

Phytoextraction and recovery of rare earth elements using willow (*Salix* spp.)

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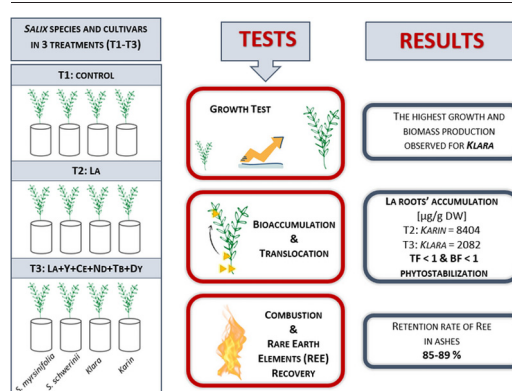
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## HIGHLIGHTS

- Phytoextraction of rare earth elements (REEs) investigated by *Salix*
- *Salix* did not exhibit effects of REEs phytotoxicity.
- REEs are efficiently enriched in bottom ashes during combustion of *Salix*.
- *Salix* could be a suitable candidate to accumulate REEs from various sources of wastewater.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Soil and water contaminations are caused by rare earth elements (REEs) due to mining and industrial activities, that threaten the ecosystem and human health. Therefore, phytoremediation methods need to be developed to overcome this problem. To date, little research has been conducted concerning the phytoremediation potential of *Salix* for REEs. In this study, two *Salix* species (*Salix myrsinifolia* and *Salix schwerinii*) and two *Salix* cultivars (Klara and Karin) were hydroponically exposed to different concentrations of six-REE for 4 weeks. The treatments were: T1 (Control: tap water), T2 (La: 50 mg/L) and T3 (La 11.50 + Y 11 + Nd 10.50 + Dy 10 + Ce 12 and Tb 11.50 in mg L<sup>-1</sup>). The effects of the REE on *Salix* growth indicators (height, biomass, shoot diameter and root length), concentrations of REE in the produced biomass, and accumulation of REE in different parts of the *Salix* (stem, root, and leaf) tissues, were determined. In addition, the retention of REE in ashes following *Salix* combustion (800 and 1000 °C) was determined. The result indicates that with La and REE exposure, the height growth, dry biomass, shoot diameter and root length of all *Salix* remained equivalent to the control treatment excluding Klara, which displayed relatively higher growth in all parameters. Further, among the REE studied, the highest La concentration (8404  $\mu\text{g g}^{-1}$  DW) and La accumulation (10,548  $\mu\text{g plant}^{-1}$ ) were observed in Karin and Klara root respectively. Translocations and bioconcentration factors were discovered at <1 for all *Salix*, which indicates their phytostabilization potential. The total REE concentrations in

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bottom ashes varied between 7 and 8% with retention rates between 85 and 89%. This study demonstrates that *Salix* are suitable candidates for REE phytostabilization and the remediation of wastewater sites to limit metals percolating to the water layers in the ecosystem.

## 1. Introduction

Rare earth elements (REEs) are metals of the lanthanoid series in the periodic table and are commonly found in soils and waters. They are becoming increasingly important in several modern technologies for example, automobiles, wind turbines, electronic equipment, electric vehicles, precision-guided weapons, and audio equipment and computers (Carpenter et al., 2015). The high-tech industry in Europe is suffering from a shortage of critical raw materials (CRMs) including the REEs including cerium Ce, dysprosium Dy, erbium E, europium Eu, gadolinium Gd, holmium Ho, lanthanum La, lutetium Lu, neodymium Nd, praseodymium Pr, promethium Pm, samarium Sm, scandium Sc, terbium Tb, thulium Tm, ytterbium Yb, and yttrium Y, and is almost totally dependent on imported CRMs (Haque et al., 2014).

Globally, there is a need for the transition to a circular, sustainable, low carbon, resource-efficient, and competitive green economy (European Commission, 2020). The earth's crust in Northern Europe, especially in Sweden and Finland, contains different REEs, that are usually evenly distributed in old alkaline bedrock at low concentrations. The highest REE potential in Finland is related to Devonian and Proterozoic carbonatites (Sokli, Korsnäs) and alkaline rocks (Otanmäki, Lamujärvi, Iivaara) and their finite zones and weathering products, and moderate potential in kaolinitic saprolites (Tana Belt and Virtasalmi), hydrothermal ore deposits, rapakivi granites, granite pegmatite association and granites with high thorium content (Sarapää et al., 2013). According to Mathieux et al. (2017), the recovery and utilization of the resources depend on several factors such as amount, the concentration of the valuable commodities, mineralogy, and the re-processing technology (commercially available and economically viable). The potential toolkit for the metal recovery mainly relies on conventional ex-situ methods like excavation, land moving, physical separation and chemical extraction using multiple solvents, which are not optimal techniques for the sustainable and cost-effective recovery of REEs from natural deposits or recycled metals (Royen and Fortkamp, 2016).

Industrial and municipal wastewaters and sludges contain high concentrations of REEs (Cánovas et al., 2017; Kegl et al., 2020). The risks of REE pollution due to mining and processing as well as from the improper disposal of materials containing these compounds could potentially lead to elevated levels in the environment (Carpenter et al., 2015). Mleczeck et al. (2018) reported that REE concentrations within the next few years may be associated with a major new form of environmental pollution and this increase may pose a threat to both plant and human health. Therefore, the recycling and management of post-mining areas, soil, and water containing studied REEs to improve the supply of these critical raw materials are vital aspects to improve a circular economy (Haque et al., 2014). Recently, inventories of CRM resources of both extractable wastes (e.g., industrial wastes, abandoned mines, mine slags, tailings) and new geological deposits have been made (Blengini et al., 2019).

Phytoremediation could provide environmentally sound, socially acceptable, and cost-effective tools to improve the circular use of metals to secure supply. In phytoremediation, plants are used to extract organic and inorganic impurities from the soil and water and translocate them through plant roots into the biomass (Mohsin et al., 2019; Li et al., 2021). To date, phytomining has been proven effective in Ni recovery using metal hyperaccumulator plants such as *Alyssum* (Li et al., 2003) and *Sebertia acuminata* (Jaffré et al., 1976) but it could potentially be extended to include other valuable elements such as REEs. Phytomining is the process of commercial metal phytoextraction in which plants are grown to accumulate high metal concentrations. It is a low-cost and environmentally friendly

method to recover dispersed metals from soils and waters (Mohanty, 2016). It is considered an aesthetic, safe and non-destructive technology with high public and commercial acceptance (Sheoran et al., 2013). Nicks and Chambers (1998) reported that the potential use of phytoremediator plants in the mining industry generates revenue by extracting commercial heavy metals as bio-ore. Phytomining can also help in soil remediation and the restoration of mine-degraded lands.

Willows (*Salix* spp.) have shown great potential for phytoextraction, as they accumulate metals, nutrients and are resilient to metal toxicity (Mohsin, 2016). In addition, they are capable of producing large amounts of biomass suitable for bioenergy and biofuel processes, and this turns phytoremediation using these crops into a promising profitable venture (Faubert et al., 2021). Willows are fast-growing, able to re-sprout after harvesting, have efficient transpiration and high nutrient uptake capacity (Kuzovkina et al., 2008). High transpiration rate, wide-spreading and extensive root systems make willows ideal for absorbing, degrading and detoxifying contaminants. Willow plantations also offer several environmental and economic benefits such as landscaping and ecosystem restoration and thus provide an excellent example promoting the benefits of bioeconomy (Delplanque et al., 2013).

Currently, the mechanism of REE accumulation in woody plants is poorly understood. Mikołajczak et al. (2017) have investigated the phytoremediation potential of REEs by five herbaceous plant species (*Achillea millefolium* L., *Artemisia vulgaris* L., *Papaver rhoeas* L., *Taraxacum officinale*, and *Tripleurospermum inodorum*) growing in four areas located in Germany in close proximity to a road with varied traffic intensity and observed a significant REEs accumulation in plant biomass. Another study was conducted by Wu et al. (2013), who investigated *Phytolacca americana* L. potential to uptake and translocate La and Y via the chelating role of amino acids. Phytomining technology has widely been implemented for Ni phytoextraction, however, very little attention has been paid regarding the phytomining potential of REEs. Worldwide, about five plant families, for example, Blechnaceae, Gleicheniaceae, Juglandaceae, Phytolaccaceae and Thelypteridaceae have been recognized as REEs hyperaccumulators (Jalali and Lebeau, 2021). The results of extensive laboratory testing with specially prepared aqueous solutions (artificial wastewater) have indicated that recovery of REEs from wastewater could be an interesting and viable option (Li et al., 2013; Kegl et al., 2020).

To date, little is known about the accumulation of REEs in short-rotation woody plants. Therefore, the first two objectives of the present study are (i) to determine the effects of high concentrations of REE on the growth traits of different *Salix* cultivars in hydroponic conditions, and (ii) to identify potentially suitable *Salix* cultivars for sequestering REE. Furthermore, a process to recover REE from the biomass in an enriched form needs to be developed. For this purpose, combustion experiments were carried out on the *Salix* cultivar samples to determine the concentrations and compositions of ash fractions, to assess how REE might be recovered for industrial use by a combustion process in a biomass boiler plant.

## 2. Materials and methods

### 2.1. Plant materials, experiment set-up and hydroponic solutions

Two *Salix* species and two *Salix* cultivars were tested hydroponically in an REE mixture in a greenhouse at School of Forest Sciences, University of Eastern Finland (UEF), Joensuu (63° 39' N, 26° 2' E). The environmental condition of the greenhouse was maintained as a day/night period of 16/8 h, day/night temperatures of 24 °C/16 °C, relative humidity of 70% and a light intensity of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . *Salix* species (*Salix myrsinifolia* and *Salix schwerinii*) and

*Salix* cultivars Klara (*S. viminalis* × *S. schwerinii* × *S. dasyclados*) and Karin (*S. schwerinii* × *S. viminalis*) × *S. viminalis*) × *S. burjatica*) were grown in tap water amended with different concentrations of REE for 4 weeks in September 2018.

