Cantrell and Byrne 1987; Cheisson and Schelter 2019). Thus, free LREE ions would be more likely to adsorb to cell walls or precipitate (e.g. with phosphate) in the xylem. In *P. americana*, it has been suggested that asparagine, glutamate and histidine may be involved in the transfer of La and Y through the xylem (Wu et al. 2013). Similarly, Yuan et al. (2017) showed the important role of citrate in REE translocation in the same plant. In tomato, glutamate plays this role (Wu et al. 2009, 2013), whereas in wheat, REE translocation is mediated by citrate, malate and oxalate in the xylem sap (Ding et al. 2005, 2006). Overall, these data suggest the importance of several amino and organic acids in REE translocation in plants. However, future studies using transgenic lines with modulated amino or organic acid synthesis or transport are needed to fully validate these hypotheses.

In fern species, a different mechanism could be involved in the LREE enrichment observed in fronds. In *Dicranopteris dichotoma*, Wang et al. (2003) identified a low molecular weight LREE-binding peptide (Wang et al. 2003). Similarly, Lai et al. (2006) found another peptide specifically binding LREEs in the REE-accumulator fern *Pronephrium simplex* (Lai et al. 2006). These peptides may be involved in reducing LREE toxicity in plants. They may be ubiquitous in fern species that accumulate REEs and, therefore, could explain the LREE enrichment observed in pteridophytes. However, we cannot rule out a lower abundance of organic acids in fern species than in angiosperms, which precludes a relative HREE enrichment in fronds. In addition, angiosperms possess a xylem consisting of well-developed vessels and tracheids, while pteridophytes only have a tracheid-based xylem (Pittermann et al. 2011). Such differences in the water-conducting tissues, in particular in their structural composition (cell wall, organic ligands), could be a possible explanation for the enrichment of LREEs versus HREEs in the shoots of different plant lineages.

The ionome analysis showed that higher concentrations of several macro- and micro-elements were found in REE accumulating ferns. Indeed, the REE concentration was found to be positively correlated with most elements analysed, except for Mo and Cu. This positive correlation with Fe, as well as Al, Mg, K and P, has also been reported in beech, spruce and oak (Brioschi et al. 2013). In *Carya*, accumulation of Mg, Ca, Mn and Fe was also positively associated with the foliar concentration of REEs (Wood and Grauke 2011). In *D. erythrosora*, we also observed an increase in Mn content together with REE accumulation. Furthermore, putative shared entry pathways in the roots for REEs, Ca and Al ions have been suggested in *P. americana* (Yuan et al. 2017). These observations support the existence of common uptake pathways for REEs and essential elements.

Additionally, the availability of elements in soils is highly pH dependent. The availability of elements such as Al, Fe, Zn, Cu, B and Mn, as well as REEs, increases at low pH values, while the availability of elements such as Mo decreases at lower pH values (Fageria et al. 2004). The modified availability of these

elements arising from soil pH could explain the correlations observed between REEs and essential elements. [Wiche et al. \(2016\)](#) observed an increase in Fe, Mn and P content, as well as REEs, in barley and white lupine ([Wiche et al. 2016](#)). The authors suggested that these elements could result from the dissolution of REE-phosphates, carbonates and Fe-Mn oxyhydroxides under the influence of root exudates ([Wiche et al. 2016](#)). This hypothesis was confirmed by [Shan et al. \(2002\)](#), who demonstrated that the bioavailability of REEs in soil is affected by soil pH and by low molecular weight organic acids ([Shan et al. 2002](#)). In addition, they highlighted the involvement of organic matter, Fe-, Mn- and Al-oxides, on the adsorption and desorption behaviour of REEs. Therefore, local acidification, as well as release of root exudates by the plants into the rhizosphere, could contribute to the increased availability and subsequent uptake of essential elements, together with REEs, by plants. A similar REE concentration pattern has also been observed between roots of *D. dichotoma* and soluble REEs in the soil ([Wei et al. 2001](#)). This similarity emphasises that soil-soluble REEs can be closely inter-related with the REE uptake by plants ([Liu et al. 2017](#)). However, in the present study, we did not find any difference in the CaCl₂-extractible fraction of REEs in the substrate of the REE accumulating (*D. erythrosora*) and the non-REE accumulating (*D. filix-mas*) species. Also, the pH of the substrate for the two species was similar, at least in the bulk substrate, which indicated that there was no strong difference in the acidification potential between the two species. Further investigation will be needed to better understand the contrasting behaviour of these two species in term of accumulation of REEs and other elements.

