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Chelate-induced accumulation of rare earth elements in plants grown on soil and ash-based growing media

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Abstract. Phytoextraction is a phytoremediation technique that uses plants to remediate contaminated areas. The aim of the study was to investigate the differences between the use of two doses of chelate (5 mM and 10 mM): CA, EGTA, and EDTA on three selected plant species which grew on two substrates (soil with increased REE content, ash). The study focused on the following rare earth elements (REE) representatives: lanthanum (La), cerium (Ce), europium (Eu), and gadolinium (Gd). Three plant species were included in the study: common yarrow (*Achillea millefolium* L.), red clover (*Trifolium pratense* L.) and autumn fern (*Dryopteris erythrosora* (D.C.Eaton) Kuntze). The plant were grown on two substrates, the main components of which were soil with increased REE content and ash. Plant samples, divided into aboveground part and underground part, were analyzed by ICP-MS. The obtained REE concentrations in plant tissues ranged from 0.02 to 60.20 mg kg^{-1} (La), 0.05 to 62.22 mg kg^{-1} (Ce), 0.01 to 45.91 mg kg^{-1} (Eu), and 0.02 to 63.60 mg kg^{-1} (Gd). To determine the ability of plants to phytoextract REE, two factors were calculated: the translocation factor (TF) and the bioconcentration factor (BCF). The highest TF value was obtained for *D. erythrosora* and *A. millefolium*, when they were grown on substrate with ash. In the experiment, the BCF index value was not higher than 1. In general, the effect of chelates on REE accumulation was plant-specific. The application of CA resulted in the most efficient REE accumulation by plants.

Keywords: rare earth elements, phytoextraction, contaminated areas, chelate, phytoremediation, common yarrow, red clover, autumn fern

INTRODUCTION

Rare earth elements (REE) have a wide range of applications. They are used, among others, in industry, agriculture, modern technologies or so-called "green" technologies. As a result of their increasingly common use, they might be released into the environment in the form of waste. Due to the mining sector associated with the extraction of rare metals, their impact on the environment and soil pollution is increasing (Lima, Ottosen, 2021). The waste can be a secondary source of their transfer to the environment.

REE accumulation in soil can potentially create a risk of toxic effects on living organisms or even humans in certain cases. It has been shown that REE can negatively affect the level of brain intelligence, which can ultimately result in memory loss. REE can also enter the placenta and blood during pregnancy, which can lead to birth defects (Wu et. al., 2013; Adeel et al., 2019).

According to the International Union of Pure and Applied Chemistry (IUPAC), REE are a group of 15 lanthanides with atomic numbers from 57 to 71 and 2 scandiums with atomic numbers 21 and 39, namely lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu), scandium (Sc) and yttrium (Y) (Tao et al., 2022).

REE are not as rare in the Earth's crust as their name suggests. The most abundant element is Ce, since its average concentration is about 66.5 mg kg^{-1}. However, REE deposits are scattered in the Earth's crust in low concentrations (Dinh et al., 2022). Natural resources of REE are limited so these elements are treated as critical raw materials. Therefore there is an increasing interest in recovery of metals, including REE, from industrial waste such as ash or REE enriched soils.

In the case of plants, REE are accumulated in the order root > leaf > stem > flower > fruit. However, near polluted areas, the metal content on the leaf surface is higher due to dust deposition (Yin, 2021). Phytoextraction is a process where plants extract contaminants from soil or water, then transport and store them in aboveground tissues. The most effective plants for this purpose are hyperaccumulators. These are plant species that can accumulate larger amounts of metals in their aboveground parts without toxic effects on them. For REE hyperaccumulators, the threshold concentration is 100 to 1000 mg kg^{-1} (Dinh et al., 2022; Deepika, Haritash, 2023). This is an environmentally friendly and inexpensive method, useful in soil remediation, but the main limitation is that it is inefficient. Therefore, ways to improve the method are being sought (Zhang et al., 2024). Adding chelates in phytoremediation methods can accelerate metal removal from soil or waste. Chelating agents act as a chemical bond, which results in the formation of metal chelate complexes. These chelates contribute to the increased solubility and plant availability of metals (Wu et al., 2013; Salifu et al., 2024). It has been shown that chelators can increase the rate of absorption and translocation of metals by up to 45% from roots to aboveground plant parts in the process of phytoremediation. Both synthetic and natural compounds can be used as chelators. The synthetic ones include: ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA), and the natural ones: histidine, citric acid, malic acid and citrate (Beiyuan et al., 2021; Zulkernain et al., 2023). Chelators such as EDTA or amino-acids (AA) act by increasing the bioavailability of heavy metals and rare earth elements. Low molecular weight organic compounds, including malic acid, citric acid, histidine and citrates, are secreted by roots into the soil and then act as chelating agents enabling the release, translocation and accumulation of heavy metals (Rabbani et al., 2024). Chelator-induced solubility of metals can pose an environmental risk related to leaching of metals to groundwater. Therefore, it would be justified to perform such induced phytoextraction under controlled conditions, for example in containers without leakage of soil solution. Such a technology would enable use of constructed substrates, produced on a basis of industrial waste combined with some organic materials to improve plant growth conditions.

In order to fill some knowledge gaps, the aim of the study was to demonstrate the differences between the use of two doses of chelators (5 mM and 10 mM): CA, EGTA, and EDTA on three selected plant species which grew on two substrates. The plant species were used: *Achillea millefolium* L., *Trifolium pretense* L., *Dryopteris erythrosora* (D. C.Eaton) Kuntze). In this study, the following REE representatives were measured in plant tissues: lanthanum (La), cerium (Ce), europium (Eu), gadolinium (Gd). The substrates were used based on: soil with increased REE content and power plant ash.

MATERIALS AND METHODS

Experimental design

The pot experiment was conducted in pots in the greenhouse of the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy (Poland). The experiment was conducted under controlled conditions from the beginning of May to the end of September 2024.

Two substrate variants were used as media to grow the plants:

– soil enriched with $LaCl_3$, $CeCl_3$, $EuCl_3$ and $GdCl_3$ (substrate 1). All these elements were added to the soil as water solutions at rate 100 mg of an element per kg of soil. Additionally compost was added to the soil to improve plant growth conditions and to provide nutrients. The compost came from the GWDA company in Piła. It consisted of 30.2% organic matter and the pH was 6.2. It was produced on the basis of a mixture of sewage sludge and selectively collected green municipal, food and agricultural waste. The compost is certified as a soil improver. Soils had been left for 1 month after adding the REE and the compost to let them react with soil before the experiment was started. Total weight of substrate 1 in pot was 2 kg (95% soil and 5% compost).

– a substrate prepared on a basis of ash from a power plant located in Upper Silesia (substrate 2). Peat was added to the substrate to lower pH of ash and GWDA compost to further improve plant growth conditions. The peat added was a commercial product. The substrate had been left for 1 month after mixing ash with peat and compost to let them react. Total weight of substrate 2 in pot was 1.66 kg (30% ash, 50% peat, 20% compost). In substrate 2 the weight of the substrate was lower compared to substrate 1 due to the final volume of this substrate.

Three plant species were then grown in the pots: red clover (*Trifolium pratense* L.) and common yarrow (*Achillea millefolium* L.) were sieved and autumn fern (*Dryopteris erythrosora* (D.C.Eaton) Kuntz) was planted from seedlings. *T. pratense* is a plant belonging to the Fabaceae family, cultivated in most regions with a temperate climate in Europe and around the world. There are studies on the resistance of *T. pretense* to the accumulation of large amounts of heavy metals and other pollutants (Dluhosova et al., 2018; Cakaj et al., 2023; Zhou et al., 2024). *A. mille-*

folium (family: Asteraceae) is a perennial herbaceous plant that occurs almost all over the world. This plant can grow even in poor soils, if not very acidic, and does not have high requirements for temperature or moisture (Syso et al., 2016; Ali et al., 2017). According to the available literature, *D. erythrosora* (family: Dryopteridaceae) is a fern native to Japan, which under natural conditions can accumulate high concentrations of REE from the soil and can be considered as a natural REE hyperaccumulator (Ozaki et al., 2000; Yoo et al., 2017).