*Salix* were grown in a randomized design with three treatments and four repetitions (Table 1). A total of 108 *Salix* cuttings (Length: 20 cm) were collected from three-year-old willow plantations (diameter 10–20 mm) in Siikasalmi, Eastern Finland. In total, 12 plastic water containers were set up (Height = 18 cm; Diameter = 40 cm; Length = 70 cm) and filled with 5 L of tap water. In each container, twelve *Salix* cuttings were added as 3 cuttings from each *Salix* species and cultivars. Chloride salts of REE were added in all the treatments. The control treatment (T1) consisted of tap water (without REE). In La treatment (T2), dose was added as La (50 mg/L of water) and in REE-mixture treatment (T3) doses for each element were added as La: 11.50 + Y 11 + Nd 10.50 + Dy 10 + Ce 12 and Tb 11.50 mg/L of water. Such concentrations were selected based on preliminary analysis in mining wastewater and phosphogypsum solution near the YARA mining site in Finland (unpublished study). Therefore, we investigated this experiment to get preliminary knowledge to establish phytoremediation field trials at a commercial scale using *Salix* in Finland. A Finnish fertilizer (Kekkilä-kesälannoite) containing N (17 g), P (4 g) and K (25 g) was added in all treatments at a rate of 200 mg/L of water to maintain nutritional stability in the growing *Salix*. During the four-week growth period, the solution was renewed four times every week to protect the *Salix* from algal bloom.

## 2.2. *Salix* biomass combustion process

Combined samples of hydroponically grown *Salix*, containing a mixture of roots, stems, and leaves, were oven-dried (24 h at 105 °C) and then homogenized by milling with a centrifugal knife mill equipped with a 0.5 mm screen. The concentrations of REE and 21 other ash-forming elements in the homogenized *Salix* sample are presented in Table 2. Small portions (550–850 mg) of the homogenized sample were combusted at two reaction temperatures, 800 °C and 1000 °C, in a fixed-bed, batch, tube reactor. Tests were performed in duplicate and triplicate at 800 °C and 1000 °C respectively. The reactor setup features an insertion probe that enables fast transfer of samples between a cool zone and a hot zone, which is maintained at the target reaction temperature with electric heating elements. A detailed drawing and description of the reactor setup have been given previously (Lane et al., 2020a). The reactor heats biomass samples to temperatures within 10 °C of the target reaction temperature after less than 10 min of inserting the sample and quenches hot samples to less than 100 °C within 10 min of retracting the sample. Samples were initially heated in a flow (2 L min<sup>-1</sup> @ STP) of diluted air, containing 2% O<sub>2</sub> and 98% N<sub>2</sub>, for 15 min before switching the reactor atmosphere to air. The reason for initially heating the samples in diluted air was to minimize temperature overshoot caused by the ignition of evolved volatiles during the initial devolatilization phase of fuel particle burnout. The samples were heated for a total duration of 45 min at 800 °C and a total duration of 30 min at 1000 °C. These heating times were sufficiently long for complete burnout of the organic component of the *Salix* biomass. The standard ash content of the homogenized *Salix* sample was determined by measuring sample weight loss following two consecutive stages of air combustion (250 °C

**Table 1**  
Scheme of the experiment.

Treatment	Code	<i>Salix</i>	Code
Control (without REE)	T1	<i>Salix myrsinifolia</i>	V1
La (single-La dose)	T2	<i>S. schwerinii</i>	V2
REE (mixture of six-REE)	T3	Klara ( <i>S. viminalis</i> × <i>S. schwerinii</i> × <i>S. dasyclados</i> )	V3
		Karin ( <i>S. schwerinii</i> × <i>S. viminalis</i> ) × <i>S. viminalis</i> ) × <i>S. burjatica</i> )	V4

**Table 2**

Concentrations of six REEs and other inorganic elements in a homogenized sample of *Salix*. All values are reported on an oven-dry (105 °C) basis.

REE (mg kg <sup>-1</sup> )	Inorganic elements (mg kg <sup>-1</sup> )		
Dy	1224	K	19,922
Ce	1160	Si	8062
Nd	1060	P	7618
Tb	881	Ca	6824
La	728	Mg	1457
Y	617	Fe	1340
Gd	8	Na	619
Er	<5	Mn	215
Eu	<5	Ti	165
Ho	<5	Zn	147
Lu	<5	Al	106
Pr	<5	Cu	64
Sc	<5	As	35
Sm	<5	Sr	18
Tm	<5	Sb	13
Yb	<5	Ba	7
		Cd	<5
		Co	<5
		Cr	<5
		Pb	<5
		V	<5

for 1 h and then 550 °C for 2 h) in a muffle furnace. The used method is closely based on the ISO 18122 international standard.

At the end of each experiment, combustion ashes were removed from the reactor, weighed to the nearest 0.01 mg, and then analyzed for concentrations of six-REE (Ce, Dy, La, Nd, Tb, and Y) and other inorganic elements. The retentions of REE in bottom ashes after combustion were calculated by mass balances according to Eq. (1).

$$R_i(\%) = \left( \frac{W_a}{W_b} \right) \left( \frac{C_{a,i}}{C_{b,i}} \right) \times 100 \quad (1)$$

where,

$R_i$  is the retention of REE  $i$  in weight percent,  $W_a$  and  $W_b$  are the weights (mg) of the ash and willow biomass respectively, and  $C_{a,i}$  and  $C_{b,i}$  are the concentrations (mg kg<sup>-1</sup>, dry basis) of REE  $i$  in the ash and willow biomass respectively.

## 2.3. Determination of REE concentrations in the *Salix* biomass and combustion ashes

The total concentrations of REE in the combustion ashes and the homogenized *Salix* sample were determined by acid (HNO<sub>3</sub>, HF, and H<sub>3</sub>BO<sub>3</sub>) digestion of samples in a microwave oven (210 °C) followed by inductively coupled plasma mass-spectrometry (ICP-MS). A NeXION 350D instrument (PerkinElmer, USA) was used for the analysis of the digestion solutions. Detailed descriptions of the digestion method and ICP-MS instrument setup have been reported previously (Lane et al., 2020a,b). The non-homogenized *Salix* samples, consisting of individual species and cultivars of *Salix*, were solubilised by low-temperature (500 °C) ashing in a muffle furnace (to decompose organic material), followed by acid digestion of the low-temperature ashes according to EPA method 3051 (EPA, 1996).

The concentrations of REE were quantified by external calibration with a multi-element calibration standard (TraceCERT Periodic Table Mix 3, Sigma Aldrich). The calibration standard was diluted in such a way that the concentrations of acids in the standard matched the concentrations of acids in the solutions containing the unknown samples. An internal standard, U-238, was added online to samples to compensate for matrix effects and instrument drift. Monoatomic interferences were prevented by selecting isotopes for analysis that are free of isobaric overlap with other elements. Polyatomic interferences were suppressed by tuning the ICP-MS instrument in such a way that reduces the formation of double-charged ions and oxides, and by operating the instrument in kinetic energy

discrimination mode. No interferences were observed that required the instrument to be operated in dynamic reaction cell mode. Method blanks and certified standard reference materials, BCR 176R (MSW incineration fly ash) and NIST 1633c (coal fly ash) were measured in addition to the unknown samples for each batch of analysis. This was done to check for contamination and to confirm the accuracy of the analysis batch.

#### 2.4. Powder X-ray diffraction (PXRD) analysis

Willow ashes were prepared at 800 °C for crystalline phase analysis using powder X-ray diffraction (PXRD). A Bruker D8 Advanced X-ray diffraction meter setup with Bragg-Brentano geometry was used to acquire the diffractogram. The PXRD specimen was prepared by loading powdered ash into a single crystal, silicon sample-holder. The diffraction pattern was recorded from 5° 2θ to 90° 2θ, without sample rotation, using the locked-couple technique, with a step-size of 0.025° 2θ, and with a collection time of 100 s per step. Crystalline phases were identified using Bruker Eva plus and MATCH phase recognition software.

#### 2.5. Analysis of growth parameters and rare earth elements (REE) in *Salix* biomass

After the growth period, plant height and root length were measured by a ruler and stem diameter was measured by a digital vernier caliper. Fresh weights of each *Salix* tissue from each treatment were recorded and then oven-dried for 72 h at 80 °C. Later, dry weights of the *Salix* were then noted.

The accumulation of REE in all *Salix* cultivars was calculated using the following equations (Ehsan et al., 2013; Farid et al., 2020):

$$\text{REEs accumulation (g plant}^{-1}) = \text{REEs concentration in each } Salix \text{ tissue (gg}^{-1}) \times \text{dry biomass of the respective tissue (g)} \quad (2)$$

#### 2.6. Evaluation of the REE phytoextraction capability of *Salix*

To evaluate the REE phytoextraction capability of *Salix*, the bioconcentration factor (BF) from accumulation, translocation factors from concentration (TF) and accumulation (TF') were calculated at the end of the experiment (Wu et al., 2010; Redovniković et al., 2017; Xu et al., 2019).

$$BF = A_p / C_s \quad (3)$$

where,  $A_p$  represents REE accumulation in the *Salix* plant and  $C_s$  the designed amount of REE in the solution

$$TF = C_s / C_r \quad (4)$$

where,  $C_s$  represents REE concentration in the shoots and  $C_r$  REE concentration in the roots.

$$TF' = A_s / A_r \quad (5)$$

where,  $A_s$  represents REE accumulation in the shoots and  $A_r$  REE accumulation in the roots.

#### 2.7. Statistical analysis

SPSS v19.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. The values are expressed as the means ± standard error (SE) of the mean. Before the analysis, Levene's test was run to check the homogeneity of variance across all *Salix*. The differences between *Salix* were determined using one-way analysis of variance (ANOVA) followed by the Tukey (HSD) test at the level of significance  $p < 0.05$ .

### 3. Results

#### 3.1. Absolute mean height, dry biomass production, stem diameter and root length among *Salix* after different treatments

In the study, visual observations demonstrated that *Salix* did not show any symptoms of REE toxicity in terms of wilting, curling, and necrosis. The survival rate was 100% under La and six-REE exposures (data not shown).