Conclusion

The increasing use and spread of REEs have encouraged the scientific community to find environmentally friendly remediation techniques, such as phytoremediation, and to better understand the REE transfer mechanisms in plants. In this study, we identified new fern species that can accumulate REEs in their fronds. The high REE accumulation capacity identified in many species suggests a common putative trait of REE accumulation in ferns, as reported for the angiosperm genus *Carya*. We also report a close relationship between REE accumulation and the homeostasis of essential elements in ferns, which supports either the common uptake pathways and/or the behaviours in soils. Further metabolomic analyses of root exudates and the recognition of molecular determinants, such as transporters to be identified through comparative transcriptomics, will be needed to identify the putative ligands or proteins underlying the REE-accumulation trait. It will be particularly interesting to reveal the genetic and physiological determinants for LREE enrichment in pteridophytes versus HREE enrichment in angiosperms. Finally, the conservation of the LREE accumulation trait among these different fern species is of great interest for phytoremediation purposes since appropriate species can be chosen according to the area to be remediated. In the future, as it has already been done for other metal accumulating plants ([Visioli et al. 2015](#)), the isolation of rhizospheric

and endophytic microorganisms (bacteria, fungi) will enable the establishment of microorganism-assisted phytoremediation. The inoculation of REE-accumulating plants with such organisms could improve the accessibility of nutrients to plants (Rengel and Marschner 2005) and, at the same time, increase the efficiency of REE phytoextraction.

Supplementary material

Table S1: REE concentrations (mg kg⁻¹ DW) in the fronds of ferns exposed to 2000 mg.kg⁻¹ REEs and grown for 6 months for the second screening (mean ± SD, n= 3).

Genus	species	Sample name	REE	La	Ce	Sm	Gd	Yb	Y
<i>Polypodium</i>	<i>cambricum</i>	Pca	3,63 ± 1,80	1,47 ± 0,57	0,77 ± 0,38	0,30 ± 0,17	0,17 ± 0,12	0,70 ± 0,44	0,30 ± 0,17
<i>Polypodium</i>	<i>interjectum</i>	Pin	2,00 ± 1,31	0,93 ± 0,81	0,47 ± 0,40	0,10 ± 0,10	0,07 ± 0,06	0,33 ± 0,06	0,10 ± 0,00
<i>Polypodium</i>	<i>vulgare</i>	Pvu	4,57 ± 1,63	2,23 ± 0,71	1,07 ± 0,40	0,27 ± 0,12	0,13 ± 0,06	0,60 ± 0,35	0,23 ± 0,06
<i>Polystichum</i>	<i>aculeatum</i>	Pac	46,8 ± 13,6	22,0 ± 12,5	9,17 ± 1,53	4,97 ± 0,50	3,33 ± 0,21	3,37 ± 1,37	4,00 ± 0,46
<i>Polystichum</i>	<i>neolobatum</i>	Pne	70,5 ± 32,8	41,6 ± 23,4	13,1 ± 5,41	5,57 ± 2,17	3,50 ± 1,23	3,47 ± 0,25	3,30 ± 0,89
<i>Athyrium</i>	<i>niponicum</i>	Ani	187 ± 72,9	98,2 ± 43,1	64,8 ± 23,8	10,5 ± 5,28	4,50 ± 1,87	6,33 ± 6,06	2,80 ± 1,06
<i>Athyrium</i>	<i>filix-femina</i>	Afi	270 ± 72,5	127 ± 29,5	84,7 ± 17,5	28,1 ± 11,6	14,0 ± 6,12	7,47 ± 3,83	9,20 ± 4,50
<i>Dryopteris</i>	<i>crassirhizoma</i>	Dcr	25,9 ± 17,5	13,3 ± 9,05	6,85 ± 4,74	2,50 ± 1,70	1,15 ± 0,78	1,20 ± 0,71	0,75 ± 0,49
<i>Dryopteris</i>	<i>coreano-montana</i>	Dco	51,6 ± 8,30	23,2 ± 3,58	12,5 ± 1,71	5,57 ± 0,61	3,03 ± 0,47	4,87 ± 1,72	2,47 ± 0,51
<i>Dryopteris</i>	<i>cyadina</i>	Dcy	52,0 ± 25,4	29,8 ± 19,8	11,7 ± 3,78	4,10 ± 1,00	1,93 ± 0,47	3,03 ± 0,72	1,43 ± 0,29
<i>Dryopteris</i>	<i>filix-mas</i>	Dfi	12,1 ± 3,59	4,97 ± 1,62	2,70 ± 0,82	1,17 ± 0,31	0,67 ± 0,12	1,87 ± 0,64	0,80 ± 0,17
<i>Dryopteris</i>	<i>lepidopoda</i>	Dle	27,3 ± 19,0	9,97 ± 8,62	5,20 ± 4,37	3,07 ± 2,61	2,30 ± 1,97	4,60 ± 1,50	2,17 ± 1,63
<i>Dryopteris</i>	<i>carthusiana</i>	Dca	28,3 ± 6,63	10,4 ± 1,78	5,30 ± 0,82	2,00 ± 0,26	1,17 ± 0,25	8,33 ± 4,94	1,07 ± 0,40
<i>Dryopteris</i>	<i>erythrosora</i>	Der	173 ± 5,50	73,5 ± 2,65	42,8 ± 1,90	18,8 ± 0,53	16,3 ± 0,42	8,78 ± 0,34	13,1 ± 0,29