The chemical characteristics of the substrate components are presented in Table 1. The soil was alkaline (pH 7.1) but after mixing with compost and reacting pH dropped to 6.2. Soil carbon (C) content was low – 1.19%. The contents of REE of interest were as follows: 8.4 mg kg^{-1}, 18.5 mg kg^{-1}, 0.24 mg kg^{-1} and 1.47 mg kg^{-1} for La, Ce, Eu and Gd, respectively. The power plant ash used to prepare the substrate 2 was alkaline (pH 8.5) and it contained 20.6% of C. The ash was relatively rich in Fe (1.40%) and Al (1.56%) and it contained 798 mg Ba kg^{-1}, 893 mg Zn kg^{-1}, 11.2 mg Cd kg^{-1} and 117 mg Pb kg^{-1} to mention potentially toxic trace elements. The contents of REE in ash were as follows: 8.9 mg kg^{-1}, 18.7 mg kg^{-1}, 0.54 mg kg^{-1} and 1.91 mg kg^{-1} for La, Ce, Eu and Gd, respectively.

The final content of elements in the substrates is presented in Table 2.

One month after establishing plant growth, chelators were added to the soil as aqueous solutions. Three chelators were used in the experiment: citric acid (CA), egtazic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA). The chelators were used at two concentration levels: 5 and 10 mM per kg of soil. CA is classified as a low molecular weight organic acid, it is an easily biodegradable natural chelate. It has a high capacity to chelate heavy metals in the soil and due to the small size of its molecules it is absorbed by plant roots at a faster rate compared to EDTA (Ibrahim, 2023). EGTA is a chelate that is biodegradable and non-toxic to organisms. It has high efficiency, and when used in phytoextraction of heavy metals, it does not show negative effects on growth and yield (Mohrazi et al., 2023). EDTA is a non-biodegradable chemical compound that contributes to the increase of the solubility of heavy metals in the soil solution, which results in increased bioavailability of heavy metals and their uptake by plants. However, the use of EDTA as a chelator is associated with a risk to the environment due to the leaching of metals when applied to soil in situ (Poursattari, Hadi, 2022). Plants growing in substrates without the use of chelators were used as controls. The experiment was watered with distilled water according to current needs. The experiment was performed in triplicate.

Plant and soil analyses

After 5 months, the plants were cut and the underground parts were gently separated from the growing media. The

Table 1. Content of elements [mg kg^{-1}] and other chemical properties of the soil (before adding REE solutions) and the power plant ash.

Element	Soil	Power plant ash
Li	3.25	11.31
Be	0.19	0.83
Al	4967.47	15669.29
V	14.19	35.53
Cr	10.59	46.60
Mn	225.16	6313.97
Fe	7244.23	14030.51
Co	3.20	15.05
Ni	7.58	53.49
Cu	6.75	498.03
Zn	48.39	892.71
As	2.71	9.30
Se	0.19	1.08
Sr	1.08	36.12
Mo	0.24	3.69
Ag	0.14	4.77
Cd	0.24	11.17
Sn	0.04	22.68
Sb	0.04	16.48
Ba	31.73	797.73
La	8.42	8.90
Ce	18.52	18.65
Eu	0.24	0.54
Gd	1.47	1.91
Tl	0.04	0.39
Pb	12.91	117.42
Bi	0.09	6.29
Na	40.31	8769.68
Mg	941.25	10300.19
K	1768.18	18454.72
Ca	1167.80	130492.47
pH in H_2O	7.13	8.55
EC [µS cm^{-1}]	99	11080
Total nitrogen [%N]	0.09	0.12
Total carbon [%C]	1.19	20.60

Table 2. Contents of the tested REE: La, Ce, Eu and Gd [mg kg^{-1}] in the substrates used in the experiment.

Substrate	La	Ce	Eu	Gd
1 95% soil, 5% compost	103.55	107.52	90.01	100.54
2 30% ash, 20% compost, 50% peat	3.21	6.35	0.21	0.68

plants were divided to aboveground parts and undergrounds parts. For *A. millefolium*, the aboveground parts included: stem, leaves, inflorescence, while the underground parts included: tap roots and fascicle roots. On the other hand, for *T. pratense*, the aboveground parts included: shoots, leaves, flowers, and the underground parts: roots. For *D. erythrosora*, the aboveground parts included: leaves, and the underground parts included: rhizomes and adventitious roots. The samples were then thoroughly rinsed first in tap water and then in distilled water. Subsequently the samples were dried in an oven at 50 ºC for 2 days. Then the plants were weighed on a laboratory scale, the aboveground and underground parts separately. The dried samples were ground in an electric mill and subjected to further laboratory analyses. In order to determine the content of elements in the dry mass of plants 0.5 g of separately aboveground parts and underground parts were weighed to be digested in concentrated HNO_3 in Teflon PFA vessels in a microwave-accelerated reaction system (MarsXpress; CEM Corp., Matthews, NC, USA). The prepared liquid samples were analyzed using ICP-MS (Agilent 7500ce). As a certified reference material, soya bean flour (INCT-SBF-4) and mixed Polish herbs (INCT-MPH-2) were used. Then the samples were mineralized in a microwave oven.

The soil samples were dried in a dryer at 50 ºC for 4 days, then sieved through a 2 mm sieve and homogenized. The substrate samples were analyzed for pH in water (1:5 substrate – water v/v rate).

Two indices were calculated to assess the chelate-assisted phytoextraction intensity. The bioaccumulation factor (BCF) was calculated based on the following equation:

$$BCF = C_{havvested\ tissue}/C_{soil/substrate}$$

where:

$C_{havvested\ tissue}$ is the concentration of metal in collected plant tissues,

$C_{soil/substrate}$ is the concentration of metal in soil or substrate.

The translocation factor (TF) was calculated as follows:

$$TF = C_{shoot}/C_{root}$$

where:

C_{shoot} is the concentration of metal in shoots,

C_{root} is the concentration of metal in roots.

These factors characterize the ability of plants to tolerate and accumulate metals (Takarina, Pin, 2017).

Statistical analysis

The statistical evaluation of the obtained results was performed using the Statistica v. 13.1 program. The results analyzed were the average of 3 repetitions. The experiment included the following factors: chelate and plant species for the substrates. The results obtained in the experiment were analyzed using factorial analysis of variance (ANOVA). The significance of differences was assessed using the Tukey's (HSD) test (significance level α=0.05).

RESULTS

Plant growth

The effect of individual chelates on plant growth and yield was varied and dependent on the plant species and substrate. Biomass growth data for three plant species are presented in Figure 1.

In pots planted with *A. millefolium* growing on substrate 1, the greatest loss of biomass was observed after the application of 5 mM EGTA and 5 mM EDTA. A decrease in biomass of 22% (for 5 mM EGTA) and 21% (for 5 mM EDTA) was noted. In the case of growth on substrate 2, *A. millefolium* responded with a decrease in growth after the application of CA at a concentration of 10 mM (by 19%). On the other hand, the application of 10 mM EDTA stimulated its growth to the greatest extent (by 15% compared to the control).

In the case of *T. pratense* grown in substrate 1, all added chelates significantly increased its growth except for 10 mM CA, where the increase in biomass was insignificant. The greatest growth-stimulating effect was observed after the addition of EDTA at both doses: with 5 mM EDTA, a 34% increase in biomass was noted, and with 10 mM EDTA an increase of 51%. No decrease in growth was observed in *T. pratense* grown in substrate 1. For substrate 2, the obtained biomass was significantly lower compared to substrate 1. For *T. pratense* grown in substrate 2, a slight increase in biomass was noted after the use of 5 mM CA and 5 mM EDTA, an increase of 11% and 15%, respectively.

In the case of *D. erythrosora* growing on medium 1, a significant increase in biomass was noted after the application of chelates: 5 mM EGTA, 5 mM EDTA and 10 mM EGTA, an increase of 22%, 32% and 22%, respectively. For substrate 2, the greatest significant effect was noted after the application of 5 mM and 10 mM EDTA, biomass increased by about 40%.

Change of substrate pH

Tables 3 and 4 show the pH values of both substrates used in the experiment after plant harvest. Analysis of variance did not show statistical significance for pH for plant species and substrates. The initial pH value of substrate 1 was 6.2, while for substrate 2 produced with ash as a base it was 7.5.