The absolute mean height among all *Salix* (V1-V4) varied from 52 to 110 cm in all treatments (T1-T3). Among all treatments T1-T3, cultivar V3 produced the highest (110 cm) and V4 lowest (52 cm) absolute mean height (Fig. 1A). Cultivars V3 and V4 in treatment T2 showed a significantly (about 13–52%) higher absolute mean height when compared to their respective cultivars in treatment T1. The same kind of increase (6%) in mean absolute height was observed with cultivar V4 in treatment T3 when compared to control treatment T1. Otherwise, when comparing treatment T3 to control treatment T1, there was a significant decrease in mean absolute height for species V1 (20%), V2 (32%) and cultivar V3 (26%).

The mean dry biomass production (leaves, shoots, and roots) varied from 2.33–7.78 g among all *Salix* (V1-V4) in treatments (T1-T3). The cultivar V3 in treatment T2 showed the highest biomass production (7.78 g) among all the *Salix*. On the other hand, the lowest biomass (2.33 g) was observed for cultivar V4 in treatment T1 (Fig. 1B). In the treatment T1, species V1 showed a 42–29% increase in dry biomass when compared to its respective cultivars in treatments T2 and T3, whereas for species V2 the dry biomass increased by 29 and 35% in comparison to treatments T2 and T3 respectively. When cultivar V3 in T2 is compared with its respective cultivar in treatments T1 and T3, the dry biomass production decreased around 28–57% respectively in T1 and T3 treatments, and when cultivar V4 in treatment T1 is compared with its respective cultivar in treatments T2 and T3, the dry biomass production increased in T2 (8%) and in T3 (73%).

The highest stem diameter (7 mm) was noticed for cultivar V3 in treatment T2 (Fig. 1C). Cultivar V3, produced the highest stem diameter in all treatments T1-T3. Importantly, when cultivar V3 in treatment T2 was compared with all *Salix* (V1-V4) in treatment T1, the stem diameter was 54%, 49%, 37% and 83% higher for V1, V2, V3, and V4 respectively.

The study shows that the highest root length (24.43 cm) was observed in cultivar V3 in treatment T2. and the lowest (12.83 cm) in species V2 in treatment T3 (Fig. 1D). When treatment T1 is compared to other treatments, the root length of species V2 decreased 13% and 23% in treatment T2 and T3 respectively. In the case of cultivar V3, a 10% increase and 6% decrease were observed in root length in treatments T2 and T3 respectively. However, species V1 and cultivar V3 produced an almost similar root growth in all treatments as no difference was found for these *Salix* between all treatments (T1-T3).

Noticeably, cultivar V3 produced the highest absolute mean height, dry biomass amount, stem diameter and root length in La treatment T2 among all *Salix* as compared with treatments T1 and T3.

#### 3.2. Rare earth elements (REE) concentration in *Salix* tissues

##### 3.2.1. REE concentration in *Salix* leaves

The highest La concentration was noticed in species V2 in treatments T2 and T3, whereas in treatment T1 a small concentration of La and other REE were observed in all *Salix* compared to treatments T2 and T3 (Table 3A).

When compared with La concentrations in control treatment T1, La concentration in *Salix* (V1-V4) in treatment T2 was comparatively higher 85% (V1), 1493% (V2) (1493%), 181% (V3), and 140% (V4). Instead, when *Salix* in treatment T2 were compared with their respective *Salix* in treatment T3, significant increases of 18% and 57% were noted for species V1 and cultivar V3 respectively. However, a decrease of around 79% and 32% was observed for species V2 and cultivar V4 respectively.

Furthermore, REE concentration differences in *Salix* (V1-V4) in treatment T3 with respect to the treatment T1 was observed as V1 (La: +56%), (Y: +1100%), (Ce: +75%), (Nd: +100%), (Tb: +214%) and



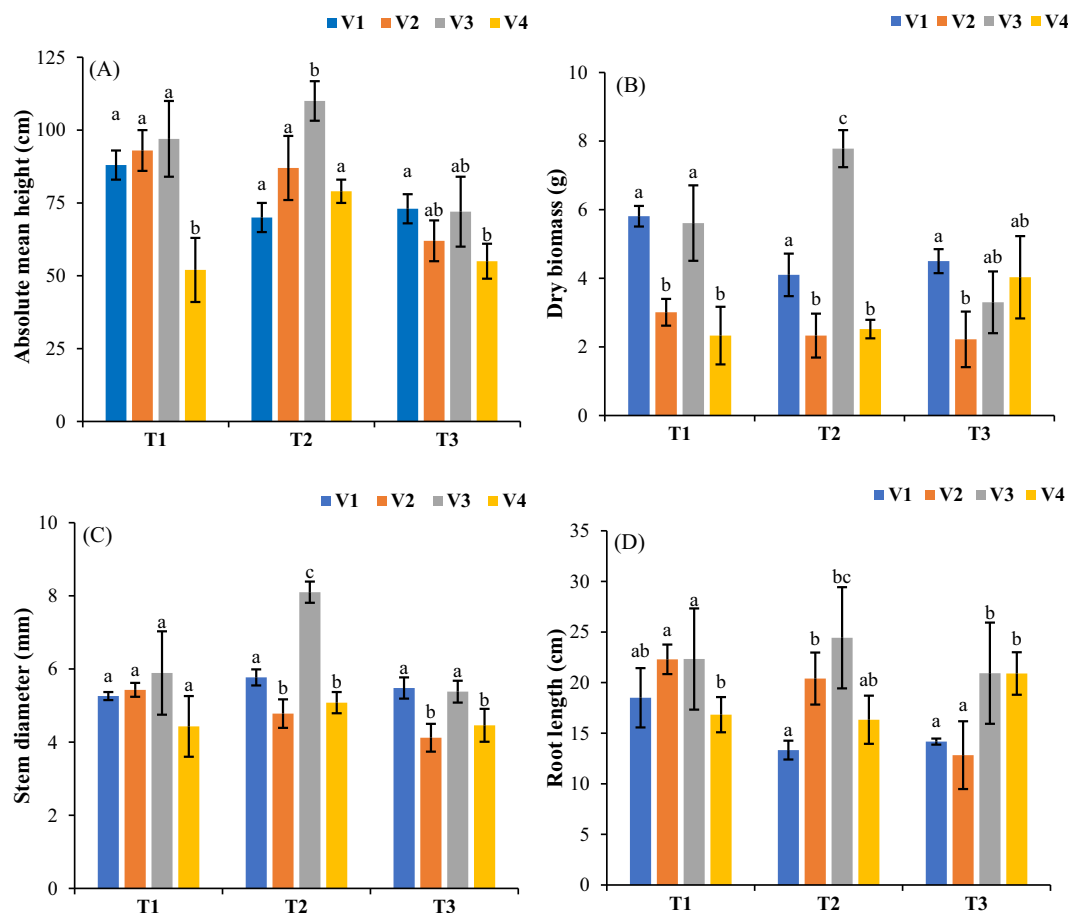


Fig. 1. A–D. Effects of different rare earth elements concentrations on the *Salix* A) absolute mean height, B) dry biomass, C) stem diameter and D) root length. Values are the mean ( $\pm$ SE) within each treatment. Different small letters indicate the significant differences at  $p < 0.05$  within *Salix*.

(Dy: +750%), V2 (La: +7650%), (Y: +8150%), (Ce: +3471%), (Nd: +3085%), (Tb: +4860%) and (Dy: +5480%), V3 (La: +78%), (Y: +817%), (Ce: -46%), (Nd: -30%), (Tb: +36%) and (Dy: +431%), V4 (La: +258%), (Y: +672%), (Ce: -15%), (Nd: -10%), (Tb: +69%) and (Dy: +354%).

### 3.2.2. REE concentration in *Salix* stem

The cultivar V3 showed the highest concentration of REEs when compared to species V1 and V2 and cultivar V4 in control treatment T1. In treatments T2 and T3 with increased La concentration, species V2 and cultivar V4 showed the highest concentrations in stems respectively.

Table 3A

REE concentration ( $\mu\text{g g}^{-1}$  DW) in the leaves of four *Salix* (V1-V4) under different treatments (T1-T3). The values are expressed as mean  $\pm$  SE ( $n = 3$ ). Values shown by dissimilar letters within the cultivars in each treatment are significantly different at  $p < 0.05$ .

Treatment	Cultivars	La	Y	Ce	Nd	Tb	Dy
T1	V1	0.66 $\pm$ 0.17 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.08 $\pm$ 0.02 <sup>a</sup>	0.06 $\pm$ 0.04 <sup>a</sup>	0.07 $\pm$ 0.05 <sup>a</sup>	0.04 $\pm$ 0.03 <sup>a</sup>
	V2	0.16 $\pm$ 0.07 <sup>b</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	0.07 $\pm$ 0.02 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>
	V3	0.32 $\pm$ 0.14 <sup>b</sup>	0.17 $\pm$ 0.04 <sup>b</sup>	0.26 $\pm$ 0.04 <sup>b</sup>	0.20 $\pm$ 0.09 <sup>b</sup>	<b>0.36</b> $\pm$ 0.12 <sup>b</sup>	0.16 $\pm$ 0.08 <sup>b</sup>
	V4	<b>0.67</b> $\pm$ 0.16 <sup>a</sup>	<b>0.22</b> $\pm$ 0.08 <sup>b</sup>	<b>0.32</b> $\pm$ 0.08 <sup>b</sup>	<b>0.29</b> $\pm$ 0.11 <sup>b</sup>	0.33 $\pm$ 0.26 <sup>ab</sup>	<b>0.24</b> $\pm$ 0.09 <sup>b</sup>
T2	V1	1.22 $\pm$ 0.91 <sup>ab</sup>					
	V2	<b>2.55</b> $\pm$ 1.13 <sup>a</sup>					
	V3	0.90 $\pm$ 0.31 <sup>b</sup>					
	V4	1.61 $\pm$ 0.91 <sup>ab</sup>					
T3	V1	1.03 $\pm$ 0.35 <sup>a</sup>	0.60 $\pm$ 0.09 <sup>a</sup>	0.14 $\pm$ 0.04 <sup>a</sup>	0.12 $\pm$ 0.03 <sup>a</sup>	0.22 $\pm$ 0.04 <sup>a</sup>	0.34 $\pm$ 0.04 <sup>a</sup>
	V2	<b>12.40</b> $\pm$ 4.31 <sup>b</sup>	<b>3.30</b> $\pm$ 1.21 <sup>b</sup>	<b>2.50</b> $\pm$ 1.32 <sup>b</sup>	<b>2.23</b> $\pm$ 1.12 <sup>b</sup>	<b>2.48</b> $\pm$ 1.04 <sup>b</sup>	<b>2.79</b> $\pm$ 1.18 <sup>b</sup>
	V3	0.57 $\pm$ 0.27 <sup>a</sup>	1.56 $\pm$ 0.35 <sup>c</sup>	0.14 $\pm$ 0.08 <sup>a</sup>	0.14 $\pm$ 0.09 <sup>ac</sup>	0.49 $\pm$ 0.18 <sup>c</sup>	0.85 $\pm$ 0.16 <sup>c</sup>
	V4	2.40 $\pm$ 1.98 <sup>a</sup>	1.70 $\pm$ 0.34 <sup>c</sup>	0.27 $\pm$ 0.02 <sup>a</sup>	0.26 $\pm$ 0.04 <sup>c</sup>	0.56 $\pm$ 0.09 <sup>c</sup>	1.09 $\pm$ 0.33 <sup>c</sup>

Bold values indicate the highest accumulation among *Salix*.