Table S2: Ionome (macro- and micro-elements, mg kg⁻¹ DW) of the fronds of ferns exposed to 2.000 mg.kg⁻¹ REEs and grown for 6 months for the second screening (mean ± SD, n=3).

Genus	species	Sample name	Al	Mn	Fe	Ni	Cu	Mo	Zn	B	Ca	K	Mg	Na	P	S
<i>Polypodium</i>	<i>cambricum</i>	Pca	19,9 ± 24,7	59,5 ± 18,7	60,7 ± 27,9	0,30 ± 0,17	2,60 ± 0,17	4,83 ± 3,35	16,1 ± 1,62	48,3 ± 20,5	14721 ± 4548	19747 ± 7569	4246 ± 311	5546 ± 2906	1643 ± 252	1443 ± 212
<i>Polypodium</i>	<i>interjectum</i>	Pin	3,90 ± 6,75	30,1 ± 15,2	35,0 ± 10,5	0,00 ± 0,00	2,63 ± 0,42	1,20 ± 0,66	16,1 ± 4,18	35,1 ± 16,9	8622 ± 3786	14211 ± 6243	2718 ± 984	3583 ± 510	2690 ± 1183	1498 ± 309
<i>Polypodium</i>	<i>vulgare</i>	Pvu	0,00 ± 0,00	37,7 ± 8,70	39,8 ± 8,23	0,00 ± 0,00	3,67 ± 0,72	4,67 ± 1,67	24,0 ± 9,42	43,2 ± 9,24	11450 ± 2127	18393 ± 9751	3341 ± 301	3421 ± 1088	2652 ± 341	1954 ± 139
<i>Polystichum</i>	<i>aculeatum</i>	Pac	35,7 ± 7,04	22,6 ± 7,49	53,7 ± 6,06	0,43 ± 0,25	4,80 ± 1,01	0,10 ± 0,00	32,0 ± 6,92	30,6 ± 1,25	13254 ± 1674	12217 ± 3384	3241 ± 233	4105 ± 1709	2918 ± 408	3059 ± 236
<i>Polystichum</i>	<i>neolobatum</i>	Pne	47,3 ± 15,8	14,1 ± 5,30	39,7 ± 6,54	0,17 ± 0,15	3,17 ± 0,25	0,70 ± 0,20	32,5 ± 4,76	36,6 ± 2,67	13680 ± 1608	12649 ± 2815	3081 ± 171	1022 ± 155	1660 ± 544	1899 ± 306
<i>Athyrium</i>	<i>niponicum</i>	Ani	35,7 ± 12,2	19,4 ± 6,38	55,8 ± 4,44	0,40 ± 0,46	2,93 ± 0,75	0,37 ± 0,38	35,7 ± 5,51	68,6 ± 14,5	28068 ± 10929	24748 ± 15318	4552 ± 1627	15445 ± 6736	2181 ± 660	2258 ± 562
<i>Athyrium</i>	<i>filix-femina</i>	Afi	31,0 ± 13,6	47,0 ± 15,5	55,4 ± 14,7	0,23 ± 0,25	4,00 ± 1,35	0,53 ± 0,23	37,7 ± 17,9	44,7 ± 10,3	25200 ± 12104	17366 ± 7040	3959 ± 1808	8609 ± 6889	3505 ± 1271	2384 ± 1412