For substrate 1 *A. millefolium* increased the pH to 6.6 (control) compared to the initial pH. Adding chelates to the

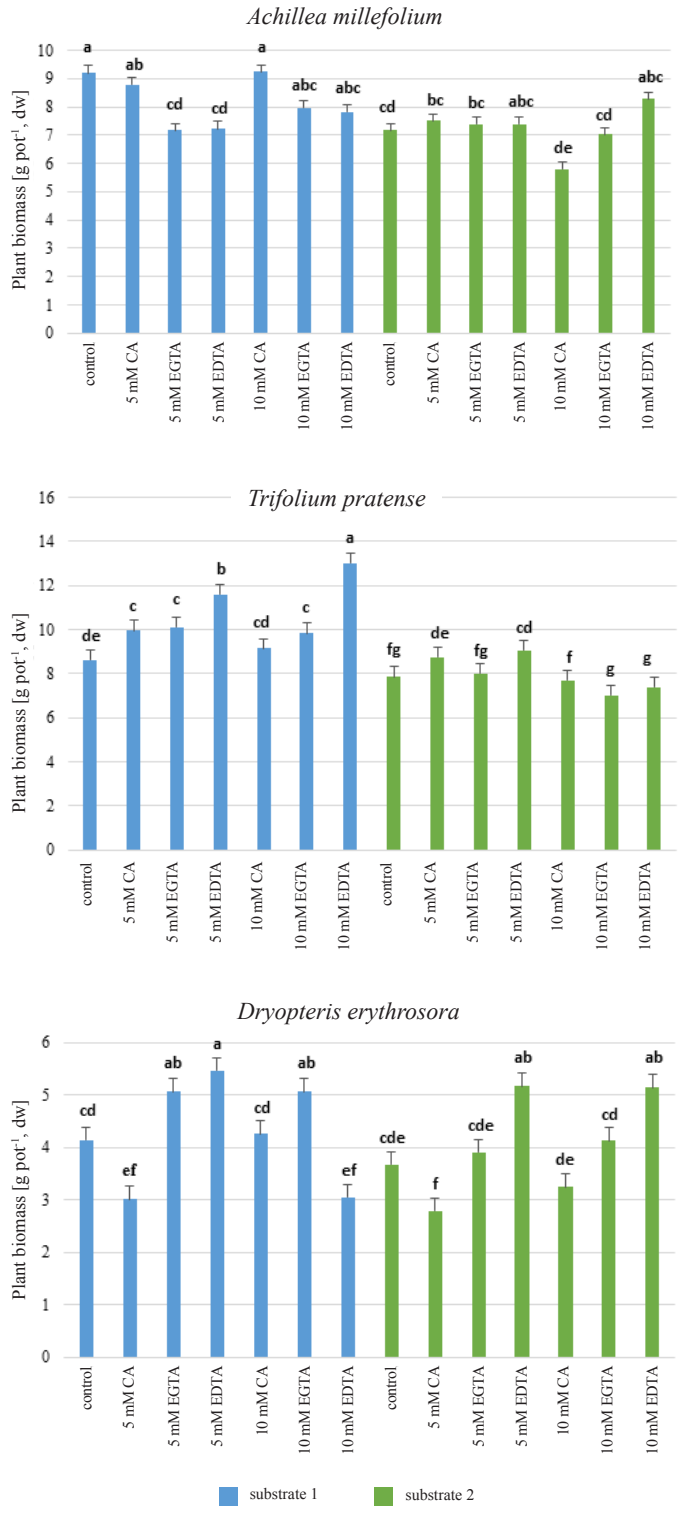

Figure 1. The total biomass production (g pot^{-1}, dw, mean± SD, *n*=3) for *Achillea millefolium*, *Trifolium pratense* and *Dryopteris erythrosora* grown on substrate 1 (95% soil, 5% compost), and substrate 2 (30% ash from a power plant, 20% compost, 50% peat). Values marked with different letters indicate significant differences within substrates and chelators for each plants species at $p<0.05$ according to Tukey's HSD test (ANOVA).

Table 3. Values of pH of the substrates used in the experiment after the plant harvest (pH in H_2O, mean± SD, *n*=3) in substrate 1 (95% soil, 5% compost).

Chelate	*Achillea millefolium*	*Trifolium pratense*	*Dryopteris erythrosora*
Control	6.6 ± 0.15	5.9 ± 0.10	6.2 ± 0.20
5 mM CA	6.2 ± 0.10	5.9 ± 0.05	5.9 ± 0.17
5 mM EGTA	5.9 ± 0.09	5.6 ± 0.10	5.9 ± 0.16
5 mM EDTA	6.2 ± 0.14	5.6 ± 0.09	6.1 ± 0.03
10 mM CA	5.5 ± 0.07	5.6 ± 0.17	5.7 ± 0.17
10 mM EGTA	5.6 ± 0.13	6.1 ± 0.14	5.7 ± 0.16
10 mM EDTA	5.6 ± 0.44	5.9 ± 0.06	5.9 ± 0.25

Table 4. Values of pH of the substrates used in the experiment after the plant harvest (pH in H_2O, mean± SD, *n*=3) in substrate 2 (30% ash from a power plant, 20% compost, 50% peat).

Chelate	*Achillea millefolium*	*Trifolium pratense*	*Dryopteris erythrosora*
Control	7.3 ± 0.19	7.4 ±0.02	7.4 ± 0.03
5 mM CA	7.3 ± 0.08	7.3 ± 0.04	7.4 ± 0.06
5 mM EGTA	7.5 ± 0.196	7.4 ± 0.04	7.6 ± 0.04
5 mM EDTA	7.3 ± 0.06	7.3 ± 0.06	7.4 ± 0.13
10 mM CA	7.2 ± 0.02	7.3 ± 0.06	7.3 ± 0.04
10 mM EGTA	7.3 ± 0.03	7.3 ± 0.03	7.3 ± 0.08
10 mM EDTA	7.4 ± 0.04	7.3 ± 0.18	7.3 ± 0.10

substrate decreased the pH for *A. millefolium* and *D. erythrosora* compared to the control. For *T. pratense*, the addition of 10 mM EGTA increased the pH by 0.2 compared to the control.

For substrate 2 all plants grown without the addition of chelates (control) decreased the pH of the substrate compared to the initial pH. For *A. millefolium*, the addition of 5 mM EGTA and 10 mM EDTA increased the pH value by 0.2 and 0.1, respectively, compared to the control. Similarly, the addition of 5 mM EGTA increased the pH in *D. erythrosora* by 0.2. In the case of *T. trifolium*, after the application of chelates, the pH was equal or lower compared to the control.

Accumulation of REE in plants tissues

The concentrations of the measured REE (La, Ce, Eu, Gd) in plant parts (aboveground and underground parts, separately) are presented in Tables 5–7. Two-way analysis of variance showed a significant effect of chelates and substrate types on the aboveground and underground parts of plants. Studies have shown that plants obtained many times higher concentrations of rare earth elements from substrate 1 than from substrate 2. It can be assumed that was driven by much lower pH and the fact that REE were added as solu-

Table 5. Concentrations of La, Ce, Eu, and Gd in above ground parts and underground parts of *Achillea millefolium* when grown on two substrates (mg kg^{-1}, mean± SD, n = 3). The substrates used were as follows: substrate 1 (95% soil, 5% compost), substrate 2 (30% ash from a power plant, 20% compost, 50% peat).