During treatment T2, La concentration in the stem correspondingly increased by 2200%, 1855%, 252% and 421% for *Salix* (V1-V4) respectively when compared to treatment T1. Meanwhile, when La concentration in all *Salix* in treatment T2 was compared with their respective *Salix* in treatment T3 there was a significant observable increase for example V1 (374%), V2 (433%) and V3 (172%) excluding V4, which with a concentration reduced by 14% (Table 3B).

There was a substantial increase observed in REE concentration in treatment T3 for the studied *Salix* as compared to their respective *Salix* in treatment T1 (Table 3B). For V1, La 384%, Y 500%, Ce 366%, Nd 420%, Tb 316% and Dy 575%; for V2 La 266%, Y 440%, Ce 175%, Nd 137%, Tb 400% and Dy 450%; for V4 La 507%, Y 1650%, Ce 560%, Nd 575%, Tb

**Table 3B**

REE concentration ( $\mu\text{g g}^{-1}$  DW) in the stem of four *Salix* (V1-V4) under different treatments (T1-T3). The values are expressed as mean  $\pm$  SE (n = 3). Values shown by dissimilar letters within the cultivars in each treatment are significantly different at  $p < 0.05$ .

Treatment	Cultivars	La	Y	Ce	Nd	Tb	Dy
T1	V1	0.13 $\pm$ 0.12 <sup>a</sup>	0.04 $\pm$ 0.03 <sup>a</sup>	0.06 $\pm$ 0.04 <sup>a</sup>	0.05 $\pm$ 0.04 <sup>a</sup>	0.06 $\pm$ 0.05 <sup>a</sup>	0.04 $\pm$ 0.03 <sup>ab</sup>
	V2	0.18 $\pm$ 0.09 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.08 $\pm$ 0.07 <sup>a</sup>	0.08 $\pm$ 0.05 <sup>ab</sup>	0.05 $\pm$ 0.04 <sup>a</sup>	0.04 $\pm$ 0.02 <sup>ab</sup>
	V3	<b>0.48 <math>\pm</math> 0.14<sup>b</sup></b>	<b>0.31 <math>\pm</math> 0.18<sup>b</sup></b>	<b>0.46 <math>\pm</math> 0.29<sup>b</sup></b>	<b>0.34 <math>\pm</math> 0.23<sup>b</sup></b>	<b>0.50 <math>\pm</math> 0.37<sup>b</sup></b>	<b>0.25 <math>\pm</math> 0.20<sup>a</sup></b>
	V4	0.14 $\pm$ 0.11 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.04 <sup>a</sup>	0.04 $\pm$ 0.03 <sup>a</sup>	0.05 $\pm$ 0.04 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>b</sup>
T2	V1	2.99 $\pm$ 1.18 <sup>ab</sup>					
	V2	<b>3.52 <math>\pm</math> 1.35<sup>a</sup></b>					
	V3	1.69 $\pm$ 0.35 <sup>b</sup>					
	V4	0.73 $\pm$ 0.25 <sup>c</sup>					
T3	V1	0.63 $\pm$ 0.10 <sup>a</sup>	0.24 $\pm$ 0.05 <sup>a</sup>	0.28 $\pm$ 0.11 <sup>a</sup>	0.26 $\pm$ 0.11 <sup>a</sup>	0.25 $\pm$ 0.07 <sup>a</sup>	<b>0.27 <math>\pm</math> 0.11<sup>a</sup></b>
	V2	0.66 $\pm$ 0.34 <sup>a</sup>	0.27 $\pm$ 0.15 <sup>a</sup>	0.22 $\pm$ 0.11 <sup>a</sup>	0.19 $\pm$ 0.09 <sup>a</sup>	0.25 $\pm$ 0.13 <sup>a</sup>	0.22 $\pm$ 0.10 <sup>a</sup>
	V3	0.62 $\pm$ 0.35 <sup>a</sup>	0.23 $\pm$ 0.06 <sup>a</sup>	0.26 $\pm$ 0.07 <sup>a</sup>	0.25 $\pm$ 0.06 <sup>a</sup>	0.32 $\pm$ 0.11 <sup>a</sup>	0.22 $\pm$ 0.03 <sup>a</sup>
	V4	<b>0.85 <math>\pm</math> 0.40<sup>a</sup></b>	<b>0.35 <math>\pm</math> 0.12<sup>a</sup></b>	<b>0.33 <math>\pm</math> 0.20<sup>a</sup></b>	<b>0.27 <math>\pm</math> 0.16<sup>a</sup></b>	<b>0.37 <math>\pm</math> 0.23<sup>a</sup></b>	0.26 $\pm$ 0.10 <sup>a</sup>

Bold values indicate the highest accumulation among *Salix*.

640% and Dy 1200%. Noticeably, cultivar V3 showed an increment of about 29% for La only, while a decrease was observed in Y: 25%, Ce: 43%, Nd: 26%, Tb: 36% and Dy: 12%.

### 3.2.3. REE concentration in *Salix* roots

In control treatment T1, the highest REE concentration in roots was observed in cultivars V3 and V4 when compared with other studied cultivars in treatment T1 (Table 3C). In treatment T2, V4 revealed the highest concentration of La followed by V1, V2, and V3. In treatment T3, cultivar V3 exhibited a larger concentration of REE than V1, V2, and V4. Compared to treatment T1, all *Salix* showed higher concentrations of REE in treatment T2 and T3.

The comparison among all *Salix* exposed to treatment T1 and T2 revealed that La concentration distinctively increased in treatment T2 when compared with respective *Salix* in treatment T1. Moreover, when *Salix* in treatment T2 were compared with respective *Salix* in treatment T3 a considerable increase was observed for La concentration such as V1 (772%), V2 (610%), V3 (100%), and V4 (574%).

## 3.3. Rare earth elements (REE) accumulation in *Salix* tissues

### 3.3.1. REE accumulation in *Salix* leaves

In treatments T1 and T2, the highest accumulation (5.59–8.11  $\mu\text{g plant}^{-1}$ ) of La was seen in species V1 respectively as compared to other *Salix* (Table 4A). On the other hand, cultivar V3 demonstrated proficiency in accumulating all elements excluding La in treatment T1. Noticeably, species V1 was found to be the best among all *Salix* in terms of accumulating La in treatment T2 and cultivar V4 was discovered to have accumulated all REE when compared to other *Salix*.

When the La accumulation in *Salix* (V1-V4) in treatment T1 was compared with respective *Salix* in treatment T2 there was an increase of 45% (V1), 564% (V2), 193% (V3) and 147% (V4). On the other hand, when

**Table 3C**

REE concentration ( $\mu\text{g g}^{-1}$  DW) in the roots of four *Salix* (V1-V4) under different treatments (T1-T3). The values are expressed as mean  $\pm$  SE (n = 3). Values shown by dissimilar letters within the cultivars in each treatment are significantly different at  $p < 0.05$ .

Treatment	Cultivars	La	Y	Ce	Nd	Tb	Dy
T1	V1	3.07 $\pm$ 1.15 <sup>a</sup>	0.42 $\pm$ 0.07 <sup>a</sup>	0.69 $\pm$ 0.10 <sup>a</sup>	0.58 $\pm$ 0.09 <sup>a</sup>	0.62 $\pm$ 0.10 <sup>a</sup>	0.45 $\pm$ 0.07 <sup>a</sup>
	V2	2.89 $\pm$ 1.10 <sup>a</sup>	0.51 $\pm$ 0.17 <sup>a</sup>	0.85 $\pm$ 0.27 <sup>a</sup>	0.69 $\pm$ 0.19 <sup>a</sup>	0.76 $\pm$ 0.28 <sup>ac</sup>	0.48 $\pm$ 0.13 <sup>a</sup>
	V3	<b>10.24 <math>\pm</math> 5.79<sup>b</sup></b>	<b>6.63 <math>\pm</math> 3.42<sup>b</sup></b>	<b>10.72 <math>\pm</math> 4.35<sup>b</sup></b>	<b>8.08 <math>\pm</math> 3.68<sup>b</sup></b>	<b>11.04 <math>\pm</math> 6.73<sup>b</sup></b>	<b>6.14 <math>\pm</math> 3.77<sup>b</sup></b>
	V4	<b>10.23 <math>\pm</math> 4.93<sup>b</sup></b>	1.34 $\pm$ 0.72 <sup>a</sup>	1.95 $\pm$ 0.93 <sup>a</sup>	1.82 $\pm$ 0.88 <sup>a</sup>	1.95 $\pm$ 1.07 <sup>c</sup>	1.55 $\pm$ 0.76 <sup>c</sup>
T2	V1	7092 $\pm$ 3483 <sup>a</sup>					
	V2	4635 $\pm$ 2393 <sup>a</sup>					
	V3	4164 $\pm$ 3756 <sup>a</sup>					
	V4	<b>8404 <math>\pm</math> 7203<sup>a</sup></b>					
T3	V1	813 $\pm$ 361 <sup>ab</sup>	606 $\pm$ 285 <sup>ab</sup>	815 $\pm$ 399 <sup>ab</sup>	710 $\pm$ 376 <sup>ab</sup>	736 $\pm$ 337 <sup>a</sup>	665 $\pm$ 376 <sup>a</sup>
	V2	652 $\pm$ 390 <sup>a</sup>	349 $\pm$ 149 <sup>b</sup>	633 $\pm$ 352 <sup>a</sup>	637 $\pm$ 375 <sup>a</sup>	455 $\pm$ 212 <sup>a</sup>	642 $\pm$ 353 <sup>a</sup>
	V3	<b>2082 <math>\pm</math> 925<sup>b</sup></b>	<b>1058 <math>\pm</math> 438<sup>a</sup></b>	<b>2013 <math>\pm</math> 946<sup>b</sup></b>	<b>1956 <math>\pm</math> 888<sup>b</sup></b>	<b>2514 <math>\pm</math> 1284<sup>b</sup></b>	<b>1159 <math>\pm</math> 447<sup>a</sup></b>
	V4	1246 $\pm$ 576 <sup>ab</sup>	890 $\pm$ 359 <sup>a</sup>	1162 $\pm$ 531 <sup>ab</sup>	1033 $\pm$ 517 <sup>ab</sup>	1101 $\pm$ 477 <sup>ab</sup>	968 $\pm$ 483 <sup>a</sup>

Bold values indicate the highest accumulation among *Salix*.