<i>Dryopteris</i>	<i>crassirhizoma</i>	Dcr	16,3 ± 8,84	39,8 ± 7,35	65,4 ± 5,73	0,25 ± 0,07	8,85 ± 5,02	0,10 ± 0,00	56,5 ± 8,63	34,5 ± 4,24	9818 ± 3236	35355 ± 16203	3624 ± 191	7007 ± 1078	3717 ± 915	3510 ± 773
<i>Dryopteris</i>	<i>coreano-montana</i>	Dco	35,3 ± 6,18	12,1 ± 3,72	62,2 ± 12,5	0,47 ± 0,21	5,17 ± 1,30	0,63 ± 0,15	33,9 ± 3,70	35,5 ± 3,11	16183 ± 2761	24716 ± 5434	4019 ± 621	5291 ± 2346	2948 ± 509	4324 ± 782
<i>Dryopteris</i>	<i>cyadina</i>	Dcy	35,0 ± 14,0	15,3 ± 8,30	61,2 ± 9,47	1,17 ± 1,27	3,93 ± 1,34	0,90 ± 0,62	32,6 ± 10,5	57,6 ± 6,92	17420 ± 4176	17105 ± 8917	3218 ± 515	5299 ± 1942	2444 ± 655	2404 ± 686
<i>Dryopteris</i>	<i>filix-mas</i>	Dfi	52,2 ± 21,6	19,7 ± 0,57	69,6 ± 12,0	0,07 ± 0,12	3,23 ± 0,31	0,40 ± 0,10	35,3 ± 3,74	51,4 ± 20,2	14284 ± 3373	19733 ± 4419	3972 ± 546	3620 ± 1217	1780 ± 126	3324 ± 295
<i>Dryopteris</i>	<i>lepidopoda</i>	Dle	27,8 ± 19,4	18,5 ± 5,04	42,1 ± 3,66	0,37 ± 0,21	5,63 ± 1,62	0,33 ± 0,25	40,2 ± 16,8	40,6 ± 13,2	15773 ± 3916	32046 ± 3587	4817 ± 1357	7124 ± 4405	4996 ± 1020	3663 ± 907
<i>Dryopteris</i>	<i>carthusiana</i>	Dca	15,4 ± 3,55	26,6 ± 10,9	53,7 ± 9,36	0,50 ± 0,30	4,17 ± 0,76	4,47 ± 0,93	32,5 ± 7,78	47,4 ± 1,04	16668 ± 2966	7024 ± 2072	7501 ± 892	2558 ± 1496	2330 ± 596	1769 ± 405
<i>Dryopteris</i>	<i>erythrosora</i>	Der	48,5 ± 15,3	33,4 ± 1,90	44,2 ± 2,33	0,56 ± 0,17	2,26 ± 0,81	1,23 ± 0,12	23,3 ± 5,55	34,5 ± 2,92	9484 ± 3302	28470 ± 3956	5751 ± 1489	3783 ± 1674	2168 ± 199	2650 ± 377

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions statement

The authors' responsibilities were as follows: NG, MLJ and DB designed the research; NG, MLJ and DB performed the research; NG analysed the data; NG, MLJ, MC, EMG and DB evaluated the data; NG, MLJ, MC, EMG and DB wrote the manuscript. All authors read and approved the final manuscript.

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