Substrate	Chelator	Aboveground parts				Underground parts			
		La	Ce	Eu	Gd	La	Ce	Eu	Gd
1	Control	1.68 ±0.24 ab	1.46 ±0.36 b	0.31 ±0.03 e	0.95 ±0.26 b	43.59 ±0.92 b	37.40 ±0.75 b	23.76 ±0.33 cd	45.74 ±2.82 b
1	5 mM CA	1.81 ±0.15 ab	1.67 ±0.16 b	0.57 ±0.02 de	1.28 ±0.12 ab	37.78 ±5.39 cd	29.29 ±5.76 c	32.41 ±4.17 b	40.15 ±2.18 bc
1	5 mM EGTA	1.58 ±0.26 c	1.78 ±0.23 b	0.67 ±0.17 cd	1.46 ±0.19 ab	57.63 ±0.26 a	53.15 ±0.68 a	41.84 ±2.65 a	63.60 ±3.43 a
1	5 mM EDTA	1.81 ±0.15 ab	1.69 ±0.09 b	1.50 ±0.22 b	3.50 ±0.54 a	41.40 ±0.82 bc	28.53 ±2.58 cd	14.76 ±1.47 e	35.61 ±0.85 d
1	10 mM CA	2.04 ±0.03 a	2.26 ±0.36 b	0.72 ±0.29 cd	1.43 ±0.69 ab	33.68 ±0.56 d	27.23 ±2.83 cd	19.76 ±0.08 d	39.70 ±1.27 c
1	10 mM EGTA	1.71 ±0.09 ab	1.74 ±1.06 b	1.05 ±0.21 c	2.12 ±0.10 a	39.11 ±0.63 bc	41.90 ±0.52 b	20.53 ±0.51 cd	34.59 ±5.09 d
1	10 mM EDTA	2.00 ±0.09 a	2.82 ±1.06 a	4.00 ±0.21 a	3.70 ±0.10 a	32.56 ±0.63 e	43.16 ±0.52 b	32.38 ±0.51 c	31.37 ±5.09 d
2	Control	0.04 ±0.02 d	0.10 ±0.03 c	0.02 ±0.01 f	0.06 ±0.03 c	0.36 ±0.01 f	0.58 ±0.0352 e	0.23 ±0.06 f	0.40 ±0.04 e
2	5 mM CA	0.05 ±0.02 d	0.11 ±0.02 c	0.03 ±0.01 f	0.06 ±0.01 c	0.19 ±0.01 f	0.12 ±0.01 e	0.12 ±0.01 f	0.21 ±0.01 e
2	5 mM EGTA	0.08 ±0.02 d	0.15 ±0.03 c	0.05 ±0.01 f	0.13 ±0.03 c	0.31 ±0.08 f	0.18 ±0.039 e	0.05 ±0.01 f	0.32 ±0.34 e
2	5 mM EDTA	0.09 ±0.03 d	0.13 ±0.05 c	0.03 ±0.02 f	0.08 ±0.05 c	0.24 ±0.03 f	0.14 ±0.05 e	0.05 ±0.01 f	0.08 ±0.01 e
2	10 mM CA	0.07 ±0.01 d	0.12 ±0.02 c	0.04 ±0.01 f	0.06 ±0.01 c	0.11 ±0.01 f	0.14 ±0.01 e	0.04 ±0.01 f	0.08 ±0.01 e
2	10 mM EGTA	0.04 ±0.01 d	0.08 ±0.01 c	0.01 ±0.0001 f	0.05 ±0.005 c	0.25 ±0.02 f	0.27 ±0.01 e	0.07 ±0.02 f	0.25 ±0.03 e
2	10 mM EDTA	0.05 ±0.01 d	0.12 ±0.03 c	0.02 ±0.01 f	0.07 ±0.02 c	0.32 ±0.03 f	0.34 ±0.04 e	0.17 ±0.03 f	0.28 ±0.08 e

Values marked with different letters (a, b, c, etc.) for each element in relation to substrates and chelators are significantly different at $p < 0.05$ according to Tukey's HSD test (ANOVA).

Table 6. Concentrations of La, Ce, Eu, and Gd in above ground parts and underground parts of *Trifolium pratense* when grown on two substrates (mg kg^{-1}, mean± SD, n = 3). The substrates used were as follows: substrate 1 (95% soil, 5% compost), substrate 2 (30% ash from a power plant, 20% compost, 50% peat).

Substrate	Chelator	Aboveground parts				Underground parts			
		La	Ce	Eu	Gd	La	Ce	Eu	Gd
1	Control	3.98 ±0.69 a	3.01 ±0.39 b	0.88 ±0.16 c	2.47 ±0.33 c	42.12 ±1.77 b	43.13 ±3.78 b	16.16 ±1.37 d	39.13 ±0.28 bc
1	5 mM CA	3.63 ±0.14 b	2.96 ±0.04 c	0.98 ±0.14 c	2.71 ±0.18 c	35.89 ±1.54 c	34.18 ±1.43 c	15.19 ±0.65 d	37.56 ±2.00 cd
1	5 mM EGTA	3.47 ±0.25 b	2.50 ±0.22 d	1.01 ±0.17 c	2.68 ±0.18 c	37.45 ±0.25 bc	35.15 ±0.68 c	22.77 ±2.65 c	34.07 ±3.42 de
1	5 mM EDTA	3.45 ±0.34 b	3.03 ±0.23 b	2.62 ±0.22 b	5.28 ±0.39 b	40.46 ±1.30 bc	33.34 ±2.12 c	23.11 ±0.30 c	33.66 ±1.04 de
1	10 mM CA	3.56 ±0.32 b	2.29 ±0.19 d	1.05 ±0.12 c	2.26 ±0.26 c	60.20 ±2.18 a	44.84 ±1.65 b	30.13 ±1.05 b	51.15 ±3.45 a
1	10 mM EGTA	2.88 ±0.05 c	4.01 ±0.15 a	3.70 ±0.18 a	5.94 ±0.01 b	60.16 ±4.72 a	62.22 ±5.15 a	45.91 ±6.40 a	43.19 ±2.76 b
1	10 mM EDTA	3.55 ±0.20 b	3.82 ±0.36 a	3.98 ±0.70 a	10.20 ±0.53 a	32.56 ±0.86 c	43.16 ±0.42 b	32.38 ±1.57 b	31.37 ±1.54 d
2	Control	0.04 ±0.01 d	0.07 ±0.01 e	0.02 ±0.01 d	0.05 ±0.02 d	0.42 ±0.03 d	0.53 ±0.02 d	0.23 ±0.03 e	0.39 ±0.04 e
2	5 mM CA	0.06 ±0.02 d	0.09 ±0.01 e	0.02 ±0.01 d	0.07 ±0.03 d	0.48 ±0.18 d	0.29 ±0.06 d	0.40 ±0.18 e	0.39 ±0.06 e
2	5 mM EGTA	0.04 ±0.02 d	0.10 ±0.01 e	0.02 ±0.01 d	0.04 ±0.01 d	0.25 ±0.05 d	0.34 ±0.06 d	0.16 ±0.06 e	0.19 ±0.05 e
2	5 mM EDTA	0.02 ±0.01 d	0.05 ±0.01 e	0.01 ±0.01 d	0.02 ±0.01 d	0.24 ±0.10 d	0.14 ±0.03 d	0.05 ±0.01 e	0.08 ±0.02 e
2	10 mM CA	0.03 ±0.03 d	0.08 ±0.05 e	0.06 ±0.07 d	0.04 ±0.02 d	0.62 ±0.13 d	0.80 ±0.13 d	0.43 ±0.03 e	0.78 ±0.09 e
2	10 mM EGTA	0.04 ±0.01 d	0.08 ±0.01 e	0.01 ±0.01 d	0.03 ±0.01 d	0.29 ±0.06 d	0.25 ±0.01 d	0.10 ±0.02 e	0.30 ±0.06 e
2	10 mM EDTA	0.02 ±0.01 d	0.05 ±0.02 e	0.01 ±0.01 d	0.03 ±0.01 d	0.32 ±0.05 d	0.34 ±0.01 d	0.17 ±0.03 e	0.28 ±0.01 e

Values marked with different letters (a, b, c, etc.) for each element in relation to substrates and chelators are significantly different at $p < 0.05$ according to Tukey's HSD test (ANOVA).

Table 7. Concentrations of La, Ce, Eu, and Gd in aboveground parts and underground parts of *Dryopteris erythrosora* when grown on two substrates (mg kg $^{-1}$, mean± SD, n = 3). The substrates used were as follows: substrate 1 (95% soil, 5% compost), substrate 2 (30% ash from a power plant, 20% compost, 50% peat).