*Salix* in treatment T2 were compared with their respective *Salix* in treatment T3, a significant increase of 20% and 79% was found for species V1 and cultivar V3 respectively, contrary to the decrease of about 47% and 91% observed for species V2 and cultivar V4 respectively.

In treatment T3, REE concentrations substantially increased in *Salix* V1, V2 and V4 with respect to their relative *Salix* in treatment T1. For V1 (La:16%, Y: 685%, Ce: 24%, Nd: 37%, Tb: 141%, and Dy: 560%), for V2 (La: 1157%, Y: 688%, Ce: 220%, Nd: 248%, Tb: 262%, and Dy: 514%) and for V4 (La: 2716%, Y: 1904%, Ce: 136%, Nd: 153%, Tb: 369%, and Dy:1366%). In contrast, there was a significant reduction noticed for V3 (La: 38%), Y: 265%, Ce: 82%, Nd: 75%, and Tb: 50% excluding Dy as it increased by 110%. Results demonstrated that *Salix* species and cultivars manifest diverse capabilities for accumulating REE in leaves when grown in single-La and six-REE solutions.

### 3.3.2. REE accumulation in *Salix* stem

Cultivar V3 presented the highest accumulation of La (3.85  $\mu\text{g plant}^{-1}$ ) in the stem in treatment T1 (Table 4B). REE accumulation ranged from 2.08–4.03  $\mu\text{g}$  in treatment T2 across all *Salix* while, in treatment T3, species V1 accumulated higher contents of REE compared to other *Salix*.

REE accumulation varied among all *Salix* and in every treatment each *Salix* performed differently. For example, when species V1 in treatment T1 was compared with its respective *Salix* in treatment T2, La accumulation was increased by 1105%. For other *Salix*, the increase was 685% (V2), 424% (V3) and 1570% (V4). Subsequently, a significant increase in La accumulation was observed for *Salix* V1 (221%), V2 (612%), and V4 (601%) in treatment T2, as compared to their relevant *Salix* in treatment T3, although for cultivar V3 La accumulation decreased by about 14%.

Furthermore, a significant increase in REE content was also noticed among *Salix* V1, V2 and V4 in treatment T3 when compared with their respective *Salix* in treatment T1. For V1 (La: 275%, Y: 343%, Ce: 268%, Nd: 105%, Tb: 247%, Dy: 400%), for V2 (La: 183%, Y: 105%, Ce: 25%, Nd:

**Table 4A**

REE accumulation ( $\mu\text{g plant}^{-1}$ ) in the leaves of four *Salix* (V1-V4) under different treatments (T1-T3). The values are expressed as mean  $\pm$  SE (n = 3). Values shown by dissimilar letters within the cultivars in each treatment are significantly different at  $p < 0.05$ .

Treatment	Cultivars	La	Y	Ce	Nd	Tb	Dy
T1	V1	<b>5.59</b> $\pm$ 3.49 <sup>a</sup>	0.48 $\pm$ 0.35 <sup>a</sup>	0.70 $\pm$ 0.46 <sup>a</sup>	0.54 $\pm$ 0.34 <sup>a</sup>	0.58 $\pm$ 0.44a	0.33 $\pm$ 0.23 <sup>a</sup>
	V2	0.28 $\pm$ 0.08 <sup>b</sup>	0.36 $\pm$ 0.14 <sup>a</sup>	0.67 $\pm$ 0.20 <sup>a</sup>	0.62 $\pm$ 0.21 <sup>a</sup>	0.48 $\pm$ 0.22a	0.50 $\pm$ 0.21 <sup>b</sup>
	V3	2.12 $\pm$ 1.96 <sup>ab</sup>	<b>1.17</b> $\pm$ 1.11 <sup>a</sup>	<b>1.74</b> $\pm$ 1.15 <sup>a</sup>	<b>1.37</b> $\pm$ 0.72 <sup>a</sup>	<b>2.44</b> $\pm$ 0.89 <sup>b</sup>	<b>1.10</b> $\pm$ 0.58 <sup>a</sup>
	V4	1.60 $\pm$ 1.12 <sup>a</sup>	0.72 $\pm$ 0.67 <sup>a</sup>	0.98 $\pm$ 0.65 <sup>a</sup>	0.91 $\pm$ 0.45 <sup>a</sup>	1.04 $\pm$ 0.65 <sup>ab</sup>	0.78 $\pm$ 0.37 <sup>a</sup>
T2	V1	<b>8.11</b> $\pm$ 2.38 <sup>a</sup>					
	V2	1.86 $\pm$ 1.48 <sup>b</sup>					
	V3	6.21 $\pm$ 1.47 <sup>ac</sup>					
	V4	3.95 $\pm$ 1.65 <sup>bc</sup>					
T3	V1	6.48 $\pm$ 2.18 <sup>a</sup>	3.77 $\pm$ 0.82 <sup>a</sup>	0.87 $\pm$ 0.29 <sup>a</sup>	0.74 $\pm$ 0.22 <sup>a</sup>	1.41 $\pm$ 0.34 <sup>a</sup>	2.18 $\pm$ 0.42 <sup>a</sup>
	V2	3.52 $\pm$ 2.37 <sup>ab</sup>	2.84 $\pm$ 2.35 <sup>a</sup>	2.15 $\pm$ 0.78 <sup>b</sup>	2.16 $\pm$ 1.04 <sup>b</sup>	1.74 $\pm$ 1.53 <sup>ab</sup>	3.07 $\pm$ 2.74 <sup>a</sup>
	V3	1.30 $\pm$ 0.47 <sup>b</sup>	4.28 $\pm$ 0.53 <sup>a</sup>	0.31 $\pm$ 0.17 <sup>c</sup>	0.33 $\pm$ 0.19 <sup>c</sup>	1.22 $\pm$ 0.26 <sup>a</sup>	2.31 $\pm$ 0.17 <sup>a</sup>
	V4	<b>45.07</b> $\pm$ 27.65 <sup>c</sup>	<b>14.43</b> $\pm$ 6.91 <sup>b</sup>	<b>2.32</b> $\pm$ 0.65 <sup>b</sup>	<b>2.31</b> $\pm$ 1.09 <sup>b</sup>	<b>4.88</b> $\pm$ 2.38 <sup>b</sup>	<b>11.44</b> $\pm$ 4.61 <sup>b</sup>

Bold values indicate the highest accumulation among *Salix*.

18%, Tb: 128%, Dy: 144%), and for V4 (La: 1858%, Y: 5050%, Ce: 1366%, Nd: 1360%, Tb: 1740%, Dy: 2433%). However, there was a significant reduction observed in the case of cultivar V3 grown in treatment T3 in comparison with treatment T2; La: 25%, Y: 56%, Ce: 68%, Nd: 58%, Tb: 65%, Dy: 48%.

### 3.3.3. REE accumulation in *Salix* root

In treatment T2, *Salix* V1-V4 presented a significant increment of La accumulation as compared to their relevant *Salix* in treatment T3; for V1: 712%, V2: 175%, V3: 327%, and V4: 1042% (Table 4C). Generally, the results revealed that all *Salix* had significantly higher REE quantities in roots from treatments T2 and T3 than in the roots from treatment T1. Consequently, a comparison among *Salix* shown that cultivar V3 performed the best of all the treatments (T1-T3) in terms of the highest REE accumulation.

### 3.4. Retention of REE in ashes following combustion of *Salix* biomass

The sample for combustion experiments was made by combining the leaves, stems, and roots from four *Salix* cultivars from treatment T3 (Table 2). Yields of ash (% w/w, dry basis) following the combustion of the homogenized *Salix* sample were 6.6%, and 6.3% at combustion temperatures of 800 °C, and 1000 °C respectively. The trend of decreasing ash content with increasing combustion temperature is largely attributed to the greater volatilization of K at high combustion temperatures. The concentrations of non-REE in the ashes prepared at 800 °C and 1000 °C, including Al, Ca, Fe, K, Mg, Mn, Na, P, Ti, and Zn, are presented in Fig. 2. This figure shows that the *Salix* ashes are largely composed of K (20–23% w/w), P (10–11% w/w), and Ca (5.4–6.2% w/w). The following elements Na, Mg, Al, Ti, Fe, Zn, and Mn were present in the ashes in relatively minor concentrations.

The retention rates and concentrations of REE (La, Y, Nd, Ce, Dy, and Tb) in the bottom ashes after combustion of the oven-dried *Salix* biomass

**Table 4B**

REE accumulation ( $\mu\text{g plant}^{-1}$ ) in the stem of four *Salix* (V1-V4) under different treatments (T1-T3). The values are expressed as mean  $\pm$  SE (n = 3). Values shown by dissimilar letters within the cultivars in each treatment are significantly different at  $p < 0.05$ .