Substrate	Chelator	Aboveground parts				Underground parts			
		La	Ce	Eu	Gd	La	Ce	Eu	Gd
1	Control	8.73 ±0.09 d	7.48 ±0.79 d	2.61 ±0.09 b	3.29 ±0.10 e	51.61 ±0.41 a	35.15 ±2.72 c	20.15 ±1.28 b	44.77 ±2.37 a
1	5 mM CA	9.67 ±1.99 c	9.08 ±1.20 c	3.60 ±0.10 a	4.99 ±0.33 c	38.36 ±1.79 b	45.11 ±3.90 a	29.74 ±2.09 a	42.00 ±0.75 ab
1	5 mM EGTA	7.98 ±0.25 de	5.99 ±0.21 d	2.53 ±0.08 b	3.42 ±0.30 e	39.37 ±5.85 b	34.24 ±0.44 c	22.87 ±1.11 b	37.36 ±1.82 b
1	5 mM EDTA	9.11 ±0.47 c	6.02 ±0.28 d	3.00 ±0.64 ab	2.82 ±0.03 f	28.35 ±6.006 c	27.00 ±5.23 d	30.30 ±1.46 a	25.40 ±2.11 c
1	10 mM CA	23.54 ±2.61 a	15.24 ±2.86 a	3.24 ±0.36 ab	7.71 ±0.98 a	49.15 ±1.64 a	40.98 ±1.83 b	21.28 ±0.55 b	42.40 ±2.99 a
1	10 mM EGTA	14.66 ±0.19 b	12.30 ±0.26 b	3.57 ±0.14 a	6.13 ±0.53 b	43.04 ±1.18 b	47.33 ±1.00 a	20.40 ±3.17 b	38.99 ±0.67 b
1	10 mM EDTA	12.08 ±1.21 bc	9.00 ±0.38 c	1.47 ±0.26 c	4.17 ±0.19 c	54.56 ±1.67 a	46.56 ±0.46 a	28.94 ±4.01 a	37.27 ±2.05 b
2	Control	0.14 ±0.02 f	0.22 ±0.04 e	0.01 ±0.01 d	0.03 ±0.01 g	0.25 ±0.03 d	0.47 ±0.01 e	0.03 ±0.01 c	0.11 ±0.03 d
2	5 mM CA	0.30 ±0.05 f	0.31 ±0.04 e	0.16 ±0.06 d	0.18 ±0.06 g	0.18 ±0.06 d	0.28 ±0.01 e	0.04 ±0.01 c	0.07 ±0.001 d
2	5 mM EGTA	0.25 ±0.03 f	0.24 ±0.04 e	0.03 ±0.01 d	0.09 ±0.07 g	0.13 ±0.02 d	0.32 ±0.01 e	0.02 ±0.01 c	0.05 ±0.01 d
2	5 mM EDTA	0.50 ±0.13 f	0.68 ±0.10 e	0.02 ±0.01 d	0.04 ±0.01 g	0.13 ±0.03 d	0.22 ±0.02 e	0.03 ±0.01 c	0.03 ±0.001 d
2	10 mM CA	0.81 ±0.31 f	0.94 ±0.22 e	0.14 ±0.10 d	0.33 ±0.04 g	0.15 ±0.02 d	0.32 ±0.02 e	0.06 ±0.02 c	0.07 ±0.01 d
2	10 mM EGTA	0.21 ±0.05 f	0.39 ±0.01 e	0.03 ±0.01 d	0.09 ±0.01 g	0.12 ±0.01 d	0.30 ±0.01 e	0.02 ±0.01 c	0.05 ±0.01 d
2	10 mM EDTA	0.36 ±0.05 f	0.55 ±0.07 e	0.04 ±0.01 d	0.07 ±0.02 g	0.18 ±0.02 d	0.36 ±0.07 e	0.06 ±0.03 c	0.11 ±0.03 d

Values marked with different letters (a, b, c, etc.) for each element in relation to substrates and chelators are significantly different at $p < 0.05$ according to Tukey's HSD test (ANOVA).

tions to the soil, creating larger pool of plant available elements. There were also substantial differences between plant species in REE accumulation in control and chelate treated substrates, with the order of accumulation ability being as follows: *D. erythrosora* > *T. pratense* > *A. millefolium*.

In addition, it was shown that the plants retained a greater amount of acquired REE in roots than in the aboveground parts of plants. It was also noted that underground parts in most cases were characterized by a decrease in REE accumulation after adding chelators compared to the control.

Comparing the accumulated REE in aboveground parts for substrate 1, the addition of EDTA at a concentration of 10 mM turned out to be the most effective additive for *A. millefolium* and *T. pratense*. The content of REE increased in *A. millefolium* comparing to the control by 0.32 mg kg^{-1}, 1.36 mg kg^{-1}, 3.69 mg kg^{-1} and 2.75 mg kg^{-1} for La, Ce, Eu and Gd, respectively; and in *T. pratense* by 3.10 mg kg^{-1} and 7.73 mg kg^{-1} for Eu and Gd, respectively. For Ce, 10 mM EGTA and 10 mM EDTA were the most effective, with an increase of 1.0 mg kg^{-1} and 0.81 mg kg^{-1}, respectively

In turn, for REE accumulation in roots of *A. millefolium* and *T. pratense* the most effective addition was EGTA: 5 mM rate for *A. millefolium* and 10 mM for *T. pratense*. The REE content in *A. millefolium* underground parts increased compared to the control by 14.04, 15.75, 18.08 and 17.86 mg kg^{-1} for La, Ce, Eu and Gd, respectively, after adding 5 mM EGTA to the soil. The dose of 10 mM EGTA stimulated increase in content of La, Ce, Eu in *T. pratense* by: 18.04, 19.09, 29.75 mg kg^{-1}, respectively.

In the case of substrate 2, no significant differences in REE accumulation were observed compared to the control (Tables 5 and 6). Additionally, plants accumulated lower amounts of metals in substrate 2 compared to substrate 1.

Dryopteris erythrosora accumulated higher amounts of REE than the other two plant species. In the case of cultivation in substrate 1, the accumulation of La, Ce and Gd in the aboveground parts was most effectively stimulated by 10 mM CA (an increase of 14.81 mg kg^{-1}, 7.76 mg kg^{-1} and 4.42 mg kg^{-1} was observed for La, Ce and Gd, respectively, compared to the control). For Eu, the same effect was obtained using of 5 mM CA and 10 mM EGTA.

Analyzing the REE content in the underground parts of *D. erythrosora* grown on substrate 1, no significant increase in the accumulation of La and Gd was noted after adding chelates to the substrate. For Ce, a significant increase was noted after the use of 5 mM CA, 10 mM EGTA and 10 mM EDTA compared to the control (an increase of 9.96 mg kg^{-1}, 12.18 mg kg^{-1}, 11.41 mg kg^{-1}, respectively). On the other hand, for Eu, a significant increase was noted for *D. erythrosora* treated with 5 mM CA and 5 mM and 10 mM EDTA, an increase of 9.59, 10.15 and 8.79, respectively. For the underground parts, similarly to the other plants, no significant differences in REE accumulation were noted.

The translocation (TF) and bioconcentration (BCF) factors are shown in Figures 2 and 3. TF greater than 1 was obtained only for *D. erythrosora* and *A. millefolium*, when they were grown on substrate 2. The highest TF in the entire experiment was attributed to the application of CA at a concentration of 10 mM equal to 5.40 for La (*D. erythrosora*). The highest TF for Gd (4.24) for *D. erythrosora* was calculated for the same chelate at 10 mM rate. The application of 5 mM EDTA for *D. erythrosora* gave the highest TF for Ce (2.98) whereas 5 mM CA gave the highest value for Eu (4.00). In case of *A. millefolium* grown on substrate 2, TF = 1 was obtained only after adding 5 mM EGTA (1.00) and 10 mM CA (1.00) for Eu and 5 mM EDTA (1.00) for Gd to the substrate (Figure 2).

There were very clear differences in TF values between the substrates tested and the plant species. For the substrate 2, TF values increased in the following order: *D. erythrosora* > *A. millefolium* > *T. pratense*. In case of substrate 1, the greatest TF values were reported for *D. erythrosora* whereas two other plant species in general had similar values (Figure 2).

BCF > 1 was not observed in the experiment. The highest BCF = 0.68 was obtained for *T. pratense* for Eu after application of 10 mM CA (substrate 2). The highest BCF index for La was obtained in substrate 1 for *D. erythrosora* after application of 10 mM CA (BCF = 0.28). For the same plant in substrate 1, the highest indices were obtained for Ce, for which BCF was 0.19 after application of 5 mM CA, 10 mM CA and 10 mM EDTA. The highest BCF index for Gd (BCF = 0.41) was obtained for *D. erythrosora*, which was grown in substrate 2 with 10 mM CA (Figure 3).

DISCUSSION

High biomass growth or the ability of plants to accumulate higher concentrations of metals without toxic effects on the plant are the conditions that a plant must meet in order to be effective in phytoremediation or phytomining. When using phytoremediation techniques, it is important to ensure that the plant used is capable of creating a sufficient biomass. Plants that accumulate metals are harvested and then subjected to a pyrolysis to recover the metals. It has been proven that lanthanides (especially La and Ce) can stimulate the growth of certain plant species. For example, by promoting nitrogen metabolism and other metabolic pathways (He et al., 2022). In this experiment the largest amount of biomass was obtained for *T. pratense*. This plant is usually grown as a fodder plant due to its high biomass growth and high protein content. Higher biomass was collected for plants grown on substrate 1 compared to substrate 2. This could have been due to stimulation of growth after prior adding REE in easily soluble form and overall better growth conditions than present in substrate 2 (Grčman et al., 2001).

Evaluating direct effects of the applied chelates on plant growth, it is difficult to draw general conclusions. The ef-

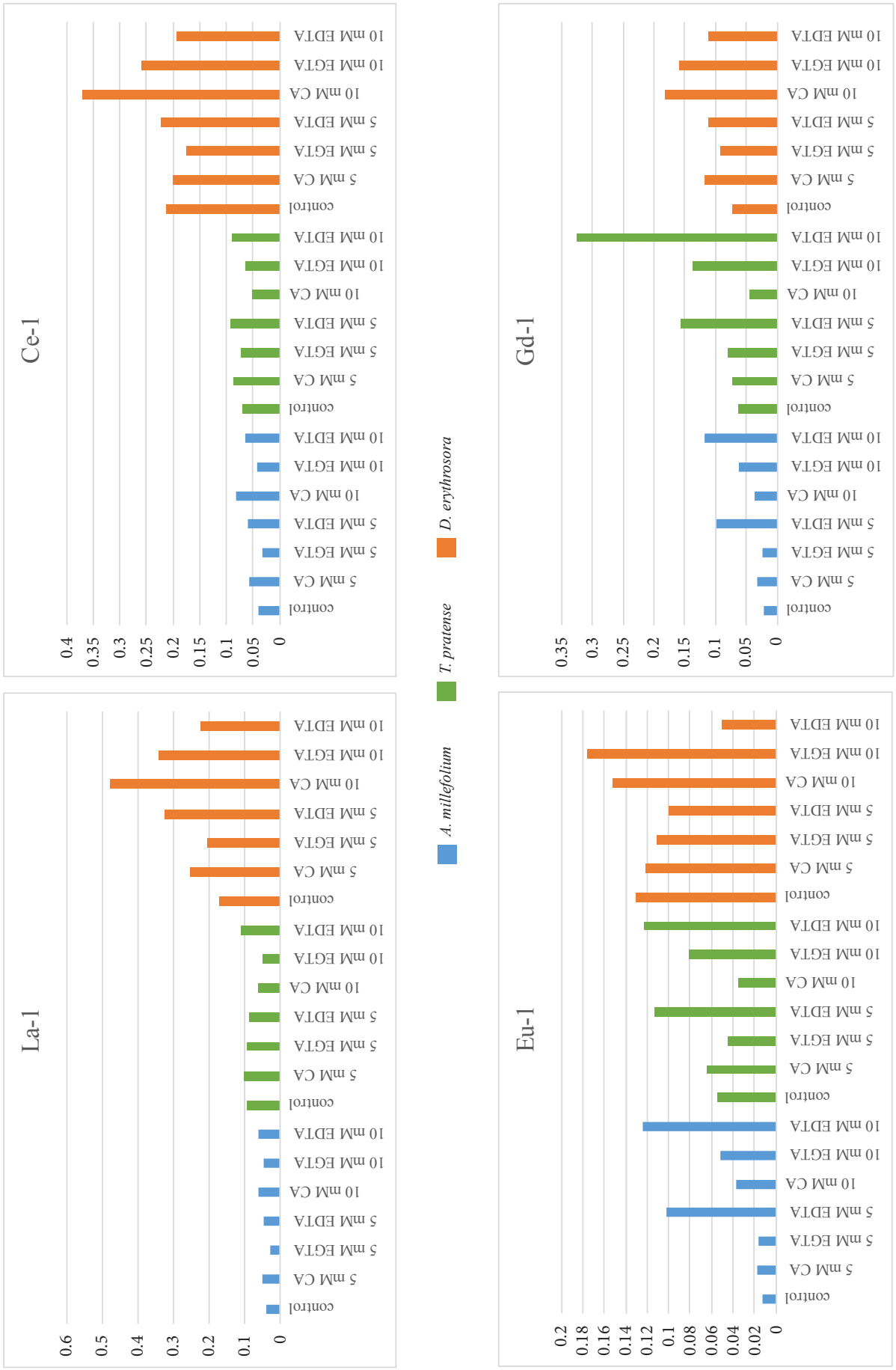
Ce-1
La-1
Eu-1
Gd-1
A. millefolium
T. pratense
D. erythrosora
control
5 mM CA
5 mM EGTA
5 mM EDTA
10 mM CA
10 mM EGTA
10 mM EDTA

Figure 2

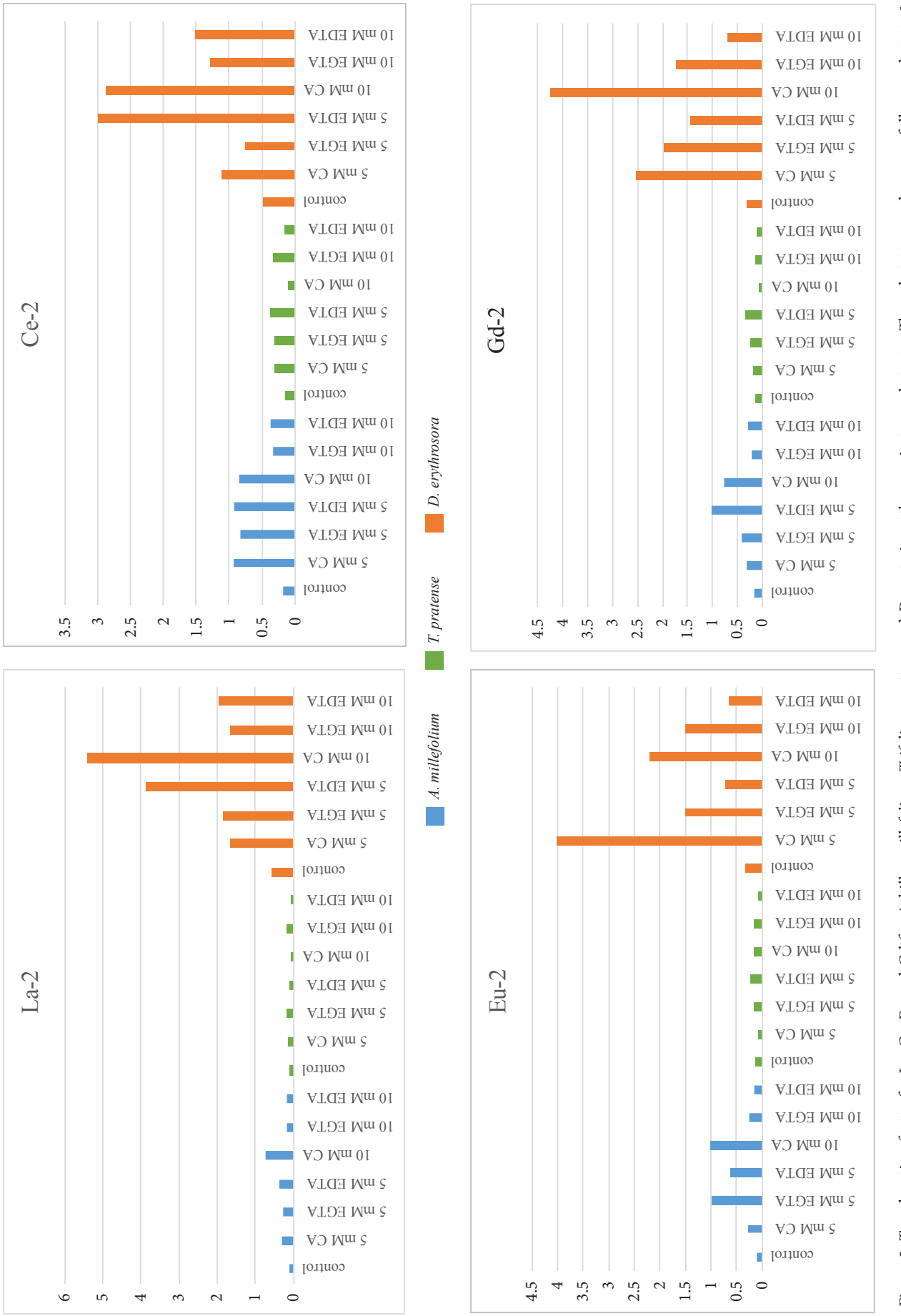

Figure 2. Translocation factor for La, Ce, Eu and Gd for *Achillea millefolium*, *Trifolium pratense* and *Dryopteris erythrosora* in two substrates. The substrates used were as follows: substrate 1 (95% soil, 5% compost), substrate 2 (30% ash from a power plant, 20% compost, 50% peat). The markings show BCF for: La-1, Ce-1, Eu-1, Gd-1 – REE in substrate 1, La-2, Ce-2, Eu-2, Gd-2 –REE in substrate 2.