Treatment	Cultivars	La	Y	Ce	Nd	Tb	Dy
T1	V1	0.97 $\pm$ 0.75 <sup>a</sup>	0.32 $\pm$ 0.28 <sup>a</sup>	0.45 $\pm$ 0.40 <sup>ab</sup>	0.37 $\pm$ 0.35 <sup>ab</sup>	0.42 $\pm$ 0.38 <sup>ab</sup>	0.32 $\pm$ 0.31 <sup>ac</sup>
	V2	0.93 $\pm$ 0.47 <sup>a</sup>	0.35 $\pm$ 0.30 <sup>a</sup>	0.54 $\pm$ 0.45 <sup>ab</sup>	0.53 $\pm$ 0.34 <sup>a</sup>	0.32 $\pm$ 0.26 <sup>ab</sup>	0.29 $\pm$ 0.24 <sup>a</sup>
	V3	<b>3.85</b> $\pm$ 3.11 <sup>a</sup>	<b>2.46</b> $\pm$ 2.18 <sup>a</sup>	<b>3.77</b> $\pm$ 3.27 <sup>b</sup>	<b>2.78</b> $\pm$ 2.34 <sup>a</sup>	<b>4.03</b> $\pm$ 3.70 <sup>b</sup>	<b>2.08</b> $\pm$ 1.37 <sup>b</sup>
	V4	0.17 $\pm$ 0.05 <sup>b</sup>	0.02 $\pm$ 0.01 <sup>b</sup>	0.06 $\pm$ 0.02 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>b</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>c</sup>
T2	V1	11.69 $\pm$ 2.33 <sup>a</sup>					
	V2	18.80 $\pm$ 8.90 <sup>ab</sup>					
	V3	<b>20.21</b> $\pm$ 4.65 <sup>b</sup>					
	V4	2.84 $\pm$ 0.89 <sup>c</sup>					
T3	V1	<b>3.64</b> $\pm$ 0.96 <sup>a</sup>	<b>1.42</b> $\pm$ 0.44 <sup>a</sup>	<b>1.66</b> $\pm$ 0.77 <sup>a</sup>	<b>1.50</b> $\pm$ 0.73	<b>1.46</b> $\pm$ 0.56 <sup>ab</sup>	<b>1.60</b> $\pm$ 0.75 <sup>ab</sup>
	V2	2.64 $\pm$ 2.09 <sup>a</sup>	0.72 $\pm$ 0.32 <sup>a</sup>	0.68 $\pm$ 0.39 <sup>a</sup>	0.63 $\pm$ 0.40	0.73 $\pm$ 0.40 <sup>ab</sup>	0.71 $\pm$ 0.40 <sup>ab</sup>
	V3	2.88 $\pm$ 1.55 <sup>a</sup>	1.06 $\pm$ 0.15 <sup>a</sup>	1.19 $\pm$ 0.17 <sup>a</sup>	1.15 $\pm$ 0.15	1.40 $\pm$ 0.35 <sup>a</sup>	1.08 $\pm$ 0.13 <sup>a</sup>
	V4	3.33 $\pm$ 2.31 <sup>a</sup>	1.03 $\pm$ 0.17 <sup>a</sup>	0.88 $\pm$ 0.34 <sup>a</sup>	0.73 $\pm$ 0.27	0.92 $\pm$ 0.34 <sup>b</sup>	0.76 $\pm$ 0.16 <sup>b</sup>

Bold values indicate the highest accumulation among *Salix*.

are presented in Fig. 3. The figure shows greater than 80% retention rates for REE in the bottom ashes, indicating that volatilization of all REE is minor at most during combustion. The figure also shows that all the analyzed REE were recovered from the residual ashes in similar proportions, i.e., they were not separated from each other during combustion and the combustion temperature has an insignificant impact on the extent of release of the REE below 1000 °C. Thus, the REE were substantially enriched in the ash residues following combustion. There was a 13-fold enrichment of the REE following combustion at 800 °C and a 14-fold enrichment of the REE following combustion at 1000 °C. Due to the low volatility of the REE, it is expected that the REE will largely report concentrate in, bottom ashes rather than fly ashes, when *Salix* is combusted in industrial-scale processes. The total REO concentrations of the residual ashes were 15 and 17% for heat treatments at 800 °C and 1000 °C, respectively.

The (Powder X-ray diffraction) spectrum for the combustion ash prepared at 800 °C (Fig. 4) showed that the main crystalline phases in the ash are  $\text{K}_2\text{SO}_4$  and rare earth metal oxides (REOs), specifically cerium oxide ( $\text{CeO}_2$ ), dysprosium oxide ( $\text{Dy}_2\text{O}_3$ ), lanthanum oxide ( $\text{La}_2\text{O}_3$ ), neodymium oxide ( $\text{Nd}_2\text{O}_3$ ), terbium oxide ( $\text{Tb}_2\text{O}_3$ ) and yttrium oxide ( $\text{Y}_2\text{O}_3$ ). The diffraction patterns for the REOs are complete and match well with all analyzed REOs. However, peaks in diffraction patterns of REOs are close to each other, and thus only broad peaks are observed instead of individual peak patterns, covering all the REOs. Probable minor crystalline phases in the ash include diopside ( $\text{CaMgSi}_2\text{O}_6$ ), bredigite ( $\text{Ca}_{14}\text{Mg}_2(\text{SiO}_4)_8$ ), potassium phosphate ( $\text{K}_4\text{P}_2\text{O}_7$ ), and calcium magnesium aluminum silicate ( $\text{CaO}\cdot\text{MgO}\cdot\text{Al}_2\text{O}_3$ ).

## 4. Discussion

Currently, substantial use of REEs in modern technology and their phytomining has received significant attention globally. However, few research have been published to date on REEs accumulation in short-

**Table 4C**

REE accumulation ( $\mu\text{g plant}^{-1}$ ) in the roots of four *Salix* (V1-V4) under different treatments (T1-T3). The values are expressed as mean  $\pm$  SE (n = 3). Values shown by dissimilar letters within cultivars in each treatment are significantly different at  $p < 0.05$ .

Treatment	Cultivars	La	Y	Ce	Nd	Tb	Dy
T1	V1	5.76 $\pm$ 1.86 <sup>a</sup>	0.84 $\pm$ 0.17 <sup>a</sup>	1.36 $\pm$ 0.22 <sup>a</sup>	1.15 $\pm$ 0.18 <sup>a</sup>	1.24 $\pm$ 0.25 <sup>a</sup>	0.89 $\pm$ 0.16 <sup>a</sup>
	V2	3.91 $\pm$ 1.57 <sup>a</sup>	0.96 $\pm$ 0.28 <sup>a</sup>	1.60 $\pm$ 0.43 <sup>a</sup>	1.29 $\pm$ 0.29 <sup>a</sup>	1.41 $\pm$ 0.46 <sup>a</sup>	0.91 $\pm$ 0.19 <sup>a</sup>
	V3	<b>16.97 <math>\pm</math> 8.35<sup>b</sup></b>	<b>11.25 <math>\pm</math> 6.53<sup>b</sup></b>	<b>18.19 <math>\pm</math> 14.44<sup>b</sup></b>	<b>13.71 <math>\pm</math> 10.65<sup>b</sup></b>	<b>18.75 <math>\pm</math> 17.26<sup>a</sup></b>	<b>10.43 <math>\pm</math> 9.81<sup>a</sup></b>
	V4	4.55 $\pm$ 2.02 <sup>a</sup>	0.90 $\pm$ 0.51 <sup>a</sup>	1.40 $\pm$ 0.82 <sup>a</sup>	1.32 $\pm$ 0.80 <sup>a</sup>	1.28 $\pm$ 0.73 <sup>a</sup>	1.17 $\pm$ 0.77 <sup>a</sup>
T2	V1	10,147 $\pm$ 5232 <sup>a</sup>					
	V2	2846 $\pm$ 2067 <sup>b</sup>					
	V3	<b>10,548 <math>\pm</math> 8170<sup>ab</sup></b>					
	V4	9837 $\pm$ 7860 <sup>ab</sup>					
T3	V1	1249 $\pm$ 543 <sup>ab</sup>	916 $\pm$ 401 <sup>ab</sup>	1285 $\pm$ 665 <sup>a</sup>	1138 $\pm$ 642 <sup>a</sup>	1127 $\pm$ 410 <sup>a</sup>	1075 $\pm$ 646 <sup>a</sup>
	V2	1033 $\pm$ 856 <sup>ab</sup>	500 $\pm$ 368 <sup>ab</sup>	977 $\pm$ 780 <sup>a</sup>	1002 $\pm$ 816 <sup>a</sup>	667 $\pm$ 504 <sup>a</sup>	983 $\pm$ 773 <sup>a</sup>
	V3	<b>2468 <math>\pm</math> 842<sup>b</sup></b>	<b>1273 <math>\pm</math> 386<sup>b</sup></b>	<b>2356 <math>\pm</math> 364<sup>b</sup></b>	<b>2307 <math>\pm</math> 450<sup>b</sup></b>	<b>2880 <math>\pm</math> 1224<sup>b</sup></b>	<b>1416 <math>\pm</math> 335<sup>a</sup></b>
	V4	861 $\pm$ 432 <sup>a</sup>	622 $\pm$ 238 <sup>a</sup>	849 $\pm$ 409 <sup>a</sup>	780 $\pm$ 410 <sup>a</sup>	769 $\pm$ 344 <sup>a</sup>	763 $\pm$ 383 <sup>a</sup>

Bold values indicate the highest accumulation among *Salix*.

rotation *Salix* biomass (Midula et al., 2017; Mleczek et al., 2018). Further research about the toxic effect of REEs on different fast-growing bioenergy crops and their accumulation are therefore needed to validate the observed results.

#### 4.1. Rare earth element (REE) effects on the *Salix* growth parameters

The results revealed that a single La and mixture of six-REE doses showed a positive influence on all *Salix* growth parameters. All *Salix* (V1-V4) produced an applicable growth, amount of biomass, shoot diameter, and root length under La and REE exposures when compared to the control treatment. However, cultivar V3 demonstrated the best performance in all growth parameters.