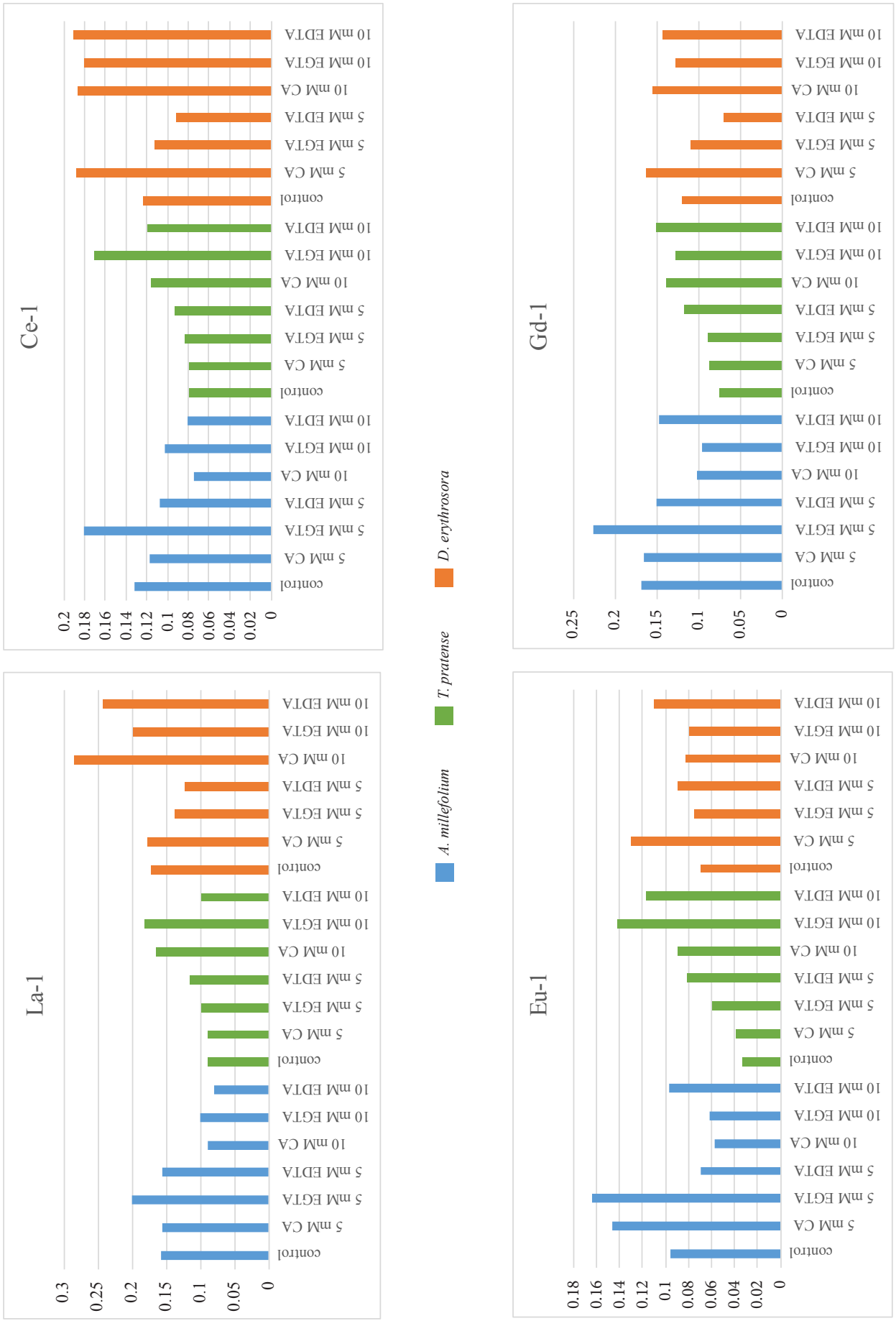
Ce-1
La-1
Gd-1
Eu-1
A. millefolium
T. pratense
D. erythrosora
control
5 mM CA
5 mM EGTA
5 mM EDTA
10 mM CA
10 mM EGTA
10 mM EDTA

Figure 3

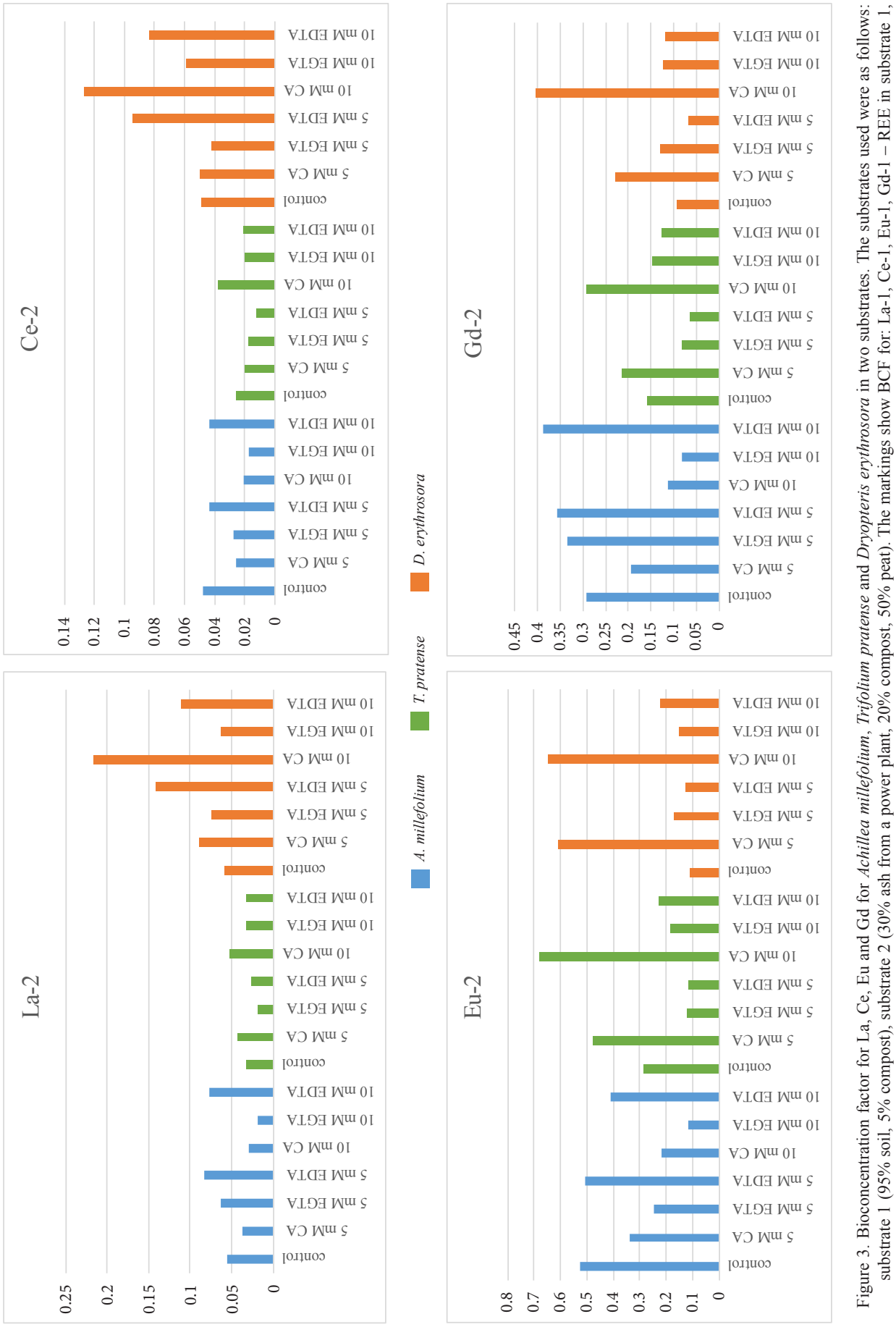

Figure 3. Bioconcentration factor for La, Ce, Eu and Gd for *Achillea millefolium*, *Trifolium pratense* and *Dryopteris erythrosora* in two substrates. The substrates used were as follows: substrate 1 (95% soil, 5% compost), substrate 2 (30% ash from a power plant, 20% compost, 50% peat). The markings show BCF for: La-1, Ce-1, Eu-1, Gd-1 – REE in substrate 1, La-2, Ce-2, Eu-2, Gd-2 – REE in substrate 2.

fects was rather plant specific. Literature search provides ambiguous information on EDTA impact on plant growth since both toxic and protective effects have been reported (Saleem et al., 2020). Our data suggests that the doses of chelates applied (5 and 10 mM) are in general not harmful to the tested plant species, however the sensitivity of plants can be diverse.

Soil pH directly affects plant development by determining the availability of nutrients and metal toxicity to plants. Adequate availability of macronutrients for plants occurs in the range of pH 6–7 (Remigio et al., 2020). In the experiment, the pH of the substrates ranged from 5.5 to 7.6. In the studies conducted by Cao et al. (2001) it was shown that the release of La, Ce and Gd gradually increases with decreasing pH.

In accordance with this theory, it was observed that plants accumulated higher amounts of REE in substrate 1, which had a lower pH compared to substrate 2. Some plants prefer more acidic environments and may contribute to lower soil pH due to the release of organic acids into the substrate or through root exudates as observed in the case of the fern *D. erythrosora* (Shan et al., 2003).

Two substrates were used in the experiment: one consisting of soil (substrate 1) and the other whose main component was ash (substrate 2). Due to the global use of coal-based energy, environmental problems are increasing, including the creation of areas where fly ash (FA) landfills are located. FA waste disposal is characterized by a high degree of absorption of water, energy and land surface. It is also the cause of pollution associated with the atmosphere and water bodies. FA, compared to soil, has a lower water retention capacity, so it can cause water stress more quickly (Yadav et al., 2021).

This may result in slower plant growth and reduced biomass production. In this experiment, plants growing on substrate 2 had lower biomass compared to plants growing on substrate 1, however the differences can be of complex character, involving overall physical and chemical growth conditions, direct effects of chelates and chelate-driven availabilities of nutrients and micronutrients.

For substrate 1, the REE content in plant tissues ranged from 0.31 to 63.60 mg kg^{-1}, while for substrate 2, the REE ranged from 0.01 to 0.94 mg kg^{-1}. The determined REE content was higher in substrate 1 than in substrate 2.

There were substantial differences between levels of REE in aboveground and underground parts between the substrate 1 and substrate 2. Addition of REE salts have created much larger pool of plant available REE in soil. It is commonly observed that elements added to soil as salts or salt solutions are in short-term much more easily absorbed by plants as those present in soil minerals or sorbed by soil components (Dong et al., 2021). Both in control and chelate treated substrates the greatest ability to bioaccumulate REE showed *T. pretense.*

Most of the REE were accumulated in underground parts and not transferred further to aboveground parts. According to Ramos et al. (2016) this is because REE absorbed by roots encounter an apoplastic barrier on their way to the xylem. This results in difficulties during the translocation of lanthanides to other plant organs. As a result, the order of REE accumulation in plant tissues is as follows: roots > stems > leaves > flowers > fruits > seeds. Similarly, in the study of Lihong et al. (1999) showed that the application of EDTA increased REE bioaccumulation in wheat (*Triticum aestivum* L.) seedlings, metal both in roots and tops (stem and leaves). However, still higher REE content was measured in roots than in tops. It is assumed that in the chemically assisted phytoremediation method by adding chelating substances the content of metals taken up by the plant increases. In this study, the use of chelators: CA, EGTA and EDTA had a small influence of the studies plants. In the studies of Ozaki et al. (2000) after the addition of chelating reagents: NTA, lactic acid and succinic acid to the medium containing Y, Ce, Pm, Eu, Gd, Lu and Yb content in *D. erythrosora* increased compared to the control. Nawaz et al. (2022) investigated the usefulness of *Brassica napus* in phytoremediation of Ni using two chelators: 10 mM CA and 1.5 mM EDTA. The studies showed greater metal accumulation by EDTA-treated plants as compared to CA. Additionally, it was shown that the addition of chelators alleviated the toxic effects of Ni on canola. Other studies using chelating agents show that enrichment of soil with histidine, malic and citric acids increased the concentration of light REE in the natural REE hyperaccumulator *Dicropteris dichotoma* by 21–78%, as compared to the control (Shan et al., 2003).

Translocation factor (TF) is a parameter indicating the efficiency of a plant in transferring metals from roots to shoots. In order for a plant to be classified as most useful in phytomining techniques, the ratio of metal content in shoots to roots should be greater than 1. If TF is lower than 1, metal accumulation is predominant at the root level (Takarina, Pin, 2017).

In general, the effect of chelates on REE accumulation was plant-specific. *D. erythrosora* that accumulated more REE then other species, also differently responded to the chelates tested. Both in case of substrate 1 and 2. So independently on the level of soluble REE in a substrate, 10 Mm CA stimulated REE accumulation most efficiently for this plant. This can be treated as a positive result due to lower potential toxicity of CA than that represented by EDTA or EGTA. In study Ibrahim (2023) examines how citric acid affects the phytoextraction capabilities of pumpkin (*Cucurbita pepo* L.) in soils contaminated with heavy metals. The application of citric acid significantly enhances plant growth, biomass, and the uptake of heavy metals, suggesting its potential as an effective agent in phytoremediation strategies.

The differences between plant species in TF values were greater than the differences between the chelates used. Our TF data indicate that translocation from underground to aboveground parts for *D. erythrosora* exceeded

the other species in this respect. The highest differences between *D. erythrosora* in terms of TF index values can be seen for substrate 2.

TF factor higher than 1 was obtained in the experiment only for two plant species*: D. erythrosora* and *A. millefolium* growing in substrate 2. The highest TF was obtained for D*. erythrosora* using 10 mM CA. It was proven that the REE concentration in soils and wastes depends on the parent substrate material. To determine the transfer of REE from the substrate to the plants, the bioaccumulation factor (BCF) was calculated. In the experiment, the BCF index value was not higher than 1.

Different effects of chelates on REE bioconcentration index were observed for the plant species, which confirms differences in physiology of REE uptake and transport between those diverse plants. *D. erythrosora* responded with increased BCF for all elements to addition of 10 mM doses of CA, EDTA and EGTA. This indicates the potential for further enhancement of REE bioaccumulation by the fern through optimisation of chelate dosing and application strategy.

CONCLUSIONS

The results of the study showed that the addition of chelates can contribute to increased REE accumulation in plant tissues but to small extent. In addition, the use of plants in phytoremediation techniques is one of the limitations, because the uptake and accumulation of REE by plants depends on, among others, environmental factors or the plant species and its morphology. Therefore, it is necessary to search for suitable plants that will be able to accumulate REE in their aboveground parts and chelating compounds and their appropriate doses in order to enhance the bioaccumulation process.

From a practical point of view, *D. erythrosora* can be considered the most suitable species for use in chelate-assisted REE phytoextraction, despite the lowest total biomass than other plants tested. However, the significantly higher REE content in the aboveground parts and the highest REE translocation to the aboveground parts, expressed by the TF index, suggest that further process optimization should include this plant.

The fern positively responded to chelate addition with increased biomass. This observation combined with the recorded translocation factor above 1, characteristic for intensive transfer of REE from roots to shoots, indicates a potential for the REE bioaccumulation enhancement, as compared to that observed in our study. It seems that there is room for enhancing effectiveness of the entire process through optimisation of ash substrate chemical composition and optimal combination of chelates, substrates and plant species. We can assume that the assisted bioaccumulation process would be more efficient in case of ashes richer in REE. It seems that further research shall focus on tailor-made combinations of chelates and ash substrate compositions (for example additions of organic or pH affecting materials to ashes) for specific characteristics of pre-selected ashes. Since various REE respond to chelates differently, further optimisation can be achieved by selecting optimal chelates and their doses depending on the chemical composition of the ash. TF of fern in many cases increased after chelate addition, therefore it can be assumed that certain modification of the substrate, for example lowering pH, in order to create better growth conditions and greater REE phytoavailability, would greatly enhance the REE amounts transferred from the growing media to plant tissues.

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