Biomass production is an important parameter to utilize the plants for phytoremediation (Yang et al., 2014; Mohsin, 2016). Controversy lingers among the scientific community about the positive effects of REEs on plant growth. Our findings are in agreement with Guo et al. (2013), Ma et al. (2014) and Shtangeeva (2014), who reported plant growth improvement and a significant increase in the biomass yield of different crops under REE exposure. Conversely, Thomas et al. (2014) observed a decrease in growth, root function, and nutritional uptake for two Canadian native plants *Asclepias syriaca* and *Desmodium canadense* exposed to REE doses. Furthermore, d'Aquino et al. (2009) observed a decrease in the seed germination rate in *Triticum durum* at low doses of La (0.01 mM) and REEs (La, Ce, Pr, Nd, and Gd) (0.1 mM), while root growth was improved under low doses and decreased with high doses of La (1 mM) and REE (10 mM). Noticeably, positive effects have also been reported with low concentrations of REE, whereas negative effects become apparent as doses are increased (Carpenter et al., 2015). Lower concentrations of REEs might promote growth development and biomass production of vascular plants (Tyler and Olsson, 2005; Liu et al., 2021) which is also in congruence to

the physiological results such as height, diameter, and root growth and biomass production observed in this study. Increase in *Salix* growth and biomass might be due to the inducement of cell proliferation (de Oliveira et al., 2015), very efficient photosystem-II photo chemistry, and electron transport rate by REEs (Yuan et al., 2017), or high nutrient uptake (Hoeber et al., 2017). REEs as micro-fertilizers have been widely used in agriculture in China, Australia, Korea, Japan, the Philippines, and Switzerland since 1990, and it has been stated that REEs can significantly enhance crop production and quality (Yuan et al., 2018). However, the use of REEs in agriculture and forestry in order to increase different plant yields should be further investigated under different concentrations to understand the behavior of these elements on plant productivity.

#### 4.2. Rare earth elements (REE) concentration, accumulation, and translocation in *Salix* tissues

In the study, we observed higher concentration and accumulation of REE in the *Salix* roots, followed by the stem and leaves (Tables 3A, 3B, 3C, 4A, 4B, and 4C). Our findings are consistent with Tyler's (2004) notion, who reported that a higher concentration of REEs in above-ground biomass of vascular plants is generally low as compared to the roots. Researchers (Fu et al., 1998; Zhang et al., 2002; Thomas et al., 2014; Carpenter et al., 2015; Liu et al., 2021) found a higher accumulation of REEs in the plant roots than in the shoots on a dry biomass basis in greenhouse conditions. Carpenter et al. (2015) reported that REEs can be accumulated by plant roots due to the similar ionic radii that they share with calcium and REEs may replace calcium molecules in several physiological processes involving proteins and enzymes, including root growth, photosynthesis, and flowering.

In this regard, the following reasons can be considered to support the results observed in this study, for example, Ramos et al. (2016) reported that plants have a range of features that affect REEs redistribution, especially those related to the presence of apoplastic barriers. Primarily, the apoplastic barriers situated in the roots are the first obstacles for these elements to reach the xylem and, consequently, they hinder their translocation to aboveground plant organs; due to this fact, the contents of REEs found in different plant organs are observed sequentially as roots > stems > leaves (Brioschi et al., 2013). Another study by De Franca et al. (2011) also observed a similar REEs accumulation pattern in plants (*Alsophila sternbergii* and *Pachystroma longifolium*) and citrus species with REE-spiked soils, which is consistent with our results. Likewise, Telesiński et al. (2011) observed that the higher REEs accumulation in plant roots than in stem and leaves is possibly due to relatively low permeability through the endodermis. El-Mahrouk et al. (2019) stated that the metal tolerance of plants and accumulation of metals in plant biomass are species-dependent and clone-dependent. Dos Santos Utmazian et al. (2007) documented plant biomass, metal tolerance, and metal accumulation patterns in roots and leaves in twenty different clones of willow species. Furthermore, de Oliveira et al. (2015) demonstrated that inside the plants, most REEs are bonded to cell walls and some elements cross the cell membrane, accumulate in organelles

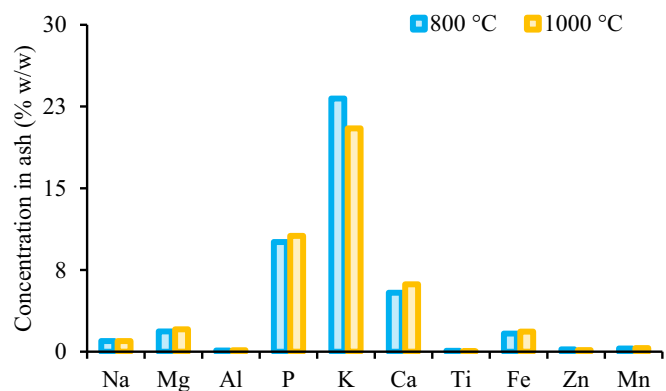


Fig. 2. Concentrations of non-rare earth elements in the *Salix* combustion ashes prepared 800 °C and 1000 °C.



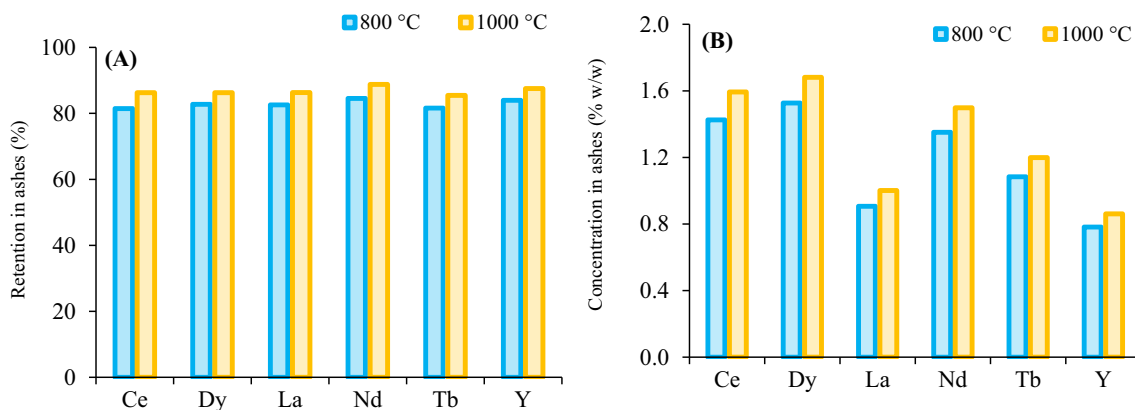


Fig. 3. Retention A) and B) concentrations of the REEs in the *Salix* combustion ashes at two temperatures 800 °C and 1000 °C.

as crystals such as “REEs oxalates” or even in root cortical tissues, which also avoids translocation to plant shoots. Moreover, roots generally exude a wide range of metabolites including organic acids, amino acids, phenolics, sugars, and other compounds that can accelerate heavy metal uptake via the root hairs (Walker et al., 2003). Higher REE concentration in roots indicates that the absorbed elements have probably been subjected to several precipitation complexation and inactivation processes in their root tissues which enables them to translocate elements to the shoots (Souri et al., 2019). Nevertheless, the metal accumulation in plants generally depends on the plant species/cultivars abilities, the amount and chemical form of metal, the presence of other elements causing synergistic or antagonistic interactions, pH level, redox potential, and conductivity of soil/water (Drzewiecka et al., 2012).

Phytoremediation potential of different willow (*Salix* spp.) populations has been investigated extensively by several researchers under heavy metals polluted environments, but little attention has been paid concerning REEs. For example, Salam et al. (2019) has investigated phytoremediation potential of four *Salix* varieties (*S. Schwerin*, *S. myrsinifolia*, Klara and Karin) in a greenhouse under Cu and Zn contaminated soils and observed Klara as an effective candidate for phytoextraction. Cao et al. (2018) has tested the phytoremediation ability of seven willow species/clones, (*S. jiangsuensis* “J172”, *S. integra* “Yizhibi”, *S. mongolica*, and four clones of *S. matsudana* (clone IDs 14, 25, 89, and 102) under Cu stress in a hydroponic experiment and reported that *S. matsudana* 89 and *S. matsudana* 25 have high Cu

accumulation capacity than the other species. Wang et al. (2016) examined the phytoremediation potential of three *Salix* species (*S. fragilis*, *S. matsudana* and *S. babylonica*) under Cd, Cu, Pb and Zn stress hydroponically and reported that *S. fragilis* is more susceptible to heavy metals pollution.

Phytoremediation capacity is strongly associated with tissue metal accumulation and biomass production (Yang et al., 2014). To determine phytoextraction or phytostabilization potential, it is essential to examine the allocation of metals between root (below ground) and shoot (above-ground) (Yang et al., 2020). Here, results revealed poor translocations of REE for all *Salix* in all treatments. Based on REE concentration, the highest TF was found in treatment T1 for species V2 (Nd: 0.26; Dy: 0.23) while in treatment T3 the TF of species V2 varied between 0.01 and 0.08 (Table 5). On the other hand, REE accumulation-based TF’ was found to be highest (>1) in species V2 in treatment T3 for Y (4.29) while overall TF’ of REE was observed (<1) in all *Salix* for treatments T1–T3 (Table 5). TF’ values increased in *Salix* for most of the metals than TF, since TF’ is generally considered a better indicator for phytoextraction which is estimated based on biomass production (Redovniković et al., 2017; Wu et al., 2010). Yuan et al. (2018) reported that vascular plants generally have a low TF of REEs. Similar low REEs TF (0.04–0.09) of various forest plants (*Pinus sylvestris*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Betula alba*, *Deschampsia flexuosa*, *Polytrichum* sp) has also been documented in northwestern Germany. Liu et al. (2021) observed TF values for a crop plant (*Boehmeria nivea* L.) in the range (0.01–0.16) under different doses of REE (0–800 μmol/L) hydroponically. Bioconcentration factor (BF) values (Table 5) were observed (<1) for all *Salix* (La: 0.002–0.01) in treatment T2. In treatment T3, BF values for all *Salix* ranged from (La: 0–0.01; Y: 0.002–0.005; Ce: 0.003–0.009; Nd: 0.003–0.01; Tb: 0.002–0.012 and Dy: 0.003–0.007). Carpenter et al. (2015) observed BF of *Asclepias syriaca* L., *Desmodium canadense* (L.) DC., *Panicum virgatum* L.) and two crop species (*Raphanus sativus* L., *Solanum lycopersicum* L.) ranging from 0.001–0.27 in REE dose-response experiments under growth chamber conditions.

This poor translocation could be due to reduction of stomatal conductance and the low transpiration rate of *Salix* caused by REE stress (Zhang et al., 2017) or the preferential storage of REE in the root cortex and cell vacuoles (Ramana et al., 2015). The largest retention of REE in *Salix* roots and less translocation to shoots may be explained by the fact that the root was in direct contact with the REEs in the nutrient solution, and the binding of REE to root surfaces, resulted in limited REE translocation to shoots (de Oliveira et al., 2015). REEs also have high affinities to carboxylic groups and can be bound in the apoplast (cell walls), which can serve as a storage pool for polyvalent cations (Yuan et al., 2017). Plants that accumulate high amounts of metals in the roots with minimal effects on growth is desirable characteristic for plants used as phytoremediators, as it suggests tolerance (Covre et al., 2020). Zhiqiang and Zhibiao (2020) reported that plants with both BF and TF values of >1 can be considered as phytoextractors;

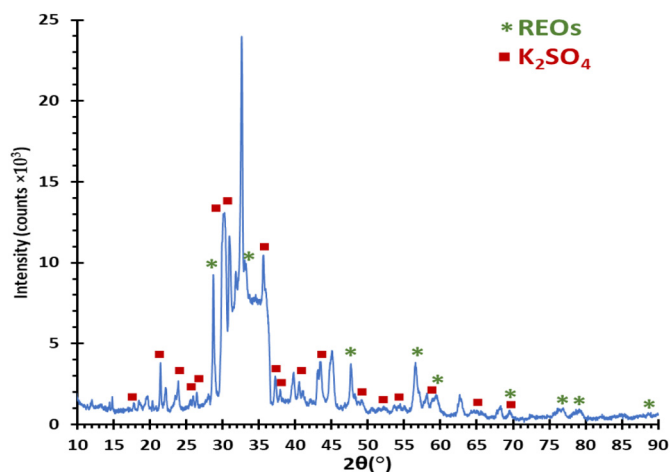


Fig. 4. Powder X-ray diffraction pattern for the ash residue produced during combustion of *Salix* at 800 °C. Peaks for main crystalline phases in the combustion ash, K<sub>2</sub>SO<sub>4</sub> and rare earth oxides (REOs), are indicated.

**Table 5**Mean values of bioconcentration factor (BF) and translocation factor (TF and TF') of *Salix* (V1–V4) under different concentration of REE in treatments (T1–T3).

Treatment	Cultivars	La			Y			Ce			Nd			Tb			Dy		
		BF	TF	TF'	BF	TF	TF'	BF	TF	TF'	BF	TF	TF'	BF	TF	TF'	BF	TF	TF'
T1	V1		0.41	<b>1.63</b>		<b>0.22</b>	<b>0.82</b>		<b>0.21</b>	0.77		0.20	0.74		0.19	<b>0.68</b>		0.18	0.65
	V2		<b>0.21</b>	0.51		0.19	0.74		0.20	<b>0.78</b>		<b>0.26</b>	<b>0.99</b>		0.17	0.66		<b>0.23</b>	<b>0.94</b>
	V3		0.08	0.39		0.12	0.53		0.10	0.53		0.10	0.55		0.10	0.51		0.10	0.53
	V4		0.13	0.41		0.18	0.49		0.18	0.51		0.17	0.48		<b>0.20</b>	0.55		0.16	0.46
T2	V1	<b>0.01</b>	<b>0.01</b>	0.02															
	V2	0.002	<b>0.01</b>	0.02															
	V3	<b>0.01</b>	<b>0.01</b>	<b>0.03</b>															
	V4	<b>0.01</b>	0	0															
T3	V1	<b>0.01</b>	0	0.01	0.004	0	0.01	0.005	0	0	0.005	0	0	0.004	0	0	0.005	0	0.01
	V2	0	<b>0.08</b>	0.01	0.002	<b>0.02</b>	<b>4.29</b>	0.004	<b>0.01</b>	0	0.004	<b>0.01</b>	0	0.002	<b>0.01</b>	0.01	0.004	<b>0.02</b>	0.01
	V3	<b>0.01</b>	0	0	<b>0.005</b>	0	0.01	<b>0.009</b>	0	0	<b>0.01</b>	0	0	<b>0.012</b>	0	0	<b>0.007</b>	0	0
	V4	0	0.02	<b>0.21</b>	0.002	0	0.05	0.003	0	<b>0.01</b>	0.003	0	<b>0.01</b>	0.003	0	<b>0.02</b>	0.003	0	<b>0.03</b>

Bold values indicate the highest BF, TF, and TF' among *Salix*. TF: based on REE concentration and TF': based on REE accumulation.

however, even if plants accumulate higher amounts of REEs in their roots, their shoots can also be useful for phytostabilization (Nadgórska-Socha et al., 2015). Our results demonstrated a remarkable ability of *Salix* to accumulate REE expressively in the roots rather than the shoots, which could be due to their dense root system (McIvor and Desrochers, 2019), and so it could be efficiently used in the remediation of wastewater (rhizofiltration) or contaminated sites to limit metal percolating to the water layer in the ecosystem (phytostabilization). Nevertheless, the duration of the current experiment was very short; therefore, the REE accumulation in roots and translocation to the shoot system are two rather complicated processes and still require more elucidation in the long run.

#### 4.3. Recovery of REE from *Salix* combustion ashes

To our knowledge, this is the first information concerning REE recovery from *Salix* by a combustion process. To date, phytomining research has been focused on hyperaccumulator *Alyssum* species for Ni recovery (Li et al., 2013) hyperaccumulating *Dicranopteris linearis* (Jally et al., 2021) and *Dicranopteris dichotoma* for REE recovery (Chour et al., 2018; Qin et al., 2019). Here, we carried out a preliminary estimation of *Salix* capability for recovering REE by analyzing ashes formed while combusting *Salix*. In this context, the proposed method for accumulation and recovery of REEs by *Salix* can potentially be utilized at phosphogypsum mining sites and wastewater plants globally.

The sum of REE oxide concentrations in the *Salix* ashes at two temperatures ranged from 8.4–9.3%, which is comparable with some of the highest grade REE-ores in the world (Goodenough et al., 2016). However, as indicated by the Powder X-ray diffraction patterns, in this work the REE are mainly present as REOs including CeO<sub>2</sub>, Dy<sub>2</sub>O<sub>3</sub>, Nd<sub>2</sub>O<sub>3</sub>, and Tb<sub>2</sub>O<sub>3</sub> (Fig. 4), whereas in mined ores the REEs commonly occur in other mineral forms, e.g., carbonates, phosphates, and silicates (Goodenough et al., 2016). The presence of REE as oxides in combustion ashes is in agreement with a previous study (Niu et al., 2015). According to Seregin and Dai (2012), the lower REEs oxide concentration limit for the economically feasible recovery of REEs from coal-derived fly ashes is 1000 ppm. Perämäki et al. (2019) reported an average REEs concentration of 530 ppm for conventional biomass combustion fly ashes in Finland and suggested this concentration as economically profitable for REEs recovery. The authors of the study also developed a hydrometallurgical method for separating REEs from combustion ashes (Patent: Väisänen et al., 2013). In this study, the high REE retentions and concentrations were measured from the bottom ashes (Fig. 3), demonstrating the potential of the phytomining concept in the extraction of REE. Moreover, it has been demonstrated that, by combusting the biomass, REEs can be efficiently enriched in bottom ashes, where they were predominantly present as oxides. It must be kept in mind that combustion ash compositions, also including concentrations of elements other than REEs, may vary greatly depending on biomass source, and the composition of the growing medium, i.e., soil or water

(Perämäki et al., 2019). In our study, the *Salix* ashes contained valuable plant macronutrients, particularly P (10%), K (23–20%), and Ca (5–6%) respectively at 800 and 1000 °C (Fig. 2). Thus, after recovering REEs from the ash matrix, the residual fraction has the potential to be utilized as a mineral amendment or inorganic fertilizer to enhance crop productivity (Väättäinen et al., 2011).

## 5. Conclusions

Metal toxicity symptoms were not observed in any *Salix* species or cultivars. Klara produced the highest absolute mean height (170 cm) and mean dry biomass (7.78 g) in all treatments. Considering the REE concentration, *Salix schwerinii* showed the highest concentration of La in leaves (12.40 µg g<sup>-1</sup> DW) in T3 (six-REE treatment) and stem (3.52 µg g<sup>-1</sup> DW) in T2 (single La dose treatment). Similarly, in the roots, Karin showed the highest concentration of La (8404 µg g<sup>-1</sup> DW) in treatment T2. In terms of REE accumulation, La accumulation (45.07 µg plant<sup>-1</sup>) was found to be the highest in Karin leaves in treatment T3 while Klara accumulated the highest amount of La both in stem (20.21 µg plant<sup>-1</sup>) and roots (10,548 µg plant<sup>-1</sup>) in treatment T2. Besides La, all *Salix* also accumulated Y, Nd, Ce, Tb, Dy in small to medium quantities. Overall, the pattern of REE concentrations and accumulation in *Salix* were observed as roots > stem > leaves. TF, TF' and BF were <1 for all *Salix* for each REE except species V2 that revealed TF' > 1 for Y. In the bottom ash formed at 1000 °C, the concentrations of individual REE metals varied between 1 and 1.68% and the total REE content was 8%, similarly, at 800 °C the concentrations varied between 0.90 and 1.52% and the total REE content was 7%. The results demonstrate that *Salix* could be a suitable candidate for the remediation of phosphogypsum mines as well as industrial and mining wastewater sites.

#### CRedit authorship contribution statement

**Muhammad Mohsin:** Investigation, Data curation, Writing and original draft preparation. **Mir Md Abdus Salam:** Data analysis, Writing, review & editing. **Nicole Nawrot:** Writing, review & editing. **Erik Kaipainen:** Investigation, Data curation. **Daniel Lane, Niko Kinnunen, Mikko Heimonen, Arja Tervahauta:** Investigation, Resources, Writing and original draft preparation. **Arja Tervahauta, Ari Pappinen and Suvi Kuittinen:** Conceptualization, Methodology. **Ari Pappinen and Suvi Kuittinen:** Supervision. **Ewa Wojciechowska, Arja Tervahauta, Olli Sippula, Ari Pappinen, Suvi Kuittinen:** Writing, review & editing. **Ari Pappinen:** Funding acquisition.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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